Contents

Lecture

1-3 Current Obstacles to Organ Transplant in Middle Eastern Countries
Faisal A. M. Shaheen,
Muhammad Ziad Souqiyyeh

4-8 The Global Registry: Hope for the Future
Behrooz Broumand

9-12 Public Health Safety and Transplant With Increased-Risk Organs: Striking the Balance
Ramesh Batra, Nitin Katariya, Winston Hewitt, Amit Mathur, Sudhakar Reddy, Adyr Moss, Dorry Segev, Andrew Singer

13-17 Current Status of Organ Transplant in Islamic Countries
Ahad J. Ghods

18-22 Trends in ABO-Incompatible Kidney Transplantation
Atsushi Aikawa, Kazuhide Saito, Kota Takahashi, the Japanese ABO-Incompatible Transplantation Committee

23-29 Eight-Year Outcomes of “The Cairo Kidney Centre Sequential Protocol”
Tarek Fayad, Emad William, Nasr Tarwik, Boulos Habashy, Hazem S. Abou-Youssef, Sameh Shokry, Soha Khalil, Ahmed Morsy, Rashad Barsoum

30-32 Role of Coronary Artery Calcium Score in Identifying Occult Coronary Artery Disease in Patients Evaluated For Deceased-Donor Liver Transplant - A Preliminary Report
Eren Taydas, Mohammad U. Malik, Abhina Dhinra, Stuart Russell, Matthew Chacko, Andrew M. Cameron, Saleh Alqahtani, Ahmet Gurakar

33-36 Organ Transplantation in Tunisia
Aziz El Matri, Taieb Ben Abdallah

37-45 Insulin Gene Therapy for Type 1 Diabetes Mellitus
Andrew M. Handorf, Hans W. Sollinger, Tausif Alam

46-54 How Evolution Tells Us To Induce Allotolerance
Walter Gottlieb Land

55-58 Organ Procurement: Should We Teach Undergraduate Medical and Nursing Students?
Serge Korjian, Yazen Daaboul, Antoine Stephan, Sola Aoun Bahous

Oral Presentation

59-63 Results of Pediatric Liver Transplant: A Single-Center Experience
Gokhan Moray, Tugan Tezcaner, Aydincan Akdur, Figen Ozcan, Atilla Sezgin, Mahir Kirnap, Sedat Yildirim, Gulnaz Arslan, Mehmet Haberal
<table>
<thead>
<tr>
<th>Page Range</th>
<th>Title</th>
<th>Authors</th>
</tr>
</thead>
<tbody>
<tr>
<td>64-70</td>
<td>Major Vascular Complications in Living-Donor Liver Transplant Recipients: Single Center Team Experience</td>
<td>Refaat Kamel, Yasser Hatata, Mohamed Taha, Karim Hosny, Ayman Amin</td>
</tr>
<tr>
<td>71-74</td>
<td>Treatment of Biliary Complications After Liver Transplant: Results of a Single Center</td>
<td>Sedat Yildirim, Ebru Hatice Ayvazoglu Soy, Aydincan Akdur, Mahir Kirnap, Fatih Boyvat, Feza Karakayali, Adnan Torgay, Gokhan Moray, Mehmet Haberal</td>
</tr>
<tr>
<td>75-82</td>
<td>Contrast Patterns of Cytomegalovirus and Epstein-Barr Virus Infection in Pediatric Living-Donor Liver Transplant Recipients</td>
<td>Hanaa Nafady-Hego, Hamed Elgendy, Shinji Uemoto</td>
</tr>
<tr>
<td>83-89</td>
<td>IL-17 mRNA Expression and Cytomegalovirus Infection in Liver Transplant Patients</td>
<td>Afsoon Afshari, Ramin Yaghobi, Mohammad Hossein Karimi, Mojtaba Darbouy, Negar Azarpira, Bita Geramizadeh, Seyed Ali Malek-Hosseini, Saman Nikeghbalian</td>
</tr>
<tr>
<td>90-94</td>
<td>Travel for Transplantation in Iran: Pros and Cons Regarding Iranian Model</td>
<td>Shahrzad Ossareh, Behrooz Broumand</td>
</tr>
<tr>
<td>95-99</td>
<td>An Analysis of Outcomes of Liver Retransplant in Adults: 12-Year’s Single-Center Experience</td>
<td>Mohamed Rabei Abdelfattah, Mohammed Al-Sebayel, Dieter Broering</td>
</tr>
<tr>
<td>100-107</td>
<td>Outcome of Critically-Ill Children After Living-Donor Liver Transplant</td>
<td>Hamed Elgendy, Walid M. El Moghazy, Hanaa Nafady-Hego, Shinji Uemoto</td>
</tr>
<tr>
<td>117-123</td>
<td>Human Leukocyte Antigen-DR Mismatched Pediatric Renal Transplant: Patient and Graft Outcome With Different Kidney Donor Sources</td>
<td>Torki Al-Otaibi, Osama Gheith, Ahmed Mosaad, M.R. Naryanan Nampoory, Medhat Halim, Tarek Said, Prasad Nair</td>
</tr>
<tr>
<td>124-126</td>
<td>Results of Liver Transplant in Elderly Patients: A Single Center Experience</td>
<td>Aydincan Akdur, Cihan Fidan, Ebru Ayvazoglu Soy, Mahir Kirnap, Feza Yarbug Karakayali, Adnan Torgay, Sedat Yildirim, Gokhan Moray, Mehmet Haberal</td>
</tr>
</tbody>
</table>
**133-138** Hepatitis B- and Hepatitis D-Virus–Related Liver Transplant: Single-Center Data
Serkan Öcal, Murat Korkmaz, Özgür Harmancı, Fatih Ensaroğlu, Aydıncan Akdur, Haldun Selçuk, Gökhan Moray, Mehmet Haberal

**159-164** Progression of Hepatic Histopathology in Kidney Transplant Recipients With Chronic Hepatitis C Virus Infection and Effect of Immunosuppression on the Course of Hepatitis C Virus Infection
Murat Korkmaz, Sevgül Faki, Serkan Öcal, Özgür Harmancı, Haldun Selçuk, Mehmet Haberal

**139-144** Predictors of Tumor-Free Survival After Liver Transplant in Patient With Hepatocellular Carcinoma
Alireza Shamsaeefar, Saman Nikeghbalian, Kourosh Kazemi, Saivash Gholami, Nasrin Motazedian, Nadia Motazedian, Mohammad Ebrahim Fallahzadeh, Maryam Moini, Bita Gramizadeh, Seyed Ali Malekhosseini

**145-147** Liver and Kidney Transplant in Primary Hyperoxaluria: A Single Center Experience
Gökhan Moray, Tugan Tezcaner, Figen Özçay, Esra Baskın, Aydıncan Akdur, Mahir Kırnap, Sedat Yıldırım, Gülnaz Arslan, Seyed Ali Malekhosseini

**148-155** Benefits of Transplant Procurement Management (TPM) Specialized Training on Professional Competence Development and Career Evolutions of Health Care Workers in Organ Donation and Transplantation
Melania G. Istrate, Tyler R. Harrison, Ricard Valero, Susan E. Morgan, Gloria Páez, Quan Zhou, Gábor Rébék-Nagy, Martí Manyalich

**156-158** Management of BK Virus Nephropathy in Kidney Transplant Recipients at the Royal Hospital - Clinical Audit - Oman
Fatma Al-Raisi, Nabil Moltsin, Pramod Kamble

**157-176** T-Regulatory Cells in Chronic Rejection Versus Stable Grafts
Fatima Al-Wedaie, Eman Farid, Khaled Tabbara, Amgad E. El-Agroudy, Sumaya M. Al-Ghareeb

**177-182** Spectrum of Histopathologic Diagnosis of Lymph Node Biopsies After Liver and Kidney Transplant
Eylem Akar Özkan, B. Handan Özdemir, E. Şebnem Ayva, Funda Gerçeker, Fatih Boyvat, Mehmet Haberal

**183-187** Bone Marrow Involvement by Lymphoproliferative Disorders After Solid-Organ Transplant
Eylem Akar Özkın, B. Handan Özdemir, Edu Yılmaz Akçay, Aysen Terzi, Sema Karakuş, Mehmet Haberal

**188-192** The Effect of Pretransplant Chronic Hepatitis C Virus Infection Treatment on Graft and Patient Survival in Renal Transplant Recipients
Murat Korkmaz, Sevgül Faki, Serkan Öcal, Özgür Harmancı, Fatih Ensaroğlu, Haldun Selçuk, Mehmet Haberal
193-196 Panel Reactive Antibodies in Predicting Hepatitis C Virus Treatment Outcome in Kidney Transplant Candidates
Serkan Öcal, Özgür Harmancı, Murat Korkmaz, Fatih Ensaroğlu, Turan Çolak, Halidun Selçuk, Gökhân Moray, Mehmet Haberal

197-200 Polymorphism of the CYP3A5 Gene and Its Effect on Tacrolimus Blood Level
Sreeja S. Nair, Sreeja Sarasamma, Noble Gracious, Jacob George, Thekkumkara Surendran Nair Anish, Reghunathan Radhakrishnan

201-206 Efficacy of Immunoabsorption To Reduce Donor-Specific Alloantibodies in Kidney-Transplant Candidates
Lionel Rostaing, Nicolas Congy, Alice Aarnink, Sébastien Maggioni, Asma Allal, Federico Sallusto, Xavier Game, Nassim Kamar

Poster Presentation

207-213 Oxidative Stress in Kidney Transplant Biopsies
Avneesh Kumar, Abdul Hammad, Ajay K. Sharma, Frank Mc-Cardle, Rana Rustom, Steve E. Christmas

214-218 A 10-Year Experience of Tuberculosis in Solid-Organ Transplant Recipients
Gaye Ulubay, Elif Küpeli, Ozlem Duvenci Birben, Emine Pinar Seyfettin, Mustafa Ilgaz Doğrul, Aylin Özsancak Uğurlu, Füsun Öner Eyüboğlu, Mehmet Haberal

219-222 Papanicolaou Smear Findings in Solid-Organ Transplant Recipients Compared With Normal Subjects According to the Bethesda 2001 System
Alev Ok Atılgan, Meriğ Tepeoğlu, A. Nihan Haberal, Elif Durukan, Esra Kuşcu, Mehmet Haberal

223-227 Long-Term Risk of Pulmonary Embolism in Solid-Organ Transplant Recipients
Elif Küpeli, Gaye Ulubay, Ilgaz Doğrul, Özlem Birben, Pınar Seyfettin, Aylin Özsancak Uğurlu, Füsun Öner Eyüboğlu, Mehmet Haberal

228-230 Effects of Different Trends on the Development and Outcome of Early Kidney Allograft Dysfunction
Viktor K. Denisov, Vadim V. Zakharov, Eugeny V. Onishchenko, Tatyana S. Golubova, Yana G. Mitsuk, Olga V. Zakharova

231-234 Use of Biological Prosthesis in a Patient With Kidney and Pancreas Transplant and a Giant Incisional Hernia: Case Report
Umit Ozcçelik, Halime Cevik, Huseyin Yuce Bircan, Alp Demirag

235-241 Assessment of Myocardial Mechanics in Patients with End-Stage Renal Disease and Renal Transplant Recipients Using Speckle Tracking Echocardiography
Bahar Pirat, Huseyin Bozbas, Valide Simsek, L. Elif Sade, Burak Sayın, Haldun Muderrisoglu, Mehmet Haberal

242-246 Acute Cardiac Tamponade: An Unusual Cause of Acute Renal Failure in a Renal Transplant Recipient
Naryanan Nampoory, Osama Gheith, Torki Al-Otaibi, Medhat Halim, Prasad Nair, Tarek Said, Ahmed Mosaad, Zakareya Al-Sayed, Ayman Alsayed, Jude Yagan

247-250 Effects of Hyperuricemia on Renal Function in Pediatric Renal Transplant Recipients
Cihan Fidan, Aslı Kantar, Esra Baskın, Kaan Gülleroğlu, Aydance Akdur, Gökhân Moray, Mehmet Haberal
251-255 A Single-Center Experience of Overseas Kidney Transplant for Immunologically High-Risk Patients
Cheol Woong Jung, Kwan Tae Park, Heungman Jun, Su Yeon Kim, Su Jin Kim, Myung-Gyu Kim, Sang-Kyung Jo, Wonyong Cho, Hyoung Kyu Kim

256-258 Living-Donor Kidney Transplant From Hepatitis B Surface Antigen-Positive Donors to Hepatitis B Antibody-Positive Recipients Without Hepatitis B Immunoglobulin Prophylaxis in an Endemic Country
Heungman Jun, Myung-Gyu Kim, Kwan Tae Park, Cheol Woong Jung

259-262 Evaluation of Late Antibody-Mediated Rejection (C4d-Mediated Rejection): A Single-Center Experience
Erhan Tatar, Adam Uslu, Cenk Simsek, Emver Vardar

263-265 Bone Marrow Biopsy in Patients With Renal Transplant: Spectrum of Findings and Diagnostic Use
Pelin Börcek, B. Handan Özdemir, Eylem Akar Özkân, F. Zeynep Taşlıca, Mehmet Haberal

266-268 The Role of a Single Center Experience in Azerbaijan's Nephrology Field
Hikmet Ismayilov, Shahin Qadimov, Kamil Muslimov, Khanbaba Huseynov, Fariz Babayev

269-272 Association Between Panel Reactive Antibody and Antiendothelial Cell Antibody Positivity in Kidney Transplant Patients
Bilkay Baştürk, Bircan Kantaroğlu, Z. Ayşül Noyan, Sedat Yıldırım, Çağla Sarıtürk

273-275 Fatal Outcome After Renal Transplant in a Pediatric Patient With Noonan Syndrome
Coskun Araz, Ebüri Kaval, Adnan Torgay, Gokhan Moray, Mehmet Haberal

276-279 Dermal Tophus: A Complication of Gout in a Kidney Transplant Recipient
Ebri Hatice Ayvazoğlu Soy, Emre Karakaya, Arzu Karatas Togral, Aydincan Akdar, Gokhan Moray, Mehmet Haberal

280-283 Prevalence and Outcome of Herpes Zoster Infection in Renal Transplant Recipients
Mahir Kırnaş, Aydincan Akdar, Hatice Ebri Ayvazoğlu Soy, Hande Arslan, Sedat Yıldırım, Gökhan Moray, Mehmet Haberal

284-285 Minimal-Access Kidney Transplant
Mohie E. Omar

286-289 The 2-Stage Liver Transplant: 3 Clinical Scenarios
Ender Gedik, Murat Büçakçoğlu, Emrah Otan, Hüseyin İlksen Toprak, Burak Işık, Cemalettin Aydın, Cüneyt Kayaalp, Sezai Yılmaz

290-293 Extracorporeal Membrane Oxygenation After Living-Related Liver Transplant
Ender Gedik, Muhammet Reha Çelik, Emrah Otan, Olcay Murat Dişi, Nevzat Erdil, Yaşar Bayındır, Ramazan Kuţlu, Sezai Yılmaz

294-300 Blood Glucose Regulation During Living-Donor Liver Transplant Surgery
Ender Gedik, Hüseyin İlksen Toprak, Erding Koca, Taylan Şahin, Ülkü Özgül, Mehmet Özcan Ersoy
301-305 Accuracy of Continuous Noninvasive Arterial Pressure Monitoring in Living-Liver Donors During Transplantation
Coskun Araz, Pinar Zeyneloglu, Arash Pirat, Nukhet Veziroglu, Aynur Camkiran Firat, Gulnaz Arslan

306-311 Epstein-Barr Virus DNAemia in Iranian Liver Transplant Recipients and Assessment of Its Variation in Posttransplant Lymphoproliferative Disorder Patients by Quantitative Polymerase Chain Reaction Assay
Marzieh Jamalidoust, Bita Geramizadeh, Sina Saadat, Nasr Hassanpour, Nahid Malekmansour, Sadaf Asaie, Nasrin Aliabadi, Saman Nikeshbalian, Maziar Ziaeyan

312-314 Evaluation of Safety and Efficacy of Liver Biopsy Following Liver Transplant
Mahir Kıranp, Aydün Özdemin, Ali Harman, Sedat Yıldırım, Göksal Moray, Mehmet Haberal

315-317 Efficacy of Cell Saver Use in Living-Donor Liver Transplant
Mahir Kıranp, Tuğan Tezcaner, Hatice Erden Ayvazoğlu Soy, Aydün Özdemin, Sedat Yıldırım, Adnan Torgay, Göksal Moray, Mehmet Haberal

318-322 Synthetic Graft for Reconstruction of Middle Hepatic Vein Tributaries in Living-Donor Liver Transplant
Refaat Kamel, Yasser Hatata, Karim Hosny, Khaled Amer, Mohamed Taha

323-326 Seizure as a Neurologic Complication After Liver Transplant
Eda Derle, Seda Kibaroğlu, Ruhsen Öcal, Mahir Kıranp, Mümüre Kılıç, Sibel Benli, Mehmet Haberal

327-330 Neurologic Complications After Liver Transplant: Experience at a Single Center
Eda Derle, Seda Kibaroğlu, Ruhsen Öcal, Mahir Kıranp, Ufuk Can, Sibel Benli, Mehmet Haberal

331-334 Role of Bronchoalveolar Lavage in Diagnosis of Fungal Infections in Liver Transplant Recipients
Merih Tepeoğlu, Aynur Camkiran Firat, Sekiz Kaplan, Adnan Torgay, Arash Pirat, Mehmet Haberal, Gulnaz Arslan

335-339 Postoperative Effects of Intraoperative Hyperglycemia in Liver Transplant Patients
Özgür Kömürcü, Aynur Camkiran Firat, Şerife Kaplan, Adnan Torgay, Arash Pirat, Mehmet Haberal, Gulnaz Arslan

340-345 Postoperative Pulmonary Complications in Living-Liver Donors: A Retrospective Analysis of 188 Patients
Gaye Ulubay, Balam Er Dedekarginoglu, Elif Kupeli, Ozlem Salman Sever, Füsun Öner Eyüboğlu, Mehmet Haberal

346-351 A Single-Center Retrospective Clinicopathologic Study of Endomyocardial Biopsies After Heart Transplant at Baskent University Hospital in Ankara, 1993-2014
Ayşen Terzi, Atilla Sezgin, Zeynep Tunca, Ebru Deniz, Ebru Şebnem Ayva, Nihan Haberal Reyhan, Haldun Müderrisoğlu, Binnaz Handan Özdemir

352-355 Invasive Pulmonary Aspergillosis in Heart Transplant Recipients
Elif Küpeli, Gaye Ulubay, Sevil Bayram Akkurt, Füsun Öner Eyüboğlu, Atilla Sezgin

356-360 Long-Term Pulmonary Infections in Heart Transplant Recipients
Elif Küpeli, Gaye Ulubay, Esma Sevil Akkurt, Füsun Öner Eyüboğlu, Atilla Sezgin
361-365 Gelatin for Purification and Proliferation of Primary Keratinocyte Culture for Use in Chronic Wounds and Burns
Marjan Rahsaz, Bita Geramizadeh, Maryam Kaviani, Saeed Marzban

366-370 Considerations in the Improvement of Human Epidermal Keratinocyte Culture In Vitro
Maryam Kaviani, Bita Geramizadeh, Marjan Rahsaz, Saeed Marzban

371-376 Human Leukocyte Antigen G and Renal Allograft Transplant
Eman Farid, Fatima Al-Wedaie, Khaled Tabbara, Amgad E. El-Agroudy, Sumaya M. Al-Ghareeb

377-382 Protective Effect of Artemisia asiatica Extract Against Renal Ischemia-Reperfusion Injury in Mice
Hyuk Jai Jang, Eui Kyun Jeong, Seong Su Kim, Ji Hwan Lee, Mi Young Oh, Ki Sung Kang, Hak Cheol Kwan, Kyung Il Song, Dae Woon Eom, Duck Jong Han

383-387 Human Leukocyte Antigen Cw7-Mediated Protection Against Polyoma BK virus in Renal Transplant Recipients Who Received Grafts From Antigen-Positive Donors
Osama Gheith, Torki Al-Otaibi, Zakaria Zakaria, Medhat Abdel Halim, Naryanan Nampoory
Preface

Mehmet Haberal, MD, FACS (Hon), FICS (Hon), FASA (Hon)
Founder and President, Middle East Society for Organ Transplantation
Chair, 14th Congress of the Middle East Society for Organ Transplantation

Seyed Ali Malek Hosseini, MD
Immediate Past President, Middle East Society for Organ Transplantation

The 14th Congress of the Middle East Society for Organ Transplantation and the 5th Middle East Transplant Games was held on September 10-13, 2014, in Istanbul. It was once again our great honor to host the MESOT Congress in Turkey after many years of it being held in various countries of the Middle East.

I founded MESOT in 1987 with the support and encouragement of scientists in the region, who recognized the need for a scientific forum for discussion of problems related to the field of transplantation, including medical, social and legal aspect that we faced on national, regional and international levels. Our goals were to promote and encourage education, research and cooperation in the field of organ transplantation for the purpose of advancing the art and science of transplantation, and to serve the patients of this region through the application of new knowledge and medical and technical advances. Over time, our efforts to promote and encourage research and clinical applications related to transplantation, resulted in the growth of our society, and with the development of important project, such as the MESOT Fellowship Program, we were able to move beyond national borders and provide medical care at the highest levels to nationals of all countries in the Near and Middle East, Mid-Asia, and North Africa.

The growth of organ and tissue transplantation in the Middle East has been truly inspirational. However, it cannot be denied that there are many issues left to be resolved on a global scale. Of these, the issue of organ trade and transplant tourism is perhaps the most pressing. In this light, we thought it highly appropriate to establish the theme of the 2014 MESOT Congress as “Organ Donation and Ethical Conduct.”

In keeping with previous years, we tried to provide an innovative and comprehensive overview of the latest research developments in the fields of experimental and clinical tissue and organ transplantation, as well as the recent social and ethical issues that impact the field. A total of 81 Invited Lectures, 171 Oral and 251 Poster Presentations were scheduled for the congress and leading scientists, scholars, and young faculty members the world over had the opportunity to present their work, share ideas, and discuss their research. Their invaluable contributions regarding new techniques and original insights into current research, as well as possibilities for future developments and directions, created the ideal platform for dynamic and stimulating intellectual exchanges.

The 5th MESOT Transplant Games, which took place during the Congress, was met with great interest. Close to 100 participants from 7 countries competed in running, swimming, bowling and table tennis. This occasion for transplant athletes to participate in sporting events was also an opportunity to demonstrate the physical success of transplant surgery and raise awareness of the need to increase organ donation.

With close to 1000 delegates from 55 countries, the 14th Congress of the MESOT meeting was once again an inspiring and dynamic experience. We are grateful to the members of the scientific, program and local organizing committees for their hard work and dedication, and we look forward to the next MESOT Congress for the continuation of this success.
Photograph taken during the Opening Ceremony of the 14th MESOT Congress, September 10, 2014. Left to right: Aziz El-Matri (Past President, MESOT), Ahad Ghods (Past President, MESOT), Antoine Barbari (General Secretary MESOT), Anwar Naqvi (Past President, MESOT), Marwan Masri (Past President, MESOT), Antoine Stephan (Past President, MESOT), Mehmet Haberal (Founder, Founder President, and President, MESOT), Roy Calne, Seyed Ali Malek-Hosseini (Past President, MESOT), Faisal Shaheen (Past President, MESOT), Philip O’Connell (President, TTS), Jeremy Chapman (Past President, TTS), Adibul Hasan Rizvi (Past President, MESOT).

MESOT Gala Dinner, Awards Ceremony, September 12, 2014

Opening Ceremony of the 14th MESOT Congress, September 10, 2014. Left to right, Seyed Ali Malek-Hosseini (Past President, MESOT), Mehmet Haberal (Founder, Founder President, and President, MESOT), Philip O’Connell (President, TTS).
Current Obstacles to Organ Transplant in Middle Eastern Countries

Faissal A. M. Shaheen, Muhammad Ziad Souqiyyeh

Abstract

The Middle Eastern map includes all the Arab countries, Iran, Turkey, Pakistan, and countries of Central Asia. There are common features of organ transplant in these countries such as inadequate preventive medicine, uneven health infrastructure, poor awareness of the medical community and public about the importance of organ donation and transplant, high level of ethnicity, poor government support of organ transplant, and political unrest. In addition, there is inadequate team spirit among transplant physicians, lack of planning for organ procurement and transplant centers, and lack of effective health insurance. Living-donor organ transplant is the most widely practiced type of transplant in the Middle East. Deceased-donor organ donation is not used properly because of continued debate in the medical community about the concept of death according to neurologic criteria (brain death) and inadequate awareness of the public about the importance of organ donation and transplant in many countries in this region. Continuous work is needed to provide solutions to overcome the current obstacles.

Key words: Donation, Organ transplant, Living-donor, Deceased-donor

Introduction

The Middle Eastern map includes all the Arab countries, Iran, Turkey, Pakistan, and countries of Central Asia. The population in these countries exceeds 600 million, and the countries have a unique location between Europe, Africa, and Asia. There have been several publications addressing the activities of organ donation and transplant in this region.1-3

There have been several recent resolutions of the World Health Organization and transplant community that affected organ transplant around the world and in the Middle East. The resolutions included combating transplant tourism (Declaration of Istanbul) and calling on members of the World Health Organization to adopt the principle of self-sufficiency of organ donation and transplant, including preventive measures for diseases that may cause organ failure.4

Organ donation and transplant in Middle East countries

There are common features of organ transplant in Middle Eastern countries that include inadequate preventive medicine, uneven health infrastructure, poor awareness in the medical community and public about the importance of organ donation and transplant, high level of ethnicity, poor government support of organ transplant, and political unrest in the region. The lack of team spirit among transplant physicians, inadequate planning for organ procurement and transplant centers, and lack of effective health insurance add more obstacles toward improvement in this field. Therefore, patients in the Middle East seek transplant tourism that has added risk of acquiring infections and other complications and exploitation of donors and recipients. Despite international efforts, transplant tourism has not been abolished, especially in the absence of effective alternatives and weakness of the organ donation and transplant programs.

The waiting lists for organ transplant have increased, and there is a growing gap between
supply and demand for organs in Middle Eastern countries. There is an estimated average 200 patients per million population in need of renal transplant. In addition, there is 10% to 15% annual death rate on dialysis and greater death rate for patients on the waiting lists for liver and heart transplant because of the absence of artificial means to support those patients while awaiting an organ.

**Sources of organ donation in the Middle East**

Living-donor organ donation is the most widely practiced type of donation in the Middle East and includes kidney and partial liver grafts. Living-donor organ donation predominantly is genetically related, but nongenetically related and commercial living-donor organ donation exist.

Deceased-donor organ donation has great potential in the Middle East because of the frequency of accidents. Nevertheless, this source still is not used properly because of continued debate in the medical community about the concept of brain death and inadequate awareness of the public about the importance of organ donation and transplant in many countries in this region. Data about current transplant activities in the Middle Eastern countries were collected in surveys by the Saudi Center for Organ Transplantation and the Middle East Society of Organ Donation and Transplantation (Figures 1-3).5

In addition, deceased-donor organ donation still is not implemented in 25% of Middle Eastern countries despite supportive legislation. There are 3 major factors that must be fulfilled to organize deceased-donor organ donation. These include religious and social acceptance, the presence of legislation for organ donation and transplant, and government support. There is religious acceptance for organ donation in the entire Middle East, but in some countries, there is no religious acceptance of death according to neurologic criteria and its equivalence to legal death. Therefore, legislation still is unavailable or not fully implemented in some countries such as Egypt, Morocco, Syria, Sudan, Qatar, United Arab Emirates, Yemen, and Libya. There are weak health systems and variable infrastructure in most Middle Eastern countries. Organ transplant and treatment of end-stage organ failure are not a priority in most Middle East countries because of the large expense and high technology equipment required. Furthermore, there is inappropriate allocation of resources because of weak government support in many instances.

There are very few organ procurement centers in the Middle East Society for Organ Transplantation (MESOT) countries to supervise the activities of organ donation and transplant at the national level. The Middle East countries lack an active network of
organ sharing except between few countries such as Saudi Arabia, Kuwait, and Qatar.

There are several dominant and distinctive models for practice of organ donation and transplant in the Middle East including the Turkish, Iranian, Pakistani, and Saudi models. All these programs have active living- and deceased-donor organ donation and transplant and have national procurement centers to supervise these activities. They all prohibit organ transplant tourism in their countries. These countries can be an example for other countries in the Middle East for proper practice of organ donation and transplant.

Possible solutions to obstacles for organ donation and transplant in middle east countries

The financial issue for treatment of end-stage renal disease is of utmost importance. Therefore, it may be necessary to adopt a funding system similar to the Pakistani model or medical insurance. Furthermore, organization is important to improve organ donation and transplant in any country, and efforts should be directed to establish national organ procurement centers. There should be encouragement to develop a network for organ sharing between Middle East countries and exchange of information about the experience of national programs (coordination of organ donation, scientific expertise, multicenter studies, and registry of organ failure patients). In addition, there should be consideration for a highly regulated new source of organ donation from living-donor nongenetically related donation.

In conclusion, organ donation and transplant programs in Middle Eastern countries have many obstacles. Continuous work is needed to provide solutions. Examples are available in the Middle East to guide new programs to improve performance.

References

The Global Registry: Hope for the Future

Behrooz Broumand

Abstract

In 2014, there is unanimous agreement that kidney transplant is the optimal treatment for most patients who have end-stage renal failure. Increasing organ shortage is the main obstacle that delays transplant and might even cause death while the patient is on the waiting list for kidney transplant. Many innovations have been proposed to increase the number of organs for transplant in different countries such as increasing awareness about organ donation, based on different cultures and religions. Support of religious and faith leaders exists for procurement of organs for transplant from patients with brain death or circulatory death. In the past decade, use of marginal and expanded-criteria deceased-donor transplant has been very helpful to expand the kidney donor pool. Dual kidney transplant is another procedure that may minimize the waiting list. The 1977 transport of kidneys from Minneapolis to Tehran helped change the life of a 15-year-old girl. At that time, we had the potential to change a life across 2 continents, even though our techniques were new. This should have provided the impetus to develop such a program. Presently, with progress in science, techniques, and organ shipment, it is our responsibility to reach across the globe to change the lives of many more young and adult patients waiting for kidney transplant. There are many countries in which kidneys from patients with brain or cardiac death are being discarded because of the unavailability of a transplant program in these countries, or because these countries have young transplant programs and very limited resources. If a global registry could be organized under the observation of the International Society of Nephrology and The Transplantation Society Sister Transplant Center Program, transplant teams would be able to use kidneys from patients with brain or cardiac death, with strict regulation of organ donation in accordance with World Health Organization guidelines.

Key words: Brain death donor, Cardiac death donor, Transplant commercialism, Transplant tourism, Xenotransplant

Introduction

The idea about transplant existed in ancient Iran. A griffin still exists in Persepolis that shows that the idea of xenotransplant had been present in ancient times (Figure 1). In 2014, there is unanimous agreement that kidney transplant is the optimal treatment for most patients who have end-stage renal failure. Increasing organ shortage is the main obstacle that delays transplant and might even cause death while the patient is on the waiting list for kidney transplant.
treatment for most patients who have end-stage renal failure. Increasing organ shortage is the main obstacle that delays transplant and might even cause death while the patient is on the waiting list for kidney transplant.

Many innovations have been proposed to increase the number of organs for transplant in different countries such as increasing awareness about organ donation, based on the different cultures and religions. Support of religious and faith leaders exists for procurement of organs for transplant from patients with brain death or circulatory death. In the past decade, use of marginal and expanded-criteria deceased-donor transplant has been very helpful to expand the kidney donor pool. Dual kidney transplant is another procedure to minimize the waiting list.

With living-donor kidney allografts, the most important step is to reassure and encourage relatives to donate their kidney. Another helpful policy has been the use of paired kidney donors. In addition, living-unrelated donors constitute transplant commercialism.

Since 1988 in Iran, when deceased-donor transplant was not possible, the government funded regulated living–unrelated-donor transplant, which is being replaced by transplant from brain dead donors. In 2013, forty-eight percent of the transplants in Iran were from brain dead donors. Despite these tactics to provide organs, the waiting list still is expanding.

In July 2013, there has been some promising news that partially may satisfy the shortage of organs for all types of transplant including kidney allografts. The first good news was about the development of a new national policy for organ donation and transplant in China.1,2 The promise was that the new national policy will improve the disturbing situation in China. It was disappointing to observe daily demonstrations of Chinese people in front of Transplant Week in San Francisco in July 2014 asking for help from Participants of the 2014 World Transplant Congress to join and support their struggle to stop “a crime against humanity” such as, slaughtering of bodies of prisoners to provide organs for transplant in China, and that “this barbaric human rights abuse must be stopped” (Figures 2 and 3).

At the Fourteenth Congress of the Middle East Society of Organ Transplantation on September 10, 2014, a pamphlet was circulated by Doctors Against Forced Organ Harvesting to promote ethics in medicine in China. In March 2014, Chinese officials stated that China will continue using organs from prisoners, and organs will be accounted for and entered in the newly designed Computerized Organ Transplant Registry System. These unpleasant facts proved that, regretfully, the first good news was not trustworthy or reliable.

The second promising news was the Human Transplantation Bill of Wales. The Welsh assembly
proposed a new system of presumed consent, in which individuals are presumed to have consented to donate their organs after death unless they have specifically opted out. This bill will become enforced in Wales in 2015, and hopefully this will increase the number of available organs for transplant after 2015.

Increased mortality on the transplant waiting list is a multidimensional disaster. Another extremely disturbing outcome, beyond increased mortality as a result of the organ shortage, is organ trafficking and transplant tourism, which are shameful for the medical profession. The struggle to overcome this unwanted outcome was addressed in the 2008 Istanbul Declaration, which has had continuous follow-up by responsible, dedicated scientists who are involved in transplant.

There are few other solutions considered by physicians involved in transplant to overcome the organ shortage for transplant. In China, part of problem contributing to the shortage of deceased donors is cultural, because brain death criteria are not widely accepted. Global collaboration between scientific, general, and renal health care organizations is required to decrease the organ shortage for transplant and stop transplant tourism and commercialism.

I propose a new project to address the increasing shortage of organs for transplant for the future, based on our experience in Iran during the past 55 years.

In 1968, the first deceased-donor kidney transplant was performed in Shiraz, Iran, and this was the first deceased-donor kidney transplant performed in the Middle East. In the early 1960s, the art of renal replacement therapy was young throughout the world including Iran. Gradually, by expanding dialysis units and the numbers of patients under maintenance hemodialysis, the need for transplant became more obvious.

In the Middle East and Iran during that decade, there were a few trained renal doctors. There were few institutes to train experts in the field. The field of renal replacement therapy was young and unknown to the public. Therefore, the need for renal replacement therapy, dialysis, and transplant was not adequately appreciated, and the pace of expansion of the field of transplant was very slow. Only a few of the patients who had end-stage renal disease and who were being dialyzed in Tehran had adequate financial support and related donors, and those patients often would travel to the United Kingdom for transplant.

There were few patients who had end-stage renal disease and who had transplant performed in Tehran and Shiraz. In 1975, several trained nephrologists, urologists, and general surgeons familiar with kidney transplant returned to Iran. The result was an increased performance of living-related donor transplant per year in 3 universities and Ministry of Health hospitals in Tehran: Dariush Kabir, Beh Avar, and Tajrish Shohada Hospitals.

With the expansion of dialysis units, increased numbers of end-stage renal disease patients, and higher knowledge of the public about the advantages of organ transplant, the demand for kidney transplant gradually increased. The shortage of experts, facilities, and suitable volunteer donors still were major obstacles to meet the demand.

Before the April 5, 2000, the 1985 Brain Death Act was not approved by the Iranian parliament, and there were no transplants from living-unrelated donors; the so-called Regulated living-unrelated donors paid by Iranian government was not yet accepted. From mid-‘s 1975, a committee known as The National Dialysis and Transplant Committee was established, composed of experts from the national blood bank, interested nephrologists, and surgeons. This committee contributed to the expansion of transplant facilities. The human leukocyte antigen profile of all patients undergoing maintenance dialysis was detected by the Iranian national blood bank. Therefore, a small national registry was created.

To expand the transplant program, the committee contacted colleagues at Euro Transplant in Leyden, Holland, and provided them the human leukocyte antigen profiles of patients waiting to be transplanted in Iran. This cooperation had phenomenal outcome. From 1975 to 1980, there were 14 deceased-donor kidneys sent to Tehran, Iran via Euro Transplant. The most impressive was a kidney flown from Hennepin County, Minneapolis via Chicago and Frankfurt, to Tehran. This story was published in the Eugene Register-Guard, April 18, 1977 (Figure 4):
“It’s difficult to find recipients for a donor with his A-B blood type,” a spokesman for the center said. “We have a computer system for finding recipients but none was waiting for this type in the United States.”

“So we called Euro Transplant and made arrangements to ship them to Germany. Dr. Robert Christian Andersen removed the kidneys from the donor about 1 a.m. Wednesday and they were flown out of Chicago about 5 p.m. that day to Frankfurt, Germany.”

“The doctor there did not want to use them because we had shipped them on ice in a preservative solution. Some surgeons don’t like to transplant kidneys that have been on ice very long.”

“So Euro Transplant called Tehran and arranged to send the kidneys there. They reported that a Dr. Nikbin and a team of surgeons transplanted the kidneys into two recipients in operations that took several hours on Thursday.”

Euro Transplant reported the kidneys began functioning immediately. A doctor on the National Dialysis Committee in Iran said 120 persons in Iran were on the waiting list to undergo kidney transplant operations.

Incidentally, I was the doctor from the National Dialysis Committee. At that time, I was in charge of the committee and practicing as a nephrologist at Beh Avar Hospital of the Ministry of Health where the transplant was performed. A photograph showed me in the operating room taking those kidneys out of preservative solution on ice (Figure 5).

When the transplant team in Beh Avar Hospital and the Iranian national blood bank registry were informed that 2 kidneys with ABO group AB+ were flown from Frankfurt to Tehran, a search revealed 2 patients listed in the Iranian national blood bank registry that had ABO group AB+, including a 15-year-old girl who was receiving maintenance dialysis in Kerman, a city in southern Iran, for 11 months.

The 15-year-old girl who had end-stage renal failure was evaluated for transplant. Her father previously had been evaluated for kidney donation to his daughter. The father and daughter were haplotype-identical for human leukocyte antigen but a positive reaction was observed in the mixed lymphocyte culture, so it was decided that the father would not be a suitable donor. We called her to come to Tehran from Kerman, and she arrived at Tehran Mehrabad airport at the same time as the kidney from Frankfurt. Both kidney and recipient moved quickly to Beh Avar Hospital and the transplant was performed successfully by an urologist and vascular surgeon, with immediate postoperative recovery of renal function. The recipient was maintained only with prednisolone and azathioprine.

After transplant, the 15-year-old recipient was followed regularly in my office. Her main problem was hypertension, which was poorly controlled with methyldopa. Her serum creatinine was < 1.5 mg/dL until 1983, when it started to rise slowly. In 1985, at 8 years after kidney transplant, the recipient developed chest pain. She was evaluated and had 3-vessel coronary artery disease that led to her death 14 years after transplant, when her serum creatinine was 4.3 mg/dL.
This recipient had been transplanted with a kidney after almost 70 hours of cold ischemia. The kidney had been rejected by a German transplant team because the transportation was not ideal. Although we had much less facilities and resources in Iran, the transplant was performed with a good outcome.

I am reporting this unusual case to cast hope for the future. The lesson to be learned from the journey of that kidney is that possibilities would be much greater when we start worldwide cooperation and a global registry. Many more patients across the world would benefit from cross-continent transplants, and we could decrease the organ shortage problem, even if just slightly.

At present there are many countries in which kidneys from patients who die from brain or cardiac death are discarded because of the unavailability of a transplant program in these countries, or because they have young transplant programs and very limited resources. If a global registry could be organized under the observation of the International Society of Nephrology and The Transplantation Society Sister Transplant Center Program, the transplant teams would be able to use these kidneys, with strict regulation of organ donation in accordance with World Health Organization guidelines.

I am certain that it would take time to develop such a system adequately. This may seem like a cumbersome initiative, with an unknown or possibly small effect on the shortage of kidneys for transplant. However, when fully developed, it would have a tremendous effect on the lives of end-stage renal disease patients across the globe.

Another important aspect of this plan would be that it will leave no room for transplant commercialism and tourism, and would halt organ trafficking. Our experience in Iran during the past 3 decades showed that increasing the numbers of transplants from brain death donors was an effective barrier to the sale of kidneys.7

In addition, globalization of the organ registry and transplant may help reach underserved and underdeveloped programs that are run by our dedicated colleagues across the globe in areas with poor facilities and resources. This, in turn, would help improve reciprocity, education, and growth of these programs across the globe and increase organ procurement and efficiency.

The 1977 transport of the kidneys from Minneapolis to Tehran helped to change the life of a 15-year-old girl and provided hope for that young girl. At that time, we were so inexperienced in our techniques, but we had the potential to change a life across 2 continents. This should have provided the impetus to develop such a program. Now that science, techniques, and organ shipment have progressed, it is our responsibility to advance programs across the globe to change the lives of many more young and adult patients who need a renal transplant.

References

Public Health Safety and Transplant With Increased-Risk Organs: Striking the Balance

Ramesh Batra,1 Nitin Katariya,1 Winston Hewitt,1 Amit Mathur,1 Sudhakar Reddy,1 Adyr Moss,1 Dorry Segev,2 Andrew Singer1

Abstract
There is significant variability amongst transplant centers, Organ Procurement Organizations (OPO), members of public, and patients about organs from Public Health Service increased risk donors. This has therefore required regulatory bodies like Centers for Disease Control and Prevention to formulate policies for transplant centers and OPOs to minimize risk of infectious transmission to recipients of solid-organ transplants from such donors.

Key words: Antibody test, Hepatitis B virus, Hepatitis C virus, Human immunodeficiency virus, Nucleic acid test, Outcomes, Survival

Introduction
Since its inception, transplant medicine has evolved tremendously in selection and treatment of donor and recipients for transplant. The donor selection criteria have expanded from experiences of transplant centers across the world to meet the growing need for organ transplant. In the past decade (2002-2012), there has been a progressive increase of 3000 more solid-organ transplants in the United States (11% increase), but during this decade the list of those awaiting transplant increased by 40000 more patients (33%). This growing divergence between the number of transplants and number of patients listed awaiting transplant has served as an impetus to expand organ acceptance criteria. These efforts have required balancing the risk of lower donor organ quality with the benefit of minimized waiting list mortality and decreased waiting times. Transplants from expanded criteria donors and donation after cardiac death donors have progressively increased to bridge the gap, and transplant centers have learned how to optimize use of these organs to further increase the expanded donor criteria. In addition to these efforts, there was a subset of organ donors who frequently were not considered for donation: donors deemed at higher risk of infectious transmission through transplant.

The Centers for Disease Control and Prevention (CDC) has endeavored to minimize human immunodeficiency virus (HIV) transmission with recommendations in 1983, 1985, and 1994. In 2013, the Public Health Service (PHS) expanded these guidelines to include screening for hepatitis B virus (HBV) and hepatitis C virus (HCV) in addition to HIV for increased-risk donors.

The 1994, CDC guidelines defined high-risk donors for HIV transmission based on detailed medical and social history from the next of kin and the pre- or posttransfusion blood specimen testing when there was no evidence of hemodilution (Table 1). These guidelines for donor screening were revised in June 2013 by PHS to include the risk of HCV and HBV infection and transmission, in addition to HIV. The PHS guidelines also reduced the time of increased scrutiny between high-risk behaviors to organ donation from 5 years to 12 months.

Serologic testing and window period
Organ procurement organizations (OPOs) are required by United Network for Organ Sharing
(UNOS) to perform serologic antibody testing. However, the accuracy of the testing is limited by the inability to detect infections in the window period, which is the period between the acquisition of infection and antibody formation. Nucleic acid testing (NAT) reduces this window period markedly and increases the sensitivity of screening for HIV, HBV, and HCV (Table 2).4

Humar and associates performed a risk-benefit analysis of NAT screening for organ donors, and they concluded that NAT offered an advantage to minimize the risk of HIV, HCV, and HBV transmission and increase organ use by screening only the high-risk donors.4 This benefit was greatest with respect to HCV infection due to the substantial reduction in the period. There was insufficient evidence to recommend routine NAT screening for HIV, HCV, and HBV in all potential organ donors. Furthermore, universal NAT testing potentially could be counterproductive for average-risk donors (donors with no identified behavioral risk factors) due to the disadvantages that might include false positive results, increased costs, and organ loss due to withdrawal of consent, logistics, and donor instability.

Table 1. Centers for Disease Control and Prevention High-Risk Donor Criteria

<table>
<thead>
<tr>
<th>1994 CDC High-Risk Donors Guidelines</th>
<th>2013 PHS Increased-Risk Donors Guidelines</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Men who have had sex with another man in the preceding 5 years</td>
<td>1. Men who have had sex with another man in the preceding 12 months</td>
</tr>
<tr>
<td>2. Persons who report nonmedical intravenous, intramuscular, or subcutaneous injection of drugs in the preceding 5 years</td>
<td>2. Persons who report nonmedical intravenous, intramuscular, or subcutaneous injection of drugs in the preceding 12 months</td>
</tr>
<tr>
<td>3. Persons who have engaged in sex in exchange for money or drugs in the preceding 5 years</td>
<td>3. Persons who have engaged in sex in exchange for money or drugs in the preceding 12 months</td>
</tr>
<tr>
<td>4. Persons with hemophilia or related clotting disorders who have received human-derived clotting factor concentrates</td>
<td>4. Persons who have had sex in the preceding 12 months with any person described in categories 1-3 or with a person known or suspected to have HIV infection</td>
</tr>
<tr>
<td>5. Persons who have had sex in the preceding 12 months with any person described in categories 1-4 or with a person known or suspected to have HIV infection</td>
<td>5. People who have been on hemodialysis in the preceding 12 months</td>
</tr>
<tr>
<td>6. Inmates of correctional systems</td>
<td>6. People who have been in prison or correctional facility for more than 72 consecutive hours in the preceding 12 months</td>
</tr>
<tr>
<td>7. Persons who have had sex in the preceding 12 months to withdrawal of consent, logistics, and donor instability due to the disadvantages that might include false positive results, increased costs, and organ loss due to withdrawal of consent, logistics, and donor instability</td>
<td>7. People who have been newly diagnosed with, or have been treated for, syphilis, gonorrhea, Chlamydia, or genital ulcers in the preceding 12 months</td>
</tr>
<tr>
<td>8. Children aged &lt; 18 months who were born to mothers with, or at risk for, HCV infection or any breast fed child of infected mother</td>
<td>8. A child aged &lt; 18 months and born to a mother known to be infected with, or at increased risk for, HIV, HBV, or HCV infection</td>
</tr>
<tr>
<td>9. Children born to mothers with HCV infection or mothers who meet the behavioral or laboratory criteria for adult donors (regardless of their HIV status)</td>
<td>9. A child who has been breast fed within the preceding 12 months and the mother is known to be infected with, or at increased risk for, HIV infection</td>
</tr>
<tr>
<td>10. Persons with inadequate blood samples due to refusal or hemodilution</td>
<td>10. Persons with inadequate blood samples due to hemodilution</td>
</tr>
<tr>
<td>11. Persons whose history, physical examination, medical records, or autopsy reports reveal other evidence of HIV infection or high-risk behavior</td>
<td>11. Persons whose medical/behavioral history cannot be obtained or risk factors cannot be determined; the donor should be considered at increased risk for HIV, HBV, and HCV infection</td>
</tr>
</tbody>
</table>

Abbreviations: CDC, Centers for Disease Control and Prevention; HBV, hepatitis B virus; HCV, hepatitis C virus; HIV, human immunodeficiency virus; PHS, Public Health Service

Orlowski and associates surveyed OPOs; they observed that 47% OPOs performed NAT on all potential organ donors and 28% OPOs performed NAT only on donors thought to be at higher risk of infection due to behavioral history.5 Kucirka and coworkers performed a meta-analysis of a PubMed-based search for studies about incidence and prevalence of HIV and HCV in the United States or Canada based on enzyme-linked immunosorbent assay (ELISA) and NAT testing (Table 3).6,7

Transplant center viewpoint

Ison and Stosor designed an Internet-based survey of United States solid-organ transplant centers to assess the approach in acceptance of organs from high-risk donors, posttransplant surveillance of patients, and opinions about the 1994 PHS guidelines.8 They summarized that acceptance of organs from high-risk donors (OHRD) was 2-fold

Table 2. Estimates of Window Period for Different Testing Methods

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>Standard Serology Testing</th>
<th>Nucleic Acid Testing</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(Enzyme-Linked Immunosorbent Assay) (d)</td>
<td>(ELISA) (d)</td>
</tr>
<tr>
<td>HIV</td>
<td>17-22</td>
<td>5-6</td>
</tr>
<tr>
<td>HCV</td>
<td>70</td>
<td>3-5</td>
</tr>
<tr>
<td>HBV</td>
<td>35</td>
<td>20-22</td>
</tr>
</tbody>
</table>

Abbreviations: HBV, hepatitis B virus; HCV, hepatitis C virus; HIV, human immunodeficiency virus

Table 3. Risk of Window Period for Human Immunodeficiency Virus and Hepatitis C Virus in Centers for Disease Control and Prevention High-Risk Donors by Nucleic Acid Testing Screening

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>Risk of Window Period for HIV Infection</th>
<th>Risk of Window Period for HCV Infection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Injection drug users</td>
<td>4.9</td>
<td>32.4</td>
</tr>
<tr>
<td>Men who have sex with men</td>
<td>4.2</td>
<td>3.5</td>
</tr>
<tr>
<td>Commercial sex workers</td>
<td>2.7</td>
<td>12.3</td>
</tr>
<tr>
<td>Incarcerated donors</td>
<td>0.9</td>
<td>0.8</td>
</tr>
<tr>
<td>Donors exposed to HIV blood</td>
<td>0.6</td>
<td>0.4</td>
</tr>
<tr>
<td>Donors engaging in high-risk sex</td>
<td>0.3</td>
<td>12.3</td>
</tr>
<tr>
<td>Hemophiliacs</td>
<td>0.035</td>
<td>0.027</td>
</tr>
</tbody>
</table>

Abbreviations: HCV, hepatitis C virus; HIV, human immunodeficiency virus

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Humar and associates performed a risk-benefit analysis of NAT screening for organ donors, and they concluded that NAT offered an advantage to minimize the risk of HIV, HCV, and HBV transmission and increase organ use by screening only the high-risk donors.4 This benefit was greatest with respect to HCV infection due to the substantial reduction in the period. There was insufficient evidence to recommend routine NAT screening for HIV, HCV, and HBV in all potential organ donors. Furthermore, universal NAT testing potentially could be counterproductive for average-risk donors (donors with no identified behavioral risk factors) due to the disadvantages that might include false positive results, increased costs, and organ loss due to withdrawal of consent, logistics, and donor instability.

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greater for liver (52%) than kidney or small bowel (25%). They also observed that transplant programs were more likely to accept OHRDs with history of injectable drug use or incarceration than other risk factors mentioned in the behavioral patterns of the PHS high-risk donors.

Posttransplant surveillance for recipients of OHRD by serology or NAT was performed routinely for HIV, HBV, and HCV in most transplant centers. Most respondents agreed that high-risk donors should not be excluded from donation, but that OHRD should be considered only for recipients in whom the risk of HIV transmission was less than the transplant. The survey also highlighted that excluding transplants from OHRD likely results in increased deaths for patients awaiting transplant than from transmission of HIV, HCV, or HBV.

After a case of HIV and HCV transmission from a CDC high-risk donor to 4 transplant recipients in 2007, there was heightened concern amongst OPOs and transplant centers that led to the special informed consent by UNOS.9 Kucirka and coworkers reported outcomes of a survey of United States transplant surgeons after this reported case.10 They observed that the response to this highly publicized case was, that 42% surgeons would decrease use of high-risk donors and 17% would increase use of NAT screening, given concerns raised regarding donor HIV and HCV transmission.

Transplant patient viewpoint
The Johns Hopkins transplant group qualitatively analyzed patient viewpoint about CDC high-risk kidney transplants.11 They observed that patients were unfamiliar with the CDC definition of infectious high-risk donors; this and the unpreparedness for transplant resulted in poor acceptance of kidneys from infectious high-risk donors. The lack of appropriate information available to patients regarding infectious high-risk donors, in addition to the social stigmas associated with HIV and HCV, had a large effect on patient decision making. However, after providing patients with adequate information about infectious high-risk donors in focus groups (about the risk of transmission, serologic testing, and the period of HIV, HBV, and HCV), 79% patients reported willingness to consider infectious high-risk donors kidneys. Most patients would be most affected by their own nephrologist’s opinion (the nephrologist who supervises their dialysis care) rather than the recommendations of the transplant nephrologist or transplant surgeon.

Consent
As of August 18, 2008, UNOS policy required transplant centers to obtain special informed consent from patients accepting PHS high-risk organs for transplant, but did not indicate any specific requirements for disclosure of the behavioral risk factors of the donor.12 This led to variability in the consent process and the type of information shared with the patient. Halpern and colleagues recommended standardization of the consent process, rather than organ-specific consent as stated by UNOS, which may help maintain autonomy of the patients while making decisions regarding high-risk organs.13 They concluded that subdivision of high-risk donors based on behavioral criteria would not help patients make a well-informed decision, but would create a social bias to certain behavioral categories of donors.13 They also recommended that UNOS should allow for prospective consent for all patients on the waiting list for the high-risk donors, which would allow sufficient time for patients to analyze the information, further support their autonomy, and avoid last-minute decision making at the time of organ offer. It was suggested that there should be a safeguard mechanism for patients who decline the consent originally and change their mind in the future.

Outcome
In 2011, 10% deceased-organ donors in the United States satisfied the 1994 guidelines for high-risk donors for transmitting infectious diseases and were referred to as CDC high-risk donors. With the revised PHS guidelines of 2013 to identify high-risk donors for transmission of HIV, HCV, and HBV, this cohort now constitutes 20% deceased donors in the United States. Grafts recovered from these donors are healthier, younger, and higher in quality. Kucirka and associates analyzed 29,950 deceased donors who were reported to UNOS from July 2004 to May 2008; they summarized that 12% kidney donors in the PHS/CDC high-risk category were expanded criteria donors, compared with 25.6% for non–high-risk donors (P < .001).14 Therefore, despite the real but small risk of HIV, HBV, and HCV transmission through transplant, the use of organs from PHS high-
risk donors minimizes wait list mortality and likely improves transplant outcomes.

References

Current Status of Organ Transplant in Islamic Countries

Ahad J. Ghods

Abstract

Objectives: The Organization of Islamic Cooperation consists of 57 member states whose people are mainly followers of the Islamic religion. During the past several decades, organ transplants have been increasingly used for the treatment of end-stage organ failures worldwide. This study is to investigate the current status of organ transplant in Islamic countries.

Materials and Methods: For data collection a literature review was carried out. Information from international registries was used and key persons from some countries were contacted.

Results: In all 5 Islamic countries of North Africa, living-donor kidney transplant was performed. Tunisia was the only country with deceased-donor organ transplant in North Africa. In 22 Islamic countries of sub-Saharan Africa, living-donor kidney transplant was performed only in Sudan and Nigeria. Deceased-donor organ transplant was illegal and nonexistent in this region. In all 14 Islamic countries of the Middle East, living-donor kidney transplant was an established practice. Turkey, Iran, and Saudi Arabia had the highest rates of organ transplant activity. In 2013, Turkey performed the highest rate of living-donor kidney and liver transplants, and Iran performed the highest rate of deceased-donor kidney and liver transplants. For 7 Islamic countries of Central Asia, organ transplant was nonexistent in Afghanistan and Turkmenistan; in the other 5 countries, a limited number of living-donor kidney or liver transplants were performed. In all 6 countries located in South and Southeast Asia, living-donor kidney transplant was performed. Only Malaysia had a limited-scale deceased-donor transplant program. Albania in the Balkans, and 2 countries (Suriname and Guyana) in South America, were also member states of the Organization of Islamic Cooperation; in these countries, only few living-donor kidney transplants were performed.

Conclusions: The organ transplant rates, especially for deceased-donor transplant, in most Islamic countries were less than expected. Some of the causes of low transplant activity included lack of public education and awareness, lack of approval and support by Islamic scholars, and lack of government infrastructure and financial resources.

Key words: Central Asia, Middle East, North Africa, Southeast Asia, Sub-Saharan Africa

Introduction

Islam the world’s second largest religion after Christianity, has 1.62 billion adherents, and is the predominant religion in the Middle East, North Africa, sub-Saharan Africa, Central Asia, South Asia, and Southeast Asia. The highest percentage of the world Muslim population (62%) lives in South and Southeast Asia, followed by 19.9% in the Middle East and North Africa and 15% in sub-Saharan Africa. Only 2.7% and 0.3% of the world Muslim population live in Europe and the Americas.

The Organization of Islamic Cooperation is the second largest organization after the United Nations and includes 57 member states whose people are mainly followers of the Islamic religion. These 57 Islamic countries that make a substantial portion of the world’s developing countries are located in the Middle East (14 members: Turkey, Iran, Saudi Arabia,
Lebanon, Qatar, Kuwait, Iraq, Syria, Jordan, Yemen, Bahrain, Oman, United Arab Emirates, and Palestine), North Africa (5 members: Egypt, Libya, Tunisia, Algeria, and Morocco), sub-Saharan Africa (22 members: Benin, Burkina Faso, Cameroon, Comoros, Chad, Djibouti, Gabon, Gambia, Guinea, Guinea Bissau, Ivory Coast, Mali, Mauritania, Mozambique, Niger, Nigeria, Senegal, Sierra Leone, Somalia, Sudan, Togo, and Uganda), Central Asia (7 members: Turkmenistan, Uzbekistan, Kyrgyzstan, Kazakhstan, Tajikistan, Azerbaijan, and Afghanistan), and South and Southeast Asia (6 members: Pakistan, Maldives, Bangladesh, Indonesia, Malaysia, and Brunei). Albania is a Muslim majority country in Southeast Europe, and 2 countries (Suriname and Guyana) in South America also are member states of the Organization of Islamic Cooperation.3

Many poor and developing Islamic countries of sub-Saharan Africa and South Asia are in the category of low human development index.4 Some Islamic countries of the Middle East that have remarkable material resources (such as crude oil and natural gas reserves) also are lagging far behind international averages in socioeconomic development such as education, health, and living standards.

During the past several decades, organ transplants have been increasingly used for the treatment of end-stage organ failure worldwide. The aim of this study was to investigate the current status of organ donation and transplant in these 57 Islamic countries. There is a proven correlation between frequency of organ transplant and human development index; therefore, it was anticipated that lower human development index in most Islamic countries would be associated with limited transplant activities or nonexistent transplant programs.

Materials and Methods

For data collection, a literature review was performed for organ donation and transplant in each of 57 Islamic countries. The availability of information varied markedly, and some countries had no reported data. Few countries had national registries. Information was used from international registries such as Global Observatory on Donation and Transplantation5 and International Registry in Organ Donation and Transplantation (Spanish group).6 Key persons from some countries were contacted to obtain needed information.

Results

The results of the study showed that in 5 Islamic countries of North Africa, living-donor kidney transplant was an established practice. Egypt and Tunisia had the highest rates of living-donor kidney transplant in this region: 13.8 per million population (pmp) in Egypt and 13.2 pmp in Tunisia.7 In Egypt, organ donation from deceased donors was legalized in 2010. However, because of ongoing religious debate about the definition of brain death, this law had not yet been activated, and deceased-donor transplant was rare in this country; transplant activity predominantly involved living-donor kidney and liver transplants.8 In 2013, there were 500 living-donor kidney (6.0 pmp) and 250 living-donor liver transplants (3.0 pmp) performed in Egypt (Professor M. A. Bakr, personal communication). Between 1991 and 2013, there were 2140 liver transplants performed, and only 2 liver transplants were from deceased donors.9 Tunisia had the highest rate of deceased-donor organ transplants in North Africa. In 2010, thirty of 132 kidney transplants (23%) were from deceased donors.7 Since the Tunisian revolution in December 2010, the deceased-organ donation rate declined from 3.0 pmp in 2010 to 0.6 pmp in 2011 and 0.1 pmp in 2012. However, the living-donor organ transplant rate remained stable at 10.2 pmp (in 2010), 9.2 pmp (in 2011), and 11.3 pmp (in 2012).10 In Algeria, the number of kidney transplants increased since 2007, reaching 137 kidney transplants (3.6 pmp) in 2011; only 2% of all transplants were from brain-dead donors (Dr. Benhocine, personal communication). In Libya and Morocco, few kidney and liver transplants were performed. In Libya, all transplants were from living donors (2.8 pmp in 2012); in Morocco, the living-donor transplant rate was 0.4 pmp and deceased-donor transplant rate was 0.1 pmp in 2011.10

In the 22 Islamic countries that are located in sub-Saharan Africa, transplants were limited to living-donor kidney transplant and were performed only in 2 countries: Sudan (186 kidney transplants in 2013) and Nigeria (14 kidney transplants in 2012). The other 20 Islamic countries of sub-Saharan Africa were lacking even a small national kidney transplant program. Deceased-donor transplant was nonexistent because of a lack of minimum infrastructure and also was illegal because of great opposition from Islamic scholars. In Sudan, where the highest rate of
living-donor kidney transplant of this region was performed, discussion about brain death and deceased-donor transplant showed great opposition from Islamic scholars.\textsuperscript{11,12} In addition, organ transplant was not a health priority in Islamic countries of sub-Saharan Africa because millions of people in these countries lived in severe poverty and death caused by wars, crimes, infections, and malnutrition.\textsuperscript{13}

In all 14 Islamic countries of the Middle East, living-donor kidney transplants were performed. Living-donor liver transplants were performed in Turkey, Iran, Saudi Arabia, Jordan, and Iraq.\textsuperscript{6,9} In several Islamic countries of the Middle East, deceased-donor organ transplant was an established practice. In 2013, deceased-donor kidney transplants were performed in Turkey, Iran, Saudi Arabia, Lebanon, and Kuwait; deceased-donor liver transplants were performed in Turkey, Iran, Saudi Arabia, and Lebanon; heart transplants were performed in Turkey, Iran, Saudi Arabia, and Lebanon; pancreas transplants were performed in Turkey, Iran, Saudi Arabia, and Kuwait; and lung transplants were performed in Turkey, Iran, and Saudi Arabia.\textsuperscript{6}

Turkey had the highest organ transplant activity of all Islamic countries, especially living-donor organ transplant. In 2013, a total of 2944 kidney transplants (39.3 pmp) were performed in Turkey, including 2359 kidney transplants (31.5 pmp) from living and 585 kidney transplants (7.8 pmp) from deceased donors. In addition, in 2013, a total of 1248 liver transplants (16.5 pmp) were performed, with 959 liver transplants (12.8 pmp) from living and 289 liver transplants (3.7 pmp) from deceased donors. Since 2009, there was a marked and steady increase in the annual number of living-donor kidney and liver transplants in Turkey; in 2013, > 80% all kidney and > 75% all liver transplants were performed from living donors.\textsuperscript{6}

Iran had the highest rate of deceased-donor organ transplant among all Islamic countries. In 2013, a total of 2670 kidney transplants (34.6 pmp) were performed in Iran; 1501 kidney transplants (19.5 pmp) were from living and 1169 kidney transplants (15.1 pmp) were from deceased donors. In addition, in 2013, a total of 592 liver transplants (7.7 pmp) were performed, including 39 liver transplants (0.5 pmp) from living and 553 liver transplants (7.2 pmp) from deceased donors. Deceased-donor organ transplant steadily increased in Iran; in 2013, > 43% all kidney and > 93% all liver transplants were from deceased donors.\textsuperscript{6} One of the transplant teams in Iran had the highest deceased-donor organ transplant activity. The Shiraz transplant team performed the first liver transplant and 80% all deceased-donor liver transplants in the country. This transplant team currently was performing almost all kidney transplants from deceased donors (Dr. Malek Hosseini, personal communications).

Saudi Arabia had an active organ transplant program that was under the supervision of the Saudi Center for Organ Transplantation (SCOT). In 2013, a total of 558 kidney transplants (20.6 pmp) were performed, including 462 kidney transplants (17.1 pmp) from living and 96 kidney transplants (3.5 pmp) from deceased donors. In addition, a total of 158 liver transplants (5.8 pmp) were performed in Saudi Arabia in 2013, of which 109 liver transplants (4 pmp) were from living and 49 liver transplants (1.8 pmp) were from deceased donors.\textsuperscript{14} Kuwait also had an active organ transplant program with kidney transplants from living and deceased donors. In 2013, a total of 66 kidney transplants (18.2 pmp) were performed in Kuwait, including 16 kidney transplants (4.4 pmp) from deceased donors.\textsuperscript{15}

Turkey, Iran, Saudi Arabia, Lebanon, and Kuwait had more active transplant programs than other Islamic member states because all these 5 countries were in the category of high human development index and had some available infrastructure for organ transplant. The human development index significantly correlated with organ transplant activity, especially organ transplant from deceased donors. In addition, these countries had the Brain Death Law and Organ Transplant Act, and most importantly, in these countries the concept of brain death and organ donation was approved and well supported by religious leaders and Islamic scholars. The Iran transplant program was the best example. For 11 years between 1989 and 2000, deceased-donor kidney, liver, and heart transplants were performed in Iran only by Fatwa from the Supreme Religious Leader, in the absence of an organ transplant law; in Islamic countries, approval from religious leaders was superior to organ transplant law.\textsuperscript{16} In contrast, there were some important Islamic countries (Egypt, Pakistan, and Indonesia) where the Organ Transplant Act had been passed but Islamic scholars had not
well supported the law, and deceased-donor organ transplant remained on a much smaller scale or was almost nonexistent.

In the 7 Islamic countries of Central Asia, organ transplant was nonexistent in Turkmenistan and Afghanistan. The other 5 countries (Uzbekistan, Kyrgyzstan, Kazakhstan, Tajikistan, and Azerbaijan) had specific transplant legislation but very small living-donor transplant programs. Azerbaijan had the highest transplant activity in this region, performing kidney and liver transplants from living donors. In 2013, there were 69 kidney transplants (7.3 pmp) and 19 liver transplants (2 pmp) performed in Azerbaijan. In Kazakhstan, in 2013, there were 130 kidney transplants (7.6 pmp) and 19 liver transplants (1.1 pmp) performed. The first kidney transplant was performed in Tajikistan in 2009, Uzbekistan in 2010, and Kyrgyzstan in 2012, and currently a very limited number of living-donor kidney transplants were being performed (Dr. Hikmet, personal communication). Until several months ago, many patients from the countries of Central Asia were traveling to Iran for transplant. Iran banned transplant operations on foreigners to stop transplant tourism, and patients from these countries currently could not be transplanted in Iran.

In all 6 Islamic countries located in South and Southeast Asia, living-donor kidney transplants were performed. In Pakistan, at the Sind Institute of Urology and Transplantation (SIUT), a medical institution founded by Dr. Adib Rizvi, ethical living-donor kidney transplant was provided free-of-cost for all patients. By the end of 2013, a total of 4140 kidney transplants were performed in SIUT including 392 kidney transplants (2.3 pmp) in 2013. Similar transplant activity also was performed by other transplant teams. The deceased-donor transplant program was undeveloped and almost nonexistent in Pakistan. By 2010, in addition to 26 kidneys received from Euro Transplant, only 5 deceased-donor kidneys were procured locally. Since passing the law in 2010 to stop organ commerce, commercial transplant decreased successfully but deceased-donor organ transplant remained nonexistent. In Bangladesh, few living-donor kidney and some living-donor liver transplants were being performed. In Brunei, 3 to 5 living-donor kidney transplants (7.5-12.5 pmp) were performed every year. In Indonesia, the most populous Islamic country with a population of 238 million, between 1977 and 2013 a total of 689 kidney and 20 liver transplants were performed, including 79 living-donor kidney transplants (0.3 pmp) in 2013. Despite the Brain Death Law and Organ Transplant Act, only 1 deceased-donor kidney transplant was performed in Indonesia (Dr Gunawan, personal communication).

Malaysia had an established deceased-donor organ transplant program. In 2012, there were 52 kidney transplants (1.8 pmp) from living donors, 81 kidney transplants (2.9 pmp) from deceased donors, and 7 deceased-donor liver transplants (0.25 pmp) performed. The number of deceased-donor kidney and liver transplants started to decrease in Malaysia because of restriction of commercial organ donation in China, and the new kidney transplant rate decreased from 6 to 7 pmp in the early 2000s to 3 pmp in 2012.

Albania, a Muslim-majority country in Southeast Europe, was a member state of the Organization of Islamic Cooperation. By 2012, Albania had not established its own national kidney transplant program and was lagging far behind neighboring Balkan countries in organ donation and transplant. The 2 countries Suriname and Guyana in South America also were member states of the Organization of Islamic Cooperation; in both countries, few living-related donor kidney transplants were performed.

Discussion

This study showed that organ donation and transplant rates in most Islamic countries were less than the expected rates. It also showed large disparities in transplant rates between countries. Some countries such as Turkey, Iran, Saudi Arabia, and Kuwait had successful deceased-donor transplant programs. Therefore, there was a question about the reasons that prevented establishing successful programs in other Islamic countries. Some of the causes of low transplant activity in Islamic countries were lack of public education and awareness, lack of approval and support by Islamic scholars, lack of government infrastructure and financial resources, and absence of the Brain Death and Organ Transplant Law. Another in-depth study is needed to evaluate the causes of low transplant activity in Islamic countries.

To adopt strategies to increase organ donation and transplant, Islamic countries should be stratified
in 3 groups. (1) In countries with successfully developed deceased-donor transplant programs, such as Turkey, Iran, Saudi Arabia, and Kuwait public awareness should be increased. (2) In countries with undeveloped deceased-donor transplant programs, especially in populous countries such as Indonesia, Egypt, Pakistan, and Bangladesh, Islamic scholar approval and support should be obtained, and public awareness should be increased. (3) And in countries with severe poverty, wars, crimes, and infections such as Islamic countries of sub-Saharan Africa, a deceased-donor transplant program is not a health priority, and living-donor kidney transplant programs may be established.

References

The ABO-incompatible living-donor kidney transplantation was developed in Japan in 1989. Currently, most transplant physicians and surgeons have noted that outcomes are unexpectedly excellent, and no hyperacute rejections have been reported since 2001. In the registry of the Japanese ABO-Incompatible Kidney Transplantation Committee, the data of 2434 ABO-incompatible living-donor kidney transplants were collected from 120 Japanese kidney transplant centers. Overall patient and graft survival rates were 97% and 94% at 1 year, 93% and 86% at 5 years, 90% and 71% at 10 years, and 73% and 52% at 20 years. The patient survival and graft rates in 2001 to 2012 were 93% and 81%, which were significantly better than 83% and 55% reported in 1989 to 2000. The addition of novel immunosuppressive treatments has improved results. Azathioprine has been replaced by mycophenolate mofetil since 2000 to 2001, and basiliximab and rituximab were introduced in 2002 and 2004. The titer of antidonor blood group antibody before transplantation was not correlated with graft survival in 2001 to 2012. De novo antibodies against vascular endothelium of peritubular and glomerular capillaries seemed to be more important than natural antibodies against red blood cells. Therefore, recipients with antidonor blood group antibody titers < 1:128 did not require antibody-removal procedures such as plasmapheresis or immunoadsorption. In particular, children (regardless of their peritoneal dialysis status) do not need to be catheterized for plasmapheresis or immunoadsorption. It is better to avoid the risks of catheterization and antibody removal procedures in children with end-stage renal failure.

Key words: ABO-Incompatible kidney transplantation, Antidonor blood group antibody, Outcome

Introduction

ABO-incompatible living-donor kidney transplantation (ABOi) has been popular in Japan since 1989. This procedure was not accepted for a long time because the associated hyperacute rejection was believed to occur because of an incompatible blood group between the donor and recipient. However, no institution has reported intraoperative hyperacute rejection since 2001. Antibody-mediated rejection (AMR) usually occurs within 2 weeks of ABOi. The outcomes of ABOi have improved markedly since 2001 compared with 1989 to 2000. We started using mycophenolate mofetil (MMF) instead of azathioprine in 2000 to 2001 and introduced basiliximab in 2002 and rituximab in 2004, thereby avoiding splenectomy after 2004. These recent developments in immunosuppression may result in less acute rejection, including AMR, with ABOi.

Natural antibodies against red blood cells were measured and considered to be an index for AMR. However, ABO blood group antigens against renal tissue were different from those against red blood cells. Therefore, a de novo antibody against renal tissue appeared to be the risk factor for AMR rather than the titer of natural antibodies against red blood cells. The Japanese ABO-Incompatible Transplantation Committee

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Committee suggested that antibody removal, such as plasmapheresis and/or plasma exchange before ABOi may be avoided if the antidonor blood group antibody (ADBGAb) titer is \( \leq 1:64 \). It is beneficial not to place a catheter for antibody removal in children who have no vascular access.

**Number and outcomes of ABO-incompatible living-donor kidney transplantations in Japan**

There were 14 cases of ABOi performed in 1989, and since then, the annual number of ABOi has increased until 2011. In 2011, there were 293 ABOi performed but the number decreased to 196 in 2012 (Figure 1). A total of 2434 cases of ABOi were registered with the Japan ABO-Incompatible Transplantation Committee. The rate of ABOi (n = 196) in living-donor kidney transplantations in Japan (n = 1431) is 13.7%. An immunosuppression desensitization protocol using MMF was initiated in 2001. Basiliximab was introduced for ABOi in 2002. Rituximab replaced splenectomy in 2004.

Overall patient and graft survival rates were 97% and 94% at 1 year, 93% and 86% at 5 years, 90% and 71% at 10 years, and 73% and 52% at 20 years (Figure 2). The patient and graft survival rates in 2001 to 2012 (n = 1985) were 98% and 96% at 1 year, 95% and 90% at 5 years, and 93% and 81% at 10 years. These rates were significantly better than 92% and 82% at 1 year, 86% and 70% at 5 years, and 83% and 55% at 10 years in 1989 to 2000 (n = 449) (Figures 3 and 4). The graft survival rate in 1989 to 2000 remarkably decreased within 1 year of transplantation (Figure 4). In contrast, the graft survival rate in 2001 to 2012 did not decrease as much as that in 1989 to 2000 (Figure 4). The higher incidence of AMR in 1989 to 2000 appeared to be a cause of poor graft survival within 1 year of ABOi. The use of MMF and rituximab may have contributed to better graft survival in 2001 to 2012.

The graft survival rates of the recipients with a preoperative ADBGAb immunoglobulin G (IgG) titer \( \geq 1:64 \) were significantly worse than those with titer \( < 1:64 \) in 1989 to 2000 (Figure 5). In contrast, patients with a preoperative ADBGAb IgG titer \( \geq 1:64 \) were not significantly different from those with titer \( < 1:64 \) in 2001 to 2012 (Figure 6). New immunosuppression protocols, including treatment with MMF and rituximab, may have resulted in better outcomes in 2001 to 2012.

Tanabe and associates reported that the graft survival of ABOi exceeded that of ABO-compatible
living-donor kidney transplantation (ABOc). This may explain why the incidence of de novo donor-specific antibody was significantly higher in ABOc than ABOi. Desensitization using rituximab may suppress donor-specific antibody in ABOi.

Advances in immunosuppression for ABO-incompatible living-donor kidney transplantation

In 1989, immunosuppression for ABOi consisted of azathioprine and/or cyclophosphamide, steroids, cyclosporine, and splenectomy. Azathioprine was replaced by MMF in 2001, and basiliximab was introduced in 2002. Rituximab was introduced in 2004 to avoid the need for splenectomy. Preoperative desensitization using MMF and rituximab to suppress ADBGAb for ABOi appeared to be more effective than antibody-removal procedures. Ramos and associates reported that plasmapheresis and intravenous immunoglobulin (IVIG) could not suppress splenic B-cell subsets, but using rituximab with these procedures could almost deplete them. In our desensitization protocol, MMF (20-30 mg/kg/d), a calcineurin inhibitor (cyclosporine, 6 mg/kg/d or tacrolimus, 0.2 mg/kg/d), methylprednisolone (8 mg were given for 10 d), and rituximab (100 mg) was given twice 10 days and 1 day before ABOi. After such desensitization, de novo ADBGAb IgG was suppressed to a lower titer until 1 year after transplantation.

The drug mycophenolate mofetil (MMF) is a stronger agent to suppress antibody formation than azathioprine; therefore, the incidence of acute rejection, including AMR, was much lower after using MMF instead of azathioprine. Basiliximab is an interleukin 2 receptor monoclonal antibody that suppresses T-cell–mediated rejection. The incidence of acute cellular rejection used to be higher with ABOi than ABOc. However, the incidence of acute cellular rejection decreased after the introduction of MMF and basiliximab. Tacrolimus is used more frequently than cyclosporine for ABOi; however, graft survival was not different between patients using cyclosporine or tacrolimus. Recent postoperative immunosuppression for ABOi consists of MMF, tacrolimus or cyclosporine, and a steroid, with induction using basiliximab.

Necessity of antibody removal procedures and intravenous immunoglobulin before and after transplantation

The natural antibodies against red blood cells appear to be different from the de novo antibodies against the vascular endothelium of peritubular and glomerular capillaries. This is likely because the blood group antigens (band 3 and bands 4 and 5) against red blood cells are different from those against the vascular endothelium of peritubular and glomerular capillaries (such as platelet endothelial cell adhesion molecule-1, plasmalemma vesicle-associated protein, and von Willebrand factor). Takahashi and coworkers reported that all 4 patients who experienced acute AMR showed C4d deposition in their peritubular capillaries in the biopsy on the episode but no deposition at the biopsy 1 hour after recirculation in the graft during the operation. None of the 5 patients with C4d deposition at the 1-hour biopsy had an episode of acute AMR. Therefore, acute AMR in these patients was not induced by natural antibodies remaining after pretransplant antibody removal, but was associated with antibodies produced de novo after transplantation. The Japan ABO-Incompatible Transplantation Committee suggested that preoperative antibody removal procedures, such as plasmapheresis, plasma exchange, and immunoabsorption, may not be
required in ABOi recipients with a low ADBGAb titer (< 1:128).

We studied 10 ABOi recipients, including 2 children (aged 4 and 7 y), without pre- and posttransplant antibody removal. The titers of ADBGAb IgG in all recipients were < 1:128. The patients were given MMF (20-30 mg/kg body weight for 10 d) and rituximab (100 mg at 10 days and 1 day before ABOi twice). Postoperative immunosuppression was based on triple therapy with cyclosporine or tacrolimus, MMF, and methylprednisolone. The maximum titers of ADBGAb IgG before transplantation were 1:8 in 2 recipients, 1:1 in 1 recipient, and 0 in 7 recipients. No recipients had clinical acute rejection, including AMR, but 1 recipient had subclinical borderline rejection determined by the biopsy performed according to protocol at 3 months and 1 year after transplantation. Mean follow-up was 21.2 ± 12.3 months (range, 11-48 mo). This may suggest that it is not necessary to perform pretransplant antibody removal in ABOi recipients who have a titer < 1:128. As previously mentioned, the graft survival in the recipients with a preoperative ADBGAb IgG titer ≥ 1:64 was not different from graft survival in recipients with a titer < 1:64 in 2001-2012.

Posttransplant antibody removal procedures for ABOi were routinely performed in most transplant centers globally except Japan. We have not performed posttransplant plasmapheresis following ABOi since 1990 at Toho University. This procedure should be avoided except in severe AMR following ABOi. In pediatric ABOi, it is beneficial not to insert a catheter for antibody removal in children without arteriovenous fistula. Most younger children scheduled for preemptive kidney transplantation or on peritoneal dialysis do not have an arteriovenous fistula. There were 2 children (aged 4 and 7 y) on peritoneal dialysis who had ABOi without pre- and posttransplant antibody removal procedures at our center. Their titers of ADBGAb were ≤ 1:2 both before and after transplantation. They have had no clinical or subclinical rejection, including AMR, and have maintained good renal function 0.26 and 0.50 μmol/L (0.30 and 0.57 mg/dL) for 1 year since ABOi.

The IVIG was used routinely worldwide for desensitization with ABOi except in Japan. It does not appear necessary to use IVIG for ABOi, but it is necessary to use IVIG for human leukocyte antigen-incompatible kidney transplantation.

Pediatric ABO-incompatible living-donor kidney transplantation

In the Japanese ABO-Incompatible Transplantation Committee registry, 89 children aged < 16 years were registered before 2012. Overall, patient and graft survival rates in the 89 pediatric cases of ABOi were 99% and 94% at 1 year, 99% and 93% at 3 years, 97% and 90% at 5 years, 97% and 80% at 10 years, and 81% and 68% at 20 years after transplantation (Figure 7). In contrast, graft survival rates in 2129 adult cases of ABOi (age > 16 y) were 93% at 1 year, 89% at 3 years, 85% at 5 years, 70% at 10 years, and 50% at 20 years after transplantation (Figure 8). Graft survival rates were significantly better in the pediatric than adult cases of ABOi (P ≤ .05) (Figure 8).

Based on these good results, ABOi is indicated in children with end-stage renal failure. Although deceased-donor kidney transplantation is beneficial for children in any country, ABOi should be considered when the patients want preemptive kidney transplantation or the waiting time for deceased-donor kidney transplantation is very long.
Conclusions

The ABOi has been popular in Japan since 1989. Most transplant physicians and surgeons had concerns about hyperacute rejection and did not expect good long-term outcomes with ABOi. However, long-term graft survival with ABOi is excellent and not inferior to that with ABOc. Pretransplant desensitization with rituximab and MMF appeared to be important in the avoidance of AMR. Pretransplant antibody removal procedures usually are not required because de novo ADBGAb against the endothelium of peritubular and glomerular capillaries appears to be different from natural ADBGAb against red blood cells. Post-transplant antibody removal should not be performed except for the treatment of severe AMR. Pediatric ABOi should be considered because the outcomes are better in children than adults are. Antibody removal should be avoided in children who are on peritoneal dialysis, are waiting for preemptive kidney transplantation, and have very low ADBGAb titer.

References

Eight-Year Outcomes of “The Cairo Kidney Centre Sequential Protocol”

Tarek Fayad,1,2 Emad William,2,3 Nasr Tawfik,2,4 Boulos Habashy,2 Hazem S. Abou-Youssef,5 Sameh Shokry,2 Soha Khalil,2 Ahmed Morsy,6 Rashad Barsoum1,2

Abstract

Objectives: To describe the long-term results of a previously developed a sirolimus-based sequential immunosuppression protocol for kidney transplant comprising 2 phases: sirolimus + cyclosporine + prednisolone for 3 months followed by sirolimus + prednisolone + mycophenolate mofetil with steroid minimization the first year. Two-year outcomes of patients on this protocol (group A) showed equivalent patient and graft survival, yet with significantly better function, compared with those on cyclosporine + mycophenolate mofetil + prednisolone (group B).

Materials and Methods: We report the 8-year outcomes in the same cohort (76 patients in group A and 37 in group B).

Results: 42% switched from group A to B versus 43% switching from B to A. Intent-to-treat patient survivals at 5 and 8 years were 88% and 85.5% for group A, and 78% and 73% for group B. Death-censored graft survivals were 93% for group A and 95% for group B. Graft function was significantly better at 8 years, with 91% of group A patients compared with 50% in group B having estimated glomerular filtration rates > 45 mL/min/1.73 m², and a significantly lower incidence of chronic allograft nephropathy in the former. Secondary parameters including blood pressure control, new onset diabetes mellitus, proteinuria and other drug-related adverse events showed no significant differences between the groups.

Conclusions: The sirolimus-based sequential immunosuppression protocol was well tolerated in 58% of patients. The intent-to-treat and patients-on-therapy analyses revealed that it was equivalent to the widely used cyclosporine + mycophenolate mofetil + prednisolone protocol regarding patient and graft survival. It is associated with better graft function and lower incidence of chronic allograft nephropathy in 8 years’ follow-up. The incidence of drug-related adverse reactions was not statistically different from those in the comparator.

Key words: Kidney transplant, Sirolimus, Sequential immunosuppression, Calcineurin inhibitor toxicity

Introduction

The introduction of the mammalian target of rapamycin inhibitor (mTORi) sirolimus (SIR), into the arena of experimental immunosuppression has provided outstanding theoretical rationale for its use in solid-organ transplant in humans. Its main benefits in mice are powerful proliferation signal inhibition and promotion of tolerance.1-3 The latter specifically addresses the negative effect of calcineurin inhibitors (CNIs)4,5 on the interleukin-2 dependent tolerogenic pathway, a major drawback to their clinical use, despite significant overall advantages. Several early clinical trials have confirmed the efficacy of de novo cyclosporine (CyA)/SIR combination in the prevention of renal allograft rejection,6 yet at the expense of a significant drug-drug interaction with significant nephrotoxicity. On the other hand, SIR permits fairly safe CNI minimization, elimination, or even avoidance.
with significant preservation or even improvement of long-term graft function.\textsuperscript{7,8}

We developed a de novo SIR-based protocol in which CyA and prednisolone (P) are coadministered for the first 3 months posttransplant, and mofetil mycophenolate (MMF) + P is given thereafter. At 2 years, the mean graft function in 76 patients who received this protocol was significantly better than in 37 control patients who received CyA + MMF + P. There were no significant differences in patient or graft survival between the 2 groups.\textsuperscript{9}

In this study, we report the 8-year outcomes of the same patients to determine if the benefits would be maintained in long-term follow-up.

Materials and Methods

This extension study included all 113 patients enrolled in the original trial who received kidney transplants at the Cairo Kidney Centre between July 2002 and July 2006.\textsuperscript{9} Their mean age upon enrollment was 44.7 years, and 39 were female. They were randomly assigned in a proportion of 2:1 to either group A (study group, 76 patients) or group B (controls, 37 patients). Table 1 shows the baseline characteristics; there were no statistically significant differences for any of the listed variables.

All patients had received transplants from live kidney donors after due evaluation and approval by the independent Hospital Ethics Committee and legal authorization by the official health authorities. All of the protocols conformed to the ethical guidelines of the 1975 Helsinki Declaration. Written informed consent was obtained from all subjects.

Table 1 shows the donor/recipient tissue type mismatches and relevant virologic characteristics, which were not statistically different between groups. No panel reactive antibodies were detected pretransplant in any recipient and crossmatches were negative.

No antibody induction was used in either group. Group A patients were assigned to our sequential SIR-based protocol. They had received preoperative cyclosporine for 48 hours, intraoperative induction with pulse methyl prednisolone, and prophylactic immunosuppression with oral SIR + CyA + P for 3 months.

The CyA dosage was tailored targeting a blood level of 600 ng/mL 2 hours after administration, and the SIR dosage was tailored to a trough target level of 5 to 10 ng/mL. Unless an acute rejection occurred, CyA was replaced by MMF, and P was gradually withdrawn or minimized to 0 to 5 mg/day by the end of the study at the attending physician’s discretion (Table 2). If an acute rejection occurred, the switch was delayed for 3 more months.

Group B patients were assigned to a conventional P + CyA + MMF from the beginning. The target CyA C2 level was 1600 ng/mL 2 hours after dosage and

| Table 1. Main Baseline Characteristics of the Patients Enrolled in the Original Study\textsuperscript{9} |
|---------------------------------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
|                                | Total | Arm A | Arm B |
| n                               | 113   | 76    | 37    |
| Demographic                     |       |       |       |
| Age (y)                         | 45 ± 15.3 | 44 ± 15.0 |
| Male sex                        | 74    | 47, 61.8% | 27, 73.0% |
| Original disease                |       |       |       |
| ADPKD                           | 3, 2.7% | 3, 3.9% | 0, 0.0% |
| CGN                             | 20, 17.7% | 15, 19.7% | 5, 13.5% |
| Nephrosclerosis                 | 25, 22.1% | 18, 23.7% | 7, 18.9% |
| Diabetes                        | 28, 24.8% | 15, 19.7% | 13, 35.1% |
| Failed transplant               | 7, 6.2% | 5, 6.6% | 2, 5.4% |
| Other                           | 19, 16.8% | 10, 13.2% | 9, 24.3% |
| Unknown                         | 11, 9.7% | 10, 13.2% | 1, 2.7% |
| Preemptive                      | 30, 26.5% | 23, 30.3% | 7, 0.0% |
| HLA mismatches/6                | 3     | 3 ± 0.89 | 2 ± 1.0 |
| Pretransplant infection         | 30, 27% | 19, 25% | 11, 29.7% |
| HCV                             | 100, 88% | 68, 89.5% | 32, 86.5% |

**Abbreviations:** ADPKD, autosomal dominant polycystic kidney disease; CGN, chronic glomerulonephritis; CMV, cytomegalovirus; HCV, hepatitis C; HLA, human leukocyte antigen.
gradually reduced as shown in Table 2. Like group A, P was gradually withdrawn or minimized to 0 to 5 mg/day by the end of the study at the attending physician’s discretion.

In the original study, the primary endpoints were patient and graft survival at 2 years. Graft failure was defined as either death or return to dialysis. The secondary endpoints were early and late graft functions, liver functions, number of drugs needed to control blood pressure at 130/80 mm Hg, proteinuria, other complications, and adverse reactions as described in the original publication.9

In this extension study, all patients were followed-up for 6 more years at quarterly intervals for 3 years and biannually thereafter. We were tolerant to noncompliance by including data within 8 weeks before or after the assigned follow-up dates. Primary follow-up parameters were patient and graft survival and graft function according to estimated glomerular filtration rate as calculated with the modification of diet in renal disease equation 4. Secondary parameters included clinical evaluation including blood pressure and peripheral edema; measurement of urinary protein/creatinine ratio; measurements of fasting blood sugar and glycated hemoglobin; blood cholesterol (with determination of high- and low-density lipoproteins) and triglycerides; peripheral blood hemoglobin, red cell counts and indices, white cell total counts and differentials, and platelet count; “liver enzymes” alanine aminotransferase, aspartate aminotransferase, gamma glutamyl transpeptidase, and alkaline phosphatase; and CyA level 2 hours after administration and/or SIR trough blood levels where applicable. Other routine or individually customized follow-up studies were available but were not included in the extension study.

Graft biopsies were obtained according to clinical indications. Acute rejection was defined and classified according to contemporary Banff criteria. While chronic histopathologic abnormalities were described as “chronic allograft nephropathy” in the original study, they were classified by an interstitial fibrosis/tubular atrophy (IFTA) score in the extension study.

All data were subjected to intent-to-treat (ITT) analysis. Because the drop-out rate was relatively high, further analysis was made by a patients-on-therapy (POT) per protocol. Statistical analyses were carried out using SPSS software (SPSS: An IBM Company, version 20, IBM Corporation, Armonk, NY, USA). Qualitative comparisons were made using chi-square or Fisher exact test while quantitative data were compared using the t test. Time-to-events (death or graft failure) were studied using Kaplan-Meier survival analyses and were compared using log-rank tests. In all tests, a P value less than .05 was considered significant.

**Results**

By the end of July 2012, twenty-two patients (16 from group A [21%] and 6 from group B [16%]) were lost to follow-up. The difference between the 2 groups was not significant (P = .542).

Thirty-two patients from group A (42%) were converted to the control protocol (B) for the reasons shown in Table 3 and Figure 1. Sixteen patients from group B (43%) were switched to the SIR + MMF + P based on evidence of functionally significant, biopsy-confirmed CyA toxicity. The time to conversion is shown in Figure 2. There was no statistically significant difference between the 2 groups (P = .855, log-rank).

<table>
<thead>
<tr>
<th>Table 3. Causes of Conversion from Protocol A to B</th>
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<tbody>
<tr>
<td><strong>Reason</strong></td>
</tr>
<tr>
<td>Acute rejection</td>
</tr>
<tr>
<td>Proteinuria</td>
</tr>
<tr>
<td>LL edema due to lymphatic obstruction</td>
</tr>
<tr>
<td>Activation of HCV viremia</td>
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<tr>
<td>Financial causes</td>
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<tr>
<td>Lymphocele</td>
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<tr>
<td>Surgery (infected herniorrhaphy wound)</td>
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<tr>
<td>Pregnancy</td>
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<tr>
<td>Rectal ulcers</td>
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<td>Travel unavailability</td>
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</tbody>
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**Abbreviations:** HCV, hepatitis C virus; LL, lower limb

**Figure 1.** Proctoscopic Image of SIR-Induced Rectal Ulcers

**Abbreviations:** SIR, sirolimus

The patient had complained of prolonged rectal bleeding of unknown origin. A few days after converting the patient to protocol B, the bleeding stopped and the ulcers healed.
After 8 years, 8% of patients in group A and 44% in group B were still receiving steroids ($P = .034$) at average daily doses of 3.75 and 8.13 mg.

Twenty-one patients had died since enrollment (including those reported in the original study) (Figure 3), including 11 from group A (14.5%) and 10 from group B (27%) (Table 4). The difference was statistically insignificant both by ITT and POT analysis (log-rank values 0.150 and 0.663).

Of those who remained alive, 5 patients from group A (6.5%) and 2 from group B (5.4%) lost their grafts. There was no statistically significant difference in graft survival censored-for-death at 8 years either by ITT or POT analysis (log-rank values 0.585 and 0.455) (Figure 4).

Graft function at 8 years was numerically better in group A (mean 61.9, SD 17.95 mL/min/1.73 m$^2$) compared with group B (mean 47.7, SD 27.64 mL/min/1.73 m$^2$), but the difference was not statistically significant ($P = .069$). However, when graft function was stratified into chronic kidney disease stages, significantly more patients from group A were in stages II and IIIa (Figure 5).

The relative frequencies of secondary follow-up parameters are shown in Table 5. None of these showed a statistically significant difference between the 2 groups by ITT or POT analysis. However, the incidence of chronic allograft nephropathy/IFTA was 13% in group A and 38% in group B ($P < .003$) by ITT analysis.

**Discussion**

The study participants were kept on the same protocol to which they were initially assigned or switched during the first 2 years.$^9$ Less than one-fifth of patients were lost to follow-up, without significant difference between the 2 groups, so there was no effect on the analysis. Likewise, the proportion of patients switching from 1 group to the other was almost identical.

Because there was no significant difference in patient- or graft-survival between the 2 groups, the
outcomes of ITT and POT analyses showed the same trend. It is noteworthy that the frequency of SIR discontinuation in our study was similar to that in the 5-year follow up of the SPIESSER study (46%). However, they reported less frequent CyA discontinuation (30%), which may be attributable to the use of smaller doses than in our series.8

The 2-year trends remain unchanged at 8 years. Patient and graft survival were not significantly different, while graft function was significantly better in the SIR group, despite the lower exposure to corticosteroids.

Eight-year patient survivals calculated by ITT were 85.5% and 73% in groups A and B, respectively ($P = .150$). The mean (95% confidence interval [CI]) patient survival time for protocol A was 7.18 years (range, 6.70-7.66 y), compared to 6.53 years (range, 5.70-7.37 y) for protocol B. By POT analysis, the patient survival rates were 86% and 81% in groups A and B ($P = .663$). The mean patient survival time (95% CI) for group A was 7.10 years (range, 6.40-7.79 y) compared with 6.894 years (range, 5.83-7.95 y) for group B. The numeric advantage of POT outcomes may be explained by the selection bias of excluding complicated cases that required switching between protocols. Results similar to our ITT analysis were reported in a recent study of 1967 living-donor transplants using different immunosuppressive protocols; their 10-year graft survival rate was 77.8%.10

The long-term numeric survival advantage of the SIR-based protocol in our study is supported by the 10-year data on 592 patients, where patient survival was 80% in patients receiving a combination of 80%
reduced CyA + SIR, 73% received a combination of full-dose CyA + SIR, and only 68% on conventional CyA and MMF.11

On the other hand, no positive effect of SIR on patient survival was observed in other trials. For example, in a large historical cohort of United States kidney transplant recipients with 8-year follow-up,12 the risk of death was highest in patients who received mTORs without CNIs (3237 patients), intermediate in those who received a combination of mTORs and CNIs (10 510 patients), and least in those on CNI without mTORi (125 623 patients). A higher risk of death also was reported in a recent meta-analysis of data on 5876 patients from 21 clinical trials, including our initial publication.13 In contrast to our cohort, there was no account of pretransplant cardiovascular risk factors or dormant infections, which may explain the discrepancy with those 2 studies.12,13 Nevertheless, this particular issue obviously calls for further careful evaluation.

Because of a lack of consensus on patient survival outcomes under SIR treatment, we opted to limit graft survival data analysis to surviving patients. Our 8-year graft survival censored for patient death were 93% and 95% in groups A and B by ITT (P = .585). The mean 95% CI graft survival time for protocol A was 7.58 (95% CI: 7.23-7.94), and 7.57 (95 CI: 6.99-8.15) for group B. Death-censored graft survivals by POT analysis were 95.5% and 90.5% for groups A and B (P = .455). The mean 95% CI graft survival time for group A was 7.64 years (95% CI: 7.16-8.12 y) compared with 7.24 years (95% CI: 6.24-8.24 y) for group B. These results compare favorably to those of Isakova and associates (2013) who reported a 5-year graft survival of 88.9% in patients maintained on SIR-based immuno-suppression.12

On the other hand, graft function was significantly higher in group A despite lower steroid exposure. Thus, the breakdown of estimated glomerular filtration rate at 8 years by ITT analysis showed that 26.3% of patients were in stage II chronic kidney disease compared with 18.9% of group B patients (P = .037). These observations are strikingly close to those reported in the 5-year SPIESSER study follow-up, where the proportion of patients with glomerular filtration rate > 60 mL/min/1.73 m² was 31% in the SIR group versus 15% in the CyA group (P = .047).8

The mean estimated glomerular filtration rates in our study were 61.9 ± 17.954 mL/min/1.73 m² and 47.7 ± 27.645 mL/min/1.73 m² in groups A and B, a numeric difference that did not reach statistical significance (P = .069). The difference was even more impressive in the POT analysis, which showed that 91.7% of group A compared with 62.5% of group B had an estimated glomerular filtration rate > 60 mL/min/1.73 m². Again, this difference did not reach statistical significance, likely because of the relatively small numbers of patients who completed 8 years on the same protocol.

Analysis of the secondary endpoints at 8 years yielded similar results as those reported at 2 years. Thus, the cumulative incidences of biopsy-proven acute rejection episodes at 8 years were 30% in group A and 32% in group B (ie, averages of 3.75% and 4%/y). This reflects the usual decline in the incidence of biopsy-proven acute rejection with time; the respective values were 6.6% and 9.45%/year in our initial 2-year report.9

Most cases of acute rejection were classified as Banff IA or IB, and very few cases were IIA. Two cases in group A developed acute vascular rejection, 1 in the first 2 weeks and the other 5 years after transplant. Only 1 patient in group B experienced acute vascular rejection. So, the numeric advantage of SIR + CyA in preventing acute rejection was lost on CyA withdrawal. This is not unexpected as many studies have documented the importance of CNIs in the prevention of this complication, particularly during the early posttransplant course. In fact, an average biopsy-proven acute rejection rate as low as 2.7%/year was achieved in another study when a SIR + low-dose CyA was continued for 10 years.11 In the final analysis, we believe that the advantage of preserved glomerular filtration rate achieved in our protocol outweighs the significance of a 1%/year reduction in the chances of acute rejection.

This view is further supported by the observation that the incidence of IFTA (including CNI toxicity) was significantly lower in group A (13%) than group B (38%) at 8 years (P = .003). Patients-on-therapy analysis revealed the same trend, with IFTA rates of 11% and 24% in groups A and B. However, because of the small number of cases, this difference did not reach statistical significance (P = .271). Similar observations were made in many other studies with long-term mTORi use, including SIR or everolimus.14,15
The incidence of new onset diabetes mellitus was more common in group A, affecting 15% of ITT patients compared with 11% in group B ($P = .5$). The diabetogenic effect of SIR was even more pronounced in the POT analysis, with new onset diabetes occurring in 20% of group A patients versus 5% of group B patients; although this is an impressive numerical difference, it was not statistically significant ($P = .148$).

The 8-year analysis did not show statistically significant differences in the number of drugs needed to control blood pressure, the incidence of edema, wound healing, anemia, hyperlipidemia, infections, or malignancy. Interestingly, there was also no difference in the incidence of proteinuria because SIR exposure was minimized by immediate withdrawal upon the detection of increasing protein excretion crossing the albumin/creatinine ceiling of 500 mg/g. Proteinuria regressed in most of these patients with the use of an angiotensin-converting enzyme inhibitor.

**Conclusions**

This 8-year analysis confirms the advantages of our SIR-based sequential protocol in living-donor renal transplant. While it proved equivalent to more commonly used CNI-based protocols with regard to patient and graft survival, the clear benefit was sustained superior graft function. Based on the current knowledge, this would predict a lower incidence of cardiovascular complications, and longer patient and graft survival.

However, the incidence of adverse events often has been a compelling reason for withdrawal in favor of a CNI protocol. It appears that only the lucky 58% of patients who do not develop significant adverse events are those who will benefit from the advantages associated with the SIR protocol.

The safe use of mTORis requires familiarity with its adverse events and drug-drug interactions. Patients must be carefully monitored for proteinuria, hyperlipidemia, hyperglycemia, bone marrow suppression, and other adverse events.

Although we did not notice any decline in patient survival at 2 or 8 years, the recently raised concerns about a possible increase in mortality risk need to be carefully resolved before recommending the wider use of our protocol. Trials are underway in our institution to try different CNIs and mTORis to identify the most optimal protocol with regard to combinations and timing.

**References**

Role of Coronary Artery Calcium Score in Identifying Occult Coronary Artery Disease in Patients Evaluated For Deceased-Donor Liver Transplant – A Preliminary Report

Eren Taydas, Mohammad U. Malik, Abhinav Dhingra, Stuart Russell, Matthews Chacko, Andrew M. Cameron, Saleh Alqahtani, Ahmet Gurakar

Abstract

Coronary artery disease may affect cirrhotic patients regardless of age and etiology of the underlying liver disease. Early identification of coronary artery disease is important to achieve the best posttransplant outcomes and survival. The coronary artery calcium score can be used as a screening tool to supplement the results of cardiac stress tests to identify a subgroup of patients who may benefit from further investigation with coronary arteriogram. Arteriogram is an invasive test and may cause renal compromise and risk of bleeding associated with coagulopathy. The present retrospective study showed that coronary artery calcium score > 250 Agatston units may help select the subgroup of patients who will benefit from further investigation with cardiac catheterization, and determining this score may limit the risks of catheterization.

Key words: Atherosclerosis, Cardiac catheterization, End-stage liver disease, Cardiac disease screening, Arteriogram alternative

Introduction

Cardiac evaluation is an integral part of evaluating a liver transplant candidate and is an important component of the preoperative evaluation before liver transplant. Early identification of coronary artery disease (CAD) is important to enable the achievement of better posttransplant outcomes and higher survival rates. However, identification of occult CAD in asymptomatic candidates is a challenge. Cardiac stress tests and transthoracic echocardiography are integral parts of the evaluation but have lower sensitivity and specificity than arteriogram. In some transplant programs, pretransplant troponin levels have been used to supplement cardiac stress testing, but the troponin level does not have wide application because its contribution is not well established. Cardiac catheterization remains the best option for investigating the presence and extent of CAD.

The application of cardiac catheterization as a standard investigative tool to search for occult CAD in liver transplant candidates has been limited by the risk of renal compromise and the invasiveness of the procedure, especially when other risk factors are present such as diabetes mellitus, hypertension, obesity, nonalcoholic steatohepatitis (NASH), and smoking. Recently, there has been some literature published suggesting that the coronary artery calcium (CAC) score may be highly predictive of coronary heart disease event risk across all age groups, and its use as a risk-stratification tool has been suggested. In addition, preliminary experience with application of CAC score in a liver transplant program has been reported.

The purpose of this study was to identify a cutoff CAC score as a screening method that would predict advanced CAD in patients undergoing evaluation for liver transplant, in the absence of known history of CAD. Advanced CAD was defined as coronary artery obstruction ≥ 50% identified during a coronary arteriogram. The secondary purpose was to...
investigate the role of specific liver disease as a risk factor for advanced CAD in liver transplant candidates.

**Materials and Methods**

We investigated the role of CAC score in predicting the presence of CAD in patients aged > 40 years undergoing evaluation for liver transplant and who had ≥ 1 of the following major cardiac risk factors: diabetes mellitus, hypertension, extensive smoking history, body mass index > 35 kg/m², and diagnosis of NASH. The CAC score was reported as a noninvasive screening tool for coronary heart disease, and the only potential risk was exposure to radiation from the computed tomography scan. After Institutional Review Board approval, the electronic medical records of patients at the Johns Hopkins Liver Transplant Program between January 1, 2011, and May 1, 2013, were retrospectively reviewed.

The computerized tomography (CT) scan of the heart was obtained in the radiology department, without the administration of intravenous contrast, for quantification of calcification in the coronary arteries, reported as the Agatston score. A reported score of 0 signified no calcium load in the coronary arteries and no evidence of CAD.

**Results**

There were a total of 175 patients. The CAC score was obtained on 66 patients who had a history of diabetes mellitus, hypertension, smoking, or NASH. There were 40 males and 26 females (mean age, 57.8 ± 0.7 y). At the time of evaluation, the mean Model for End-Stage Liver Disease (MELD) score was 15.2 ± 6.8, and mean CAC score was 379.6 ± 639.8. There were 3 main diagnoses as the primary cause hepatic failure: patients who had hepatitis C virus cirrhosis (33 patients), alcoholic cirrhosis (14 patients), or NASH (12 patients); the other 7 patients had alternate causes of cirrhosis including hepatitis B virus, autoimmune liver disease, primary sclerosing cholangitis, and primary biliary cirrhosis (Figure 1). After the patient had a discussion with an experienced transplant cardiologist, cardiac catheterization was performed by a single experienced interventional cardiologist in 20 patients (30%).

There were 8 patients (40%) who had coronary obstruction < 50%. The other 12 patients (60%) had maximum obstruction ≥ 50% (range, 50%-90%). Mean total CAC score was 750.3 ± 714 Agatston units in patients with < 50% stenosis). Mean total CAC score was 883.8 ± 659.7 Agatson units in patients with coronary obstruction reported as ≥ 50% noted at cardiac catheterization.

There was no statistical difference between the groups in extent of CAD and mean CAC score (P > .05). However, receiver operating characteristic curve analysis showed 91% sensitivity and 50% specificity at a calculated CAC score cutoff of 243 Agatston units for the identification of occult coronary artery disease (Figure 2).

In the 14 patients with alcoholic cirrhosis, 7 patients were catheterized and 3 patients had coronary obstruction ≥ 50%. In the 12 patients with

![Figure 1. Indications for Liver Transplant in Study Patients](image)

**Abbreviations:** HBV, hepatitis B virus; HCV, hepatitis C virus; NASH, nonalcoholic steatohepatitis

![Figure 2. Receiver Operating Characteristic Curve](image)

The analysis showed 91% sensitivity and 50% specificity at a calculated coronary artery calcium score cutoff of 243 Agatston score for the identification of occult coronary artery disease.
NASH, 4 patients were catheterized and 2 patients had coronary obstruction ≥ 50%. In the 33 patients who had hepatitis C virus, 9 patients underwent cardiac catheterization and all 9 catheterized patients had coronary artery obstruction ≥ 50%.

Discussion

Early identification of CAD is essential to be able to achieve better posttransplant outcomes and survival. The CAC score can be used as a screening tool to supplement traditional cardiac stress tests. The application of CAC scoring has been proposed to screen larger populations. According to our preliminary experience obtained from this retrospective analysis of the data, CAC score > 250 Agatston units can be helpful in identifying liver transplant candidates who would benefit from further investigation with cardiac catheterization. Catheterization still is considered the best test to investigate the presence of CAD, but it may be limited because of possible renal compromise. Application of the CAC score as a screening tool can potentially eliminate the risks of routine invasive cardiac catheterization. In addition, the CAC score may identify a subgroup of patients who may benefit from catheterization in an effort to identify advanced CAD, evidenced by coronary obstruction ≥ 50%. Renal function must be reviewed in detail prior to the decision to perform an arteriogram, especially in patients who have higher MELD score and elevated serum creatinine level. The risks and benefits of coronary catheterization should be discussed in a multidisciplinary way among transplant hepatologists, transplant surgeons, and cardiologists experienced with liver transplant patients, especially considering the planned liver transplant.

The underlying liver disease also should be taken into consideration. In this small cohort, there was a significant overrepresentation of hepatitis C virus in individuals with coronary obstruction ≥ 50%. This observation should be studied further in a larger cohort.

References

Organ Transplantation in Tunisia

Aziz El Matri,¹ Taieb Ben Abdallah²

Abstract

Kidney transplants were first performed in Tunisia in 1986, and transplants soon extended to other organs including the heart, liver, and pancreas. Live-related donor and deceased-donor kidney transplants were both begun in the summer of 1986. An organ procurement and transplant law was passed in March 1991, and the National Centre for Advancement of Organ Transplantation was created in 1995. The number of transplantation units has increased to 7 throughout the country, and the yearly transplant number has progressively increased to 139 in 2010, including 20% from deceased kidney donors. Despite these gains, the need continues to grow.

Heart transplants began in January 1993, and Tunisia and Jordan are currently the only Arab countries where it is practiced. However, only 16 patients have received a heart transplant as of 2004, and the number of recipients has decreased in the past 10 years. Liver transplants are rare in other Arab countries, but began in Tunisia in January 1998. Over 10 years, 38 patients benefited from this procedure. After a few years of stagnation, the number of liver transplants is increasing. While all types of transplantation are needed, kidney transplantation is a priority in Tunisia. The target is to perform 400 transplants annually, which would require a long-term strategy to provide full financial coverage using the National Health Insurance Funds in both the public and private sectors.

Key words: Kidney transplant, Liver transplant, Heart transplant, Transplantation in Tunisia

Introduction

The first organ transplantation surgeries in Tunisia were kidney transplants in 1986, followed by hearts in 1993, livers in 1998, and lungs in 2012. After the 1991 enactment of the organ procurement and transplant law, transplants spread from the Departments of Urology and Nephrology at the University Hospital Charles Nicolle of Tunis to the Principal Military Hospital of Tunis, and then to other university hospitals (Sfax, Monastir, Sousse, Marsa, and Rabta). We present here an historical overview and the current situation of organ transplantation in Tunisia.

Materials and Methods

Historical data were collected by interviewing the actors involved in the first transplants and studying the literature.¹⁻³ Medical, surgical, and immunologic data were mainly provided by the National Centre for the Advancement of Organ Transplantation⁴⁻⁵ and the Global Observatory on Donation and Transplantation.⁶

Results

Legal and organizational aspects

When the first organ transplant was performed, the only available legal text was a decree dated July 19,
1952, authorizing the retrieval of organs from deceased donors for diagnostic or therapeutic purposes. At that time, it was promulgated to allow autopsies and authorize corneal procurement from deceased donors. However, after consultation with the legal and religious authorities including Mufti Sheik Mokhtar Sellami, who did not object, it was considered that this decree was sufficient to permit solid-organ transplants. This considerable responsibility was taken on by transplant teams and surgeons. On March 25, 1991, law No. 91-22 was passed to regulate organ donation, including procurement from both living and deceased donors based on the presumed consent principal. However, although it was not specified in the law, the medical staff decided, after a long debate, to limit living donors to blood relatives. This changed in 2000, when the medical community decided to extend the procurement to emotionally related donors.

The National Centre for Advancement of Organ Transplantation supervised by the Tunisian Ministry of Health was created after law No. 95-49 was passed in June 1995. Its aim was to promote the advancement of organ donation; supervise and coordinate organ activity in the country; and ensure effective oversight, traceability, and surveillance.

Kidney transplantation

The first kidney transplants

On June 4, 1986, a young male received a kidney donated from his brother. The surgery was carried out at the Department of Urology in the University Hospital Charles Nicolle by Prof. Sadedine Zmerli and his collaborators Mounir El Ouakdi, Mohsen Ayed, and Habib Boujnah with the attendant vascular surgeon Khaldoun Bardi and the anesthesiologist Hedi Ben Ayed. The nephrology team of the Department of Internal Medicine chaired by Prof. Hassouna Ben Ayed and including Aziz El Matri, Taieb Ben Abdallah, and Chieheb Kechrid ensured appropriate preoperative preparation of the recipient and postoperative follow-up, including immunosuppressive treatment.

On July 10, 1986, the same team and the urologist Mohamed Chebil carried out the second living-donor kidney transplant in Tunisia. On July 16, 1986, the first kidney transplant from a deceased donor was performed in the same hospital. The head of the General Surgery Department Prof. Ridha Mzabi retrieved the organ, and Sadedine Zmerli carried performed the transplant. Since then, kidney transplants have become a routine activity.

The first renal transplant using the kidney of a deceased donor at the Principal Military Hospital was performed on November 16, 1992, by the vascular surgeons Jalel El Menaa and Tahar Sraieb with the attending anesthesiologists Mohamed Dhahri and Jalel Hmida. The first kidney-pancreas transplant was carried out in the same hospital on February 9, 1993.

Afterwards, other hospitals routinely performed kidney transplants. The University Hospital of Sfax, Habib Bourguiba performed its first transplant with a living-donor kidney on April 4, 1994. The urologists were Mohamed Nabil Mhiri and Ali Bahloul; the attending anesthesiologist was Ali Karoui; the attending nephrologists were Abdelhamid Jerraya, Jamil Hachicha, and Mohamed Ben Hmida; and the immunologist was Hafedh Makni. On July 3, 1997, they performed the first deceased-donor kidney transplant. On October 11, 2004, the first preemptive transplant in Tunisia was carried out. The University Hospital of Monastir began its living donor kidney transplant program on September 22, 1995. The Urologist was Hamadi Saad, the Anesthesiologist was Mourad Gabbiche, and the Nephrologist Abdelatif Achour was from the Department of Nephrology headed by Prof. Mezri El May. The University Hospital Sahloul of Sousse started a living-donor kidney transplant program in November 2007. The team included urologists Faouzi Mosbah and Faouzi Limaiem, anesthesiologists Rachid Said and Souad Chelbi, and Nephrologist Abdelatif Achour. The living-donor kidney transplant program at the University Hospital of Tunis La Rabta was started in November 2010. The team included Urologists Y. Nouira and Mohamed Khamas, Anesthesiologist Ali Cherif, and the Nephrologists Fatma Ben Moussa and L. Ben Fatma.

Current situation

During the last 28 years, 1,492 kidney transplants have been carried out in 6 units, including 79% and 21% from living- and deceased-donors. The program has been progressing slowly, with a mean rate of 10.5 per million individuals and a peak of 13.2 per million in 2010 (Figure 1). The donors were 53% female and 47% male with a mean age of 40 ± 11.3 years. Most living donors were mothers, brothers, sisters, and
fathers of the patients. The recipients were 66.4% men and 33.4% women, with a mean age of 30.4 ± 11.5 years. The patient and graft survival rates at 20 years were 50% and 42%.4

Heart transplantation
The first heart transplant took place at the Principal Military Hospital of Tunis, on January 15, 1993. The surgical team included the late Prof. M. Fourati and his collaborators H. Thameur, T. Mestiri, A. Iouaz, and J. Menaa; the Anesthesiologists M. Dhahri, J. Hmida, and S. Cheour; and the Cardiologists M. Gueddiche and H. Chaouch. The first transplant was successful, and the patient survived 8 years. In the following 12 years, the same team carried out 16 transplants with good outcomes. However, the program has been stagnating since 2011 (Figure 2).

Liver transplantation
Tunisia’s first liver transplant was performed on January 18, 1998 at the university hospital Sahloul in Sousse by the surgeon R. Bel Haj Hamida and the attending anesthesiologist Rachid Said. They performed 2 additional transplants before the program was transferred in December 1999 to the University Hospital of La Marsa, Mongi Slim in the suburb of Tunis. The surgeon T. Khalfallah and the anesthesiologist M. S. Ben Ammar and their teams regularly carried out regularly an average of 3 transplants per year. In a 10-year period, 38 patients have received transplants, including 6 livers from living donors (Figure 3).4

2013 update
Tunisia counts 12 officially registered transplant centers including 6 for kidney, 2 for liver, 3 for heart, and 1 for lung, but few are regularly active.6 During the year, 127 kidney transplants were performed (18 and 107 from deceased and living donors, respectively, including 8 pediatric patients). There were also 1 liver and 1 lung transplant from deceased donors.6

The waiting list of the year counts 9000 patients, with 1257 ever active. A total of 121 patients were included for the first time, and 22 died before receiving an organ.

Discussion
The first organ transplants in Tunisia were kidneys and coincided with the implementation of transplantation in other Arab countries in the region.7 Transplant surgeries were quickly decentralized to other institutions and began to be performed for other organs. For renal transplants, the number of units has increased from 1 to 6. The annual number of transplants stagnated around 40, until 2005, but thanks to the extension to emotionally related living donors, the efforts of all the teams, and the coordination of the National Centre for the Advancement of Organ Transplantation, the annual number of kidney transplants reached nearly 132 in 2010, with only approximately 20% from deceased donors. However, the need for transplants continues to grow and the waiting list is longer than ever. Costs associated with dialysis have increased annually to
reach near 5% of the total national health expenditure.

Tunisia and Jordan are the only Arab countries that perform heart transplants.\(^8\) However, few are performed. There are many reasons for the low number of heart transplants, but the obstacles are not insurmountable.

The first Arab country to perform liver transplants was Saudi Arabia in 1990.\(^9\) Although a program has been developed in Tunisia, liver transplants are only carried out by a single team.

Undoubtedly, all types of organ transplants are important, but kidney transplants are a priority because of the high incidence of end-stage renal disease and the associated socioeconomic impact in a developing country. To meet the needs of the population, a target to perform 400 transplants per year would require a long-term strategy within the framework of the total reimbursement for all renal replacement therapies by the National Health Insurance Funds. This would require the expansion or creation of units, more equipment, highly motivated personnel, and the planning of an adapted budget. New ways to approach the question need to be considered such as incentive measures to motivate the medical personnel and staff, the creation of public centers specialized in transplants, and inclusion of the private sector.

**Conclusion**

The history of organ transplants in Tunisia demonstrates the competence of the professional specialists and the determination of public authorities to initiate organ donation programs. However, it is time to adopt new approaches to meet the needs of the patients and improve their quality of life.

**References**

Insulin Gene Therapy for Type 1 Diabetes Mellitus

Andrew M. Handorf, Hans W. Sollinger, Tausif Alam

Abstract
Type 1 diabetes mellitus is an autoimmune disease resulting from the destruction of pancreatic β cells. Current treatments for patients with type 1 diabetes mellitus include daily insulin injections or whole-pancreas transplant, each of which are associated with profound drawbacks. Insulin gene therapy, which has shown great efficacy in correcting hyperglycemia in animal models, holds great promise as an alternative strategy to treat type 1 diabetes mellitus in humans. Insulin gene therapy refers to the targeted expression of insulin in non-β cells, with hepatocytes emerging as the primary therapeutic target. In this review, we present an overview of the current state of insulin gene therapy to treat type 1 diabetes mellitus, including the need for an alternative therapy, important features dictating the success of the therapy, and current obstacles preventing the translation of this treatment option to a clinical setting. In so doing, we hope to shed light on insulin gene therapy as a viable option to treat type 1 diabetes mellitus.

Key words: β cell, Liver, Autoimmunity, Pancreas transplant, Minicircle

Introduction
Type 1 diabetes mellitus (T1DM) results from the autoimmune destruction of insulin-producing β cells of the pancreas. As a result, affected individuals are unable to produce adequate insulin to precisely regulate blood glucose levels. Over time, chronic hyperglycemia results in numerous complications affecting the vascular system, kidneys, retina, lenses, nervous system, and skin. In fact, diabetes is the leading cause of end-stage kidney failure, blindness, nontraumatic amputations, and a variety of debilitating neuropathies. Despite active research, there is currently no cure for T1DM.

According to the National Diabetes Statistics Report, 29.1 million people, or 9.3% of the US population, have diabetes, with T1DM accounting for roughly 5% of all diagnosed cases in the adult population. The total economic cost of diabetes is estimated to be $245 billion per year, with T1DM costing an estimated $8 billion to $14 billion per year. Thus, diabetes poses a significant burden to our economy. While there is no cure for T1DM, several therapies currently exist to control blood glucose levels and limit adverse systemic damage resulting from chronic hyperglycemia. Of these treatments, exogenous insulin therapy and whole-pancreas transplant are most common but present significant drawbacks. Hence, there is clearly a need for alternative state-of-the-art therapies. To that end, insulin gene therapy has arisen as a promising alternative with considerable potential for the long-term treatment of T1DM.

In this review, we will summarize the current therapies used to treat T1DM and discuss the inherent limitations of each treatment. We will then introduce a novel treatment for T1DM—insulin gene therapy—and review the important features required for its success, as well as the current state of this promising treatment. Lastly, we will discuss the current obstacles preventing insulin gene therapy from being a clinical reality and propose future directions critical for the translation of this therapy. In doing so, we hope to shed light on insulin gene therapy as a promising alternative to treat T1DM.
Traditional therapies for treatment of Type 1 diabetes mellitus

Exogenous insulin-based therapies

Type 1 diabetes mellitus is a chronic disease for which there is no cure. Instead, classical treatments have been aimed at obtaining an approximate restoration of deficient insulin levels. The most common form of insulin therapy involves injection of exogenous insulin, which typically requires multiple insulin shots per day. While daily insulin injections preserve life and delay the onset of secondary complications like blindness or kidney failure, they are cumbersome and inadequate at restoring optimal glucose control. In addition, exogenous insulin therapy is associated with adverse events like hypoglycemia, weight gain, and worsening diabetic retinopathy if HbA1c levels decrease too rapidly. The insufficiencies of exogenous insulin treatment arise from the fact that most insulin regimens are nonphysiologic in nature and unable to mimic normal β cell secretion in response to ever-changing blood glucose levels. To improve upon traditional insulin therapy, more physiologically mimetic insulin replacement therapies that replace prandial and basal insulin separately, insulin analogues with altered stability or activity, and insulin pumps have been used. Nonetheless, the sophistication of insulin secretion from β cells is impossible to duplicate with exogenous insulin therapy. Consequently, exogenous insulin therapy delays, but does not prevent, the onset of complications that ultimately increase morbidity and mortality.

Transplant therapies

To replace endogenous β cell function and obtain tighter control of blood glucose levels, whole-pancreas or pancreatic islet transplant surgeries have been performed. A whole-pancreas transplant was first performed in 1966, and today, over 30,000 pancreatic transplants have been completed. Pancreas transplants have demonstrated sustained insulin independence and excellent control of blood glucose for several recipients posttransplant, as evidenced by normal HbA1c, a measure of average plasma glucose over the previous 2 to 3 months. However, the usefulness of pancreas transplant therapy is limited by a severe shortage of donor organs and the need for lifelong immunosuppression, along with its associated adverse effects. Thus, this procedure is only recommended for T1DM patients who are unresponsive to conventional insulin-based therapies. Transplant of pancreatic islets was proposed as a better alternative to whole-pancreas transplant on the basis of 2 theoretical advantages: the procedure is less invasive and a single donor’s islets could serve multiple patients if the cells could be induced to proliferate ex vivo or in vivo. Islet transplant therapy has enabled patients to remain insulin independent for an average of approximately 1 year, but long-term graft survival rates have remained < 10%. Despite considerable efforts, equivalent successes like those observed with whole-pancreas transplants have not been matched by islet transplants.

Gene therapy for treatment of type 1 diabetes mellitus

Due to the inherent limitations of exogenous insulin- and transplant-based therapies, alternative strategies are being investigated to treat T1DM. The goal of future treatments will be to restore dynamic control over blood glucose levels without requiring cumbersome daily injections, major surgical operations, or lifelong immunosuppressive regimens. Additionally, future therapies must be affordable for all patients. Gene therapy has emerged as a promising alternative for the treatment T1DM that could meet all of the aforementioned criteria. Gene therapy can be broadly defined as the treatment of a disease by using therapeutic genes as drugs. These therapeutic genes are delivered to target cells using a vector, such as a virus, to restore or replace lost cellular functions. In the case of T1DM, the delivery of therapeutic genes can improve the clinical outcome of diabetic individuals by preventing the autoimmune destruction of β cells prior to the onset of disease, reprogramming non-β cells into surrogate β cells, or simply replacing the function of lost β cells. Here, we will briefly describe the first 2 gene therapy strategies and their limitations before discussing the last option—replacing the function of β cells via insulin gene therapy—in-depth. Specifically, we will cover the important features required for successful insulin gene therapy, the current obstacles preventing the therapy from reaching clinics, and important future directions.

Prevention of autoimmune β cell destruction

The idea of preventing the autoimmune destruction of β cells through the use of gene therapy is a logical
one. To do so, researchers have attempted to (1) alter the immune system so that it no longer recognizes β cell antigens as foreign or (2) modify the residual β cells so that they can withstand attack from the patient’s own immune system. For example, researchers have overexpressed IL-4 via an adenoviral vector in nonobese diabetic mice, or overexpressed the antiapoptotic gene, bcl-2, in β cells specifically, to attenuate the autoimmune attack and increase the survival of β cells. However, these approaches are severely limited for 3 reasons. First, this strategy relies on the early detection of diabetes, and often times, greater than 80% of an individual’s β cells have already been destroyed by the time they become symptomatic. Thus, efforts to protect the remaining β cells may prove futile. Second, T1DM is a multifactorial disease, making it nearly impossible to predict whether a prediabetic individual will ever succumb to the disease. Thus, early intervention can be risky and perhaps even accelerate the progression of the disease. Third, the immune system is highly evolved and its complexities are not well understood; however, what is becoming increasingly apparent are the innumerable functional redundancies that allow it to compensate for the loss of any single factor or pathway. At our current level of understanding, it seems inconceivable to selectively target the immune system to prevent the autoimmune destruction of β cells; thus, other gene therapy strategies have been explored.

Reprogramming non-β cells into β cells

The goal of reprogramming non-β cells into surrogate β cells is to create replacement cells that are as similar as possible to native β cells. Researchers have targeted several cell types with the hopes of reprogramming them into β cells, including pancreatic exocrine cells, keratinocytes, hepatic oval stem cells, adipose-derived keratinocytes, and hepatocytes. Of these, hepatocytes have been most commonly targeted due to the fact that they are closely related developmentally to β cells.

To accomplish this reprogramming, the transcription factor pancreatic and duodenal homeobox gene 1 (PDX1), which regulates pancreatic development during embryogenesis and controls β cell function in adults, has proven indispensable for the conversion of non-β cells into β cells. Ectopic expression of PDX1 has proven quite successful at converting non-β cells into β cells capable of synthesizing, processing, and secreting insulin. In conjunction with PDX1, other transcription factors like NKX6.1, Neurogenin, and NeuroD have been shown to enhance reprogramming efficiency. Importantly, many studies have found that surrogate β cells produced from reprogramming are able to ameliorate streptozotocin-induced hyperglycemia in mice. Despite these successes, the overall efficacy of reprogramming strategies relies on the long-term absence of recurring autoimmunity against newly formed β cells, which inevitably express a variety of autoantigens that ultimately led to the destruction of native β cells to begin with. While studies in nonobese diabetic mice, a model of autoimmune diabetes, provide hope that autoimmune destruction could be averted through reprogramming strategies, these studies must be performed for longer time periods to assess true efficacy. Ultimately, these strategies will require either lifelong immunosuppression or selective immunomodulation to prolong the survival of the newly generated β cells.

Insulin gene therapy

Given the autoimmune etiology of T1DM, it would be paradoxically advantageous to treat the disorder without regenerating β cells, as the risk of their autoimmune destruction is theoretically great. Instead, it may be advantageous to take a minimalist approach and simply replace the key functions of β cells without substantially altering the phenotype of the host cell. Along these lines, researchers have actively investigated the possibility of expressing insulin alone in non-β cells, a field known as insulin gene therapy.

For insulin gene therapy strategies to be successful, several criteria must be considered. First, an appropriate target organ must be selected. Ideally, the target organ would have the ability to sense and respond to continually changing blood glucose levels. In addition, it would be advantageous for the target organ to have the capacity to store insulin and secrete it in a rapid and glucose-inducible fashion. Second, an effective gene delivery method must be used that is both safe and effective at driving long-term insulin expression in the target organ. Lastly, ectopic insulin expression should be responsive to fluctuating blood glucose levels, being up-regulated during hyperglycemia and downregulated during euglycemia. The following sections will outline...
important considerations to optimize each of these criteria and summarize the current state of the field.

The most important feature that a target cell for insulin gene therapy should possess is the ability to respond to fluctuating levels of glucose. This implies that the cells express both glucose transporter-2 (GLUT2) and glucokinase (GK), enabling transport of glucose into a cell and its subsequent metabolism.24 The only cells that express both GLUT2 and GK are pancreatic β cells, hepatocytes, and cells of the hypothalamus and small intestine. Of these, hepatocytes are particularly good candidates for insulin gene therapy because they are essential regulators of glucose metabolism in response to insulin. Of course, GLUT2 and GK can be co-expressed with insulin in other cell types, but this greatly increases the complexity of gene therapy. For example, Bosch and colleagues targeted skeletal muscle cells, one of the primary targets of insulin action, and found that GK needed to be co-expressed with insulin to attain normoglycemia.25 Similarly, Hughes and coworkers found that AtT-20ins cells—anterior pituitary cells that express GK but not GLUT2—needed to be cotransfected with insulin and GLUT2 to confer glucose-stimulated insulin secretion.26,27

It would also be beneficial for the cellular target of gene therapy to be immunoprivileged, thus allowing it to evade preexisting autoimmunity. This is particularly critical, given that insulin has been shown to be one of the primary autoantigens targeted by the adaptive immune system.23 Importantly, previously published studies have shown that insulin misexpression in the liver does not cause hepatocytes to become the target of autoimmunity.28

Lastly, it would be ideal if the target cells could process proinsulin—a precursor form of insulin with greatly reduced biological activity—into mature insulin and store it in granules for immediate secretion upon hyperglycemia. For proper processing of proinsulin, the β cell prohormone convertases PC2 and PC3 are required.29 Unfortunately, these enzymes are only expressed in neuroendocrine secretory cells. To complicate matters further, few cells in the body have the capacity to store insulin (or any other protein) in secretory granules. Thus, groups have devised novel strategies to bypass these concerns. Irminger and associates expressed PC2 and PC3 in rat insulinoma cells and found that proinsulin could then be processed into mature insulin.30 Using this strategy, virtually any cell of the body could be induced to express fully processed insulin. However, misexpressing exogenous proteases in vivo can have unforeseen consequences that greatly hinder cellular activities, perhaps limiting the use of this strategy. Thus, other strategies have been used to bypass this concern.

To compensate for most cells’ inability to store insulin, Rivera and associates engineered an insulin analogue that aggregated in the endoplasmic reticulum and was only secreted upon stimulation with a synthetic small molecule drug that induces protein disaggregation, in essence turning the endoplasmic reticulum into a proinsulin storage depot. Indeed, they showed that this method led to rapid secretion of insulin and lowered diabetic hyperglycemia in mice.31 Similarly, Auricchio and associates obtained regulated secretion of insulin using a rapamycin-inducible system in vivo.32 However, when using drug-inducible systems, researchers must select the drug of choice carefully to limit unwanted adverse effects. For instance, rapamycin produces diabetes-like symptoms, such as insensitivity to insulin and decreased glucose tolerance, and as such, would not be desirable for insulin gene therapy applications.

To deliver insulin to the target cell, researchers have used several gene delivery vehicles. The ideal gene expression vector for insulin gene therapy should have the ability to target specific cells, transduce both dividing and nondividing cells, be nonimmunogenic, have reliable methods for large scale production, and most importantly, induce long-term expression of insulin. Both viral and nonviral vector-based gene delivery methods have been used for insulin gene therapy applications, with each showing successful amelioration of diabetes-associated hyperglycemia in small animal models.33-35 However, nonviral gene delivery methods are limited by their inefficient delivery to target cells and lack of chromosomal integration, which restricts the longevity of gene expression. As such, viral vectors have been used more prevalently in insulin gene therapy applications and are ultimately the future of the therapy. Adenoviruses,34,36,37 adeno-associated viruses,25,38,39 oncoretroviruses,40,41 and lentiviruses42-44 all have been used to deliver insulin to hepatocytes, but they vary in their ability to meet the essential criteria
described previously. A summary of each viral vector’s ability to meet the demands required for insulin gene therapy applications is included in Table 1. Based on the fact that lentiviral vectors can transduce both dividing and nondividing cells, are nonimmunogenic, and can induce long-term expression of insulin, they are considered the optimal gene delivery vehicle for long-term correction of T1DM and its associated complications, and indeed, they have proven successful at delivering insulin to the liver in several different studies.42-44

The most critical aspect dictating the success of insulin gene therapy strategies is the sophistication of the DNA construct. The ideal DNA construct must be able to produce a sufficient quantity of biologically active insulin in a glucose-responsive fashion. In addition, it would be beneficial if the DNA construct design restricted the expression of insulin specifically to the target cell and included additional components to precisely attenuate the amount of insulin produced as hyperglycemia subsides. A construct possessing these features would hold great utility in insulin gene therapy.

Given that most cells, including hepatocytes, do not express PC2 or PC3, the first feature that an insulin construct should possess is a proinsulin cDNA sequence capable of being processed by the target cells into mature insulin. As mentioned previously, wild-type proinsulin expressed in cells other than β cells will lack potent bioactivity due to the absence of the proinsulin processing machinery. The most commonly used strategy to overcome this problem has been to incorporate furin cleavage sequences within the preproinsulin cDNA sequence. In so doing, the modified proinsulin can be cleaved by furin—a ubiquitously expressed endoprotease. Simonson and colleagues incorporated furin cleavage sites between the B-chain and C-peptide, and between the C-peptide and A-chain of proinsulin, and found a significant increase in the secretion of fully processed insulin from rat myoblasts, which ultimately resulted in improved glucose oxidation.45 This provides the opportunity for virtually any cell type in the body to produce fully mature insulin, albeit in a constitutive and unregulated fashion.

The next feature of an ideal DNA construct would be an element that restricts expression to the targeted cell type of choice. To do so, many groups have used tissue-specific promoters, and for hepatic insulin gene therapy, a variety of liver-specific promoters have been used. Examples of liver-specific promoters include the insulin-like growth factor binding protein-1 (IGFBP-1),46 glucose-6-phosphatase (G6Pase), liver-type pyruvate kinase (L-PK),47 phosphoenolpyruvate carboxykinase (PEPCK),28 albumin,34,37 and S-14. While liver-specific transcriptional regulation of insulin gene transcription has been achieved using these promoters, significant limitations have been observed. These gene expression systems displayed relatively weak promoter activity, especially when compared with strong constitutive promoters like cytomegalovirus. As a result, the amount of secreted insulin was often insufficient to fully correct hyperglycemia. For instance, Han and colleagues used the L-PK basal promoter to obtain glucose-responsive, liver-specific insulin expression and found that it displayed dramatically lower luciferase activity than the constitutive cytomegalovirus promoter. Even after adding a series of enhancer elements upstream of the promoter, its transcriptional activity remained weaker than that of the cytomegalovirus promoter. Nonetheless, the modified L-PK promoter showed glucose-responsiveness and was able to restore normoglycemia for up to 1 month, although glucose clearance was delayed and slower than normal.47 It should be noted that 1 potential limitation of using the L-PK promoter is that it is inhibited by insulin, thus potentially creating an undesirable negative feedback on insulin expression. To avoid this scenario while still attaining liver-specific transgene expression, albumin and S14 promoters could be used.

### Table 1. Capacity of Various Viral Vectors To Meet the Needs of Insulin Gene Therapy

<table>
<thead>
<tr>
<th>Viral Vector</th>
<th>Target specific cells</th>
<th>Transduction of both dividing and nondividing cells</th>
<th>Immunogenic</th>
<th>Long-term transgene expression</th>
<th>Large-scale vector production</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adenovirus</td>
<td>-</td>
<td>+</td>
<td>++</td>
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<td>-</td>
</tr>
<tr>
<td>Adeno-associated viruses</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Oncoretrovirus</td>
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<tr>
<td>Lentivirus</td>
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Viral vectors vary in their capacity to meet the needs of specific gene therapy applications. For insulin gene therapy, lentiviruses are well-suited based on their ability to target specific cells, transduce both dividing and non-dividing cells, and induce long-term insulin expression without causing a marked immune response.
To enhance insulin expression from weak promoters, enhancer elements are commonly incorporated into the DNA construct. A useful enhancer for insulin gene therapy is the glucose-inducible responsive element. Thule and associates constructed a glucose-responsive IGFBP-1 promoter to control insulin expression in the liver by inserting glucose-responsive elements from the rat L-PK gene. In so doing, they were able to induce insulin expression in a glucose-dependent manner from cultured primary rat hepatocytes in vitro and correct streptozotocin-induced hyperglycemia in rats in vivo. However, the IGFBP-1 promoter, like the L-PK promoter, is inhibited by insulin. Conversely, our work has used an insulin expression plasmid containing 3 copies of the S14-based glucose-inducible responsive elements to confer glucose dependence to the liver-specific rat albumin promoter.

Using this system to control the expression of furin-cleavable insulin, we demonstrated that the glucose-inducible production of processed insulin was able to reduce fasting blood glucose levels, blood glucose levels of rats fed ad libitum, and peak blood glucose levels during oral glucose tolerance tests in streptozotocin-treated diabetic rats. While significantly improved, blood glucose levels of rats fed ad libitum and during the oral glucose test failed to reach that of nondiabetic rats.

To improve upon the efficiency of this initial glucose-responsive insulin construct, we incorporated additional elements known to enhance gene transcription, mRNA processing, and translational efficiency. Specifically, we tested the ability of a transcriptional enhancer from the human α-fetoprotein gene, an intron from the human growth hormone sequence that enhances RNA processing efficiency, a translation enhancer from the human vascular endothelial growth factor sequence that functions as an internal ribosomal entry site, and an intron from the 3'-untranslated region of the human albumin sequence that facilitates mRNA processing to enhance insulin production and better restore normoglycemia in streptozotocin-treated diabetic rats. In so doing, we found that incorporation of the vascular endothelial growth factor-derived translational enhancer alone resulted in a 4- to 6-fold increase in insulin production in both low and high glucose conditions. Furthermore, we found that individual incorporation of the human growth hormone intron or α-fetoprotein transcriptional enhancer enhanced insulin production. However, when both elements were incorporated together, insulin production was not further improved. Thus, we selected a DNA construct that contained the α-fetoprotein transcriptional enhancer, vascular endothelial growth factor translational enhancer, and albumin 3'-untranslated region in addition to the 3 copies of glucose-inducible responsive elements, albumin promoter and human insulin gene (Figure 1).

When testing this insulin construct for its ability to improve the overall phenotype of streptozotocin-treated diabetic rats, we were not only able to induce normoglycemia in fasting rats but also rats fed ad libitum (Figure 2). In treated diabetic rats, we were
also able to correct weight loss due to uncontrolled hyperglycemia such that the rate of weight gain matched that of healthy control rats for at least 1 month (Figure 3). In addition, intraperitoneal glucose tolerance tests demonstrated a correction of hyperglycemia within 45 minutes. Importantly, a single treatment with our DNA construct significantly improved many systemic abnormalities downstream of hyperglycemia. Specifically, it raised serum albumin levels in diabetic rats to normal and restored elevated serum levels of aspartate transaminase, alanine aminotransferase, and alkaline phosphatase to near normal. The treatment also significantly reduced hyperglycemia-associated hypertriglyceridemia and hypercholesterolemia, indicating healthy liver function. Interestingly, even after precise glycemic control was lost (~1 month), the metabolic benefits of treatment with our DNA construct persisted well beyond that, suggesting that even suboptimal insulin production can counteract much of the immediate systemic damage associated with hyperglycemia. Based upon these results, our liver-based insulin gene therapy treatment offers a promising approach to treat T1DM.34

**Future perspectives**

Many advances in hepatic insulin gene therapy have been made over the past 2 decades, fueling optimism that a treatment for T1DM may be found sooner rather than later. However, several obstacles still remain before this approach can become a clinical reality. First, it is important to optimize the hepatic delivery of vectors to drive glucose-responsive insulin production. Currently, physiologic control of blood glucose levels has been obtained using glucose-responsive, liver-specific promoters delivered via adenoviral vectors and minicircles, as well as via lentiviral vectors with strong constitutive promoters. Ultimately, it will be necessary to combine the strengths of lentiviral vectors and glucose-responsive, liver-specific promoters to obtain long-
term, dynamic correction of diabetes without the threat of hypoglycemic episodes. In fact, such a treatment could potentially provide lifelong correction of T1DM. To do so, it may be necessary to add additional elements to our current glucose-responsive insulin constructs to further enhance expression of insulin.

Second, although insulin gene therapy has yielded promising results in mouse and rat models of T1DM, these models do not always accurately replicate human disease, especially when the immune system plays a role in the pathology of the disease. Future studies, like those conducted by Bosch and colleagues, must be conducted in large animal models, such as dogs, pigs, or nonhuman primates, to provide a better indication of the efficacy of insulin gene therapy before contemplating human clinical trials.

Lastly, it will be critical to assess the long-term safety of lentiviral vector-mediated insulin gene therapy. At this time, malignant transformation associated with vector-mediated insertional mutagenesis has only been observed in 3 clinical entities, all of which occurred using first generation gammaretroviral vectors. However, with the advent of later-generation lentiviral vectors, no adverse events have been observed, and there are currently over 2000 ongoing human gene therapy clinical trials worldwide. In addition, there are several safety mechanisms, such as self-inactivating vectors, insulators, and suicide genes, which have been built into viral vectors for gene therapy applications, thereby providing many preemptive options to improve the safety of vectors. Overall, the future appears very bright for insulin gene therapy as an alternative strategy to treat T1DM.

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How Evolution Tells Us To Induce Allotolerance

Walter Gottlieb Land

Abstract
Modern immunology, in many ways, is based on 3 major paradigms: the clonal selection theory (Medawar, Burnet; 1953/1959), the pattern recognition theory (Janeway; 1989), and the danger/injury theory (Matzinger, Land; 1994). The last theory holds that any cell stress and tissue injury including allograft injury, via induction of damage-associated molecular patterns, induces immunity including alloimmunity leading to allograft rejection. On the other hand, the concept precludes that “non-self” per se induces immunity as proposed by the two former theories.

Today, the danger/injury model has been largely accepted by immunologists, as documented by a steadily increasing number of publications. In particular, overwhelming evidence in support of the correctness of the model has come from recent studies on the gut microbiota representing a huge assemblage of “non-self.” Here, harmless non-injurious commensal microbes are protected by innate immunity-based immune tolerance whereas intestinal injury-causing pathogenic microbes are immunology attacked.

The ability of the immune system to discriminate between harmless beneficial “non-self” to induce tolerance and harmful life-threatening “non-self” to induce immunity has apparently emerged during evolution: Protection of innate immunity-controlled beneficial “non-self” (eg, as reflected by microbiotas but also by the fetus of placental mammals) as well as immune defense responses to injuring/injured “non-self” (eg, as reflected by plant resistance to biotic and abiotic stress and allograft rejection in mammals) evolved under pressure across the tree of life, that is, in plants, lower and higher invertebrates as well as lower and higher vertebrates.

And evolution tells us why the overall existence of protected microbiotas really makes sense: It is the formation of the “holobiont.” - a metaorganism - that is, the host plus all of its associated microorganisms that - in terms of a strong unit of selection in evolution - provides that kind of fitness to all species on earth to successfully live, survive and reproduce. In other words: “We all evolve, develop, grow, and reproduce as multigenomic ecosystems!

Regarding reproduction, another impressive example of active immunologic protection of “non-self” refers to pregnancy in placental mammals that emerged about 400 millions of years ago. Similar to “non-self” microbiotas, pregnancy in placental mammals reflects an evolution-driven phenomenon on the basis of innate immunity-controlled tolerance induction to semiallogeneic non-injuring/non-injured “non-self” aiming to ensure reproduction!

Altogether, the lesson learned from evolution of how to avoid allograft rejection is clear: prevent allograft injury to induce allotolerance, in other words: create a “transplant holobiont.”

Key words: Innate immunity, Evolution, Immunologic tolerance
Background

The danger/injury model

Modern immunology in many ways is based on 3 major paradigms. The 2 traditional models (clonal selection theory and pattern recognition theory) both state that microbial “nonself” induces immunity, which is “the science of self/nonself discrimination.”

More recently, the danger/injury theory states that injury induces immunity. The latter model was developed from 2 sources. At 20 years ago, (1) its discovery by our group during a clinical trial in kidney transplant patients provided convincing evidence that tissue injury (referred to in that article as allograft injury) induces immunity (alloimmunity-mediated allograft rejection), and (2) its publication by Polly Matzinger as a self-coherent chain of arguments resulting in the stringent conclusion that the self/nonself discrimination theory of immune responses must be inappropriate.

After the discovery of the innate immune system in the late 1990s, the danger/injury model was modified several times. Today, its core refers to the scenario that pattern recognition receptors (PRRs) expressed on or in cells of the innate immune system recognize microbe-associated molecular patterns (MAMPs) and danger/damage-associated molecular patterns (DAMPs). The DAMPs (a term coined by those 2 groups in the early 2000s) are endogenous molecules that normally are hidden from recognition by the immune system, but are induced by any tissue injury or cell stress. As reviewed previously, DAMPs are released from dying cells or damaged extracellular matrix as high mobility group box 1 (HMGB1), nucleic acids, extracellular adenosine triphosphate (ATP), and hyaluronan; they also can be exposed on stressed cells such as major histocompatibility complex class I chain-related proteins (MICs) and injury-induced altered-self antigens (neoantigens) which bind to natural immunoglobulin M (IgM), thereby activating the complement cascade.

According to current theories, cell stress/tissue injury-induced DAMPs, following recognition by PRRs, trigger innate immune pathways, resulting in a “sterile” inflammatory response. In the presence of nonself antigens such as microbial or allogeneic antigens, dendritic cells (DCs) become activated to elicit an adaptive immune (alloimmune) response (Figure 1). The key event of an emerging adaptive alloimmune response resulting in allograft rejection is the DAMPs-induced maturation of PRRs-bearing, donor- and recipient-derived, immature DCs to allostimulatory human leukocyte antigen (HLA)/non-HLA-presenting DCs. The latter DCs are able to migrate to the secondary lymphoid tissue of the recipient to activate naïve alloreactive T lymphocytes. This has been reviewed previously.

The danger/injury model also inherently precludes that nonself per se induces immunity, but in contradistinction, induces immunologic tolerance (Figure 1). As reviewed previously, in the absence of injury, presentation of harmless nonself antigens under noninflammatory subimmunogenic conditions, promotes generation of tolerogenic DCs (tolDCs) that are able to induce regulatory T cells (Tregs) dedicated to promote induction of immunologic tolerance.

The mammalian gut microbiota

The danger/injury model currently has been widely accepted by immunologists. Recent new insights into the role of the mammalian intestinal microbiota in maintaining immune homeostasis - in terms of a fascinating inversion of the self/nonself discrimination view of immunology - have underscored

![Figure 1. Modern Version of the Danger/Injury Model in Immunology (Oversimplified Diagram)](image)

Abbreviations: DAMPs, damage-associated molecular patterns; DCs, dendritic cells; MHC, major histocompatibility complex; PRRs, pattern recognition receptors; Th1, T helper 1 cell; Th17, T helper 17 cell. Nonself antigens in the presence of injury, and in association with recognition of DAMPs by PRRs-bearing cells of the innate immune system, generate immunostimulatory DCs which induce Th1-/Th17 cells, thereby promoting immunity. Nonself antigens in the absence of injury generate tolerogenic DCs which induce regulatory T cells, thereby promoting immune tolerance.
the correctness of the concept. The new paradigm in immunology has been shifted to the recognition that it is not the molecular structure of pathogens that activates the immune system, but rather, their perturbation of and damage to generic cellular processes.

More than 100 trillion harmless commensal microorganisms (total, 1.5 kg) colonize the human oral cavity and gastrointestinal tract, outnumbering human cells by a ratio of 10:1. The composition and activity of this resident nonself play crucial roles in shaping the metabolic and regulatory networks that define good human health. Its effect on human well-being, which cannot be overemphasized, is mediated by a tremendous repertoire of microbial genes (microbiome) that perform myriad functions beneficial to the host, such as the development of gut-associated lymphoid tissues (GALTs), as reviewed previously. The microbiota play a pivotal role in the development of T cells, both within and outside the intestine, by selectively expanding and activating different T cell subsets under normal and/or pathologic conditions. In turn, intestinal innate immune responses that are induced by commensal populations regulate the composition of the mammalian microbiota, pointing to a complex interplay between the host immune system and the microbiota that is necessary for gut homeostasis. Toll-like receptors (TLRs) are crucial for maintaining tolerance to and shaping the assemblage and composition of commensal microbiota. Moreover, the mammalian gut innate immune system is able to discriminate (under the control of DCs and regulated by innate immune PRRs including TLRs and NOD-like receptors [NLRs]) between harmless nonself such as food antigens and commensal bacteria (to induce tolerance) and harmful nonself such as pathogenic bacteria, viruses, and fungi (to induce immunity). The extraordinary homeostasis is maintained by the generation of tolDCs to induce Foxp3-expressing Tregs, thereby maintaining mucosal tolerance and, in contrast, generation of immunostimulatory DCs to promote induction of Th1 and Th17 cells, thereby mounting immune responses, as reviewed previously (Figure 2). Hence, nonself antigens of gut commensals are not simply ignored, but rather, they trigger an active immunosuppressive process to maintain intestinal homeostasis. Accordingly, the immune system appears to be more a “bouncer at a nightclub,” rather than a defensive army to keep our organism “pure” from microbes. The immune system actively tolerates and recruits, farms, and protects the nonself symbionts that do not cause injury.

Figure 2. Scenario Model of the Complex Interplay Between the Mammalian Immune System and the Microbiota

Abbreviations: cT cell, conventional T cell; DAMPs, damage-associated molecular patterns; DC, dendritic cell; immunostimul., immunostimulatory; MAMPs, microbe-associated molecular patterns; PRRs, pattern recognition receptors; Th1, T helper 1 cell; Th17, T helper 17 cell; Treg, regulatory T cell

The innate immune system is able to discriminate, under the control of dendritic cells, and regulated by innate immune PRRs, between (1) harmless “nonself” (commensal bacteria) to induce Treg-mediated tolerance and (2) harmful nonself (pathogenic bacteria, viruses, and fungi) to induce Th1/Th17-mediated immunity. The extraordinary homeostasis is maintained by the generation of tolerogenic DCs to induce Tregs, thereby maintaining mucosal tolerance and, in contrast, generation of immunostimulatory DCs to promote induction of Th1 and Th17 cells, thereby mounting immune responses. Presentation of microbial antigens by DCs to T cells is illustrated in terms of signal 1; signal 2 refers to costimulatory molecules, and signal 3 refers to secretion of Th1-/Th17 cell polarizing cytokines.
The danger/injury model in light of evolution

Tolerance to/protection of nonself in the absence of dangerous injury

Evolutionary biologists tell us of the ubiquity of nonself microbial associations that is not peculiar to mammals including humans. Rather, tolerance to/protection of harmless nonself is the normal condition for all creatures across the tree of life including plants, sponges, lower and higher invertebrates, and lower and higher vertebrates. Symbiosis is the rule and not an exception in the plant and animal kingdom. This recognition of biologists began with the work of Woese and Fox who, in the late 1970s, opened a new research frontier by producing sequence-based measures of phylogenetic relations, revealing the deep evolutionary history shared by all living organisms. This innovative concept led to a rapid development and application of molecular sequencing technologies, which allowed biologists for the first time to recognize the true diversity, ubiquity, and functional capacity of microorganisms.12 This recognition, in turn, has led to a new understanding of the biology of plants, animals, and bacterial kingdoms that have coevolved and coadapted in response to environmental selective pressures over hundreds of millions of years, thereby allowing emergence of microbiomes. Animals, for example, can no longer be considered individuals in any sense of classic biology: anatomic, developmental, physiologic, immunologic, genetic, or evolutionary.12,13

Bacteria and the origin of animals

To properly cope with this issue, we first must understand the periods when nonself associations among bacteria and animals first evolved. The last common ancestor of plants and animals may have lived approximately 1 billion years ago. As reviewed previously,14 animals diverged from their protistan ancestors 700-800 million years ago, 3 billion years after bacterial life originated, and 1 billion years after the first appearance of eukaryotic cells. Thus, the current relations between protists and bacteria, from predation to obligate and beneficial symbiosis, probably were operating already when animals first appeared. Sponges (animals of the phylum Porifera) harbor hundreds of bacterial species. As early animals further diversified, animal-bacteria interactions continued to shape evolution in new ways, such as training and educating ancient animals to establish sophisticated metabolic pathways and efficient innate immune pathways that allowed immune-mediated host defenses as observed in sponges,15 cnidarians,16 and insects.17

The long history of shared ancestry and alliances between animals and microbes is reflected in their genomes. As reviewed previously,14 analysis of the large number of full genome sequences presently available reveals that most life forms share approximately one-third of their genes, including those encoding central metabolic pathways. Many animal genes are homologues of bacterial genes, mostly derived by descent, but occasionally by gene transfer from bacteria. In 37% of the 23 000 human genes, there are homologues in the Bacteria and Archaea, and another 28% of the genes originated in unicellular eukaryotes. Among these homologous genes are some genes whose products provide the foundation for signalling between extant animals and bacteria, thereby allowing communication between microorganisms and their hosts.

Tolerance to/protection of beneficial nonself microbes

In accordance with the long history of shared ancestry and alliances between animals and microbes, evolutionary biologists provide increasing evidence suggesting that induction and maintenance of tolerance to/protection of harmless (beneficial) nonself (exemplified by resident microbiotas) reflect an evolution-driven paradigm in immunology that is controlled actively by components of the innate immune system. There are 2 examples from evolution that are addressed here.

As shown in studies on transgenic Hydra polyps with an altered antimicrobial peptide (AMP) repertoire or silenced TLR/MyD88 activity, components of the innate immune system of the cnidarian Hydra (a 3-cm small animal) are involved in maintaining homeostasis between the animal and its nonself resident microbiota.18 Toll-like receptor-controlled innate immune pathways lead to secretion of AMPs with regulatory functions in host-microbe homeostasis to protect beneficial and coevolved microbes, whereas bactericidal peptides may kill pathogenic microbes (Figure 3).16,18-22 As argued in relation to observations from Hydra,18 AMPs appear to be key factors for host-bacteria coevolution. Thus, the role of TLRs in controlling the resident microbiota could date back to the earliest
multicellular organisms, because humans and Hydra share molecules involved in the TLR signalling cascade such as the Toll/interleukin-1 receptor (TIR) domain.

Similarly, innate immune recognition proteins that recognize bacteria-derived MAMPs control and maintain resident microbes in Drosophila melanogaster. Protective immune tolerance to the commensal microbiota is maintained via the peptidoglycan recognition protein SC2, a negative regulator of IMD/Relish innate immune signalling. In contrast, this pathway, when triggered by another set of recognition receptors (PGRP-LE and -LC), leads to production of AMPs that kill injury-inducing pathogenic microbes (Figure 3).19-22

Evolution tells us why the overall existence of microbiotas under the control of the innate immune system makes sense. According to the hologenome concept of evolution,23 our bodies must be understood as “holobionts” whose anatomic, physiologic, immunologic, and developmental functions evolved in shared relations between different species. The formation of the “holobiont,” a metaorganism, (the host [“self”] plus all its associated and integrated microorganisms [nonself]) provides – in terms of a strong unit of natural selection in evolution – that kind of fitness to all species on earth to successfully live, survive, and reproduce. We all develop, grow, and evolve as multigenomic ecosystems, armed with an immune system directed against sterile and infectious injuries. In fact, one may conclude that we have never been individuals!

**Tolerance to/protection of semiallologeneic nonself, the fetus in placental mammals**

Formation of host (self)-microbiota (nonself) holobionts is 1 example of how evolution works. Another example of active immunologic protection of nonself is pregnancy of eutherian placental mammals, which can be regarded as a host (self)-semiallologeneic (nonself) holobiont that emerged during the Devonian era approximately 380 million years ago,24 in an era when evolution already had created host-microbiota holobionts millions of years before placental mammals appeared. Emergence of tolerance to a nonself fetus in placental mammals

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**Figure 3. Proposed Evolutionary Model of Microbiota (oversimplified diagram based on first reports from the literature)**

**Figure 4. In the Interest of Evolution**

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**Abbreviations:** AMP, antimicrobial peptide; DAMPs, damage-associated molecular patterns; MAMPs, microbe-associated molecular patterns; MSU, monosodium urate; PGRP, peptidoglycan recognition protein; PRRs, pattern recognition receptors; TLR, Toll-like receptor

Active control of microbiota composition by PRRs of the innate immune system, exemplified by studies on Drosophila melanogaster and Hydra polyp. In Hydra, a protective response to commensal populations is supposed to be provided by TLR-controlled innate immune pathways leading to secretion of AMPs with regulatory functions, whereas DAMPs such as monosodium urate and foreign RNA have been shown to elicit a strong innate immune defense response, as reflected by upregulation of the bactericidal peptide periculin-1. In Drosophila, a protective immune tolerance to the commensal microbiota is reportedly maintained via the PGRP-SC2, a negative regulator of IMD/Relish innate immune pathway, whereas another set of recognition receptors, the PGRP-LE and PGRP-LC, trigger the IMD/Relish pathways leading to production of bactericidal AMP that can kill injury-inducing pathogenic microbes; cell death-associated DAMPs such as proteases presumably operate as ligands of PRRs (as shown in other sets of studies).19-22
may reflect the flexibility in evolution to use, as a blueprint, the more ancient innate immunity-controlled mechanisms to induce tolerance to commensal microbes, creating a “triple holobiont” in pregnant woman (Figure 4). Allogeneic pregnancy represents a physiologic situation in which tolerance to paternal alloantigens is critical for successful reproduction of placental mammals, serving survival of a species as a stringent imperative of evolution. Multiple mechanisms that operate during pregnancy to limit responses of maternal alloreactive T cells to fetal alloantigens have been described, but induction of Tregs appears to play a key role.25 There is compelling evidence in favor of an evolutionary selected mechanism of Treg-cell–mediated maternal-fetal tolerance gained during evolution of eutherian mammals. In analogy to the capacity of Tregs to mediate tolerance against nonself antigens of the microbiota,11 extrathymic generation of Tregs in placental mammals may have arisen to mitigate maternal-fetal semiallogeneic incompatibility, a scenario primarily driven by the evolutionary pressure to enforce maternal-fetal tolerance.26,27 Recent studies in human pregnancy revealed a significant increase of highly suppressive HLA-DR+ Tregs in the decidua.28

In accordance with this scenario, there is increasing experimental data suggesting that the induction of Tregs during pregnancy is controlled by the innate immune system via generation of tolDCs, a mechanism that also is described for composition and maintenance of the mammalian microbiota.29,30 As reviewed previously,31 during normal pregnancy, most human and murine decidual DCs, under the effect of pregnancy-associated hormones, present a tolerogenic phenotype and mainly produce interleukin 10. In line with this, spontaneous abortions in humans and mice are associated with an increased number of immunostimulatory DCs. The DCs are highly susceptible to hormonal stimulation by expressing receptors for progesterone, estradiol, human chorionic gonadotropin, and luteinizing hormone. Accordingly, hormonal stimulation of activated bone-marrow–derived DCs resulted in most studies in an impaired upregulation of major histocompatibility complex class II molecules and costimulatory molecules associated with a reduced capability to secrete proinflammatory cytokines. In addition, a role for PRRs in controlling these innate immune mechanisms has been addressed.32

**Immunity/resistance to nonself in the presence of dangerous injury**

As mentioned above, the danger/injury model includes the proposal that injury, via induction of DAMPs (and not nonself per se) elicits immunity. There are examples from evolution in support of this proposition, and 3 examples will be addressed briefly in the following subsections, the first dealing with plants.

**Evolutionary role of DAMPs in plants**

Plants are complex organisms. Their sessile lifestyle forces them permanently to monitor their surrounding hostile environment to identify dangerous stimuli and stressors that originate from attackers. Since plants do not have an adaptive immune system to defend against all those enemies, they initiate defense by relying solely on a sophisticated innate immune system that bears similarities with animal innate immune systems, but which probably evolved independently.33 The plant defense system recognizes microbial elicitors, such as MAMPs, herbivore-associated molecular patterns (HAMPs), and DAMPs that are released upon tissue injury caused by pathogenic microbes and other nonmicrobial inciting conditions. As reviewed previously,34 prominent examples of plant-derived DAMPs include cell wall fragments of larger plant molecules that are formed upon damage, such as oligogalacturonide fragments, resistance-inducing oligosaccharides, cell-wall-derived pectins, and endogenous proteins such as systemin and volatile organic compounds (VOCs). Certain immune responses of plants that are directed against herbivores can indirectly defend plants by changing the behavior of the natural enemies of the herbivores. An indirect defense is the emission of herbivore-induced plant volatile compounds that are specific VOCs that attract predators and parasitoids of insect herbivores to the attacked plant.35 Plant biologists previously believed that this indirect defense response was directed against nonself, such as the mucus of the caterpillar. However, this is incorrect. Early studies were performed on a mechanical artificial caterpillar (Mec Worm) that could cause permanent damage during a prolonged wounding period. The use of this device mimicked the spatiotemporal feeding patterns of living herbivores that cause the lima bean (Phaseolus lunatus) to release a blend of VOCs as an indirect
defense response that resembled what was observed after insect feeding on the same plant. These experiments demonstrated that the effect of mechanical wounding (that is, sterile injury) on the induction of defense responses during herbivore feeding was substantial. More recent studies extended these early observations. Flame wounding or applying leaf extract or solutions of sucrose or ATP to slightly wounded lima bean leaves induced an indirect defense mechanism.

**Evolutionary role of DAMPs in invertebrates**

Another evolutionary observation in support of the concept that injury induces immunity refers to studies on the cnidarian Hydra polyps that were either exposed to monosodium urate for 24 hours or to foreign dsRNA introduced by electroporation, both known as typical DAMPs released from dying mammalian cells. Exposure to these 2 DAMPs elicited a strong innate immune response, reflected by a significant up-regulation of the expression of periculin-1, a novel antimicrobial bactericidal peptide of Hydra (Figure 3).

In addition, there is increased evidence in support of the theory that the injury-inducing immunity concept has evolved in Drosophila melanogaster. In most gram-positive bacteria and fungi, recognition of MAMPs by extracellular PRRs of D. melanogaster leads to secretion of microbial/fungal cytotoxic proteases that operate as danger signals to trigger the Toll pathway via activation of the endogenous Toll ligand Spätzle. Spätzle can be regarded as a protease-induced DAMP that, in cooperation with MAMPs, elicits an innate immune response against pathogens. This theory is supported by recent reports that showed that wounding, which leads to a sterile injury-induced innate immune response in the fly, is associated with upregulation of serine protease activity.

Further support of the concept includes more recent studies in D. melanogaster that showed that cell-death-induced production of DAMPs, such as certain proteases, leads to a Toll signalling pathway through a persephone-mediated proteolytic cascade that cleaves the Toll ligand Spätzle (Figure 3). A more recent report referred to the range of wound-induced immune responses that can be modelled in flies. These wounding models have revealed the most immediate signals leading to immune cell activation, and highlighted a number of complex signalling cascades required for subsequent injury-associated inflammatory responses in flies. These recent results from studies in insects indicate that a Toll-like receptor-triggered defense pathway, elicited by injury-induced DAMPs, is highly conserved in invertebrates.

**Role of DAMPs in parturition**

Tolerance to/protection of semiallogeneic nonself, the paternal antigens, in placental mammals may represent an active evolution-driven process of the innate immune system, and not just ignorance of foreign antigens. However, evolution in reproduction continues when the fetus is mature, such as in disrupting the tolerant state. At term, evolution has solved the problem by again harnessing the function of the innate immune system by using injury-induced DAMPs. There is increasing evidence indicating that human preterm or term parturition reflects a process of disruption of the fetal tolerance state by an infectious or sterile-injury-induced maternal innate immune response.

Human parturition reportedly is initiated by an inflammatory innate immune response elicited by DAMPs derived from fetal or amniotic fluid such as HMGB1 in preterm gestation or fetal DNA at term. These DAMPs, presumably induced by mechanical stretch associated with oxidative stress to the myometrium (possibly hypoxia-induced), enter the uterine vessel circulation (a common physiologic mechanism at term prior to labor) to reach innate immune cells within the uterine wall. Thus, in term parturition, creation of sterile uterine inflammation mediated by proinflammatory mediators produced by DAMP-activated infiltrating leukocytes causes uterine contraction, labor, and delivery, as reviewed previously (Figure 5). Therefore, this is an evolution-driven event that mirrors the vital interest of evolution. It is an injury-induced innate immune process that initiates human parturition, aiming at successful reproduction of the human species, and is a process that may be interpreted as the innate immune-mediated rejection of semiallogeneic nonself. This scenario is reminiscent of injury-induced innate/adaptive immune-mediated allograft rejection.

**Outlook**

Does knowledge and information about evolution have an effect on currently applied organ transplant? Can the facts emerged during evolution be applied...
to new strategies and efforts to prevent allograft rejection and induce allotolerance?

To properly answer this question, we must understand the periods when nonself associations between bacteria and animals evolved. We must realize that organ transplant was introduced and developed by surgeons 100 years ago, a small period compared with the 800 million years when animals evolved. Thus, there is no reason to assume that, during this ultrashort period, evolution could change its ancient pattern to actively tolerate and protect noninjurious nonself under the control of innate immunity, such as in the human gut microbiota and the mammalian fetus. Furthermore, during this ultrashort period, evolution did not create an extra immune defense system that is different from the immune system that functions as an evolutionarily evolved, very efficient system to actively tolerate and protect nonself under the control of innate immunity.

Hence, tolerance to nonself in the absence of injury, and immunity to nonself in the presence of injury are evolution-driven paradigms in immunology. Therefore, allografts are not rejected because they are foreign nonself. Allografts are rejected because they are injured organs. The lesson for transplant clinicians, to be learned from evolution, about how to avoid allograft rejection is plausibly clear. It is important to prevent allograft injury to induce allotolerance and create a “transplant holobiont.” There are various strategic tools to approach this goal as has already been discussed elsewhere.

Abbreviations: DAMPs, damage-associated molecular patterns; HMGBl, high mobility group box 1; ROS, reactive oxygen species

Figure 5. In the Interest of Evolution

References


Organ Procurement: Should We Teach Undergraduate Medical and Nursing Students?

Serge Korjian,1 Yazan Daaboul,1 Antoine Stephan,2 Sola Aoun Bahous1

Abstract

Organ procurement and transplant improve health outcomes among patients with organ failure. Although many strategies have been developed to overcome the organ shortage, the worldwide rates of organ donation remain suboptimal. The lack of commitment to the health care mission of organ donation and the limited expertise of health care professionals reflect 2 major barriers to organ procurement and raise the need to teach organ procurement to health care professionals early during their undergraduate education. To accommodate the various available curricular models and to develop a homogeneous and equitable teaching methodology irrespective of the adopted design, an early step is to set clear goals and objectives for an organ procurement program. Outcomes should be matched to different academic levels and tailored to the duration of each medical and nursing curriculum. In all cases, hands-on experience leads to a better understanding of the topic, especially with the advent of simulation techniques that may be useful for training as well as testing purposes. An effective program finally requires that attainment of objectives and outcomes are systematically tested using proper evaluation tools that adequately pair with the curricular design. In conclusion, organ procurement teaching should adopt a systematic evidence-based approach that simultaneously contributes to medical and nursing education and improves organ donation rates.

Key words: Medical education, Curriculum, Organ procurement program, Organ donation, Evidence-based strategy

Introduction

Organ transplant has rapidly evolved into a success story of modern medicine and is a life-saving therapeutic option for patients with end-stage organ failure. Nonetheless, organ availability is limited. According to the International Registry in Organ Donation and Transplantation (IRODaT), the rates of deceased and living organ donors are globally suboptimal but vary from 1 to 35 per million population among different countries.1

Despite an increase in the organ donation rate with the expansion of donor criteria and advances in surgical and organ preservation techniques, there remains a substantial worldwide gap between the number of individuals on the transplant waiting list and the number of available donors.2 Unfortunately, there is ever-increasing demand, with more than 120,000 transplant candidates currently wait-listed; each day, 106 individuals are added and 18 patients die before receiving a transplant.3

The role of health care professionals

A systematic approach to understand the determinants of organ donation has been implemented since donation was first made available. Initially, observational evidence demonstrated that public awareness might influence individuals and subsequently increase organ donor rates.4-9 So too might interventions that address governmental systems and formalize the donation process and
organize activity on the provider end. Such interventions include presumed consent legislations, organ conscription, financial incentives, organ exchange mechanisms, and organizational structure development.\(^{10}\)

Beyond efforts that target donors, emerging evidence has recently demonstrated that donation does not always depend on donors’ choices. In fact, family refusal is considered a major limiting factor that significantly impedes organ donation.\(^{11,12}\) Accordingly, health care professionals (HCPs) who received no organ procurement education were initially required to build optimal family-oriented hospital environments that facilitate organ donation.\(^{13-18}\) However, HCPs frequently lack the basic knowledge of organ procurement; are sometimes unable to answer questions correctly; and often raise ethical and psychological concerns associated with donation, brain death, and circulatory death.\(^{19}\) In addition, HCPs are likely to be uncomfortable discussing organ donation or obtaining consent from family members.\(^{16,17}\) Thus, a novel strategy to shift the responsibility from individual HCPs to expert organizations was deemed necessary.\(^{15,16}\) The majority of countries currently implement organ procurement organization channeling systems where expert nonprofit organizations coordinate local or regional organ donation processes. Despite their expertise, organ procurement organizations cannot truly function without the combined role of in-hospital HCPs. The need for complementary efforts highlighted the requirement to educate and train HCPs in organ procurement. While HCP education within an organ procurement organization does not necessarily translate into better professional practice, training is necessary for priming, which makes the belief of organ donation salient among HCPs and stimulates the association between professional behavioral changes and organ donation.\(^{20}\) Equally important, trained HCPs are needed to assess medical and environmental elements that might facilitate or hinder donation and execute proper and timely hospital collaborations with organ procurement organizations to help maintain donated organs viable for transplant.\(^{13}\)

The approach to developing organ procurement programs in medical and nursing education

The cornerstones of any educational strategy are to identify the problems at hand and devise an optimal approaches and to assess the needs of the targeted learners.\(^{21}\) In the domain of organ donation, the increasing gap between organ supply and demand drives the need for strategies to increase donor identification and organ procurement. Evidence to support the implementation of educational and organizational strategies for HCPs to improve organ donation has previously been presented. Douville and associates conducted a systematic review to assess the efficacy of interventions aimed at HCPs that promote organ and tissue donation. Although they lacked robust methodologies, 5 studies demonstrated that interventions have a significant effect on HCP behaviors, namely in terms of referring potential donors, approaching donors’ families, and securing a donated organ.\(^{22}\) Another study by McGlade and associates demonstrated that a 33-hour integrated course on organ procurement and clinical care of potential organ donors improved nursing students’ knowledge of organ suitability after death, their ability to obtain consent, and their understanding of organ donation legislation and the definition of death.\(^{23}\) Accordingly, there is a need to consolidate organ transplant teachings into health care education and to educate HCPs about the intricate nuances of donor identification, approaching the family, and respecting the ethical and legal considerations of donation. In addition to being an educational concern, the positive ramifications of such strategies on public health should also be considered.

In reality, many graduating HCPs lack concrete knowledge regarding organ donation. A needs-assessment study by Anker and associates demonstrated that a significant number of medical schools in the United States and nursing schools in the state of New York provide information on organ donation in short lectures with little emphasis on discussion groups and patient interactions.\(^{24}\) The study also demonstrated that many students are not taught how to discuss donation at a routine health care visit or how to be organ donors themselves. Additionally, 20% of medical schools fail to teach students about the process of obtaining consent for donation, and approximately 12% of nursing programs fail to teach students the definitions of brain and cardiac death.\(^{24}\) Furthermore, results of a pilot study by Bardell and associates demonstrated that medical students at Queen’s University in Ontario, Canada, exhibit little knowledge of scientific and social issues related to organ donation.\(^{25}\) Medical
students lack superior knowledge of organ donation over their nonmedical undergraduate colleagues, which may reflect the minimal time allotted to organ donation issues in medical curricula. The findings of these studies demonstrate the need to devise effective educational strategies to teach medical and nursing students important knowledge and competencies before they enter the health care workforce.

As a third step in establishing an organ procurement educational strategy, the development of goals and objectives is essential to guide curricular advancing, equalize various teaching methods, and compare pre- and postintervention measures of efficacy. Learner-specific objectives in organ procurement education include proper identification of donors, knowledge of the ethics of living and deceased organ donation, and demonstration of a skillful approach to proposing donation. Once a set of objectives is described, educational strategies come into play. The most effective educational method provides congruence among cognitive, affective, and psychomotor objectives. Although cognitive objectives are more elementary with standard classroom teaching, affective and psychomotor objectives often require clinical experience, patient exposure, and simulations. For that reason, the most effective strategies for organ procurement education include a combination of basic lectures and tutorials with more interactive teaching methods. Irrespective of the adopted implementation technique, hands-on experience is crucial to secure a better grasp of the subtleties of organ procurement. The development of robust communication skills and group-based approaches to organ procurement is essential to outline real-life scenarios of interprofessional interplay. Subsequently, interprofessional education and practice should be central to teaching organ procurement and include simulated or live-situation workshops for training and testing.

Given the heterogeneity of curricula and the varied educational models such as discipline-, organ problem-, or competency-based models, the implementation of a universal integrated course on organ procurement and transplant may seem challenging. However, it is possible to ensure the achievement of all major objectives by devising proper assessment methods and tailoring an integrated course to the educational model.

Individual institutional committees are essential to adapt organ procurement programs and develop teaching strategies aligned with individual curricular designs. Although a condensed course approach has previously been reported, the longevity of the gained knowledge and competencies have not been studied. Organ procurement program can also be taught progressively, whether embedded within other courses or clerkships (eg, ethics, surgery, or public health) or as a separate topic that parallels other disciplines. For instance, introducing the concept of organ donation and procurement in an organ-based model of medical education may be accomplished gradually during modules that include different donated organs, such as hepatology, nephrology, ophthalmology, and pulmonology.

Standardized methods to assess student learning should be used to ensure that all objectives have been met. Multiple-choice questions may be effective for assessing students’ basic knowledge of organ donation and procurement, although testing understanding of ethical dilemmas and cultural ambiguities often require structured essay questions. Another approach to assessment is the use of objective structured clinical examinations that include simulation scenarios and standardized patients (actors trained to depict a clinical situation). Objective structured clinical examinations have been successfully applied in several domains of medical and nursing education and may be useful in tackling the social, ethical, and legal aspects of donation and transplant, as well as in testing student competencies that pertain to patient and family approaches.

Furthermore, long-term evaluation of organ procurement educational strategies is required to determine the efficacies of interventions and identify areas for improvement. Ideally, approaches should lead to improved rates of donor identification, donor referrals, and donor and family consent. This in turn should lead to higher rates of organ donors and successful transplants. Although this may be difficult to assess given the probable heterogeneity of implementation and the small short-term effects, intra-institutional evaluation of these measures may help guide organ procurement curricular development. Feedback assessment and evaluation create a closed loop that circulates back to the original unmet public need of effective organ procurement. In summary, the evaluation of an educational approach...
should answer the question: how effective was this particular strategy in improving organ procurement?

Conclusion

Organ donation is an important public need that requires worldwide efforts. Health care professionals play a major role in the determination of organ donation rates. Despite the association between training of HCPs and the increased rate of organ donation, HCPs often lack an understanding of their role in organ procurement. Thus, the integration of organ procurement programs into undergraduate medical and nursing curricula is necessary. Programs should include well-defined objectives and follow an evidence-based systematic approach that yields educational benefits and ultimately increases organ donation rates.

References

Results of Pediatric Liver Transplant: A Single-Center Experience

Gökhan Moray,¹ Tugan Tezcaner,¹ Aydıncan Akdur,¹ Figen Özçay,² Atilla Sezgin,³ Mahir Kırnap,¹ Sedat Yıldırım,¹ Gülnaz Arslan,⁴ Mehmet Haberal¹

Abstract

Objectives: Liver transplant is an established curative therapy for children with chronic end-stage liver disease or acute liver failure. In this study, we aimed to evaluate pediatric liver transplant in terms of outcomes, complications, and long-term follow-up results.

Materials and Methods: Pediatric patients who had liver transplant in our institution were included. We retrospectively evaluated demographic features including body weight, Child-Pugh score, etiology of liver disease, graft source, perioperative outcomes, perioperative complications, postoperative complications, and long-term results. Outcomes of treatment of complications and revision transplant were evaluated.

Results: Between September 2001 and December 2013, there were 188 pediatric liver transplants performed in our institution. Most grafts (90.9%) were obtained from living-related donors. There were 13 patients (6.9%) who had an intervention because of a hemorrhage postoperatively. Biliary leakage was observed in 33 patients (17.5%) and biliary stricture during follow-up was observed in 32 patients (17%). Thrombosis rates in the hepatic artery and portal vein were 12.3% and 0.5%. Revision transplant was performed in 11 patients (5.8%); reason for revision transplant was rejection in 50% patients. The remaining children were alive with good graft functioning after treatment of complications and revision transplant. The overall 5- and 10-year survival rates were 82.3% and 78.9%.

Conclusions: The overall outcomes of pediatric liver transplant at our center are very promising. With improved care of younger children and the combined efforts of the parents and medical team, the number of the children receiving transplants will increase in the future.

Key words: Children, End-stage liver failure, Treatment

Introduction

Liver transplant is a well-established treatment for children with chronic end-stage liver disease or acute liver failure. During the past 2 decades, advances in surgical techniques, preservation technology, immunosuppressive therapies, and infection monitoring and treatment have improved patient and graft survival.¹

Insufficient deceased-donor pool is the main problem around the world. Experience and technical improvements in living-donor surgery have led to the increase of pediatric liver transplant with excellent patient and graft survival. These techniques have expanded the potential donor pool and decreased waiting list mortality.²

Successful pediatric liver transplant reports have been published globally. A correlate of this success is that long-term complications are becoming more apparent because patient survival is reaching > 20 years. The outcomes differ slightly from those reported from other areas in the world, but the general themes are similar. A review of long-term outcomes was included in the recently published American Association for the Study of Liver Diseases guidelines for treatment.³
The aim of this preliminary study was to evaluate 1 of the largest cohorts of pediatric patients who have undergone liver transplant at a single center in Turkey.

**Materials and Methods**

We analyzed data for all pediatric recipients who underwent liver transplant between September 2001 and December 2013. The study patients were followed before living-donor liver transplant and annually after transplant. The donor data obtained from the center’s database included relationship to the recipient and graft type. The following recipient data were collected: demographic features including body weight, Child-Pugh score, etiology of liver disease, blood type, perioperative patient condition, immunosuppression protocol, postoperative complications, cause of death, and outcome at last follow-up (survival or death). Outcomes of treatment of complications and revision transplant were evaluated. During the study period, 377 liver transplants were performed in our center with 1-year minimum follow-up. The 188 transplants that involved children aged < 18 years (50.13%) were enrolled in the present study. All recipients had ABO-compatible grafts. The study was approved by the Ethical Review Committee of the Institute. All of the protocols conformed to the ethical guidelines of the 1975 Helsinki Declaration. Written informed consent was obtained from all subjects.

All biliary and vascular anastomoses were performed with loupe magnification (original magnification × 2.5) by the same surgeon. The technical details of the hepatic vein and portal vein anastomoses have been previously described. When there was a size discrepancy between the graft portal vein and the recipient portal vein, the smaller-sized portal vein was spatulated from both the anterior and posterior walls to create a wide anastomosis site. An inferior hepatic vein > 5 mm was encountered in the 2 right lobe grafts; these 2 inferior hepatic veins were anastomosed end-to-side to the inferior vena cava. In the first 51 liver transplants, we made a hepatic arterial anastomosis using a modified parachute technique.

Ultrasonographic examination of hepatic perfusion was done twice daily during the first week after surgery. In addition, routine ultrasonographic examinations were scheduled 1 month after orthotopic liver transplant and at 3-month intervals thereafter. A heparin drip infusion was begun on the day of transplant and was adjusted to maintain active coagulation time (whole blood) trough levels between 150 and 200 seconds. The heparin infusion was continued for 1 week; after that, anticoagulation therapy consisted of aspirin (40 mg daily) and dipyridamole (4 mg/kg given 3 times daily). All children received tacrolimus-based immuno-suppression. Tacrolimus blood levels were maintained between 10 and 15 ng/mL during the first month and subsequently between 5 and 10 ng/mL. Methylprednisolone (10 mg/kg) was administered intraoperatively and was continued postoperatively from 10 mg/kg, tapered to 0.1 mg/kg at the end of the first month, and stopped at the end of the third month. Children received prophylaxis against fungi, viruses, and Pneumocystis for 6 months after surgery.

**Statistical analyses**

Survival rates were estimated by Kaplan-Meier method and compared with log-rank test. Factors that affected mortality were analyzed with Cox proportional hazards regression model. The level of significance was set at .05. Statistical analyses were performed with statistical software (SPSS for Windows, Version 11.0, SPSS Inc, Chicago, IL, USA).

**Results**

Between September 2001 and December 2013, there were 17 deceased-donor (9.1%) and 171 living-donor (91.9%) liver transplants done in children. The mean body weight was 23.1 kg (range, 4.5-81.0 kg). There were 54 children (28.7%) who weighed < 10 kg, and 50 children (26.5%) were aged < 1 year. There were 110 boys (58%) and 78 girls (42%). The mean age of the children was 5.9 ± 5.5 years (range, 0.2-18 y). When these 188 children were classified according to Child-Pugh score, 21 were classified as A, 79 were classified as B, and 88 were classified as C.

Indications for liver transplant varied (Table 1). Donor selection criteria were described elsewhere. Only donors with graft-to-recipient weight ratios > 0.8 and fatty liver < 30% were accepted. The residual liver volume as assessed by computed tomography exceeded 35% total liver volume in all cases. The age of the donors ranged from 23 to 66 years. The donors were mostly the mother or father.
of the children (Table 2). The surgical technique used in the living donors was described previously. In our subjects; we used segments 2 and 3 for the left lateral segment grafts; segments 2, 3, and 4 for the left lobe grafts; and segments 5 to 8 for the right lobe grafts. There were 25 children (13.2%) who received a right lobe graft, 40 children (21.2%) who received a left lobe graft, 106 children (56.3%) who received a left lateral segment graft, and 17 children (9.1%) who received a whole liver graft.

Biliary and vascular complications were among the most common complications in our series (Table 3). Hepatic arterial thrombosis (HAT) was observed in 23 children (12.2%) and was diagnosed with routine daily Doppler ultrasonography examination. Stenosis was observed at the arterial anastomosis in 11 children. Portal vein stenosis developed in 9 children (4.7%). Hepatic vein stenosis developed in 3 children (1.5%) at 1, 6 and 14 months after liver transplant. At diagnosis, these children had ascites and elevated liver enzyme levels. Hepatic vein stenoses were treated with repeat balloon dilation and intraluminal stent placement. There were 33 children (17.5%) who developed biliary leakage, and 32 children (17%) developed biliary stenosis at the anastomotic site. Early intervention was needed in 13 children (6.9%) because of hemorrhage.

Median hospital stay was 14 days (range, 7-118 d); median stay in the intensive care unit was 2 days (range, 1-118 d). In 188 pediatric liver transplants, early mortality rate (during first 3 months after surgery) was 9.5% (18 patients). The most common cause of early mortality was sepsis in 8 patients (44.4%); other causes of early mortality were primary nonfunction in 3 patients (16.7%), acute respiratory distress syndrome in 2 patients (11.1%), intracranial hemorrhage in 2 patients (11.1%), arterial stenosis in 1 patient (5.5%), pneumonia in 1 patient (5.5%), and bleeding in 1 patient (5.5%).

In 170 pediatric liver transplants, late mortality rate (after 3 postoperative months) was 10.5% (18 patients). The most common cause of death was sepsis in 9 patients (50.0%); other causes of late mortality were graft loss in 3 patients (16.6%), thromboembolism in 2 patients (11.1%), tumor recurrence in 2 patients (11.1%), intracranial hemorrhage in 1 patient (5.5%), and necrotizing pancreatitis in 1 patient (5.5%).

Graft loss was observed in 17 patients; etiology of graft loss was chronic rejection in 6 patients (35.3%), hepatic arterial complications in 4 patients (23.5%), biliary complications in 4 patients (23.5%), and primary nonfunction in 3 patients (17.6%). Revision transplant was performed 12 times in 11 children. Of the 17 patients who had graft loss, 6 died before revision transplant. Mortality rate after revision transplant was 54.54%. Of these 6 patients, 5 patients died early after surgery because of sepsis in 2 patients, thromboembolism in 2 patients, and bleeding in 1 patient. There was death in 1 patient because of chronic rejection at 4 years after revision transplant. The overall 5-year survival rate was 82.3% and 10-year survival rate was 78.9% (Figure 1).

**Discussion**

We reviewed the outcomes of 188 pediatric liver transplant recipients, 1 of the largest pediatric liver transplant cohorts in our country. The survival rates observed in our series were excellent at 5 years
and 10 years (78.9%) after liver transplant. The present results compare favorably with recently published data from another series about liver transplant.\(^8,9\) Most grafts were obtained from living donors. A narrow organ donor pool is the most common problem that limits transplant in our country and around the world. There are rare countries that overcome the deceased-donor problem.

In our series, 38 of 188 children (20.5%) underwent liver transplant for Wilson disease, and 33 children (17.5%) biliary atresia. In the literature, Wilson disease is the most common and biliary atresia is the second most common indication for liver transplant.\(^10,11\)

Biliary and hepatic arterial complications were the most common complications in our series. The use of reduced-size grafts with more complicated surgery from living-related donors may increase the incidence of bile duct complications such as biliary stenosis and biliary leakage.\(^12\) In contrast, the disadvantage of using large-for-size grafts in infants is that insufficient tissue oxygenation and graft compression are observed in association with a high incidence of vascular complications that result in poor outcomes.\(^13\) We prefer duct-to-duct anastomosis in our center except when the indication for transplant is biliary atresia, because there are some advantages such as the possibility of performing endoscopic retrograde cholangiopancreatography in cases with leakage or stenosis. A previous study showed that there was no statistically significant difference in postoperative anastomotic biliary complications between duct-to-duct and Roux-en-Y hepaticojejunostomy biliary reconstruction in pediatric liver transplant recipients weighing < 15 kg.\(^14\)

The HAT is a severe complication following liver transplant with a high risk for short- or long-term graft dysfunction and biliary complications. Early after surgery, HAT has a mortality rate of 50%. The incidence of early HAT is 2.6% to 20% in adults, 9% to 14.9% in pediatric patients, and 30% in children aged < 1 year.\(^15\) Previous studies have demonstrated that the risk of HAT is increased in patients who weigh < 10 kg, are aged < 3 years, are female, have a graft with multiple arteries, and have a hepatic artery with a diameter < 2 mm.\(^16,17\) Although 29% patients in this study had < 10 kg body weight, HAT was observed only in 12.2%.

Portal vein thrombosis occurs in 2% to 10% pediatric recipients.\(^18\) Although often silent early after transplant, portal hypertensive clinical findings and hypersplenism are usually seen with time. Major problems in portal vein reconstruction in children include impairment of the vascular structure because of previous surgery in a child with biliary atresia or recurrent cholangitis. Another problem is the difference in the diameter of the portal vein between adults and infants. At our center, when there is a size discrepancy, the smaller-sized portal vein is spatulated (anterior and posterior walls) to make a wide anastomosis. In our series, we observed 9 cases (4.7%) of portal vein stenosis. Children with venous complications all were successfully treated by interventional radiographic techniques.

Revision liver transplant remains controversial in the setting of living-donor liver transplant because of the limitation of donors and the fact that previous reports have demonstrated poorer outcomes with revision than primary liver transplant.\(^19\) Taking into consideration the recipient mortality rate of 54.4% observed in the present study, it is necessary to determine clear indications and limitations for revision living-donor liver transplant to avoid morbidity and mortality in potential living-donor candidates.

During the past 2 decades, medical and surgical innovations have established pediatric liver transplant as the optimal therapy for patients who have acute or chronic liver disease. Our study has shown promising overall survival rates in children have no chance except liver transplant. Living-donor liver transplant has increased the donor pool and decreased pediatric waiting list mortality, and it is...
important to conduct further investigations of the most important remaining causes of death in children with liver transplant. In addition to living-donor liver transplant, there is a need for major efforts to educate the public about organ transplant and donation. In our study, only 17 children (9.1%) received a liver from a deceased donor. We hope that increased experience and refinement of this procedure will lead to further improvements in outcomes in patients undergoing liver transplant.

References

Major Vascular Complications in Living-Donor Liver Transplant Recipients: Single Center Team Experience

Refaat Kamel,1 Yasser Hatata,2 Mohamed Taha,3 Karim Hosny,4 Ayman Amin5

Abstract

Objectives: Vascular problems such as thrombosis and stenosis of the hepatic artery, portal vein, and hepatic vein are serious complications after living-donor liver transplant and can cause increased morbidity, graft loss, and patient death. The aim of this study was to assess the incidence, treatment, and outcome of recipient vascular complications after living-donor liver transplant in a single Egyptian center.

Materials and Methods: Between November 2006 and March 2014, we performed 226 living-donor liver transplants for 225 patients at Dar Al Fouad Hospital in 6th of October City in Egypt. Review of all patients with vascular complications was performed.

Results: In 20 of 225 recipients (8.9%), there were vascular complications that occurred from day 0 to 14 (mean, 5.6 ± 3.4 d). Complications included isolated hepatic artery thrombosis in 7 patients (35%), isolated portal vein thrombosis in 6 patients (30%), isolated hepatic vein stenosis in 3 patients (15%), and isolated hepatic artery stenosis in 1 patient (5%). Combined portal vein thrombosis and hepatic artery thrombosis occurred in 2 patients (10%), and combined portal vein thrombosis and hepatic vein stenosis occurred in 1 patient (5%). Complications were identified with duplex ultrasonography and confirmed with computed tomographic angiography and direct angiography when needed. Multidisciplinary treatment included percutaneous transarterial or transvenous thrombolysis with or without balloon dilation and stenting, open surgical exploration with thrombectomy, vascular revision, or retransplant. There were no intraoperative deaths, but mortality occurred in 15 of 20 patients (75%). Survival ranged from 6 days to 70 months. Preoperative portal vein thrombosis was observed in 3 of 7 patients (43%) who had postoperative portal vein thrombosis.

Conclusions: Major vascular complications in living-donor liver transplant recipients have poor outcome despite early detection and prompt multidisciplinary intervention. Preoperative recipient portal vein thrombosis is a risk factor for postoperative portal vein thrombosis.

Key words: Hepatic artery stenosis, Hepatic artery thrombosis, Hepatic vein stenosis, Portal vein thrombosis

Introduction

Vascular complications after living-donor liver transplant are serious problems that frequently result in graft loss and patient death.1 Despite marked improvements in vascular techniques during the past several decades, the overall incidence of vascular complications in adults is 8% in studies of deceased-donor liver transplant2; the incidence is 10% after living-donor liver transplant because of the smaller vessels, insufficient vessel length for reconstruction, and greater risk of a twist of the vascular pedicle.3 Close surveillance of all vascular anastomoses using duplex ultrasonography facilitates early detection and treatment of these complications before irreversible graft failure occurs. Treatment options usually include percutaneous thrombolysis, percutaneous angioplasty, surgical revascularization,
retransplant, or, less commonly, a nonoperative approach.1

The aim of this study was to assess the incidence, treatment, and outcome of vascular complications in recipients after living-donor liver transplant at a single Egyptian center (Dar Al Fouad Hospital, 6th of October City, Egypt).

Materials and Methods

Subjects
The charts of recipients who had living-donor liver transplant performed at Dar Al Fouad Hospital between November 2006 and March 2014 and who developed vascular complications were reviewed retrospectively. Analysis was performed of recipient demographics, preoperative imaging, vascular complications (type, time of occurrence, and treatment), and outcome. The study was approved by the ethics committee before this work began, and the protocols conformed with the ethical guidelines of the 1975 Declaration of Helsinki. Written informed consent was obtained from patients or their guardians including approval of the treatment protocol and anonymous use of data for research purposes.

All transplants were performed by the same surgical team. All grafts were right liver lobe grafts without the middle hepatic vein from a living donor. Hepatic vein anastomosis was performed end-to-side with continuous 5-0 polypropylene suture with 3× loupe magnification. Portal vein anastomosis was performed end-to-end with continuous 6-0 polypropylene suture, also with 3× loupe magnification. Hepatic artery anastomosis was done end-to-end with interrupted 8-0 polypropylene sutures using a microscope with 4× to 8× magnification, depending on artery size. Duplex ultrasonography examination was performed intraoperatively immediately after finishing the anastomoses to ensure vascular patency and was repeated daily within the first 3 weeks after transplant. All patients were transferred to the intensive care unit for the first 5 days after surgery and transferred to the ward for the remainder of the first 3 weeks unless there was a major complication. Graft function was monitored by detailed daily biochemical tests. All patients received heparin infusion during the first 5 days; subsequently, they received low molecular weight heparin and aspirin until the end of the first 3 weeks, and aspirin for 6 months.

The diagnosis of vascular complications was established by a minimum of 2 imaging tests and/or surgical confirmation. A multidisciplinary team including microsurgeons, transplant surgeons, interventional radiologists, and hepatologists decided on the treatment protocol.

Technique of intra-arterial thrombolysis
Diagnostic arteriography was performed with standard catheter techniques using right femoral artery access with selective catheterization of the celiac trunk using 5 French Cobra or Simon catheters. After hepatic artery thrombosis (HAT) was confirmed, a microcatheter with side holes was manipulated into the thrombus. Thrombolysis was performed with a bolus dose of streptokinase (150 000 U). If the artery did not recanalize, continuous infusion (100000 U/h) was performed for maximum 12 hours. If the angiogram after recanalization showed an underlying stricture, balloon angioplasty and stent placement were performed. The endovascular interventions were monitored using duplex ultrasonography to ensure good arterial flow in the graft during the procedures. During continuous infusion, duplex ultrasonography was performed every 2 hours. The infusion was stopped when the duplex scan showed arterial flow in the hepatic artery at the graft hilum and intrahepatic branches, and this was followed by confirmatory angiography. Follow-up was performed using daily duplex ultrasonography (every 12 hours for the first 3 days; after 3 days, once daily until hospital discharge). Successful intra-arterial thrombolysis was defined as resolution of the thrombus with delineation of the intrahepatic branches.

Technique of hepatic vein venography and venoplasty
The right internal jugular vein was used for vascular access. Pre- and postanastomotic hepatic venous pressures and pull-through pressures were measured. If a major anastomotic stenosis was identified, then a stiff 0.035-inch guide wire was passed through the stenotic segment, and this was followed by dilation with a balloon (venoplasty). An appropriately sized metallic stent was used in cases with failure of the balloon waist to expand during
inflation, persistent pressure gradient (> 5 mm Hg) during balloon dilation, and/or recurrent stenosis with persistent pressure gradient after dilation. Patients received systemic anticoagulation with heparin sodium to maintain partial thromboplastin time 1.5-fold greater than normal level immediately after the procedure.

Results

Patients

There were 226 adult living-donor liver transplant procedures performed for 225 adult recipients between November 2006 and March 2014. Vascular complications developed in 20 patients (8.9%) (Table 1). There were 2 females (10%) and 18 males (90%). The preoperative diagnosis was posthepatitis C liver decompensation in 17 patients (85%) and hepatocellular carcinoma in 3 patients (15%). Their age range was 26 to 68 years, with mean age 49.6 ± 9.4 years. Preoperative triphasic computed tomography (CT) of the abdomen showed portal vein thrombosis (PVT) in 3 of the 7 patients who developed postoperative PVT. There were 2 patients who had grade II PVT and 1 patient who had grade III PVT.4 Portal vein thrombectomy was performed in these 3 patients before hepatic graft implantation (Figure 1). The correlation between preoperative PVT and the development of postoperative PVT was statistically significant ($P = .021$) using the McNemar test.

Incidence

Vascular complications were identified by duplex ultrasonography and confirmed by hepatic CT angiography and percutaneous transarterial angiography when diagnosis was not definite. In the 20 recipients who had vascular complications, isolated HAT occurred in 7 patients (35%), isolated PVT in 6 patients (30%), isolated hepatic vein stenosis (HVS) in 3 patients (15%), and isolated hepatic artery stenosis (HAS) in 1 patient (5%). Multiple complications occurred in 3 patients: combined PVT and HAT in 2 patients (10%) and combined PVT and HVS in 1 patient (5%) (Table 2). Time of occurrence of vascular complications ranged from 0 to 14 days (mean, 5.6 ± 3.4 d) (Table 3).

Treatment

Isolated HAT was treated by percutaneous transarterial thrombolysis alone in 2 patients (Figure 2) with additional balloon dilation and stenting in 2 other patients. The reason for the stenting was the appearance of an anastomotic stricture in 1 patient (Figure 3) and a kink in the other patient after the thrombus had resolved. The stricture was caused by...
>2:1 size mismatch between the recipient and graft arteries. Another case occurred on day 1 and was treated with surgical exploration and thrombectomy, but HAT recurred and was treated with percutaneous transarterial thrombolytic infusion. In 1 patient, HAT developed during a severe rejection episode with graft failure and was treated with retransplant, but HAT recurred; the patient received percutaneous transarterial thrombolytic infusion unsuccessfully and died because of biliary problems.

Another patient had graft artery intimal dissection intraoperatively and the arterial anastomosis was repeated 6 times, finally with a saphenous vein graft, but there still was no flow because of intrahepatic arterial dissection. The patient with isolated HAS was treated with percutaneous transfemoral balloon angioplasty and stent.

The 6 patients with isolated PVT were treated with surgical exploration and thrombectomy through a small transverse venotomy in the accessible main portal vein. Care was taken not to disrupt the arterial and biliary anastomoses which were anterior to the vein. Fogarty balloon catheters were used to extract intrahepatic thrombi proximally and distally. However, some segmental branches remained occluded (Figure 4), and thrombolytic therapy was considered life-threatening because of the abnormal coagulation profile.

In the 3 patients who had isolated HVS, 1 patient had a redundant hepatic vein anastomosis inverted into the vein lumen and underwent percutaneous transjugular hepatic vein stent placement (Figure 5). The other 2 patients had received middle hepatic vein dominant grafts with small right hepatic veins.

**Table 3. Time of Occurrence of Vascular Complications**

<table>
<thead>
<tr>
<th>Complication</th>
<th>No. of Patients</th>
<th>Onset (d)</th>
<th>Range (d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HAT</td>
<td>7</td>
<td>5.5 ± 4.75</td>
<td>0-14</td>
</tr>
<tr>
<td>PVT</td>
<td>6</td>
<td>7.2 ± 2.92</td>
<td>4-11</td>
</tr>
<tr>
<td>HVS</td>
<td>5</td>
<td>4.6 ± 0.57</td>
<td>4-5</td>
</tr>
<tr>
<td>HAS</td>
<td>1</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Multiple vascular complications</td>
<td>3</td>
<td>3.6 ± 2.88</td>
<td>2-7</td>
</tr>
<tr>
<td>All vascular complications</td>
<td>20</td>
<td>5.6 ± 3.43</td>
<td>0-14</td>
</tr>
</tbody>
</table>

*Data reported as mean ± SD (range).*

**Figure 2. Thrombolysis For Hepatic Artery Thrombosis**

(A) Selective hepatic artery angiography showing thrombosis at the arterial anastomosis (white arrow). (B) Restoration of intrahepatic arterial flow after successful intra-arterial thrombolysis (black arrow).

**Figure 3. Thrombolysis, Balloon Angioplasty, and Stenting For Hepatic Artery Thrombosis on Top of a Stenotic Anastomosis**

(A) Thrombosis at anastomotic site (arrow). (B) Restoration of normal intrahepatic arterial flow, but a tight stenosis became apparent (arrow). (C) Balloon dilation of stricture showing waist (arrow). (D) Insertion of intra-arterial bare metal stent (arrow).

**Figure 4. Portal Vein Thrombosis**

(A) Near-total thrombosis of main portal vein (arrow). (B) After surgical thrombectomy, the main and right portal veins are patent (arrow), but a wedge of graft is infarcted (arrowhead) because of failed recanalization of its segmental branch.

**Figure 5. Hepatic Vein Stenosis**

(A) Computed tomographic (CT) venography showing stenosis at junction of right hepatic vein and vena cava (arrow). (B) Percutaneous transjugular hepatic venography with delayed washout of contrast from hepatic vein (arrow). (C, D) The CT coronal and axial images after stent insertion (arrows).
and a segment 8 vein that was not reconstructed, which caused congestion, and they were treated nonoperatively.

There were 2 patients who had combined PVT and HAT. Surgical thrombectomy was done for PVT in both patients, and HAT was treated with balloon angioplasty and stent in 1 patient and saphenous vein interposition graft in the other patient. The patient who had combined PVT and HVS was treated nonoperatively (Table 4).

Prognosis
Survival ranged from 6 days (0.2 mo) to 70 months; least survival was observed in patients who had PVT and longest survival in patients who had HAT (Table 5). Survival was 70% at 1 mo, 25% at 6 mo, 25% at 1 y, and 10% at 5 y. Death occurred in 15 of 20 patients (75%) because of different causes (Table 6).

The most common cause of death was graft failure in 8 patients (40%). There were 5 patients who were considered censored because they were lost to follow-up during the study.

Discussion
Vascular complications are a major cause of morbidity and mortality after liver transplant. The complication HAT is a serious problem, with a reported incidence 3% to 5% in adults and 8% to 12% in pediatric recipients.5,6 The complication HAT usually occurs early within the first 2 weeks after transplant and is associated with major graft loss and mortality.2 In our series, the overall incidence of HAT was 4% of 226 living-donor liver transplant procedures performed. Potential risk factors for HAT have been identified in many studies and may be surgical (technical) or nonsurgical.7 We encountered an underlying anastomotic stricture in 1 patient who developed HAT (Figure 3). The arterial anatomy of both recipient and donor graft can affect the incidence of HAT. Smaller arteries are associated with higher incidence of HAT, evidenced by the higher rate in pediatrics.8 A common problem is the discrepancy in size between the graft and recipient arteries. We encountered a > 2:1 size mismatch between the recipient and graft arteries. Different techniques have been described for an anastomosis in such cases, such as spatulation of the smaller artery.7 In our series, 1 patient developed HAT because of a major kink. Before starting the anastomosis, we presently try to estimate where the final arterial position will be located after removal of the abdominal retractors and descent of the diaphragm and liver caudally, and we excise any excess artery. Intimal dissection is another risk factor that is problematic especially in the graft artery where the flap is located along the direction of blood.

<table>
<thead>
<tr>
<th>Table 4. Techniques Used to Treat Vascular Complications*</th>
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<tbody>
<tr>
<td>Treatment</td>
</tr>
<tr>
<td>------------</td>
</tr>
<tr>
<td>Percutaneous interventions†</td>
</tr>
<tr>
<td>Surgical exploration</td>
</tr>
<tr>
<td>Surgery and percutaneous interventions</td>
</tr>
<tr>
<td>Retransplant</td>
</tr>
<tr>
<td>Nonoperative follow-up</td>
</tr>
<tr>
<td>Total</td>
</tr>
</tbody>
</table>

*Abbreviations: HAS, hepatic artery stenosis; HAT, hepatic artery thrombosis; HVS, hepatic vein stenosis; PVT, portal vein thrombosis
†Percutaneous interventions included transarterial thrombolysis with or without angioplasty and stenting.

<table>
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<tr>
<th>Table 5. Survival in the Group With Vascular Complications*</th>
</tr>
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<tbody>
<tr>
<td>Complication</td>
</tr>
<tr>
<td>-------------</td>
</tr>
<tr>
<td>HAT</td>
</tr>
<tr>
<td>PVT</td>
</tr>
<tr>
<td>HVS</td>
</tr>
<tr>
<td>HAS</td>
</tr>
<tr>
<td>Multiple vascular complications</td>
</tr>
<tr>
<td>All vascular complications</td>
</tr>
</tbody>
</table>

*Abbreviations: HAS, hepatic artery stenosis; HAT, hepatic artery thrombosis; HVS, hepatic vein stenosis; PVT, portal vein thrombosis
Data reported as mean ± SD (range).

<table>
<thead>
<tr>
<th>Table 6. Outcome and Causes of Mortality in 20 Patients With Vascular Complications*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Complication</td>
</tr>
<tr>
<td>-------------</td>
</tr>
<tr>
<td>Graft failure</td>
</tr>
<tr>
<td>Small-for-size syndrome</td>
</tr>
<tr>
<td>Bilary sepsis</td>
</tr>
<tr>
<td>Chest infection</td>
</tr>
<tr>
<td>Cardiac arrest</td>
</tr>
<tr>
<td>Censored</td>
</tr>
<tr>
<td>Total</td>
</tr>
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</table>

*Abbreviations: HAS, hepatic artery stenosis; HAT, hepatic artery thrombosis; HVS, hepatic vein stenosis; PVT, portal vein thrombosis
These potential risk factors include hypercoagulable disorders (thrombophilias) in the recipient and donor, cytomegalovirus mismatch, ABO incompatibility, and the arterial reperfusion time. Prolonged operative times also are suggested as a risk factor. Another important risk factor is acute rejection episodes, which increase the capillary bed resistance and impede arterial flow. We encountered 1 patient who developed HAT because of acute rejection on postoperative day 14, for which retransplant was done because of graft failure, but HAT recurred because of recurrence of acute rejection after retransplant. Interrupted sutures are associated with fewer hepatic arterial complications. The use of magnification microscopes with continuous zoom and high magnification loupe reduces the incidence. The complication HAS is a risk factor for HAT and should be corrected when detected during routine duplex ultrasonography surveillance.

Complications related to the portal vein are less common than those of the hepatic artery but are associated with high graft loss incidence. In our series, we observed that preoperative PVT significantly correlated with posttransplant PVT (P = .021). Our protocol in patients who have preoperative PVT and who undergo thrombectomy during the transplant before portal anastomosis is to administer heparin infusion for 5 days, followed by low molecular weight heparin for three weeks and warfarin for 6 months. The 3 patients who underwent thrombectomy developed PVT despite being on the protocol; therefore, we are considering routinely inserting an inferior mesenteric vein catheter during transplant in these patients that can be used for anticoagulation or thrombolysis if PVT develops. Prompt detection and aggressive surgical treatment are required to reduce mortality and graft loss.

The complication HVS should be suspected whenever there is unexplained increasing ascites or graft dysfunction. Percutaneous transjugular angioplasty usually is an effective method to relieve hepatic vein or inferior vena cava stenosis. In 1 of our patients, there was a redundant hepatic vein internally inverted anastomosis that was treated successfully with a stent. To avoid HVS, we recommend performing a large venotomy in the inferior vena cava to anastomose to the graft right hepatic vein. We do not use anterior patches. Furthermore, we recommend reconstruction of segment 8 vein if the right hepatic vein is small, even if the graft is of adequate size, to avoid congestion.

Reported survival rates for recipients with vascular complications is 88.9% at 1 mo, 66.7% at 6 mo, and 33.3% at 12 mo. In our series, survival rate was 70% at 1 mo, 25% at 6 mo, 25% at 12 mo, and 10% at 5 y; least survival was observed in patients who had PVT and longest survival was in patients who had HAT.

In conclusion, major vascular complications in living-donor liver transplant recipients have poor outcome with major graft and patient loss. Detailed preoperative preparation, refining surgical technique and decision making, identifying risk factors, early diagnosis with routine surveillance, and prompt multidisciplinary treatment are needed to reduce the incidence of vascular complications and associated graft loss and patient mortality.

References

Treatment of Biliary Complications After Liver Transplant: Results of a Single Center

Sedat Yildirim,1 Ebru Hatice Ayvazoglu Soy,1 Aydincan Akdur,1 Mahir Kirnap,1 Fatih Boyvat,2 Feza Karakayali,1 Adnan Torgay,3 Gokhan Moray,1 Mehmet Haberal1

Abstract

Biliary complications are major sources of morbidity after liver transplant due to vulnerable vascularization of the bile ducts. Biliary complications are the “Achilles’ heel” of liver transplant with their high incidence, need for repeated and prolonged treatment, and potential effects on graft and patient survival. Although standardization of reconstruction techniques and improvements in immunosuppression and organ preservation have reduced the incidence of biliary complications, in early reports the morbidity rates are 50%, with related mortality rate 25% to 30%. Prophylaxis is a major issue. Although many risk factors (old donor age, marginal graft, prolonged ischemia time, living-donor liver transplant, partial liver transplant, donation after cardiac death, hepatic arterial thrombosis, organ preservation, chronic rejection, and other donor and recipient characteristics) do not directly affect biliary complications, accumulation of the factors mentioned above, should be avoided. However, no accepted standard has been established. Treatment strategy is a subject of debate. Recently, nonoperative treatment of biliary complications have been preferred for diagnosis and therapy, because percutaneous or endoscopic treatment may prevent the need for surgical intervention. In this study, we reviewed our treatment of early and late biliary complications after liver transplant.

Key words: End-stage liver disease, Liver transplantation, Biliary stricture, Bile leakage

Introduction

Biliary complications are major sources of morbidity after liver transplant due to vulnerable vascularization of the bile ducts. Biliary complications are the “Achilles’ heel” of liver transplant with their high incidence, need for repeated and prolonged treatment, and potential effects on graft and patient survival. Although standardization of reconstruction techniques and improvements in immunosuppression and organ preservation have reduced the incidence of biliary complications, in early reports the morbidity rates are 50% with related mortality rate 25% to 30%. Prophylaxis is a major issue. Although many risk factors (old donor age, marginal graft, prolonged ischemia time, living-donor liver transplant [LDLT], partial liver transplant, donation after cardiac death [DCD], hepatic arterial thrombosis, organ preservation, chronic rejection, and other donor and recipient characteristics) do not directly affect biliary complications, accumulation of the factors mentioned above, should be avoided. However, no accepted standard is established. Treatment strategy is a subject of debate. Recently, nonoperative treatment of biliary complications has been preferred for diagnosis and treatment of biliary complications, because percutaneous or endoscopic treatment may prevent the need for surgical intervention. In this study, we reviewed our treatment of early and late biliary complications after liver transplant.
Materials and Methods

We performed 377 liver transplants (188 pediatric and 189 adult liver transplants) between September 2001 and February 2014 in our center, including 304 LDLT (80%) and 73 deceased-donor liver transplants (DDLT) (20%). Biliary reconstruction was with hepaticojejunostomy in 65 patients (17%) and duct-to-duct reconstruction in 312 patients (83%). We retrospectively evaluated all 377 liver transplant patients for biliary complications, either early (within 6 months of liver transplant) or late (> 6 months after liver transplant) bile leakage and biliary stricture. We analyzed the treatment of 132 patients (35%) who had biliary complications.

In 125 grafts, biliary reconstruction was a duct-to-duct anastomosis over a T-tube or transhepatic catheter. All biliary anastomoses were performed with loupe magnification (original magnification ×2.5). The biliary anastomosis was performed with 6-0 or 7-0 monofilament suture for the duct-to-duct or bilioenteric anastomosis. Intraoperative hepatic blood flow and graft status were assessed with routine Doppler ultrasonography. Doppler ultrasonography of hepatic perfusion was performed twice per day during the first postoperative week. At the end of the first week, routine abdominal computed tomography scans were obtained. Liver function tests were measured daily during the first 2 weeks. Tacrolimus-based immunosuppressive therapy was used in all patients. No patient received induction therapy.

Since early symptoms of biliary complications often can be nonspecific or missing, we could diagnose bilious secretions or bile duct pathology early via routine ultrasonography. The diagnosis of a bile leakage was suspected from a perihepatic collection on ultrasonogram or marked bile drainage from a surgical drain. We performed percutaneous drainage of perihepatic bilioma in cases without a T-tube. We injected contrast through the bilioma catheter or T-tube and showed when there was any communication of the bilioma with the biliary system. Anastomotic or nonanastomotic stenoses often were associated with jaundice, increased cholestatic enzyme levels, and fever. We observed recurrent cholangitis. We performed percutaneous or endoscopic retrograde cholangiography (ERC) after noninvasive imaging studies. Endoscopic stenting, percutaneous transhepatic internal-external biliary drainage catheter, or surgical reconstruction were performed for treatment of biliary complications.

Results

In 377 liver transplant patients, 132 patients (35%) had biliary complications (leakage and/or stricture) early or late after surgery. Biliary complications were more frequent with LDLT (LDLT, 37%; DDLT, 28%), pediatric liver transplant (pediatric, 36%; adult, 34%), and duct-to-duct anastomosis (duct-to-duct, 38%; hepaticojejunostomy, 15.9%). Early postoperative complications were observed in 69 patients with bile leakage (18%) and 30 patients with biliary stenosis (8%). Late complications were observed in 3 patients with bile leakage (0.8%) and 38 patients with biliary stenosis (10%).

The treatment of early bile leakage included drainage guided by ERC, transhepatic percutaneous biliary drainage (PBD), or surgical revision. The PBD (internal and/or external catheters) was the first choice of treatment for bile leakage. We performed 52 PBD procedures for early bile leakage. In 4 patients, PBD failed and surgical revision was performed. The ERC was performed in 17 patients for early bile leakage. In 10 patients, ERC failed, and further treatment included percutaneous treatment (8 patients) or surgery (2 patients). In 15 patients who had early bile leakage, surgery was the first choice, and in 2 patients, further percutaneous interventions were performed at follow-up.

The treatment of 30 early biliary strictures was by ERC-guided drainage, PBD, or surgical revision. The PBD (internal and/or external catheters) with balloon dilation was the first choice of treatment for early biliary stricture. We performed 32 PBD procedures for early biliary stricture. In 1 patient, PBD failed, and surgical revision was performed. There were 7 ERC procedures performed for early biliary stricture; all these ERC procedures failed, and the patients were treated with percutaneous treatment (8 patients) or surgery (2 patients).

The treatment of 3 patients who had late bile leakage was by ERC-guided drainage, PBD, or surgical revision. We performed PBD procedures for 1 patient; in follow-up, multiple percutaneous procedures were required, and he had biliary anastomosis revision for biliary stenosis. In addition to this patient, 1 patient had successful surgery and 1 patient had successful ERC for late bile leakage.
The treatment of 38 late biliary strictures was by ERC-guided drainage, PBD, or surgical revision. The PBD (internal and/or external catheters and balloon dilation) was the first choice of treatment for late biliary stricture. We performed 28 PBD procedures for late biliary stricture; this failed in 3 patients, and surgical revision was performed. Six ERC procedures were performed for late biliary stricture; in 5 patients, ERC failed, and treatment was with percutaneous treatment. In 3 patients who had late biliary stricture, surgery was the first choice, and all patients were successfully treated without further interventions.

There were 52 PBD procedures performed for early bile leakage. Late biliary strictures developed in 19 patients who had early bile leakage who were treated with PBD (19 of 38 patients [50%] with early bile leakage treated with PBD). Each of these patients had ≥ 3 PBD procedures during follow-up at 1-month intervals. After this prolonged treatment that included multiple interventions, 4 patients had revision surgery after failed repeated percutaneous treatment. In 21 patients, surgery was the first choice for treatment of biliary complications; 4 patients developed biliary complications at follow-up. We observed 2 patients who had bile leakage, treated successfully with PBD. There were 2 biliary strictures that developed after surgical treatment; 1 patient had surgical revision of hepaticojejunostomy, and the other biliary stricture was treated by PBD.

Discussion

Biliary complications are frequent causes of morbidity after liver transplant. Many factors related to recipient, graft, operative course, and postoperative course are associated with biliary complications (leakage or stricture). These factors include recipients with advanced recipient age and more impaired liver function, LDLT, ischemia-reperfusion injury, prolonged warm or cold ischemia time, arterial complications, type of reconstruction, and type of liver graft. It is reported in many studies that LDLT is a major risk factor for biliary complications. Therefore, graft and operative risk factors should be evaluated separately for DDLT and LDLT. Small duct size and the possibility of multiple duct orifices increase the risk of biliary complications. Devascularization of the bile duct during hilar dissection also contributes to these risk factors. Although previous studies showed that ischemia-reperfusion injury with prolonged warm or cold ischemia time and preservation methods are risk factors for biliary complications, in recent reports, ischemia-reperfusion injury seems to be associated more often with ischemic, nonanastomotic complications. In addition, preexisting bile leakage is associated with later biliary stricture.

Biliary strictures are among the most common complications after liver transplant with high mortality. However, the incidence of stricture has decreased with recent improvements. Biliary stricture can present at any time after transplant, but most present within 1 year after transplant. In various studies, the incidence of biliary stricture is higher after LDLT than DDLT due to devascularization of the bile duct, technical difficulty of biliary reconstruction (small or multiple ducts), and bile leakage from the cut surface, which causes fibrosis around the anastomosis. In our study, we revealed a significantly higher incidence of biliary stricture after LDLT than DDLT (10%). The treatment of anastomotic stricture has improved during the past 2 decades. In the primary treatment of biliary strictures, mostly endoscopic and percutaneous methods are preferred over surgery. Surgery is reserved for patients who have failed noninvasive treatment.

Bile leakage from an anastomosis or cut surface is the second most common complication after liver transplant. The incidence of bile leakage is 8.2%. Most patients are treated with an endoscopic approach or percutaneous radiography-guided drainage to divert bile away from the leakage and maintain bile drainage to the intestines.

The choice of percutaneous interventional radiology or endoscopy as the first treatment option for biliary complications may seem obvious because these methods are less invasive than surgical revision. However, when choosing the treatment modality, we must consider treatment-related morbidity and mortality, recurrence rates, quality of life, and retransplant rates. The main disadvantages of interventional techniques are the frequent use of prolonged drainage with catheters (minimum, 3-6 mo) and the need for repeated treatment sessions.
under anesthesia. In addition, retransplant rates after PBD or ERC are 0% to 20%, and in surgical series, the incidence of retransplant was 12%. The risk of complications (such as hemobilia, pancreatitis, sepsis, cholangitis, or intestinal bleeding) after percutaneous or endoscopic treatment also is ignored due to the benefit of avoiding surgical interventions. In a previous study, minor and major complication rates of PBD were 11% and 2%, Most complications are minor and amenable to medical treatment. However, they can be life threatening and cause death.

We preferred PBD for treatment of biliary complications as the first treatment option. We performed 52 PBD procedures for early bile leakage. Each of these patients had ≥ 3 PBD procedures during follow-up at 1-month intervals. After all these prolonged and multiple interventions, 4 patients had revision surgery after failed repeated percutaneous modalities. Late biliary strictures developed in 19 patients who had early bile leakage that was treated with PBD (19 of 38 patients [50%] with early bile leakage treated with PBD). In 21 patients, surgery was the first choice for treatment of biliary complications, and 4 of these patients developed biliary complications at follow-up. We observed 2 patients who had bile leakage that was treated successfully with PBD. There were 2 biliary strictures that developed after surgical treatment; 1 patient had a surgical revision of hepaticojejunostomy and the other biliary stricture was treated with PBD.

The treatment strategies differ between different centers. Many reported series showed excellent results with PBD and ERC procedures with low complication rates. When choosing the treatment modality, treatment-related morbidity and mortality, recurrence rates, quality of life and retransplant rates of the procedure should be considered. For early bile leakage, a surgical approach cannot be avoided in most patients, after prolonged treatment with repeated sessions of percutaneous or endoscopic tools.

In conclusion, on the basis of the current literature, no strong recommendation can be provided for the initial treatment of biliary complications. Further investigations with long-term follow-up should be designed to compare treatment strategies.

References
Contrast Patterns of Cytomegalovirus and Epstein-Barr Virus Infection in Pediatric Living-Donor Liver Transplant Recipients

Hanaa Nafady-Hego,1,2 Hamed Elgendy,3,4 Shinji Uemoto5

Abstract

Objectives: Cytomegalovirus and Epstein-Barr virus remain leading causes of morbidity and mortality in the living-donor liver transplant population, particularly in pediatric patients. Herein we compare the incidence, timing, and risk factors for infection in this group.

Materials and Methods: We performed a retrospective study of 344 consecutive pediatric patients who received living-donor liver transplants at Kyoto University Hospital. Patients were followed-up for maximum 7.1 ± 3.6 years (range, 0.02-13.2 y) after surgery.

Results: The mean age at the time of transplant was 3.95 ± 4.75 years (median, 1.38 y; range, 0.07-17.87 y). A total of 156 patients (45.2%) developed viral infections. Of those patients, 91 (26.5%) developed cytomegalovirus infection, and 93 (27%) developed Epstein-Barr virus. Cytomegalovirus developed at 39.3 ± 34.6 days, while Epstein-Barr virus developed 3.99 ± 3.67 years after transplant. Frequent rejection attacks (hazard ratio [HR], 1.58; 95% confidence interval [CI]: 0.14-2.18; P = .006) were an independent predictor for postoperative cytomegalovirus infection, while preoperative cytomegalovirus seropositive results (HR, 1.76; 95% CI: 1.03-2.18; P = .038), short cold ischemia time (HR, 1.0; 95% CI: 0.99-1.0; P = .02), larger graft (HR, 1.3; 95% CI: 1.00-1.73; P = .047), and new cases compared to old cases (HR, 2.27; 95% CI: 1.14-4.52; P = .019) were independent predictors for postoperative Epstein-Barr virus infection.

Conclusions: Extended surveillance of cytomegalovirus and Epstein-Barr virus DNAemia is recommended for pediatric patients receiving living-donor liver transplants, particularly infants who are at high risk, and especially those exposed to frequent attacks of rejection and those that receive larger grafts.

Key words: Hepatic grafts, Immunosuppression, Rejection, Risk factors

Introduction

Liver transplants have been successfully used to treat children with end-stage liver disease, offering the opportunity for a long, healthy life. Living-donor liver transplants (LDLT) have come to account for a substantial number of pediatric cases performed in many centers throughout the world where deceased-donor organ procurement is rare. Immunosuppressive drugs are used to prevent rejection, inhibit activation of T lymphocytes, and modulate cell proliferation and macrophage function, thereby creating an optimal environment for the development of infections. Thus, infectious complications now represent the most common cause of morbidity and mortality after transplant procedures. Early and severe viral infections are
caused by viruses of the herpes family, including Epstein-Barr virus (EBV), and cytomegalovirus (CMV). Significant scientific breakthroughs and remarkable advances in molecular diagnostics and therapeutics have reduced the incidence and severity of CMV disease and EBV-related posttransplant lymphoproliferative disorders (PTLD) after liver transplant surgery during the early postoperative period. A parallel decline in associated morbidity and mortality has followed. However, despite these improvements, CMV and EBV remain common infectious complications and continue to negatively influence the outcome of liver transplant procedures. Moreover, the widespread and prolonged use of antiviral drugs changes the natural course of CMV disease by delaying its onset. In addition, an increased incidence of antiviral drug-resistant CMV infections now exists. In contrast, experimental and clinical data demonstrate a promising role for immunotherapy in preventing and treating PTLD and advocate the role of optimal preventive and treatment strategies for EBV to reduce the incidence of PTLD.

In our current study, we highlighted the most important aspects distinguishing CMV and EBV. In addition to the difference in the timing of appearance, the increasing frequency of EBV but not CMV infection over years, possibly due to immune response, is being recognized in the pathogenesis of CMV and EBV infection after liver transplant procedures. Such findings should provide additional avenues and opportunities for improving disease management strategies. Preoperative selection of pediatric patients for LDLT should be individualized based upon clinical and laboratory conditions. These, together with the common occurrence of CMV and EBV infection in high-risk patients, should direct the search for optimal preventive strategies for CMV and EBV infection occurrence after liver transplants.

Materials and Methods

Subjects
A total of 344 consecutive pediatric patients (< 18 y) that underwent LDLT at Kyoto University Hospital between 1998 and 2011 were included in our study. This protocol conforms to the ethical guidelines of the 1975 Helsinki Declaration. Due to the retrospective nature of this study, Institutional Review Board (The Ethical Committee of the Faculty of Medicine at Kyoto University) approval was not needed.

Surgical procedure and immunosuppression therapy
The surgical techniques and preoperative management of transplant recipients at our center have been previously described in detail. In brief, the basic immunosuppression regimen consists of tacrolimus and low-dose corticosteroids. Oral tacrolimus was given every 12 hours starting 1 day before the operation, at dosages dependent on target trough levels of 10 to 15 ng/mL for the first 2 weeks and 5 to 10 ng/mL for the next 2 months. A dosage of 10 mg/kg of methylprednisolone was administered after reperfusion of the grafts and then 1 mg/kg twice a day for the first 3 days, 0.5 mg/kg twice a day for the next 3 days, and 0.3 mg/kg on day 7. From day 8, oral prednisolone (0.3 mg/kg/d) was added. In case of ABO-incompatible LDLT, additional immunosuppressive therapy was administered to inhibit humoral rejection as reported previously. Cyclophosphamide was orally administered at a dose of 2 mg/kg/d 7 days before surgery and was switched to 1 mg/kg/d of azathioprine from 1 month after surgery. In addition, prostaglandin E1 was given intravenously at a dosage of 0.01 mg/kg/min for 1 to 2 weeks after surgery. Corticosteroid pulse therapy was added weekly during the first month, and then gradually tapered during months 3 to 12 after LDLT. In case of rejection, a regimen of tacrolimus and steroid pulse therapy previously described was instituted.

Postoperative antimicrobial prophylaxis
Antiviral prophylaxis, including ganciclovir, was not administered except in cases of seronegative recipients who received allografts from CMV-seropositive donors. Oral flomoxef, an oxacefem antibiotics were started from the first postoperative day and continued for 72 hours thereafter to prevent bacterial infection. Trimethoprim and sulfamethoxazole were administered once daily as a prophylaxis against pneumocystis. Oral miconazole was administered for 7 days after transplant for antifungal prophylaxis.

Diagnosis of viral infections
Cytomegalovirus and EBV status were evaluated weekly by serology and polymerase chain reaction testing,
Viral infections were diagnosed based upon clinical findings, positive results from serologic tests, and liver biopsies.\textsuperscript{14} Infection was confirmed by detection of the viral DNA fragment in urine, buffy coat, ascites, and tissue samples using polymerase chain reaction.\textsuperscript{15} Any asymptomatic viral infection determined by serologic testing was excluded from the study. In addition, systemic lymphadenopathy was assessed, and biopsies of the lymph nodes at the body surface, including the axillary lymph nodes and inguinal lymph nodes, were performed to rule out PTLD.

### Risk factors for infections

We evaluated the risk potential of several preoperative, operative, and postoperative variables. The preoperative variables included age at the time of LDLT, gender, clinical status, and the presence of preoperative infection and ascites. A standardized height score (SD score) was calculated for each patient as follows: $Z$ score = (measured value – average value in normal population)/SD of the normal population. Laboratory variables included white blood cell count, C-reactive protein, electrolytes (calcium, phosphorus, magnesium, potassium and sodium), liver function markers (aspartate transaminase, alanine transaminase, total bilirubin, and albumin), coagulation factors (international normalized ratio, antithrombin III, platelet number, and prothrombin time), renal function markers (blood urea nitrogen and creatinine), preoperative hospital stay, ABO mismatching, and pediatric end-stage liver disease score. Operative variables included operation time, cold ischemic time, warm ischemic time, blood or blood product transfusion (packed red blood cells, fresh frozen plasma, and platelets), fluid transfusion (5\% albumin), blood loss, and graft recipient body weight ratio. Postoperative variables included surgical complications (intra-abdominal hemorrhage, bile leak, and intestinal perforation), repeat surgery, postoperative intensive care unit stay, renal dialyses, length of insertion of intravascular catheter, intra-abdominal drainage, bile drainage, and graft dysfunction. Graft dysfunction was defined as persistent abnormal liver function with serum aminotransferase levels 2 to 3 times normal, with or without elevated bilirubin, and abnormal biopsy finding. Postoperative days were divided into 2 equal groups. Data of concomitant rejection or administration of steroid pulse therapy at the time of infection were also evaluated.

### Statistical analyses

Statistical analyses were performed using the Statistical Package for the Social Science (SPSS: An IBM Company, version 16.0, IBM Corporation, Armonk, NY, USA). Data are presented as mean ± standard deviations, median, range, or percentage where appropriate. Cox proportional hazard regression model was used to evaluate the effect of different factors on the risk of CMV or EBV infections. Factors with a $P$ value $\leq .05$ were included in the multivariate analysis. A $t$ test for quantitative data and chi square test for qualitative data were used to compare differences between LDLT recipients who did and did not develop either CMV or EBV viral infections.

### Results

#### Patient population

Among 344 subjects who underwent LDLT from November 1998 to April 2011, forty-four required a second transplant, and 1 required a third. Of the study participants, 193 were female (56.1\%). The mean age at the time of transplant was $3.95 \pm 4.75$ years (median, 1.38 y; range, 0.07-17.87 y). Indications for LDLT among patients were biliary atresia ($n = 215$, 62.6\%), acute liver failure ($n = 38$, 11\%), metabolic liver disease ($n = 21$, 6.1\%), tumors ($n = 17$, 4.9\%), Alagille syndrome ($n = 13$, 3.8\%), liver cirrhosis/hepatitis ($n = 8$, 2.3\%), Byler disease ($n = 4$, 1.2\%), and others ($n = 28$, 8.1\%). The mean follow-up duration was 7.1 ± 3.6 years (range, 0.02-13.20 y). Among all recipients and patients the preoperative serology identified 277 CMV cases and 272 EBV cases. A total of 156 patients (45.2\%) developed viral infections, of those, 91 (26.5\%) developed CMV, at $39.3 \pm 34.6$ days (median, 33 d; range, 3-300 d). Hepatitis was confirmed by liver biopsy in 13 cases, 9 developed fevers of unknown cause, 2 developed pneumonia, 1 was diagnosed as having CMV enteritis, and 1 patient manifested with pancytopenia. The remaining cases were diagnosed using polymerase chain reaction testing. A serologic combination of seropositive donor (D+) and seronegative recipient (R-) for CMV was reported in 69 patients, of which, 1 developed CMV. At $39.3 \pm 34.6$ days (median, 33 d; range, 3-300 d). Hepatitis was confirmed by liver biopsy in 13 cases, 9 developed fevers of unknown cause, 2 developed pneumonia, 1 was diagnosed as having CMV enteritis, and 1 patient manifested with pancytopenia. The remaining cases were diagnosed using polymerase chain reaction testing. A serologic combination of seropositive donor (D+) and seronegative recipient (R-) for CMV was reported in 69 patients, of which, 1 developed CMV. Moreover, 55 of 85 patients that developed CMV were seropositive for CMV preoperatively. The incidence of CMV infection was significantly influence by the preoperative CMV seropositive status ($P = .008$) and
a significant trend was identified for the serologic D+/R- combination \( (P = .094) \).

A total of 93 patients (27%) developed EBV at 3.99 ± 3.67 years (median, 2.93 y; range, 0.02-10.92 y) including 4 who developed PTLD, 9 that developed hepatitis, 8 that developed fever, and 2 that developed pneumonia. A serologic combination of seropositive donor (D+) and seronegative recipient (R-) for EBV was reported in 82 patients who developed EBV, and 39 patients were seropositive for EBV preoperatively. The incidence of EBV infection developed EBV, and 39 patients were seropositive for EBV preoperatively. The incidence of EBV infection was not significantly influence by the preoperative EBV serologic state \( (P = .356) \) or D+/R- match \( (P = .432) \).

A total of 29 cases having both CMV and EBV infection were not included in risk factor analysis. The exact pattern of CMV and EBV infected patients is shown in Table 1.

### Risk factors for cytomegalovirus and Epstein-Barr virus

**Univariate analysis for factors affecting CMV infection after living-donor liver transplant**

We compared factors affecting CMV infections after LDLT with those affecting EBV infections. Correlation testing revealed that seropositivity (R+) for CMV \( (P = .017) \), preoperative low platelet count \( (P = .01) \), and preoperative elevated total bilirubin \( (P = .041) \), high preoperative total blood albumin level \( (P = .041) \), high preoperative white blood cell count \( (P = .001) \), short cold ischemic time \( (P = .002) \), larger graft recipient body weight ratio \( (P = .008) \), new cases compared to old cases \( (P < .001) \), and frequent attacks of rejection \( (P < .001) \) correlated with postoperative EBV infections (Table 2B).

### Multivariate analysis for factors affecting infection after living-donor liver transplant

Potential predictors further examined with multivariate analysis revealed that frequent attacks of rejection (hazard ratio [HR], 1.58; 95% CI: 1.14-2.18; \( P = .006 \)) independently predicted postoperative CMV infections, while preoperative low platelet count showed a trend (HR, 1.0; 95% CI: 0.99-1.00; \( P = .088 \)) (Table 3A).

Regarding EBV, CMV (R+) (HR, 1.76; 95% CI: 1.03-2.18; \( P = .038 \)) short cold ischemic time (HR, 1.0; 95% CI: 0.99-1.00; \( P = .002 \)), larger graft recipient body weight ratio (HR, 1.3; 95% CI: 1.03-1.60; \( P = .008 \)) correlated with postoperative EBV infections (Table 2B).

### Table 1. Characteristics of Cytomegalovirus, Epstein-Barr Virus, and Noninfected Cases After Pediatric Living Donor Liver Transplant

<table>
<thead>
<tr>
<th>Variable</th>
<th>CMV Infected (n = 91)</th>
<th>EBV Infected (n = 93)</th>
<th>Noninfected (n = 189)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>3.4 ± 4</td>
<td>2.6 ± 3.7*</td>
<td>4.8 ± 5.2 *</td>
<td>&lt; .001</td>
</tr>
<tr>
<td>Sex (n, %)</td>
<td>female</td>
<td>female</td>
<td>female</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>(53, 8.2%)</td>
<td>(57, 13.3%)</td>
<td>(85, 45%)</td>
<td></td>
</tr>
<tr>
<td>Indication for LDLT</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Biliary atresia</td>
<td>56 (61.5%)</td>
<td>62 (66.7%)</td>
<td>114 (60.3%)</td>
<td></td>
</tr>
<tr>
<td>FHF</td>
<td>14 (15.4%)*</td>
<td>12 (12.9%)</td>
<td>14 (7.4%)</td>
<td>.035</td>
</tr>
<tr>
<td>Metabolic liver disease</td>
<td>2 (2.2%)</td>
<td>2 (2.2%)</td>
<td>13 (6.9%)</td>
<td></td>
</tr>
<tr>
<td>Tumors</td>
<td>6 (6.6%)</td>
<td>10 (5.3%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alagille syndrome</td>
<td>3 (3.3%)</td>
<td>3 (3.2%)</td>
<td>8 (4.2%)</td>
<td></td>
</tr>
<tr>
<td>Liver cirrhosis/ hepatitis</td>
<td>1 (1.1%)</td>
<td>3 (1.6%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Byler disease</td>
<td>2 (2.2%)</td>
<td>2 (2.2%)</td>
<td>1 (0.5%)</td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>7 (7.7%)</td>
<td>12 (12.9%)</td>
<td>26 (13.8%)</td>
<td></td>
</tr>
<tr>
<td>Retransplants</td>
<td>14</td>
<td>14</td>
<td>23</td>
<td></td>
</tr>
<tr>
<td>CMV preoperative</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>positive serology</td>
<td>55 (60.4%)*</td>
<td>60 (64.3%)**</td>
<td>73 (38.6%)*</td>
<td>.008</td>
</tr>
<tr>
<td>CMV(D+/R-)</td>
<td>14 (15.4%)*</td>
<td>13 (14%)**</td>
<td>46 (24.3%)**</td>
<td>&lt; .001</td>
</tr>
<tr>
<td>EBV preoperative</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive serology</td>
<td>99 (39.1%)</td>
<td>97 (41.9%)</td>
<td>77 (40.7%)</td>
<td>.094</td>
</tr>
<tr>
<td>EBV(D+/R-)</td>
<td>54 (21.3%)</td>
<td>23 (24.7%)</td>
<td>38 (20.1%)</td>
<td>.019</td>
</tr>
</tbody>
</table>

**Abbreviations:** CMV, cytomegalovirus; D+/R-, positive donor/negative recipient; EBV, Epstein-Barr virus; FHF, fulminant hepatic failure; LDLT, living donor liver transplant

### Table 2A. Univariate Analysis of Risk Factors for Cytomegalovirus Infection in 344 Pediatric Living Donor Liver Transplant Recipients

<table>
<thead>
<tr>
<th>Variable</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CMV(R+)</td>
<td>.017</td>
</tr>
<tr>
<td>Preoperative low platelet count</td>
<td>.01</td>
</tr>
<tr>
<td>Preoperative diagnosis as fulminant hepatic failure</td>
<td>.24</td>
</tr>
<tr>
<td>Higher frequency of rejection</td>
<td>&lt; .001</td>
</tr>
</tbody>
</table>

### Table 2B. Univariate Analysis of Risk Factors for Epstein-Barr Virus Infection in 344 Pediatric Living Donor Liver Transplant Recipients

<table>
<thead>
<tr>
<th>Variable</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (&lt; 1 y)</td>
<td>.001</td>
</tr>
<tr>
<td>Prolonged preoperative hospital stay (&gt; 7 d)</td>
<td>.033</td>
</tr>
<tr>
<td>Preoperative ascites</td>
<td>.018</td>
</tr>
<tr>
<td>Prolonged intraarterial catheter insertion</td>
<td>.014</td>
</tr>
<tr>
<td>CMV(R+)</td>
<td>&lt; .001</td>
</tr>
<tr>
<td>CMV(D+/R-)</td>
<td>.024</td>
</tr>
<tr>
<td>Low preoperative albumin</td>
<td>.41</td>
</tr>
<tr>
<td>High preoperative total bilirubin</td>
<td>.1</td>
</tr>
<tr>
<td>High preoperative white blood cell count</td>
<td>&lt; .001</td>
</tr>
<tr>
<td>Short cold ischemic time</td>
<td>.002</td>
</tr>
<tr>
<td>Large graft recipient body weight</td>
<td>&lt; .001</td>
</tr>
<tr>
<td>More fresh frozen plasma</td>
<td>.008</td>
</tr>
<tr>
<td>New cases compared to old cases</td>
<td>&lt; .001</td>
</tr>
<tr>
<td>Higher frequency of rejection</td>
<td>&lt; .001</td>
</tr>
</tbody>
</table>

**Abbreviations:** CMV, cytomegalovirus; D+/R-, seropositive donor/seronegative recipient; R+, seropositive recipient
CI: 1.00-1.73, \( P = .047 \)), and new cases compared to old cases (HR, 2.27; 95% CI: 1.14-4.52, \( P = .019 \)) independently predicted postoperative EBV infections (Table 3B).

**Table 3A. Multivariate Analysis of Factors Affecting CMV Infection in 344 Pediatric LDLT Recipients**

<table>
<thead>
<tr>
<th>Variable</th>
<th>HR (95% CI)</th>
<th>( P ) value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preoperative platelet count</td>
<td>1 (0.996-1.0003)</td>
<td>.088</td>
</tr>
<tr>
<td>Higher frequency of rejection</td>
<td>1.58 (1.142-1.78)</td>
<td>.006</td>
</tr>
</tbody>
</table>

**Table 3B. Multivariate Analysis of Factors Affecting EBV Infection in 344 Pediatric LDLT Recipients**

<table>
<thead>
<tr>
<th>Variable</th>
<th>HR (95% CI)</th>
<th>( P ) value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CMV-R+</td>
<td>1.76 (1.03-3)</td>
<td>.038</td>
</tr>
<tr>
<td>Short cold ischemia time</td>
<td>1 (0.99-1)</td>
<td>.02</td>
</tr>
<tr>
<td>Large graft recipient body weight</td>
<td>1.3 (1.003-1.73)</td>
<td>.047</td>
</tr>
<tr>
<td>New cases compared to old cases</td>
<td>2.27 (1.144-4.516)</td>
<td>.019</td>
</tr>
</tbody>
</table>

**Abbreviations**: 95% CI, 95% confidence interval; CMV, cytomeglovirus; CMV-R+, seropositive recipient R+ for CMV; EBV, Epstein-Barr virus; HR, hazards ratio; LDLT, Living-donor liver transplant

**Prognosis**

In our cohort there were 47 deaths, 2 were due (4.3%) to EBV-related PTLD, 1 was due (2.2%) to EBV hepatitis, and 1 was due (2.2%) to CMV infection. A total of 50 patients had graft dysfunction, 16 of which had CMV and 12 had EBV infections. There were no significant differences among infected and noninfected cases (\( P = .213 \) and \( P = .369 \)).

**Different effect of pathology on cytomegalovirus/Epstein-Barr virus infection**

In our cohort, 37 cases were diagnosed as having FHF. The incidence of CMV/EBV infection in these patients was compared to those in patients with chronic liver failure. The results showed that CMV was detected in 40.5% of FHF patients and 24.8% of chronic liver failure patients (\( P = .035 \)), while EBV was detected in 35.1% of FHF patients and 26.1% of chronic liver failure patients (\( P = .20 \)).

**The contrast pattern of cytomegalovirus/Epstein-Barr infection in pediatric living-donor liver transplant over the 13-year period**

Studying the patterns of CMV and EBV infections from 1998 to 2011, we found that the frequency of EBV but not CMV was lower in the older cases and gradually increased since 2002.

**Discussion**

The results for pediatric liver transplants improved dramatically after development of effective immuno-suppressive drugs in the second half of the 1980s.\(^{16}\) Despite the antiviral strategies of today, CMV and EBV infection have remained the most troublesome complications after LDLT.\(^{5,17,18}\) Our results showed that 155 of pediatric patients (45.2%) had at least 1 viral infection episode after LDLT. A diagnosis of CMV infection was confirmed in 91 of these patients (26.5%), which is consistent with a previous report from our center.\(^{8}\) Other centers showed a higher incidence of infection, reporting that CMV affects up to 75% of liver transplant recipients directly or indirectly.\(^{17,19}\) A possible explanation for the discrepancy between our results and theirs is the lower target immuno-suppressive trough level at our center compared with others, which did not lead to impairment of the immune system.

On the other hand, EBV infection was diagnosed in 93 of our patients (27%), which is considered high compared to Shepherd and his colleagues.\(^{19}\) We attribute this increase to the difference in our inclusion criteria, as we included all infected cases regardless of severity, while Shepherd and associates\(^{19}\) included only severe cases. Moreover, our follow-up was longer than theirs was. Earlier reports by Singh and associates\(^{20}\) described data close to ours. Altogether, our findings may suggest that although the incidence of viral infection at our center was relatively high compared to some centers,\(^{19,21,22}\) we had a lesser frequency of CMV disease and PTLD compared to a previous report.\(^{19}\) These results may be explained by the serial real-time polymerase chain reaction screening protocol used at our center for evaluating the CMV and EBV genome that was found to be helpful for early detection and monitoring of viral infections for preemptive treatment of PTLD.\(^{23,25}\)

**Cytomegalovirus** infection was diagnosed earlier than EBV infection at our center. A possible explanation for this is that CMV infection occurs due to a severely suppressed immune status in the recipient. Uncontrolled CMV proliferation in the absence of CMV-specific immune-protective CD8+ cells after transplant favors the occurrence of CMV infection.\(^{26}\)

Moreover, the incidence of CMV infection was significantly influence by preoperative seropositivity for CMV, while EBV showed no significant difference based on preoperative seropositivity. This difference may be attributed to the fact that CMV infection occurs in the early period while the immune system
is severely suppressed that allow CMV proliferation, while EBV usually occur later after reduction of immunosuppressive drugs.

The chance of a satisfactory outcome in the management of CMV and EBV infection depends on early diagnosis and the use of strategies aimed at prevention. Early treatment as well as the prevention of CMV and EBV infections in children may be facilitated by knowledge of the primary risk factors for these infections in this population. In the current study, numerous risk factors were identified as being associated with CMV. A recipient seropositive status was associated with CMV infection and Lejungman and associates also reported that the seropositivity of patients is a major risk for CMV infection after transplant. We and others also found that FHF was a major risk factor for CMV infection, and although the mechanism of this association is not completely understood, FHF is associated with very high levels of tumor necrosis factor alpha which may directly promote viral replication.

Concomitant rejection was an independent risk factor for CMV infection and similar data have been reported previously. These data raise the possibility that immunosuppression is a modifiable risk factor for serious viral infection. Although it is difficult to assess which comes first, rejection or infection, avoiding over-immunosuppression by carefully monitoring immunosuppressant drug dosages and blood levels and limiting steroid use in pediatric patients should be an important goal. Fortunately, immunosuppression reduction is highly encouraged at our center. No less than 15% of pediatric patients (which is high compared with other centers) achieve complete withdrawal of immunosuppression and develop a state of operational tolerance.

A low preoperative platelet count was found to be an independent risk factor for CMV. This can be explained by the clinical association of FHF in those patients. Another explanation is that a low platelet count itself may be an indication for more transfusions that may lead to viral infection transmission.

 Regarding factors affecting EBV infections after LDLT, correlation testing showed a young age at the time of transplant (< 1 y) to be an associated risk for EBV infection. Guthery and his associates also reported a young age at transplant to be an independent factor for EBV infection. They also found elevated white blood cell counts to be associated with EBV infection and an abnormally elevated preoperative white blood cell count has been associated with postoperative morbidity and mortality. Frequent attacks of rejection was an independent risk for EBV infection that can be explained by the immunologic cascade triggered by the CMV viral infection, where for example release of tumor necrosis factor alpha plays a pathogenic role. These indirect effects of CMV may exert a state of enhanced immune suppression with a concurrent higher risk of EBV infection and hence PTLD. Therefore, effective prevention of CMV also may prevent EBV infections, primarily by limiting the effect of CMV on immune regulation. Hypoalbuminemia is a common problem among persons with acute and chronic liver disease at the time of hospital admission and serum albumin level is an important prognostic indicator. Among hospitalized patients, lower serum albumin levels correlate with an increased risk of morbidity and mortality. Our results showed that low preoperative serum albumin levels, more fresh frozen plasma, prolonged intravascular catheter insertion, preoperative ascites, and high preoperative serum total bilirubin level are associated risk factors for EBV, which may reflect that cases with preoperative poor medical conditions are more prone to infection with EBV after a liver transplant.

We also found elevated white blood cell counts to be associated with EBV infection and an abnormally elevated preoperative white blood cell count has been associated with postoperative morbidity and mortality. Frequent attacks of rejection was an independent risk for EBV, and similar data has been reported by Shepherd and associates. This finding can indicate that EBV can be induced by cytokine-mediated interactions due to rejection with normal cells of the immune system because we could not find any relation between EBV infection and immunosuppression. Graft-recipient-body-weight ratio also was found to be an independent factor for EBV infection in our study. This can be explained by the fact that graft recipient body weight ratio differences are usually found in younger patients who were more prone to infection. Although, we found that new cases compared to old were independently predictive of EBV infection, we found that graft survival is better in recent cases compared with old cases that may be
due to the frequent monitoring and early detection of EBV infection.

Our findings suggest that although we have a relatively low incidence of infection at our center, viral infections still remain the second cause of death after sepsis in pediatric patients following LDLT. Similar findings on the effect of CMV on mortality rates have been reported in liver transplant recipients. In our study, EBV related PTLD lead to the death of 2 patients. Mortality due to PTLD after pediatric solid-organ transplant was previously reported to be 12% to 32% and consist with our findings this incidence has been significantly reduced by EBV DNA monitoring and tapered immunosuppressive drug regimens.

Altogether, we noticed that the number of patients in our study that were infected and those that died as a result of CMV and EBV was lower than that in other centers which perform LDLT. This discrepancy can be due to many factors including the efficacy of our EBV screening program in decreasing the incidence of EBV-related PTLD as well as the fact that prophylactic antiviral therapy in D+/R-combination cases may result in better outcomes at our center. In addition, the lower target level of immunosuppression at our center and the weaning protocol in which physicians start the process of gradual decreasing immunosuppressive drugs until finally stopping them completely appears to be beneficial.

In conclusion, based on the results of the current study, it is strongly recommended that early diagnosis of CMV and EBV is essential for LDLT patients. In this regard, CMV and EBV quantitative polymerase chain reaction testing may aid in surveillance. Such surveillance should be extended, especially in the case of infants who have a bigger graft recipient body weight ratio, those with a previous history of FHF, and patients at a particularly high risk of CMV and EBV infection. Additional research should be directed at improving the sensitivity, specificity, and predictive values of polymerase chain reaction and other testing modalities for this purpose. Multicenter trials are encouraged to evaluate the role of various regimens in the prevention of CMV and EBV infection in high risk patients.

References

IL-17 mRNA Expression and Cytomegalovirus Infection in Liver Transplant Patients

Afsoon Afshari,1 Ramin Yaghobi,2 Mohammad Hossein Karimi,2 Mojtaba Darbouy,1 Negar Azarpira,2 Bita Geramizadeh,2 Seyed Ali Malek-Hosseini,3 Saman Nikeghbalian3

Abstract

Objectives: Cytomegalovirus (CMV) establishes a lifelong, asymptomatic infection in immunocompetent hosts. Interleukin-17 producing CD4+ T-cells (Th-17) are a subtype of CD4+ T-cells. The precise role of Th-17 responses during cytomegalovirus replication has not been elucidated, although recent studies suggest that infections such as murine cytomegalovirus induce a Th-17 response. Th-17 cells also have been associated with allograft rejection and autoimmune diseases. In this study, we tried to find the relation of cytomegalovirus infection and interleukin 17 (IL-17) cytokine in liver-transplanted patients.

Materials and Methods: Two groups of patients were evaluated in this study. The first group consisted of 54 cytomegalovirus uninfected liver-transplanted patients, and the second group consisted of 15 cytomegalovirus-infected patients. Three ethylenediaminetetraacetic acid-treated blood samples were collected from each patient on days 1, 4 and 7 post liver transplant. For diagnosing cytomegalovirus infection antigenemia and Taq-Man real-time polymerase chain reaction protocols were used. Also, to determine the expression level of IL-17 gene, an in-house SYBR green real-time polymerase chain reaction technique was used.

Results: Using antigenemia and also Taq-Man real-time polymerase chain reaction technique was used. Results: Using antigenemia and also Taq-Man real-time polymerase chain reaction helps find active

cytomegalovirus infection, and the load of cytomegalovirus in each patient. The first group of patients showed that IL-17 expression level was down-regulated after day 4 of sampling. But in cytomegalovirus-infected patients, IL-17 expression level was increased significantly. The results between IL-17 gene expression level between the 2 groups of patients showed that IL-17 expression level significantly increased in second group during day 4 (P = .038) and 7 (P = .009) post liver transplant.

Conclusions: Significant increase of IL-17 mRNA levels in cytomegalovirus-infected group compared with the uninfected one reinforced the role of IL-17 as a proinflammatory cytokine dealing with cytomegalovirus infection in liver transplanted patients.

Key words: Viral infection, Interleukin-17, Allograft rejection

Introduction

Orthotopic liver transplant (OLT) is mentioned as a final therapeutic protocol for end-stage liver diseases.1-3 Surveillance duration of transplanted liver is related to many factors including pathogens.4 Many of the pathogens can threaten the health of a transplanted liver, among them cytomegalovirus (CMV) can cause mortality and morbidity directly and/or indirectly influencing immunosuppression that make the patient susceptible to different super-infections.1 Cytomegalovirus is an opportunistic pathogen that can infect people worldwide (from 40% to 90%), but commonly make active infection immunocompromised patients5 like organ transplant recipients and undoubtedly have negative effect on the results of transplant.1,6,7 Active CMV infection risk in transplant recipients is around 30%
Cytomegalovirus can affect an allograft outcome by interfering cellular and humoral immune responses with changing the subpopulation of CD4+ and CD8+ T lymphocytes and also cytokines. It is known that CMV infection can induce increasing of the level of several chemokines and cytokines in liver transplanted patients leading to control of infection and sometimes this struggle renders to allograft injury or rejection. There also are reports demonstrating that both chronic hepatitis B virus (HBV) and hepatitis C virus (HCV) infections are related to virus-induced liver inflammation, infiltration, and activation of Th17 cells, and the amount of liver damage caused by the antiviral immune response.

After transplant, CD4+ and CD8+ T cells especially play an important role in controlling CMV replication. Recently, a new subset of CD4+ T cells, Th17 cells, were introduced to have prominent roles in the result of liver transplant. Th17 cells can activate specific transcription factors and produce IL-17 cytokine as its major cytokine product and also IL-21, IL-22, and IL-23 receptor, and IL-23 acts as a stabilizing agent for production of Th17 cells. Interleukin-17 has no sequence similarity to any other known cytokine and mammalian protein.

Interleukin-17 is the major proinflammatory cytokine of these cells which is a part of normal host response to infection. One study elucidated the role of Th17 in viral infection persistence by up-regulating the antiapoptotic molecules that renders survival of virus-infected cells. Also, another study showed the in situ existence of the Th17 cells in HBV-infected liver can worsen the liver physiology. Interleukin-17 can recruit neutrophils and monocytes to the site of inflammation.

The importance of Th17 cells and its important cytokine, IL-17, is detected in different inflammatory and autoimmune diseases (eg, rheumatoid arthritis, psoriasis, inflammatory bowel disease, and multiple sclerosis), and allograft rejection. Also, IL-17 by its proinflammatory action, can induce expression of many factors dealing with inflammation. Increase of IL-17 after transplant and rejection in different transplant type in human and mice is documented. In rat acute renal allograft rejection models, elevation of IL-17 proteins in day 2 after transplant has been shown. In mouse heart transplant model, using IL-17 inhibitor, reduced production of inflammatory cytokines within the graft and increased graft survival. The role of Th17 and IL-17 in liver transplant is not studied much in humans, but reported a significant increase of IL-17/IL-23 protein level in patients with acute rejection after liver transplant. Also, our recent study showed the increase of IL-17 mRNA level in the first week after liver transplant in rejected ones. As IL-17 is important in organ allograft rejection, it may have capacity for being considered as a target for anti-rejection therapy, either alone or in combination with immunosuppressive agents.

Taking together, considering the role of IL-17 as a proinflammatory cytokine in liver transplanted patients and the role of CMV in causing inflammation in transplanted patients, in this study we determined the mRNA expression level of IL-17 in CMV infected liver transplanted patients.

Materials and Methods

Study groups

The patients that were enrolled in this study were composed of 2 groups of liver transplanted patients who underwent surgery at Namazi Hospital, Shiraz, Iran, between years 2011 and 2013. EDTA-treated blood samples were collected from each studied patient in days 1, 4 and 7 intervals after the liver transplant. Using Ficol, the buffy coat and plasma were isolated from each blood sample and preserved in -80°C for further analysis. Based on experiencing CMV infection or not, studied patients were divided into infected and uninfected liver transplanted. The uninfected patients consisted of 54 patients and the infected group were composed of 15 patients. The study was approved by the Ethical Committee of Shiraz University of Medical Sciences (The study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki). For detecting CMV antigenemia technique was used for all of the samples and Taq-man real-time PCR were administrated for certifying the results of antigenemia. For determination of the mRNA expression level of IL-17 SYBR Green real-time PCR was used and β-actin was also considered for internal control.

The routine immunosuppression regimen consisted of tacrolimus or cyclosporine with mycophenolate mofetil and steroids. Drug dosage was adjusted to maintain target therapeutic blood levels of 200 mg/mL for CsA (5 mg/kg/d) or 10 mg/mL for tacrolimus.
Donors were selected on the basis of ABO blood group compatibility. HLA matching is routinely not done for liver transplant patients.

**Cytomegalovirus Quantification**

*Viral genome extraction*

Using Invisorb Spin Virus DNA Mini Kit (Invitek, Birkenfeld, Germany) CMV-DNA was extracted from plasma samples according to the manufacturer’s protocol. Before starting the extraction procedure, the internal control was added to each sample. Determination of CMV viral load was done using gensig real-time PCR kit (Primer Design Ltd TM, Advanced kit, United Kingdom). The reaction mix for PCR was performed in 20 μL total volume and was composed of 10 μL Precision TM Master Mix (Applied Biosystems Grand Iland, NY, USA), 1 μL primers and a probe targeting the glycoprotein B (gB) sequence, 1 μL primers and a probe targeting the internal control (IC) gene, 5 μL of the DNA and 3 μL DEPS water.

The program used for this reaction was included 1 cycle 95°C for 10 minutes, followed by 50 cycles of 95°C for 5 seconds and 60°C for 60 seconds using Step One Plus Real-Time thermocycler (Applied Biosystems-Grand Iland, NY, USA). This quantitative PCR assay was sensitive enough to detect as few as 10 copy of CMV genome per milliliter of body samples.

**Cytomegalovirus antigenemia protocol**

All of the patients were tested with antigenemia for detection of active infection. For executing antigenemia test on the samples, EDTA-treated whole blood samples were used and the procedure was followed through the package insert of the CMV Brite Turbo kit (IQ Products, Groningen, The Netherlands). Two hundred thousand cells were collected for Cyto-centrifuged preparations (Cytospin 3, Shandon Scientific, Cheshire, England). Then a cocktail of 2 fluorescein isothiocyanate-labeled monoclonal antibodies (C10/C11) were used for staining indirect immunofluorescence and directed against CMV lower matrix protein pp65. By acquiring fluorescence microscope, bright green stained nucleus of CMV infected polymorphonuclear cells were detected and finally the CMV antigen positive cells were counted and reported.

**RNA isolation and cDNA synthesis**

Total RNA was isolated by RNX plus (CinnaGen, Iran) from Buffy coats by using an in-house RNA extraction protocol. For certifying the purity and integrity of RNA, the optical density 260/280 and agarose gel (1%) electrophoresis measured. One microgram of each RNA sample was changed to cDNA by Reverse Transcriptase (Vivants, Subang Jaya, Malaysia) and random hexamer. This cDNA synthesis was performed in two steps. First, RNA (10 μg/μL), dNTPs (1 μL/10 mm), and random hexamer (0.2 μg/μL) were mixed and incubated at 65°C for 7 minutes and then on ice for 2 minutes. Second, M-MLV reverse transcriptase enzyme (1 μL/200 U), reverse transcriptase-buffer (2 μL/× 10), RNase inhibitor (1.3 μL/60 U), and nuclease free water were mixed and added to product of first step. Then final mix was incubated at 45°C for 90 minutes and 85°C for 5 minutes.

**SYBR green real time polymerase chain reaction**

For the quantitative analysis of IL-17 mRNA expression profile in both groups of studied patients, real-time PCR method was performed. After evaluation of the β-actin and GAPDH genes as internal controls, the β-actin gene was finally used as internal control for minor fluctuations. The primer was originally designed for transcripts of IL-17 (NM_002190.2) and β-actin (NM_001101.3) as the internal control. The primer sequences for amplification of IL-17 and β-actin transcripts were as follows: 5’-TCTGGGAGGCAGTGCCC-3’ and IL-17R: 5’-GGGCAGTGTGGAGGCTCCCT-3’, β-actin F: 5’-GGCGGCACCACCACTGTACCC-3’, and β-actin R: 5’-GACGATGGAGGGCCCGACT-3’. The PCR mixes were composed of SYBR green Premix (10 μL) by Ex Taq (Takara, Otsa, Shiga, Japan), SYBR green dye (0.2 μL), forward and reverse primers (3 PM), and template cDNA (2 μL). The temperature program for PCR condition was as follows: One cycle 95°C for 5 minutes, followed by 40 cycles of 95°C for 30 seconds, and 65°C for 20 seconds using Step One Plus real-time instrument (ABI, Step One Plus, Grand Iland, NY, USA). For checking the specificity of amplification reaction melting-curve analysis was evaluated. The results for the target genes were measured as fluorescent signal intensity and normalized to the internal standard gene β-actin.

**Statistical analyses**

For evaluating the IL-17 expression pattern between 2 sampling collection intervals between CMV infected and uninfected liver transplant patient groups using Livak method. Statistical analysis was
performed using SPSS, version 16 for Windows (SPSS, Chicago, IL, USA). The t test, Mann-Whitney U test, and ANOVA test were used to evaluate statistical association and/or difference inside and between 2 studied groups. \( P < .05 \) was considered as statistically significant.

**Results**

**Patients’ profile**

The CMV-infected liver transplant patients who termed CMV+ consisted of patients with positive results of antigenemia and also Taq-Man real-time PCR methods. The uninfected patients consisted of 54 patients whose age range was between 1 and 74 years with a mean of 34.33 ± 21.26 years old. The 36 of 54 uninfected patients (68%) were male, and 17 of 54 were female (32%). The infected patients consisted of 15 patients who experienced CMV infection. The age range in these patients was between 1.5 and 62 with a mean of 30.25 ± 15.3 years old. The infected group was composed of 7 of 15 men (46.6%) and 8 of 15 women (53.4%). The distribution frequency of the underlying disease in both uninfected and infected liver transplant patients was as follows: Hepatitis B virus infection 15 in uninfected (27.8%), and 2 infected (13.3%), auto immune hepatitis 9 in uninfected (16.7%) and 3 infected (20%), primary sclerosing cholangitis 9 in uninfected (16.7%) and 2 were infected (13.3%), cryptogenic cirrhosis 7 in uninfected (13%) and 3 infected (20%), Wilson disease 3 uninfected (5.5%) and 1 infected (6.7%), biliary atresia 3 uninfected (5.5%) and 1 infected (6.7%), hypertyrosinemia 2 uninfected (3.7%) and 1 infected (6.7%), hepatitis C virus infection 1 uninfected (1.85%), and 2 infected (13.3%), and other diseases 5 uninfected (9.25%) and 0 infected groups. The highest frequency of the underlying diseases was hepatitis B virus infection and auto immune hepatitis in uninfected and infected transplant patients. The most frequent ABO blood group also was O+ in uninfected and A+ in infected transplant patients. The details of ABO blood groups are shown in Table 1.

**IL-17 gene expression in uninfected and infected liver transplant patients**

To identify whether IL-17 had affected liver transplant outcomes, 69 liver transplant patients were selected. The analysis of results in uninfected patients showed that IL-17 expression was down-regulated after day 4 of follow-up (Figure 1). In infected patients, IL-17 expression was significantly and steadily increased during all days of follow-up (Figure 2).

**Time dependent IL-17 gene expression between infected and uninfected liver transplant patients**

The results of comparing the interleukin-17 gene between uninfected and infected liver transplant

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**Table 1. The Frequency of ABO Blood Groups in Uninfected and Infected Studied Groups**

<table>
<thead>
<tr>
<th>Blood groups</th>
<th>CMV-</th>
<th>CMV+</th>
</tr>
</thead>
<tbody>
<tr>
<td>A+</td>
<td>18 (33.3%)</td>
<td>9 (60%)</td>
</tr>
<tr>
<td>A-</td>
<td>1 (1.8%)</td>
<td>-</td>
</tr>
<tr>
<td>B+</td>
<td>14 (26%)</td>
<td>1 (6.7%)</td>
</tr>
<tr>
<td>B-</td>
<td>1 (1.8%)</td>
<td>-</td>
</tr>
<tr>
<td>AB+</td>
<td>2 (3.8%)</td>
<td>2 (13.3%)</td>
</tr>
<tr>
<td>AB-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>O+</td>
<td>17 (31.5%)</td>
<td>3</td>
</tr>
<tr>
<td>O-</td>
<td>1 (1.8%)</td>
<td>-</td>
</tr>
<tr>
<td>Total</td>
<td>54 (100%)</td>
<td>15 (100%)</td>
</tr>
</tbody>
</table>
patients are presented in Figure 3. Interleukin-17 expression level decreased in infected patients in the first day sampling time and increased significantly, on the second (day 4) and third (day 7) compared with uninfected during sampling days post-transplant (Figure 3A-3B), but this increase was significant in the fourth and seventh day of sampling ($P = .038$, 95% CI: 0.00-0.05 and $P = .009$, 95% CI: 0.00-0.09) (Figure 3B and 3C). Interleukin-17 expression level also was increased but not significantly in uninfected patients during the first ($P = .215$, 95% CI: 0.189-0.408) sampling day post-transplant. The IL-17 gene expression was increased around 140 times in infected patients compared with uninfected ones during posttransplant follow-up.

**Discussion**

Liver transplant should be mentioned as a costly and critical therapy for many irreversible liver damages and should be preserved not to be threatened by any harmful agent.\(^3\) It is a fact that organ transplant can make an inflammatory environment that many inflammatory cytokines like IL-17 are released.\(^13\) Th17 cells are a specific subset of CD4+ T cells, which control tissue-inflammation and proinflammatory cytokines.\(^12\)

Viral infections like CMV can be troublesome for liver transplanted patients. Little is known about the role of Th17 cells and IL-17 in CMV-infected patients; therefore, by considering these data, in this study, the possible association of the mRNA expression level of IL-17 gene with CMV pathogenesis was evaluated in liver transplanted patients. The rate of IL-17 expression in uninfected group showed a statistically significant increase in second sampling day (fourth day), which is followed by a decrease in the next sampling time. This expression pattern is a result of ischemic reperfusion occurring early after transplant and by removing of the inflammatory factors, the IL-17 expression reduces in day 7 of sampling.\(^4\) The other group (CMV+) showed a detectable increase in the last sampling day, which was also statistically significant.

The comparison of IL-17 mRNA expression level between both studied groups and in all 3 sampling intervals also showed a no significant increase in uninfected group in the first sampling time, which was followed by 2 statistically significant increases in second and third sampling time, which highlights the previously mentioned role of Th17 cells and its cytokine (IL-17) in the time of CMV infection in liver transplanted patients.

The transplant procedure by its own can have direct effects on expression of cytokines related to graft.\(^3\) After transplant, periods of ischemia and then reperfusion renders the release of various inflammatory mediators,\(^23,28\) which are important in T-cell differentiation and even graft destructive immune cells.\(^25,29\) One study group reported that the factors, which are released by endothelial cells during ischemia-reperfusion injury, can increase production of some cytokines like IL-17 and IFN-$\gamma$ by CD4+ T cells.\(^30\) They also reported that these factors can result in increase of Th17 activity within the graft.

Liver injuries are followed by inflammatory responses, which are composed of infiltration of innate immune cells. Infiltration of monocytes also as a result of liver injury is a critical cellular mechanism which cause inflammation and activation of profibrogenic hepatic satellite cells in humans and

![Figure 3. Comparing the IL-17 mRNA Expression Level Between Infected and Uninfected Patients](image-url)
mice. In mentioned situation, adaptive immune cells like CD8+ and CD4+ T-cells are involved in inflammation of the liver. Activation of CD4+ T-cells is followed by their differentiation into various subsets of cells like Th1, Th2, Tregs, and Th17, depending on the cytokines present in the local environment. Th17 cells and their important cytokine, IL-17, deal with inflammation and even rejection in liver transplanted patients. It is believed that Th17 cells have more potential for changing situations, rather Th1 and Th2 cells. Hanidziar and associates believed that Th17 cells present in the allograft, are more a biomarker of tissue-inflammatory state rather than a single mechanism, which is responsible for rejection.

Research shows that IL-17 is involved in defense against bacterial infections and autoimmune diseases of the host and also its role is mentioned in transplant. IL-17 increase is detected in human lung allograft rejection. Also, in mouse studies, the role of Th17 and IL-17 is mentioned. In bone marrow transplant, patients experiencing acute graft versus host disease Th17 cells increased in peripheral blood. Furthermore, Fabrega and associates showed the increase of IL-17/IL-23 protein level in human with rejected liver transplant. Recently, our previous study, also confirmed this data.

Viral infections are another threat for a newly transplanted liver and among them, CMV mortality and morbidity. We know both of the innate and adaptive immunity are responsible in viral control. In immunocompromised patients like liver transplanted ones, CD8+ T cells that are virus-specific act against CMV-related disease, which their maintenance is done by uninfected specific CD4+ T cells. Around 4% to 5% of all CD4+ and CD8+ T cells are specific for CMV in seropositive humans. It is suggested that the Th17 responses happen during CMV replication, but the details are unclear. One study as the first research on the role of IL-17 HBV-infected patients showed that Th17 population and IL-17 cytokine significantly increase. They believed that significant elevation of Th17 cells percentage express that Th17 cells play a role in HBV infection. At the same time, another study confirmed the findings of Ge and associates and showed that in the damage that is caused by HBV in liver, Th17 cells have prominent role. These findings elucidated that the population of Th17 cells, inside liver, and in the peripheral environment are elevated in HBV patients and these cells can activate dendritic cells and monocytes and make them to produce inflammatory cytokines during HBV infection. Also, in immune responses during Murine CMV (MCMV) infection models, IL-17 producing CD4+ T cells have important roles. Recently, Egli and associates demonstrated that Th17 cells are harmful in making inflammatory autoimmune diseases, and IL-17 can lead to immunopathology responses of CMV-infected hosts. Furthermore, another study claimed that lung lesions that are made by MCMV infection in animal models render to production of IL-17 cytokine that can control immune responses and make lung lesions either. Also they proposed that IL-17 may be responsible for developing MCMV pneumonia after allogenic transplant. They believed that in studying the IL-17 dynamic evolution, the most prominent part is the time that is selected for collecting of samples, in pneumonia induced by MCMV.

Putting this data together, this study on the role of Th17 in CMV infection in liver transplanted patients is barely studied. This project highlights the importance of IL-17 cytokine in immune responses against CMV infection in transplanted livers, which also needs more studies to elucidate this mechanism in details.

References

Travel for Transplantation in Iran: Pros and Cons Regarding Iranian Model

Shahrzad Ossareh,1 Behrooz Broumand2

Abstract

Transplant tourism is one of the main unacceptable aspects of medical tourism, implicating travel to another country to receive an allograft. Organ shortages in wealthier countries have persuaded patients to preclude organ waiting lists and travel to other countries for getting organs especially kidneys. On the other hand, in many countries, there is no transplant program, and hemodialysis is expensive. Hence, patients with end-stage kidney disease may have to travel to get a kidney allograft for the sake of their lives. In Iran, a legal compensated and regulated living unrelated donor kidney transplant program has been adopted since 1988, in which recipients are matched with live-unrelated donors through the Iran Kidney Foundation and the recipients are compensated dually by the government and the recipient. In this model regulations were adopted to prevent transplant tourism: foreigners were not allowed to receive a kidney from Iranian donors or donate a kidney to Iranian patients; however, they could be transplanted from donors of their own nationality, after full medical workup, with the authorization of the Ministry of Health. This was first considered as a humanitarian assistance to patients of the countries with no transplant program and limited and low quality dialysis. However, the policy of “foreign nationality transplant” gradually established a spot where residents of many countries, where living-unrelated donor transplant was illegal, could bring their donors and be transplanted mainly in private hospitals, with high incentives for the transplant teams. By June 2014, six hundred eight foreign nationality kidney transplants were authorized by Ministry of Health for citizens for 17 countries.

In this review, we examine the negative aspects of transplant for foreign citizens in Iran and the reasons that changed “travel for transplant” to “transplant tourism” in our country and finally led us to stop the program after more than 10 years.

Key words: Travel for transplant, Transplant tourism, Iranian model of kidney transplant

Introduction

Transplant tourism is one of the main unacceptable aspects of medical tourism, implicating travel to another country for receiving an allograft. People used to travel from under-developed to developed countries to receive the types of medical treatment that were not available in their home countries.1,2 However in case of organ transplant the route has mostly reversed from wealthier to underdeveloped countries for the sake of economic issues or bypassing the legal barriers.

Organ shortage in wealthier countries has persuaded a number of patients to preclude long-term organ waiting lists in the United States, Canada, Australia, Israel, Japan, Oman, Saudi Arabia, and European countries and travel to other countries for getting organs especially kidneys. On the other hand in some countries such as Tajikistan, Afghanistan, and Uganda, among many others, there is no available transplant program and hemodialysis is expensive and not covered by public health care...
systems. In these countries, patients with end-stage kidney disease may have to travel abroad to get a kidney allograft for the sake of their lives.

In most of the countries of the world buying and selling organs are illegal and allografts can be received from cadavers, live relatives, and sometimes emotionally related donors and paired kidney donation. However, in many countries, there are no strict rules for patients who have been transplanted through transplant tourism and the medical team has to take care of the recipients after returning back to home countries. The Istanbul Declaration distinguishes transplant tourism from travel for transplant. Travel for transplant is the movement of organs, donors, recipients or transplant professionals across borders for the purpose of transplant. Travel for transplant becomes transplant tourism if it involves organ trafficking and/or transplant commercialism or if the resources devoted to providing transplants to foreigners undermine the country’s ability to provide transplant services for its own population.

**Kidney transplant model in Iran**

In Iran, a legal compensated and regulated living-unrelated donor kidney transplant program has been adopted since 1988, in which recipients are matched with live-unrelated donors (LURD) through Iran Kidney Foundation and the recipients are compensated dually by government and the recipient. This model could eliminate the transplant waiting list of the country within a decade, and due to restricted kidney donation/sale by Iranians to Iranians, restriction of transplant operation to the university hospitals and nonprofit nature of the whole procedure for the transplant teams, gained some popularity. However, during the next decades, the model was not successful in this regard, and the waiting list has grown to more than 17,000 by 2011, although the latter figure includes the hemodialysis patients who have not been registered for transplant.

On the other hand, apart from the main issues that should be thoroughly examined in every organ donation program, which are beneficence, nonmaleficence, donor autonomy, altruistic donor motivation, coercion free donation, fully informed consent, and avoidance of medical paternalism, the model is not free of ethical debates, within its defined and accepted legal frameworks. The main debates are the increasing financial relation between the donor and recipient, which could have ideally been eliminated by total governmental compensation instead of a dual recipient-government payment, and the lack of a proper donor follow-up program, with little information about the future of donors.

**Foreign nationality transplant in Iran**

In the Iranian model of kidney transplant, several regulations were adopted to prevent transplant tourism: foreigners were not allowed to receive a kidney from Iranian unrelated or a deceased-donor or donate a kidney to an Iranian patient; however, they could be transplanted from volunteer living related or unrelated donors from their own nationality, after full medical workup, with the authorization of from Ministry of Health. This policy helped keep the model within its legally defined framework for many years. By June 2004, among 241 Afghan refugees with end-stage kidney disease in Iran, 62 had undergone kidney transplant, all receiving kidneys from donors of their own nationality which were living-unrelated donors in 50 cases, living-related donors in 9 cases, and a spouse or deceased-donor each in 1 case. Ghods also reported 7 ESRD patients from Azerbaijan transplanted in Hasheminejad Kidney center from Azari donors (5 LURD and 2 related donors) and considered it as a humanitarian assistance to the patients of a country with no transplant program and limited and low-quality dialysis. He also reported 4 patients from Turkey, 2 patients from India and Japan living in Iran, and 1 who came from Yemen, all transplanted from same nationality donors, except for the Japanese woman who had married an Iranian man and was an Iranian citizen. As the recipients brought their matched donors to Iran, full medical work-up was implied and Iranian donors were not used for foreign citizens, this was accepted by the Ministry of health and more private and governmental hospitals were allowed to practice “travel for transplant” in Iran. By June 2014, six hundred eight foreign nationality kidney transplants were authorized by Iranian Ministry of Health for citizens from 17 countries (Table 1 and Figure 1) (Dr. Katayoun Najafizadeh, personal communication).

However, Ghods later mentioned the brokers who arranged paid donation in Azerbaijan and admitted that “these kidney transplants are in the category of unethical commercial transplant tourism.
Table 1. Frequency of Kidney Transplants on Foreign Nationals in Iran, 2004 to 2014

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*Until June 2014.
**Including 23 cases of transplants from Afghani deceased-donors.

and should be avoided.” He denied to continue LURD transplant for foreigners and considered it as a type of medical tourism and suggested to exert pressure on Azerbaijan health authorities to establish their own kidney transplant program.8

Iran was essentially trying to provide kidney transplant to her citizens through a controlled LURD program, while expanding deceased-kidney transplant, together with helping the foreign refugees and a few neighboring countries, which had no transplant program and limited dialysis facilities.10,11,14 However, the policy of “foreign nationality transplant” gradually established a spot where residents of Oman, India, Saudi Arabia, and many other countries, where LURD transplant was illegal, could bring their LURD donors, and be transplanted mainly in private hospitals, to where foreign nationality transplant operations were gradually transferred, with high incentives for the transplant teams. Reports of cases of Omani and Saudi citizens receiving kidneys from Iranian “paid” donors had been previously published and 3 more such cases were recently discovered by Ministry of Health in 2 private hospitals, who had submitted fake Iranian ID cards to receive kidneys form Iranian donors.8,15 Doctors from India unofficially complained about the transplant of Indian citizens from LURD Indian donors in Iran and mentioned the brokers and Indian organ trade Mafia on personal emails to one of us (Dr. Behrooz Broumand). In the past few months, the concerns of neighboring countries for formation of a transplant tourism spot in Iran intensified and detection of cases of illegal Iranian to foreign citizen kidney transplant together with identification of 12 illegal organ trade bands, led the Iranian Ministry of health to forbid any kidney transplant for foreign citizens and again limit transplant operation to university hospitals with no incentives for the transplant team.15,16

**The negative aspects of foreign nationality transplant**

What were the negative aspects of transplant for foreign citizens in Iran and what changed “travel for transplant” to “transplant tourism”? The declaration of Istanbul says that “Travel for transplant becomes transplant tourism if it involves organ trafficking and / or transplant commercialism or if the resources devoted to providing transplants to foreigners undermine the country’s ability to provide transplant services for its own population.”
In Iran, Iranian kidneys were not officially sold to foreign citizens, although organ sale and hence “organ trafficking” was happening in the home country. However, with higher fees offered by foreign citizens to Iranian donors, compared to the relatively fixed “rewarding gift” that is paid by Iranian recipients and controlled by Iran Kidney Foundation, there was a risk of increasing illegal kidney sale from Iranians to foreigners. Also the transplant teams which used to receive a fixed and limited fee for service incentive from the governmental hospital, were set at the risk of being seduced by the high fees offered by foreigners, especially those from rich countries. On the other hand, although some neighboring countries were actually happy with the transplant of their citizens in Iran (like Azerbaijan, which did not set up a transplant program), others including India seemed to be concerned about the new activity that was taking place over their borders and with the organ trade mafia that was conducting this activity. So it is obvious that organ trafficking, at least form outside of Iran was happening.

Second, the Iranian resources, especially kidneys were not generally provided to foreigners. However, in addition to the increased risk of illegal kidney sale to the foreign citizens due to financial appeal, the transplant teams were spending their time on foreign citizen transplant and this can be assumed as an important country’s resource. With the increasing number of foreign transplants and more financial attractiveness of the transplant fees in private hospital, this would undermine the country’s ability to provide sufficient transplant services to its own population.

Third, the main strong points of Iranian model of LURD transplant have been the full informing of transplant complications to the donors, clear agreements between the donor and the recipients about the “rewarding gift,” and consent of the donor first-degree relatives (father in case of single donors and spouse in case of married ones), together with full payment according to agreements (no cheating), although the mere obligation to donate under economic “coercion,” dual donor-governmental compensation (instead of full governmental) and lack of a regulated donor follow-up registry, are the main concerns with this model. With donors found and negotiated outside our country’s regulations, none of the mentioned conditions could be expected to be met, and although full medical workup was routinely done and the transplant procedure was a “clean” one regarding donor infections and operation conditions, the model was not working for the foreigners within its legal frame works.

Conclusion

We are delighted to inform that this activity, as a form of transplant tourism, was stopped on August 14, 2014, after 608 transplants for foreign citizens had been performed, with a number of cases of illegal Iranian to foreign citizen kidney sales. According to our experience, it seems that it is very hard or impossible to legalize any form of live-unrelated kidney transplant program for foreigners even in the context of a legal live-unrelated kidney transplant program, as in our country. Due to monetary nature of such programs and organ shortage in general, deviations from any defined regulations will finally happen and the unethical issues especially organ trafficking mafias, will establish. We insist on maintaining on banning any kind of organ trafficking and transplant tourism and not to try to establish or legalize such programs in response to worldwide organ shortage.

References

An Analysis of Outcomes of Liver Retransplant in Adults: 12-Year’s Single-Center Experience

Mohamed Rabei Abdelfattah,1,2 Mohammed Al-Sebayel,1 Dieter Broering1

Abstract

Objectives: Liver retransplant is the only therapeutic option for irreversible liver graft failure. Its incidence varies between 5% and 22% worldwide. Liver retransplant - despite some recent improvement - is associated with significantly poorer outcome compared with the primary transplant.

The purpose of this study was to assess the outcome of liver retransplant compared with primary liver transplant, compare the outcome of early and late liver retransplant, and to evaluate the outcome of liver retransplant within different predictive index categories.

Materials and Methods: We retrospectively reviewed adult patients who had liver retransplant from May 2001 to December 2013. Patients were divided into 2 groups: group A (early liver retransplant), retransplant within 30 days; and group B (late liver retransplant), retransplant > 30 days after primary liver transplant.

Results: After 460 primary adult liver transplants, 17 liver retransplants (3.7%) were performed in 16 adults. Mean patient survival after liver retransplant was 29.5 ± 31.9 months. The 1-, 3-, and 5-year patient survival was 68.8%, 51.6%, and 38.7%. Mean graft survival following liver retransplant was 27.9 ± 32.1 months, and 1-, 3-, and 5-year graft survival was 51.5%, 34.3%, and 22.9%. Patient and graft survival was significantly better in group B than group A at 1 year and 3 years. There were 9 liver retransplants (52.9%) with predictive index category IV; 7 retransplants (41.2%) with predictive index category III; and 1 retransplant (5.9%) with predictive index category II. Patient survival was significantly higher for predictive index category III than IV at 1 year and 3 years.

Conclusions: Patient and graft survival are lower after liver retransplant than primary liver transplant. Graft and patient survival are better in late than early liver retransplant and in predictive index category III than IV.

Key words: Complications, End-stage liver disease, Liver transplant, Survival

Introduction

Liver transplant is an established treatment for its approved indications. Currently, 1- and 5-year survival rates after liver transplant are 90% and 70%. However, many patients (10%-19.4%) have graft loss after primary liver transplant.1,2 Liver retransplant is the only therapeutic option for irreversible liver graft failure. The incidence of retransplant varies between 5% and 22% worldwide.3-6

The situation of liver retransplant is complex. It involves financial and ethical issues. It is associated with higher costs and it results in a missed opportunity for primary transplant in another patient. This has importance due to the increasing donor organ shortage.7,8 Moreover, despite some recent improvements, liver retransplant is associated with significantly poorer outcome than primary
transplant; based on data from United Network for Organ Sharing, graft survival at 1, 3, and 5 years after liver retransplant was 82.6%, 74.2%, and 56.0% and after primary liver transplant was 92.4%, 86.1%, and 69.9%.1 This difference in outcome has been confirmed in numerous reports.9-14

It is important to distinguish between liver retransplant performed for early or late indications. Early after primary liver transplant (within the first 30 d), the indications usually are urgent, and this carries special concern regarding evaluation to exclude cases with refractory sepsis, brain herniation, or multiorgan failure. However, early liver retransplant is technically easier, especially within 1 week, than late liver retransplant.3 Late after transplant, the situation is much more complicated because patients usually have comorbidities that contribute to medical complexity such as renal failure, recurrent infection, and use of immunosuppression. Furthermore, liver retransplant is technically demanding due to distorted anatomy and vascular adhesions.3

It has been noted that outcome of liver retransplant is variable. Factors were identified that were associated with poor outcome; the value of some of these factors was ascertained, and some risk models were created.15,16 The purpose of this study was to review data from > 12 years about the outcome of liver retransplant in adults at our center.

Materials and Methods

We retrospectively reviewed our database for cases of liver retransplant for adults from May 2001 to December 2013. Data were collected including demographic data; primary transplant data including type of graft, urgency of transplant, Model for End-Stage Liver Disease (MELD) score before transplant, and donor risk index (DRI) for cases transplanted from a deceased donor; data related to retransplant including interval to retransplant, indication for retransplant, type of graft, urgency of retransplant, MELD score before retransplant, intensive care unit stay, mechanical ventilation, DRI for cases transplanted from a deceased donor, and operative details; and data related to outcome of retransplant including patient survival and graft survival. Liver retransplant procedures were divided into 2 groups: group A (early liver retransplant) included cases transplanted within 30 days after primary liver transplant, and group B (late liver retransplant) included patients who were transplanted > 30 days after primary liver transplant.

Results

Overall and demographic data
From May 2001 until the end of December 2013, there were 460 liver transplants for adult patients performed at our center. Our database revealed 17 liver retransplants for 16 adult patients (retransplant rate, 3.7%) including 10 males (62.5%) and 6 females (37.5%); 15 patients had 1 liver retransplant and 1 patient had 2 liver retransplants.

Primary transplant data
Primary liver transplants were right lobe living-donor liver transplant (LDLT) in 9 patients (56.25%) and whole organ deceased-donor liver transplant (DDLT) in the other 7 patients (43.75%). In their first liver transplant, most patients had elective admissions; 1 patient was admitted to the intensive care unit (6.25%), 2 patients were inpatients (12.5%), and 13 patients were admitted electively to the hospital to undergo primary liver transplant (80.25%). The MELD score for patients before primary liver transplant ranged from 7 to 34 points (mean, 21.2 ± 8.2 points). Indication for liver transplant was end-stage liver disease secondary to hepatitis C virus (HCV) cirrhosis in 7 patients (43.75%), hepatitis B virus (HBV) cirrhosis in 5 patients (31.25%), autoimmune hepatitis in 2 patients (12.5%) and Budd-Chiari syndrome or cryptogenic liver cirrhosis in 1 patient each (6.25%). In addition to these indications, 3 patients had concomitant hepatocellular carcinoma (18.75%). There were 15 patients who had their primary transplant at our institution; the 2 other patients had their primary liver transplant in the United States, and in these 2 patients, deceased-donor data could not be retrieved, and DRI was not calculated. In the other 5 DDLT cases, DRI was < 2 with low risk from the deceased-donor perspective (DRI range, 1.2-1.9 points; mean, 1.46 ± 0.32 points). Therefore, good liver graft quality at the primary liver transplant was present in all retransplanted cases.

Overall data on liver retransplant
There were 15 patients who had 1 liver retransplant and 1 patient who had 2 liver retransplants. There
were several different indications for liver retransplant in adult patients (Figure 1).

The overall mean MELD score for patients before the retransplant was 27.5 ± 8.1 points (range, 11-38.3 points). Retransplant was done using whole organ DDLT in all procedures except in 1 procedure that was performed with a right lobe LDLT (the patient’s daughter was aged < 18 years at primary liver transplant later on; she donated the right lobe of her liver to retransplant her father when she was above 18 years.).

Patient survival after liver retransplant was mean 29.5 ± 31.9 months (range, 9-3063 d). Graft survival following liver retransplant was mean 27.9 ± 32.1 months (range, 0-3063 days). The 1-, 3- and 5-year patient and graft survival rates were determined using Kaplan-Meier method (Figure 2).

Comparative data on early versus late liver retransplant
There were 9 procedures (56.25%) in group A (early liver retransplant) and 8 procedures (43.75%) in group B (late liver retransplant). Indications for liver retransplant differed between the 2 groups (Table 1).

In the 9 patients in group A, 1 patient had 2 liver retransplants. Indications for retransplant in this group included graft loss secondary to early vascular complications in 4 patients (44.4%); small-for-size syndrome (SFSS) in 4 patients; and primary nonfunction (PNF) (11.1%) in 1 patient. In group B; all patients had 1 liver retransplant, and the different indications for retransplant were tabulated (Table 1).

The MELD score in group A ranged from 16 to 36 points (mean, 29.3 ± 6.3 points) and in group B from 11 to 38.3 points (mean, 25.4 ± 9.8 points) (Table 2). In group A, all patients were retransplanted using whole-organ DDLT; in group B, patients were retransplanted using whole-organ DDLT except for 1 patient who was retransplanted using right lobe LDLT. Operative time, operative blood loss, and intraoperative transfusion requirements were significantly less in group A than group B (Table 2).

Patient survival in group A (early liver retransplant) ranged from 9 to 3063 days (median survival, 29.8 ± 38.6 mo) and in group B (late liver retransplant) ranged from 51 to 1945 days (median survival, 29.3 ± 26.5 mo). The 1-, 3-, and 5-year patient survival was determined using Kaplan-Meier method for both groups (Figure 3). Using log-rank test, patient survival was significantly different between both groups (log-rank value = 1.89).

Graft survival in group A (early liver retransplant) ranged from 9 to 3063 days (median survival, 26.5 ± 36 mo) and in group B (late liver retransplant)
ranged from 51 to 1945 days (median survival, 29.3 ± 26.5 mo). The 1-, 3-, and 5-year graft survival was determined using Kaplan-Meier method for both groups (Figure 4). Using log-rank test, graft survival was significantly different between both groups (log-rank value = 7.85).

Liver retransplant
Liver retransplants were retrospectively assessed according to predictive index categories (PIC) proposed by University of California at Los Angeles (UCLA) workers. The categories were PIC IV in 9 liver retransplants (52.9%), PIC III in 7 retransplants (41.2%), and PIC II in 1 retransplant (5.9%). There were no liver retransplants in the PIC I category. Patient survival following liver retransplant differed according to the PIC category, with PIC III (log-rank value = 15.88) associated with significantly better patient and graft survival than PIC IV (log-rank value = 22.54). Kaplan-Meier method was used to compare patient and graft survival between PIC III and PIC IV categories (Figure 5 and 6).

Discussion
The incidence of liver retransplant varies between 5% and 22% worldwide. Our liver retransplant rate was lower than this range (3.7%). This can be attributed to the severe shortage of deceased-donor grafts in Saudi Arabia, especially at the beginning of the liver transplant program in Saudi Arabia. This is compounded by the absence of a national allocation policy, and might have been affected by the learning stage, which may have limited the performance of retransplants.

The SFSS was the most common cause of retransplant in our series (23.5%), followed by early hepatic artery thrombosis (HAT) (17.6%), recurrence of original disease (17.6%), chronic rejection (11.8%), late vascular complications (11.8%), and, rarely, PNF (5.9%) and biliary complications (5.9%). Bramhall and coworkers\(^2\) reported HAT as the most common indication for retransplant (31.6%), followed by chronic rejection (22.4%), recurrent disease (13.2%), and PNF (10.7%). The HAT was not the most common reason for our retransplants. This can be attributed to routine use of operative microscopy for hepatic artery anastomosis at our center and failure to retransplant cases of HAT in a timely fashion because of the lack of a national allocation policy at the start of the liver transplant program in Saudi Arabia. The reason that SFSS was the commonest indication for retransplant at our center was the lack of reconstruction of anterior segment veins in right lobe LDLT, especially at the start of our liver transplant program, which led to the frequent occurrence of anterior segment congestion and SFSS.

Operative time, operative blood loss, and intraoperative transfusion requirements were significantly lower in early than late retransplants. These findings are consistent with many previous reports and may be attributed to distortion of anatomy and presence of dense, vascular adhesions and portal hypertension.
Our data showed better 1- and 3-year patient and graft survival in late than early retransplants. At 5 years, the early group had slightly better patient and graft survival than the late group. This can be explained partially by worsening of survival in the late group due to development of graft cirrhosis secondary to recurrence of HCV.

Recently, workers at UCLA developed the PIC scoring system in which patients were stratified into 4 classes that were highly predictive of patient and graft survival. The authors recommended reserving liver retransplant for patients with PIC I to III but not patients with PIC IV. The present data confirm better survival of patients with PIC III than PIC IV, similar to data from the UCLA researchers. Evaluation of the details showed that patient survival after liver retransplant in our cohort paralleled the UCLA data (50% and 25% at 5 years for PIC III and PIC IV in our data compared with 49% and 22% in the UCLA data). However, our 5-year graft survival after liver retransplant was slightly worse than reported by the UCLA workers for PIC III (UCLA, 43%; present study, 26.7%) and slightly better for PIC IV (UCLA, 19%; present study, 22.2%). This can be attributed to the smaller sample size in the present study.

In summary, liver retransplant is the only therapeutic option for irreversible liver graft failure. However, organ shortage and logistic reasons may prevent patients from timely retransplant. Our patient and graft survival were lower after liver retransplant than primary liver transplant, especially when early liver retransplant was considered. This mandates the application of the risk stratification model to predict optimal retransplant procedures. The PIC categories were relevant retrospectively for predicting outcome after liver retransplant.

References

Abstract

Objectives: The outcome of children who had living-donor liver transplant was analyzed according to their status before transplant, and we analyzed the outcome of critically ill patients.

Materials and Methods: This was a retrospective analysis of children who received primary living-donor liver transplant at Kyoto University Hospital. According to the criteria of the United Network for Organ Sharing, we divided patients into 3 groups: Group A patients had been admitted to the intensive care unit before living-donor liver transplant; Group B patients were hospitalized but did not require intensive care unit stay; and Group C patients were living at home and underwent elective transplant.

Results: A total 685 patients met inclusion criteria. Children in Group A were younger than Group B and received liver grafts from younger donors than Group B and C. Group A patients had marked impairment in liver and renal function and coagulation profile and needed higher volumes of fresh frozen plasma transfusions. Group A patients had significantly worse outcomes and early patient death than the other group; Group A patient survival was 68.3%, 63.2%, 60.1%, and 56.1% at 1, 5, 10, and 15 years after living-donor liver transplant \( (P < .0001) \). Group A had worse graft survival than other groups \( (P < .0001) \), and Group A graft survival was 68.3%, 65.9%, 54.1%, and 49.9% at 1, 5, 10, and 15 years. Low gamma-glutamyl transpeptidase was an independent risk factor for patient death in Group A \( (hazard\;ratio,\;1.004;\;95\%;confidence\;interval,\;1.0-1.007)\; \( (P < .05) \). Group A patients had a higher rate of multidrug-resistant hospital-acquired infections.

Conclusions: Children who were admitted to the intensive care unit prior to living-donor liver transplant had marked impairment of pretransplant laboratory parameters and worse outcome than other groups.

Key words: End-stage liver disease, Infection, Intensive care unit, Pediatric, Risk factors

Introduction

Although better immunosuppressive agents and surgical techniques partially account for increasing survival with living-donor liver transplant (LDLT), advances in pre- and postoperative care also have helped improve survival. Liver transplant has been accepted as the standard therapy for patients with end-stage liver disease. As a result, there has been a substantial increase in the number of patients placed on waiting lists for transplant.\(^1,2\) Infection after pediatric LDLT is a major cause of morbidity and mortality, especially in critically-ill patients, as described in several studies.\(^3,6\)

To our knowledge, the outcome of severely ill children after LDLT has not been adequately
reported. Therefore, the objective of this retrospective cohort study was to compare the outcomes of critically ill children who underwent LDLT with the outcomes of patients who were less ill, as defined by the United Network for Organ Sharing criteria.7 Patients were considered severely ill when they had been admitted to an intensive care unit (ICU) before the liver transplant (termed ICU-bound); patients who had been admitted to a hospital but not an ICU, and patients who had been living at home at the time of transplant, were considered less ill. Patient survival, graft survival, and risk factors for survival and infectious complications were assessed.

**Materials and Methods**

This study included children who received primary LDLT at Kyoto University Hospital between June 1990 and April 2011. Inclusion criteria were primary liver transplant with standard techniques for end-stage liver disease, both chronic and acute fulminant hepatic failure. Patients who had retransplant and auxiliary liver transplants were excluded. According to United Network for Organ Sharing criteria, we divided patients into 3 groups: Group A patients had been admitted to the ICU before LDLT (ICU-bound); Group B patients were hospitalized but did not require ICU stay; and Group C patients were living at home and underwent elective transplant. All clinical and laboratory data were collected from the patient charts, and patient and graft survival were assessed.

**Surgical procedure**

Techniques for donor and recipient operations have been described previously.8,9 The left lateral segment was the primary choice. However, when the estimated graft recipient weight ratio (GRWR) was > 4%, a monosegment graft was used.10,11 For larger recipients, graft selection was extended to the left lobe and right lobe according to GRWR and the residual liver volume in the donor after heptectomy.

**Immunosuppression**

Immunosuppression consisted of combination therapy with tacrolimus and steroids.13 The tacrolimus started orally from the day before the operation, then continued postoperatively. Target tacrolimus trough serum levels initially were > 10 ng/mL, decreasing gradually to 6 to 8 ng/mL a few months after LDLT. Methylprednisolone therapy was used for induction and switched to oral prednisolone therapy 1 week after LDLT. Steroid therapy was routinely tapered by 3 to 6 months after transplant when graft function was maintained. In cases of ABO-incompatible LDLT, additional immunosuppressants and preconditioning regimens were given to inhibit humoral rejection; treatment included prostaglandin E1, cyclophosphamide, azathioprine, mycophenolate mofetil, and plasma exchange.14

**Clinical data**

All clinical and laboratory data were retrieved from the patient charts. The values used for analysis were from the last records before LDLT.

**Risk factors for patient survival**

We evaluated the risk potential of several preoperative, operative, and postoperative variables. The preoperative variables included age at LDLT, sex, preoperative clinical status, presence of preoperative infection, ascites, laboratory variables (white blood cell count, C-reactive protein, electrolytes [sodium, potassium, calcium, phosphate, magnesium], liver function tests [aspartate aminotransferase, alanine aminotransferase, total bilirubin, gamma-glutamyl transpeptidase (GGTP), and albumin], coagulation factors [international normalized ratio, antithrombin III level, platelet count, and prothrombin time], renal function tests [blood urea nitrogen and creatinine levels]), preoperative hospital stay, ABO-mismatching, and pediatric end-stage liver disease score. Operative variables included operative time, cold ischemia time, warm ischemia time, blood or blood product transfusion (packed red blood cells), fresh frozen plasma, platelets, fluids, 5% albumin), blood loss, and GRWR. Postoperative variables included surgical complications (intraabdominal hemorrhage, bile leakage, or intestinal perforation), repeat surgery, (duration of insertion of intravascular catheter, intraabdominal drainage, or bile drainage), or graft dysfunction. Graft dysfunction was defined as persistent abnormal liver function with serum aminotransferase levels at 2 to 3 times normal, with or without elevated bilirubin level, and abnormal biopsy findings. Data about concomitant rejection or administration of steroid pulse at the time of infection also were evaluated.
Antimicrobial prophylaxis
The patients received flomoxef, an oxacephem antibiotic, 1 hour before the operation, and this was continued for 72 hours after surgery. Trimethoprim and sulfamethoxazole were administered once daily as prophylaxis against *Pneumocystis*. Miconazole was administered for 7 days after transplant as antifungal prophylaxis. Antiviral prophylaxis, including ganciclovir, was not administered except in seronegative recipients who received an allograft from *cytomegalovirus*-seropositive donors.

The protocol for the evaluation of infections during the posttransplant hospital stay included testing for endotoxin (gram-negative bacteria), D-glucan (*Pneumocystis carinii* and fungi except *Cryptococcus neoformans*), *cytomegalovirus* DNA (polymerase chain reaction), and Epstein-Barr virus DNA (semiquantitative polymerase chain reaction) in peripheral blood once weekly and additionally when there was a suspicion of infection, and culture of blood, any wound discharge, urine, stool, pharyngeal swabs, sputum, and bile ≥ twice weekly.

Definition of infections
Bacterial and fungal infections were defined using the criteria proposed by the Centers for Disease Control and Prevention.15,16 Primary bacteremia was defined as bacteremia with no physical, radiographic, or pathologic evidence of a definite infection source. Catheter-related blood stream infection was defined when the same organism was cultured from the catheter tip and blood culture.17 Secondary bacteremia was defined when blood cultures and cultures of samples collected from the suggested infection site showed the same organism. Surgical site infection included cholangitis, peritonitis, intraabdominal abscess, and wound infection.18

Confirmed fungal infections were diagnosed according to positive culture and body temperature > 38°C. Probable or possible fungal infections were diagnosed when there was a positive finding for D-glucan antigen or *Aspergillus* antigen, or when the patient was febrile despite the administration of broad-spectrum antibiotics.19

Viral infections were diagnosed by clinical findings and the detection of viral DNA or RNA fragments by quantitative real-time polymerase chain reaction.20 In addition, systemic lymphadenopathy was assessed, and biopsy of the lymph nodes at the body surface, including the axillary and inguinal lymph nodes, was performed to exclude posttransplant lymphoproliferative disorder.

Postoperative treatment of infection
Bacterial infection initially was treated empirically with vancomycin (40-50 mg/kg/d) (recommended trough level < 10 mg/L), and antibiotics were adjusted or changed according to the identified organism. In patients who had sepsis, the tacrolimus dose was decreased or completely withdrawn, and liver biopsy was performed to exclude the possibility of rejection.

Fungal infection was treated with antifungal agents such as miconazole or fluconazole. Amphotericin B was added when there was no improvement. Wide spectrum antibiotics were stopped. The immunosuppression drugs were decreased and liver biopsy was performed. In addition, micafungin sodium (50-150 mg/d) was administered.

Treatment of viral infection varied. *Cytomegalovirus* disease was treated with ganciclovir (10 mg/kg/d intravenous for 2 weeks, and tapered to 5 mg/kg/d) until clinical resolution occurred, and tacrolimus dosage was minimized. Epstein-Barr virus disease was treated with acyclovir (60 mg/kg/d) or vidarabine (10 mg/kg/d) and reduction in the dosage or complete cessation of tacrolimus. When Epstein-Barr virus DNA accounted for > 10^5 copies/μg DNA, tacrolimus was temporary stopped until Epstein-Barr virus DNA level decreased to < 10^4 copies/μg DNA.

Statistical analyses
Kruskal-Wallis test was used to compare continuous variables between the 3 groups, followed by pairwise comparisons when a significant difference was identified. Chi-square test was used to evaluate associations between the 3 groups for categoric variables. Overall patient survival was described by Kaplan-Meier method and compared using log-rank test. The outcome was defined as graft failure or patient death after LDLT, and analysis for risk factors was done using Cox proportional hazards regression model. Hazard ratios and 95% confidence intervals were assessed. Data analysis was performed with statistical software (SPSS for Windows, Version 16.0, SPSS Inc., Chicago, IL, USA). Values of *P* ≤ .05 were considered significant.
Results

Patient population
In 1354 subjects who underwent LDLT in the study sample (from June 1990 to April 2011), a total 685 patients met the inclusion criteria (mean follow-up, 7.52 ± 5.13 y). The mean age for all patients was 4.06 ± 4.61 years (median, 1.66 y; range, 0.12-17.87 y), and 414 recipients were females (60.4%). Group A included 74 patients who had been admitted to the ICU before LDLT; Group B had 354 children who were living at home and underwent an elective transplant. There were significant differences between patients of the 3 groups (Table 1). In Group A, children were younger than Group B and received liver grafts from younger donors than Group B and C. Biliary atresia was the main indication for LDLT in all groups, but the proportion of biliary atresia was lower in Group A (31%) than Group B or C (Table 1). Fulminant hepatic failure constituted 32% Group A patients. Group A patients had marked impairment in liver and renal function and coagulation status as indicated by the high bilirubin, creatinine, blood urea nitrogen, prothrombin time, and international normalized ratio. However, there were comparable values for albumin and liver enzyme levels between the groups (Table 1). Intraoperative variables and postoperative complications were studied, and we observed that Group A had significantly higher mean transfusion volume of fresh frozen plasma than Group B or C (Table 2).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group A (Intensive Care Unit-Bound) (n = 74)</th>
<th>Group B (Hospitalized) (n = 354)</th>
<th>Group C (At Home) (n = 257)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>4.5 ± 5.3</td>
<td>3.6 ± 4.5</td>
<td>5.4 ± 5</td>
</tr>
<tr>
<td>Female sex, No. (%)</td>
<td>38 (51)</td>
<td>223 (63)</td>
<td>153 (59)</td>
</tr>
<tr>
<td>Donor age (y)</td>
<td>32.27 ± 7.76</td>
<td>33.29 ± 7.40</td>
<td>35.25 ± 6.59</td>
</tr>
<tr>
<td>Recipient weight (kg)</td>
<td>18.3 ± 16.7</td>
<td>14 ± 12.6</td>
<td>18.3 ± 13.8</td>
</tr>
<tr>
<td>Recipient height (cm)</td>
<td>91.6 ± 35.9</td>
<td>85.5 ± 31.4</td>
<td>97.7 ± 30.9</td>
</tr>
<tr>
<td>GRWR</td>
<td>2.6 ± 1.5</td>
<td>2.7 ± 1.3</td>
<td>2.1 ± 1.1</td>
</tr>
<tr>
<td>Liver disease, No. (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Biliary atresia</td>
<td>23 (31)</td>
<td>260 (73.4)</td>
<td>190 (73.9)</td>
</tr>
<tr>
<td>Alagille syndrome</td>
<td>2 (2.7)</td>
<td>8 (2.3)</td>
<td>12 (4.7)</td>
</tr>
<tr>
<td>Byler disease</td>
<td>-</td>
<td>2 (0.6)</td>
<td>10 (3.9)</td>
</tr>
<tr>
<td>Metabolic liver disease</td>
<td>8 (10.8)</td>
<td>13 (3.7)</td>
<td>12 (4.7)</td>
</tr>
<tr>
<td>Cirrhosis/hepatitis</td>
<td>5 (6.8)</td>
<td>9 (2.5)</td>
<td>6 (2.4)</td>
</tr>
<tr>
<td>Tumors</td>
<td>13 (1.7)</td>
<td>10 (3.9)</td>
<td></td>
</tr>
<tr>
<td>Others</td>
<td>12 (16.2)</td>
<td>45 (12.7)</td>
<td>8 (3.2)</td>
</tr>
<tr>
<td>Fulminant hepatic failure</td>
<td>24 (32)</td>
<td>13 (3.7)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Graft type</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Monosegment, left lateral segment, left lobe, right lobe</td>
<td>1, 29, 9, 2</td>
<td>28, 240, 39, 9</td>
<td>5, 171, 62, 10</td>
</tr>
<tr>
<td>ABO-incompatibility Id, compatible, incompatible</td>
<td>50, 13, 11</td>
<td>222, 72, 54</td>
<td>169, 59, 28</td>
</tr>
<tr>
<td>Platelet count (× 10^12/L)</td>
<td>93.02 ± 65.15</td>
<td>157.70 ± 99.84</td>
<td>178.41 ±120.38</td>
</tr>
<tr>
<td>Prothrombin time(s)</td>
<td>17.38 ± 6.18</td>
<td>15.11 ± 3.84</td>
<td>13.28 ± 2.32</td>
</tr>
<tr>
<td>International normalized ratio</td>
<td>2.64 ± 1.57</td>
<td>2.13 ± 5.54</td>
<td>1.36 ± 0.50</td>
</tr>
<tr>
<td>Aspartate aminotransferase (IU/L)</td>
<td>173.2 ± 205</td>
<td>171.4 ± 163</td>
<td>170.0 ± 113.1</td>
</tr>
<tr>
<td>Alanine aminotransferase (IU/L)</td>
<td>109.8 ± 159.5</td>
<td>102.2 ±104.4</td>
<td>112.6 ± 83.7</td>
</tr>
<tr>
<td>Albumin (g/dL)</td>
<td>3.50 ± 0.76</td>
<td>3.42± 0.59</td>
<td>3.52 ± 0.62</td>
</tr>
<tr>
<td>Total bilirubin (mg/dL)</td>
<td>24.35 ± 17.94</td>
<td>13.09 ± 11.77</td>
<td>7.62 ± 7.3</td>
</tr>
<tr>
<td>Creatinine (mg/dL)</td>
<td>0.33 ± 0.21</td>
<td>0.21 ± 0.12</td>
<td>0.24 ± 0.17</td>
</tr>
<tr>
<td>Blood urea nitrogen (mg/dL)</td>
<td>17.78 ± 15.75</td>
<td>8.79 ± 5.81</td>
<td>8.86 ± 3.53</td>
</tr>
<tr>
<td>White blood cell count (× 10^12/L)</td>
<td>6.5 ± 4.4</td>
<td>7.2 ± 4.9</td>
<td>7.7 ± 7.4</td>
</tr>
<tr>
<td>C-reactive protein (mg/L)</td>
<td>1.5 ± 1.6</td>
<td>1.7 ± 3.5</td>
<td>1.7 ± 2.1</td>
</tr>
<tr>
<td>Lactate dehydrogenase (IU/L)</td>
<td>363.65 ± 158</td>
<td>337.6 ± 146.8</td>
<td>352.8 ± 220.1</td>
</tr>
<tr>
<td>GGTP (IU/L)</td>
<td>149.6 ± 148.8</td>
<td>195.4 ± 266.7</td>
<td>233.8 ± 361.1</td>
</tr>
<tr>
<td>Cholesterolase (U/mL)</td>
<td>120.2 ± 100.2</td>
<td>122 ± 100.7</td>
<td>113.7 ± 89.9</td>
</tr>
</tbody>
</table>

Abbreviations: GGTP, gamma-glutamyl transferase or gamma-glutamyl transpeptidase; GRWR, graft recipient body weight ratio

*Continuous variables are presented as mean ± SD.

Patient and graft survival
Patients who received ICU care before LDLT (Group A) had significantly worse outcome than the other groups. Group A had patient survival 68.3%, 63.2%, 60.1%, and 56.1% at 1, 5, 10, and 15 years after LDLT (P = .001) (Figure 1). Group B and C had better patient survival than Group A, and patient survival was similar for Group B and C (P = .799). In Group B, patient survival was 87.6%, 85.9%, 82.7%, and 81.4% at 1, 5, 10, and 15 years; in Group C, patient survival was 89.4%, 87.1%, 82.4%, and 76.1% at 1, 5, 10, and 15 years (Figure 1). A remarkable feature of critically ill patients (Group A) was the poor outcome and the
pattern of patient loss, because early patient loss accounted for decreased survival to 80% in the first month after LDLT.

Group A had markedly worse graft survival than other groups ($P = .001$) (Figure 2). Group A had graft survival 68.3%, 65.9%, 54.1%, and 49.9% at 1, 5, 10, and 15 years. In contrast, Group B had graft survival 89%, 85.9%, 80.1%, and 72.6% at 1, 5, 10, and 15 years, and Group C had graft survival 89.1%, 86.8%, 81.2%, and 73.8% at 1, 5, 10, and 15 years (Figure 2).

### Risk factors for patient survival after living-donor liver transplant

A univariate proportional hazards regression model was used to examine potential risk factors for association with patient survival. Univariate analysis revealed 4 potential significant risk factors for poor patient survival, including female sex, pretransplant platelet count, pretransplant lactate dehydrogenase level, and pretransplant GGTP level ($P < .05$) (Table 3).

The 4 potential risk factors derived from the univariate analysis were further assessed by multivariate analysis. The multivariate analysis revealed that low GGTP level was the only variable that had independent prognostic significance (Table 3).

### Analysis of infection after pediatric living-donor liver transplant

The incidence and rate of hospital-acquired infection was 51% (2.5) in Group A, 61% (1.3) in Group B, and 54% (1.2) in Group C. Bacterial infection contributed to 64% infectious episodes in Group A, 63% in Group B, and 52% in Group C. Fungal infection was detected in 7.4% infectious episodes in Group A, 4% in Group B, and 6% in Group C. Viral infection was detected by polymerase chain reaction in 28% patients in Group A, 33% in Group B, and 42% in Group C (Tables 4, 5, and 6).

With respect to timing of infection, bacterial and fungal infections occurred at 13 ± 9 days in Group A, 12 ± 12 days in Group B, and 14 ± 12 days in Group C. Viral infections occurred at 18 ± 16 days in Group A, 22 ± 19 days in Group B, and 22 ± 13 days in Group C. The most common isolate was methicillin-resistant *Staphylococcus aureus* (MRSA) in Group A, *Pseudomonas aeruginosa* in Group B, and *Enterococcus* in Group C (Table 4).

### Discussion

This study is an important update about our experience with pediatric patients who underwent LDLT. It showed that GGTP had independent prognostic significance for recipient survival. Female sex, pretransplant platelet count, and lactate dehydrogenase were associated with poor patient survival. The ICU-bound children before transplant...
had the worst outcome after LDLT; they had higher infection rates and were complicated by fungal and multidrug resistant bacterial infections.

In this study, we examined the outcome of severely ill children who had been admitted to an ICU before liver transplant. Children who had been admitted to a hospital but not an ICU, and children who had been living at home before transplant, were considered less ill.

The ICU-bound children were younger than hospitalized children who were and received liver grafts from younger donors than the other 2 groups. The most common indication for LDLT in most children was biliary atresia, but this was less frequent in ICU-bound children. Critically ill children had marked impairment in liver and renal function and coagulation profile; they received higher volumes of fresh frozen plasma transfusion and had a higher incidence of biliary leakage after LDLT. Patients who received ICU care before LDLT also had a significantly worse outcome than other groups.

The remarkable feature of critically ill patients was the poor outcome and also the pattern of patient loss, because early patient loss accounted for a decrease in survival to 80% in the first month after LDLT. In another study that examined patient and graft survival after LDLT in adult recipients who received ICU care before transplant, there was no
difference in survival between the patient groups; in that study, factors that may have affected outcome such as age, sex, race, cause of disease, and cold ischemia time were similar between the patient groups. We observed that low pretransplant platelet count was associated with poor outcome in our children, and another study reported that low posttransplant platelet count may predict early posttransplant survival.

The enzyme GGTP is a microsomal enzyme that is distributed widely in human tissues that are involved in secretory and absorptive processes, particularly the bile canaliculi. This enzyme also may help screen for biliary complications in patients who have orthotopic liver transplant. In this study, we observed that GGTP had independent prognostic significance for recipient survival. In another study after orthotopic liver transplant, an early increase in GGTP correlated with better outcomes. An experimental study found that GGTP is an early and sensitive marker in ethanol-induced liver injury in rats.

We observed a high pretransplant bilirubin level in our cohort. Majority of our children who had biliary atresia were scheduled for LDLT due to failure of Kasai operation. In consistent with previous 2 studies of children who had the Kasai operation for correction of biliary atresia, and those children in the pretransplant scenario exhibited malnutrition and hyperbilirubinemia.

We observed that female sex was associated with greater morbidity, similar to a previous study from Kyoto that reported that female sex and high GRWR were independent risk factors for hepatic artery thrombosis after LDLT. We observed a high pretransplant bilirubin level in our cohort. Majority of our children who had biliary atresia were scheduled for LDLT due to failure of Kasai operation. In consistent with previous 2 studies of children who had the Kasai operation for correction of biliary atresia, and those children in the pretransplant scenario exhibited malnutrition and hyperbilirubinemia.

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**Table 4C. Site of Infection and Frequency of Isolated Organisms in At Home Patients**

<table>
<thead>
<tr>
<th>Infection Site</th>
<th>Organism</th>
<th>Group C (At Home) (n = 257) Number of Isolates/Number of Total Isolates</th>
</tr>
</thead>
</table>
| Respiratory tract infection | Gram-positive cocci: MRSA 4/20 MRCNS 1/1 Gram-negative bacilli: 3/6 Fungi: Candida albicans 1/5
| Bloodstream infection | Gram-positive cocci: Enterococcus species 2/5 Enterococcus faecalis 2/4 Enterococcus faecium 1/2 MRSA 3/8 MRCNS 2/4 Other gram-positive cocci 1/5 Gram-negative bacilli: Pseudomonas aeruginosa 3/8 Escherichia coli 2/3 Other gram-negative bacilli: 1/5 Fungi: Candida albicans 2/6
| Gastroenteritis | Gram-negative bacilli: Salmonella enteritidis 1/2

**Table 5. Types of Bloodstream Infection**

<table>
<thead>
<tr>
<th>Type of Bloodstream Infection</th>
<th>Group A (Intensive Care Unit-Bound) (n = 74)</th>
<th>Group B (Hospitalized) (n = 354)</th>
<th>Group C (At Home) (n = 257)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary bloodstream infection</td>
<td>3</td>
<td>9</td>
<td>5</td>
</tr>
<tr>
<td>Secondary bloodstream infection due to</td>
<td>7</td>
<td>17</td>
<td>8</td>
</tr>
<tr>
<td>Surgical site infection</td>
<td>5</td>
<td>11</td>
<td>7</td>
</tr>
<tr>
<td>Urinary tract infection</td>
<td>1</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Respiratory tract infection</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Catheter</td>
<td>0</td>
<td>3</td>
<td>1</td>
</tr>
</tbody>
</table>

**Table 6. Frequency and Rate of Diagnosed Infections**

<table>
<thead>
<tr>
<th>Organism</th>
<th>Group A (Intensive Care Unit-Bound) (n = 74)</th>
<th>Group B (Hospitalized) (n = 354)</th>
<th>Group C (At Home) (n = 257)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacterial episodes (MDRO, %)</td>
<td>61 (33%)*</td>
<td>162 (23%)</td>
<td>82 (18%)</td>
</tr>
<tr>
<td>Fungal episodes</td>
<td>7</td>
<td>10</td>
<td>9</td>
</tr>
<tr>
<td>Viral episodes</td>
<td>27</td>
<td>86</td>
<td>65</td>
</tr>
<tr>
<td>Epstein-Barr virus</td>
<td>13</td>
<td>47</td>
<td>24</td>
</tr>
<tr>
<td>Cytomegalovirus</td>
<td>14</td>
<td>39</td>
<td>41</td>
</tr>
</tbody>
</table>

**Incidence of infection** (infection rate): 51% (2.5) 61% (1.3) 54% (1.2)

**Abbreviation:** MDRO, multidrug resistant organisms

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(MRWCS, methicillin-resistant coagulase-negative staphylococci; MRSA, methicillin-resistant *Staphylococcus aureus*.
weeks after liver transplant. The lower incidence of bacterial infection in our center than previously reported may be attributed to the lower trough level used in our patients and lower associated immunosuppression. Although the predominant infections in our study were in the abdomen (43%) and bloodstream (26%), similar to other studies, the major isolated pathogens were MRSA, a condition that necessitates special attention with respect to careful drug monitoring to avoid further emergence of drug-resistant organisms.

In conclusion, critically ill children who were admitted to ICU prior to LDLT had worse outcome than other children did. They had marked impairment in liver and renal function and coagulation profile, received more fresh frozen plasma transfusions, and had greater incidence of biliary leakage after surgery. The pretransplant GGTP level had independent prognostic significance for recipient survival. Female sex, pretransplant platelet count, and pretransplant lactate dehydrogenase were associated with poor patient survival. The ICU-bound children had higher infection rates and were complicated with fungal and multidrug resistant bacterial infections.

References


Large-for-Size Liver Transplant: A Single-Center Experience

Aydincan Akdur,1 Mahir Kirnap,1 Figen Ozçay,2 Atilla Sezgin,3 Hatice Ebru Ayvazoglu Soy,1 Feza Karakayali Yarbug,1 Sedat Yildirim,1 Gokhan Moray,1 Gulnaz Arslan,4 Mehmet Haberal1

Abstract

Objectives: The ideal ratio between liver transplant graft mass and recipient body weight is unknown, but the graft probably must weigh 0.8% to 2.0% recipient weight. When this ratio > 4%, there may be problems due to large-for-size transplant, especially in recipients < 10 kg. This condition is caused by discrepancy between the small abdominal cavity and large graft and is characterized by decreased blood supply to the liver graft and graft dysfunction. We evaluated our experience with large-for-size grafts.

Materials and Methods: We retrospectively evaluated 377 orthotopic liver transplants that were performed from 2001-2014 in our center. We included 188 pediatric transplants in our study.

Results: There were 58 patients < 10 kg who had living-donor living transplant with graft-to-body-weight ratio > 4%. In 2 patients, the abdomen was closed with a Bogota bag. In 5 patients, reoperation was performed due to vascular problems and abdominal hypertension, and the abdomen was closed with a Bogota bag. All Bogota bags were closed in 2 weeks. After closing the fascia, 10 patients had vascular problems that were diagnosed in the operating room by Doppler ultrasonography, and only the skin was closed without fascia closure. No graft loss occurred due to large-for-size transplant. There were 8 patients who died early after transplant (sepsis, 6 patients; brain death, 2 patients). There was no major donor morbidity or donor mortality.

Conclusions: Large-for-size graft may cause abdominal compartment syndrome due to the small size of the recipient abdominal cavity, size discrepancies in vascular caliber, insufficient portal circulation, and disturbance of tissue oxygenation. Abdominal closure with a Bogota bag in these patients is safe and effective to avoid abdominal compartment syndrome. Early diagnosis by ultrasonography in the operating room after fascia closure and repeated ultrasonography at the clinic may help avoid graft loss.

Key words: End-stage liver disease, Infant, Pediatric, Treatment

Introduction

Liver transplant is the best treatment for end-stage liver disease in children. Advances in surgical techniques, intensive care, and immunosuppressive therapy allow this complex procedure to be performed in young and small infants. The ratio between the liver graft mass and recipient body weight (GBWR) is very important for living-donor liver transplant. The ideal GBWR is unknown, but it is believed that the graft must weigh 0.8% to 2.0% recipient body weight.1 When this ratio > 4%, the graft is termed a large-for-size graft (LFSG), and problems caused by an LFSG are termed large-for-size syndrome (LFSSS). This condition is observed especially in recipients weighing < 10 kg.2 In this study, we evaluated our experience with LFSG.

Materials and Methods

We retrospectively evaluated 377 orthotopic liver transplants that were performed from 2001-2014 in...
our center. We included 188 pediatric liver transplants in our study. Data for recipients with body weight < 10 kg were reviewed retrospectively. Data included age, sex, etiology of liver disease, Child-Pugh score, pediatric end-stage liver disease (PELD) score, survival, and postoperative treatment of LFSS.

In all patients, intraoperative Doppler ultrasonography was performed after closure of the abdomen, and Doppler ultrasonography was performed twice daily at the clinic during the first 7 days after surgery. After transplant, all patients were treated with the same immunosuppression protocol including tacrolimus, mycophenolate mofetil, and prednisolone. No protocol liver biopsy specimens were obtained, and biopsies were performed only for investigation of biochemical abnormalities such as elevated serum transaminase or bilirubin levels.

Results

There were 58 patients with body weight < 10 kg (mean weight, 7.8 ± 1.44 kg; range, 4-10 kg) and GBWR > 4%. All patients had living-donor living transplant. There were 35 male and 23 female patients. The most common indication for liver transplant was biliary atresia in 30 patients (51.7%). The other indications for liver transplant were progressive familial intrahepatic cholestasis type 2 (9 patients) and type 1 (2 patients), neonatal hepatitis (3 patients), Alagille syndrome (3 patients), Crigler-Najjar syndrome type 1 (4 patients), cryptogenic cirrhosis (1 patient), hepatoblastoma (2 patients), hepatic metastasis of cystic neuroblastoma (1 patient), cytomegalovirus hepatitis (1 patient), fulminant hepatitis A (1 patient), and tyrosinemia type 1 (1 patient). The mean Child-Pugh score was 8.9 ± 1.76 and PELD score was 24.1 ± 9.2.

The LFSS occurred in 18 patients. After closing the fascia in 10 patients, we diagnosed vascular problems (kinking) in the operating room by Doppler ultrasonography, and only the skin was closed without fascia closure. After the skin was closed, Doppler ultrasonography was repeated and the patients were taken to the intensive care unit. There were 5 patients who were reoperated for hernia at 1 year after transplant. The families of other 5 patients did not want a hernia operation. In 2 patients, the abdomen was closed with a Bogota bag at the first operation. There were 5 patients who were reoperated due to vascular problems and abdominal hypertension, and the abdomen in these patients was closed with a Bogota bag. All abdomens were closed in 2 weeks. In 1 patient, we reduced the graft volume using a stapler after the vascular anastomosis was completed.

No vascular thrombosis or graft loss occurred due to LFSS. Early after surgery, only 1 patient had a major abdominal infection and died from sepsis. Graft survival was 95% at 5 years and 89% at 10 years. There was no major donor morbidity or donor mortality.

Figure 1. Abdominal Closure With Bogota Bag Technique

Figure 2. Graft Reduction

(A) Marking the surface. (B) Reduction with stapler. (C) Bleeding control.
Discussion

In LDLT or split liver transplant, GBWR < 0.8% is associated with a higher incidence of postoperative liver dysfunction.\(^3\-^5\) In pediatric liver transplant, the use of an LFSSG (GBWR > 4%) may cause graft damage such as vascular complications and necrosis due to an insufficient blood supply to the graft.\(^6\,^7\)

The main problem with an LFSSG may include the risk of abdominal compartment syndrome due to the small size of the recipient abdominal cavity, size discrepancies in vascular caliber, insufficient portal circulation, and disturbance of tissue oxygenation.\(^8\)

Some liver transplant centers reduce the graft into a monosegmental or hyperreduced graft. The graft reduction can be performed in situ, during the donor operation, or as a back table procedure with use of segment II or III as a graft.\(^9\)-\(^13\) In our series, we performed reduction after the vascular anastomosis was finished in 1 patient who weighed only 5 kg. An advantage of this technique is that when the liver is placed in the abdominal cavity, reduction is not performed when it is not required. Another advantage is that bleeding areas can be seen after the anastomosis is completed and easily controlled. In addition, this avoids a prolonged donor operation time that may occur when graft volume reduction is performed during the donor operation.

Reduction of the graft has several disadvantages for both donor, in case of in situ reduction, and recipient. In situ reduction in the donor requires a longer operating time and has an increased risk of biliary leakage and bleeding. For the infant recipient, there are increased risks of biliary leakage from the parenchymal surface, impaired venous drainage, and longer cold ischemia time with graft reduction outside the body.\(^13\) To avoid this complication risk, we chose the Bogota bag technique or skin closure without fascia closure in our patients. The major complication of the skin closure technique was the development of hernia, which required a second operation but did not affect graft survival. In our series, we repaired the hernias at 1 year after liver transplant. We did not use synthetic grafts, and all hernias were repaired with anatomic herniorrhaphy techniques.

Some studies have reported severe vascular problems, early graft loss, and graft necrosis because of direct pressure on the liver parenchyma using large grafts.\(^14\,^15\) In our study, we did not have any graft loss, graft necrosis, or vascular thrombosis due to LFSS. However, vascular kinking was observed in 15 patients, and this was treated with Bogota bag technique or skin closure without fascia closure.

In conclusion, skin closure alone or abdominal closure with a Bogota bag in these patients is safe and effective to avoid abdominal compartment syndrome. Early diagnosis by intraoperative ultrasonography after fascia closure and repeated postoperative ultrasonography in the clinic may help avoid graft loss.

References

Study of the Risk Factors and Complications of Diabetes Mellitus After Live Kidney Donation

Mohammed M. Abuelmagd,1 Ayman M. Nagib,1 Megahed M. Abuelmagd,2 Ayman F. Refaie,1 Yasser A. Elhindi,3 Mohammed F. Ahmed,3 Mohammed H. Ali,3 Hanzada M. Elmaghribi,1 Mohammed A. Bakr

Abstract

Objectives: Kidney donors, similar to the general population, are at risk for development of type 2 diabetes mellitus. The course of donors who develop type 2 diabetes mellitus has not been well studied. This work is aimed at estimating the incidence of diabetes after kidney donation, and study some risk factors and some complications of diabetes mellitus after donation.

Materials and Methods: The material of this record based work comprised the records 2267 donors who donated 1 of their kidneys between 1976 and 2014 in the Urology and Nephrology Center, Mansoura University, Egypt, and regularly followed up at its outpatient clinic. There were 388 donors included in the study and their medical records were revised.

Results: Postdonation weight gain and family history of diabetes mellitus were statistically significant on both the development of diabetes mellitus, high, very high albuminuria, and/or decreased creatinine clearance. Metformin and insulin use seemed to significantly reduce the protein excretion, and creatinine clearance decline in the studied group.

Conclusions: There is a significant effect of the family history of diabetes mellitus on the development of high, very high albuminuria, and/or decreased creatinine clearance.

Key words: Family history of diabetes mellitus, Postdonation albuminuria, Donor exclusion criteria, Postdonation diabetes mellitus, Postdonation obesity

Introduction

Living kidney donation has become an essential part of transplant practice. Historically, this has been attributed to the shortage of deceased donor kidneys and the growing waiting list of potential recipients. However, kidney transplant from a living donor has become the treatment of choice for many patients and their families, offering optimum patient and graft survival.1

Studies over the last 60 years have provided considerable evidence regarding the ability of the kidneys to make and release glucose under various physiologic conditions. Yet traditionally, the kidneys have not been considered an important source of glucose (except during acidosis or after prolonged fasting), with most clinical discussions on glucose dysregulation centering on the intestine, pancreas, liver, adipose tissue, and muscle.2 However, the significance of the kidneys’ contribution to glucose homeostasis, under both physiologic and pathologic conditions, has become well-recognized, and is thought to involve functions beyond glucose uptake and release. Besides the liver, the kidney is the only organ capable of generating sufficient glucose (gluconeogenesis) to release into the circulation, and it is also responsible for filtration and subsequent reabsorption or excretion of glucose.3 These findings have provided considerable insight into the myriad of pathophysiologic mechanisms involved in the development of hyperglycemia and type 2 diabetes mellitus (T2DM).4
Diabetes mellitus is an absolute contraindication to living donation. Prospective donors with an increased risk of T2DM because of family history, ethnicity, or obesity should undergo a glucose tolerance test and the only considered as donors if this is normal. Yet it is unknown whether developing T2DM after donating a kidney leads to a higher risk of experiencing acceleration in glomerular filtration rate (GFR) decay when compared to nondiabetic donors or diabetics with 2 kidneys. Some transplant centers decline kidney donors with a strong family histories of T2DM because of theoretical concerns regarding the possible additive effect of hyperfiltration that is instigated by diabetes and reduction in renal mass.

**Materials and Methods**

**Subjects**
Subjects of this record-based work comprised the records 2267 donors who donated their kidneys between 1976 and 2014 at the Urology and Nephrology Center, Mansoura University, and regularly followed-up at its outpatient clinic. In this study, we tried to look up donors’ paper and/or electronic files. A total of 344 donors were declared dead by a verified hospital patient information system data. A total of 1535 donors did not meet the least frequency of follow-ups required for the inclusion (at least the data of 2 visits per year for the last 3 years until March 2014). After excluding donors who did not fulfill the inclusion criteria, 388 donors were included in this study. The following data were extracted from the donors’ files.

**Preoperative data**
The center policy is to accept living-related donors at the age range between 21 and 60 years, and to include donors with a family history of diabetes mellitus up to the start of this and other corresponding studies. Also, the data regarding the preoperative body mass index (BMI) were extracted, donors with BMI ranging between 25 and 30 kg/m^2 were included. However, donors with a BMI between 31 and 40 kg/m^2 were encouraged to lose weight before donation and we did not accept any donor with a BMI > 40 kg/m^2 into the preparation program.

**Postoperative data**
Serial monitoring of the data regarding donors’ BMI, serial monitoring of blood pressure data, identification of the type of antihypertensive treatment if present, serial measurement of fasting blood sugar levels donors with fasting blood glucose between 110 and 126 were considered prediabetics. Also, donors with fasting blood sugar above 126 were considered diabetics and both were considered to have disturbed glucose homeostasis, identification of the type of antihyperglycemic treatment (if present), serial monitoring of postdonation albumin creatinine ratio, serial calculations of the estimated creatinine clearance by the application of the (Chronic Kidney Disease Epidemiology Collaboration) (CKD-EPI) equation, serial monitoring of lipid profile postdonation, and the result of the fundus examinations for the diabetic donors only.

**Statistical analyses**
Statistical analyses were performed with SPSS software (SPSS: An IBM Company, version 16.0, IBM Corporation, Armonk, NY, USA). The data was analyzed using the t test for comparison of continuous data, and the chi-square test for comparison of simple proportions. P value less than .05 were considered statistically significant.

**Results**
Among the 388 studied donors 43 became diabetic at a mean interval of 6.9 ± 5.8 years postdonation. Among them there were 19 men and 24 women. Their mean age was 48.39 ± 7.66 years at the time of diagnosis of Diabetes mellitus (DM) and they were significantly (P < .0001) older than the studied 345 nondiabetic donors at the time of the initiation of this study. The mean average BMI at their serial follow-ups was significantly (P = .024) higher in diabetics when compared to the 345 nondiabetic donors. Among the 43 studied diabetic donors there were 25 donors who developed high albuminuria (albumin to creatinine excretion ratio between 30 to 300 mg/g), high albuminuria (albumin to creatinine excretion ratio above 300 mg/g) and or decreased estimated glomerular filtration rate (eGFR) below 70 mL/min at a mean interval of 10.1 ± 4.6 years postdonation and 6 ± 3.9 years after the diagnosis of DM, among them there were 14 then and 11 women. All of the 25 diabetic donors with high albuminuria, very high albuminuria, and or decreased eGFR below 70 mL/min had varying degrees of diabetic retinopathy (Tables 1, 2, and 3). The highest
frequency of DM was among the > 30 kg/m² BMI index group (Table 4). Both frequencies of DM and high albuminuria, very high albuminuria, and or decreased eGFR below 70 mL/min were significantly higher \((P = .005, P = .001)\) among donors with a positive family history of 2 or more family members with DM. The significance was noticeably higher \((P = .001)\) among donors with high albuminuria, very high albuminuria, and or decreased eGFR below 70 mL/min (Table 5).

Among the studied 388 donors there also were 60 donors with prediabetes (impaired fasting glycemia fasting blood sugar between 6.94 and 7.77 mg/dL and or impaired glucose tolerance, 2 hours postprandial between 140 and 199 mg/dL). There was no significant difference between their average BMI and the diabetic group. And their mean age was also not significantly lower than the diabetic group. In comparison, between the studied diabetic versus the prediabetic group, the urinary albumin creatinine ratio was significantly lower \((P = .015)\) in the prediabetic group but the range in this group denoted the presence of microalbuminuria also in prediabetic donors. A percentage of 17 (32.6%) of diabetic donors were high albuminuric and 16 (26.7%) of prediabetic donors were high albuminuric. However, 8 of diabetic donors (18.6%) had severely increased albuminuria (very high albuminuria), and only 1 of prediabetes did (1.7%) (Table 6).

In comparison, between diabetic donors with microalbuminuria, very high albuminuria, and diabetic donors with normal urinary protein excretion, the BMI index was significantly \((P = .04)\) higher in the very high albuminuric and the high albuminuric groups than the normalalbuminuric group. Also, the mean age was not significantly higher in the very high albuminuric and the high albuminuric groups than the normalalbuminuric group (Table 7). In comparison between diabetic donors on insulin containing regimens versus diabetic donors on other regimens, the estimated clearance was not significantly higher \((P = .44)\) but the urinary albumin creatinine ratio was significantly lower in the insulin group \((P = .01)\). In comparison, between diabetic donors on metformin-containing regimens versus diabetic donors on other (nonmetformin) oral hypoglycemic agents, the estimated clearance was significantly higher \((P = .003)\) and urinary albumin creatinine ratio was significantly lower \((P = .04)\) in the metformin group

| Table 1. Demographic and Clinical Characteristics of the Studied 388 Live Kidney Donors |
|---------------------------------|---------------------------------|---------------------------------|-----------------|-----------------|-----------------|-----------------|
| Demographic Characteristics     | Value                           | Demographic Characteristics     | Value                           | Demographic Characteristics     | Value                           |
| Donor age at last follow-up     | M ± SD (y)                       | Donor gender                     | M/F (%) (Male)                  | Donor age at time of donation    | M ± SD (y)                       |
| M ± SD (y)                      | 50.1 ± 10.5 (27-74)              | M/F (%) (Male)                   | 146/242 (37.6%)                | M ± SD (y)                      | 41 ± 10.15 (21-61)               |
| Age at diagnosis of DM          | M ± SD (y)                       | Age at diagnosis of high albuminuria, very high albuminuria and or decreased eGFR below 70 mL/min | M ± SD (y) | 48.39 ± 7.66 (23-66) | M ± SD (y)                      | 19/24 (39.5%) |
| Donors with high albuminuria, very high albuminuria and or decreased eGFR below 70 mL/min | M/F (%) (Male) | 14/11 (56%) | Interval between donation and development of diabetes | M ± SD (y) | 6.9 ± 5.8 (1-22) | Interval between diagnosis of DM and development of high albuminuria, very high albuminuria, and or decreased eGFR below 70 mL/min | M ± SD (y) | 10.1 ± 4.6 (4-21) | Interval between diagnosis of DM and development of high albuminuria, very high albuminuria, and or decreased eGFR below 70 mL/min | M ± SD (y) | 6 ± 3.9 (1-14) |
| Donors with high albuminuria, very high albuminuria, and or decreased eGFR below 70 mL/min | M/F (%) (Male) | 14/11 (56%) | Interval between donation and development of diabetes | M ± SD (y) | 6.9 ± 5.8 (1-22) | Interval between diagnosis of DM and development of high albuminuria, very high albuminuria, and or decreased eGFR below 70 mL/min | M ± SD (y) | 10.1 ± 4.6 (4-21) | Interval between diagnosis of DM and development of high albuminuria, very high albuminuria, and or decreased eGFR below 70 mL/min | M ± SD (y) | 6 ± 3.9 (1-14) |
| Donors with high albuminuria, very high albuminuria, and or decreased eGFR below 70 mL/min | M/F (%) (Male) | 14/11 (56%) | Interval between donation and development of diabetes | M ± SD (y) | 6.9 ± 5.8 (1-22) | Interval between diagnosis of DM and development of high albuminuria, very high albuminuria, and or decreased eGFR below 70 mL/min | M ± SD (y) | 10.1 ± 4.6 (4-21) | Interval between diagnosis of DM and development of high albuminuria, very high albuminuria, and or decreased eGFR below 70 mL/min | M ± SD (y) | 6 ± 3.9 (1-14) |

Abbreviations: BMI, body mass index

| Table 2. Frequency and Characteristics of the Studied Diabetic Versus Nondiabetic Donors |
|---------------------------------|---------------------------------|---------------------------------|-----------------|-----------------|-----------------|-----------------|
| Variable                        | Diabetic Donors                 | Nondiabetic Donors              | P Value         | Diabetic Donors | Nondiabetic Donors | P Value         |
| Frequency, (%)                  | 43 (11%)                       | 345 (89%)                      | < .0001         | 41 (10.1%)      | 349 (89.9%)     | .548            |
| Predonation BMI                 | M ± SD (y) Kg/m²                | 29.2 ± 2.3 (22-34)             | 28.5 ± 3.4 (23-33) | .488            | 28.5 ± 3.4 (23-33) | .488            |
| BMI at last follow-up           | M ± SD (y) Kg/m²                | 34.1 ± 5.9 (26-52)             | 31.3 ± 4.1 (23-42) | < .0001         | 31.3 ± 4.1 (23-42) | < .0001         |

Abbreviations: BMI, body mass index

| Table 3. Frequency of the Studied Chronic Complications of Diabetes Mellitus (Diabetic Retinopathy and Nephropathy) Among the Diabetic Group |
|---------------------------------|---------------------------------|---------------------------------|-----------------|-----------------|-----------------|-----------------|
| Chronic Complication            | Diabetic Donors                 | Diabetic Donors                 | P Value         | Diabetic Donors | Diabetic Donors | P Value         |
|                                | Free From the Studied Chronic Complications | Frequency, (%) | With the Studied Chronic Complications | Frequency, (%) |
|                                | M ± SD (y)                      | 18 (41.8%)                      | 25 (58.1%)      | .198            | 25 (58.1%)      | .198            |
|                                | M ± SD (y)                      | 11 (25.6%)                      | 32 (74.4%)      | < .0001         | 32 (74.4%)      | < .0001         |

Abbreviations: eGFR, estimated glomerular filtration rate

| Table 4. Frequency of Diabetes Mellitus in Different Current Body Mass Index Groups of the Studied Donors |
|---------------------------------|---------------------------------|---------------------------------|-----------------|-----------------|-----------------|-----------------|
| Current BMI group, Kg/m²        | Frequency of Diabetes Mellitus Among the Studied Donors (n = 388) | Frequency/Total Number of the Groups, (%) |
|                                |                                |                                |                                |                                |                                |
| 18-25 n = 122                  | 5 (4.1%)                       |                                |                                |                                |                                |
| 25-30 n = 149                  | 9 (6%)                         |                                |                                |                                |                                |
| > 30 n = 117                   | 29 (24.7%)                     |                                |                                |                                |                                |

Abbreviations: BMI, body mass index
were on insulin alone. No donors were on insulin + metformin.

Abbreviations: CKD-EPI, chronic kidney disease epidemiology collaboration

11 donors were on insulin plus (nonmetformin) oral hypoglycemic drugs, 4 donors were on other (nonmetformin) oral hypoglycemic drugs alone, 3 donors only were on insulin alone. No donors were on insulin + metformin.

Table 5. Frequency of Diabetes Mellitus and High Albuminuria, Very High Albuminuria and/or Decreased Estimated Glomerular Filtration Rate Below 70 mL/min Among Diabetic Donors With a Family History of Diabetes Mellitus Compared With Diabetic Donors With No Family History of Diabetes Mellitus

<table>
<thead>
<tr>
<th>Variable</th>
<th>Diabetic Donors With A Family History of Diabetes Mellitus (%)</th>
<th>Diabetic Donors With No Family History of Diabetes Mellitus (%)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>DM (n = 43)</td>
<td>28 (64.3%)</td>
<td>15 (35.7%)</td>
<td>.005</td>
</tr>
<tr>
<td>High albuminuria, high albuminuria, and/or decreased eGFR below 70 mL/min n = 25</td>
<td>18 (72%)</td>
<td>7 (28%)</td>
<td>.001</td>
</tr>
</tbody>
</table>

Abbreviations: eGFR, estimated glomerular filtration rate; DM, diabetes mellitus

Table 6. Comparison Between the Different Groups of Disturbed Glucose Homeostasis Donors

<table>
<thead>
<tr>
<th>Variable</th>
<th>Diabetic Donors</th>
<th>Donors with Prediabetes</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frequency, (%)</td>
<td>43/388 (11%)</td>
<td>60/388 (15.4%)</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>BMI at last follow-up</td>
<td>34.4 ± 4.3 (26-52)</td>
<td>34.2 ± 5.3 (29-51)</td>
<td>.4</td>
</tr>
<tr>
<td>Age at time of the study M ± SD (y)</td>
<td>53.9 ± 9.3 (38-74)</td>
<td>51.4 ± 6.5.4 (37-66)</td>
<td>.134</td>
</tr>
<tr>
<td>Creatinine Clearance by CKD-EPI equation M ± SD (y) mL/min/1.73m²</td>
<td>65.8 ± 23 (31-122)</td>
<td>84.6 ± 16.3 (55-126)</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Urinary albumin creatinine ratio median (range), mg/g</td>
<td>32 (3-6700)</td>
<td>24 (5-350)</td>
<td>.015</td>
</tr>
<tr>
<td>Percentage of high albuminuria</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>urinary albumin creatinine ratio between 30-300 mg/g frequency, (%)</td>
<td>17 (32.6%)</td>
<td>16 (26.7%)</td>
<td>.742</td>
</tr>
<tr>
<td>Percentage of very high albuminuria (Severely increased albuminuria) urinary albumin creatinine ratio above 300 mg/g frequency, (%)</td>
<td>8 (18.6%)</td>
<td>1 (1.7%)</td>
<td>.0083</td>
</tr>
</tbody>
</table>

Abbreviations: BMI, body mass index; CKD-EPI, chronic kidney disease epidemiology collaboration

Table 7. Comparison Between High Albuminuric and Very High Albuminuric and Normaloalbuminuric Diabetic Donors

<table>
<thead>
<tr>
<th>Variable</th>
<th>High Albuminuric Diabetic Donors (%)</th>
<th>Very High Albuminuric Donors (%)</th>
<th>Normaloalbuminuric Diabetic Donors (%)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frequency, (%)</td>
<td>17/43 (32.6%)</td>
<td>8/43 (18.6%)</td>
<td>18/43 (41.8%)</td>
<td>.07</td>
</tr>
<tr>
<td>Average BMI at serial followups M ± SD, kg/m²</td>
<td>37.8 ± 4.3</td>
<td>39.2 ± 4.8</td>
<td>26.3 ± 6.77</td>
<td>.04</td>
</tr>
</tbody>
</table>

Abbreviations: BMI, body mass index

Table 8. Comparison Between Diabetic Donors on Different Antidiabetic Protocols

<table>
<thead>
<tr>
<th>Variable</th>
<th>Diabetic Donors on Insulin</th>
<th>Diabetic Donors Not on Insulin</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frequency, creatinine clearance by (%)</td>
<td>14*/43 (32.6%)</td>
<td>29/43 (67.4%)</td>
<td></td>
</tr>
<tr>
<td>CKD-EPI equation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M ± SD (range), mL/min/1.73m²</td>
<td>68.2 ± 16.9 (56-118)</td>
<td>67.2 ± 23 (31-122)</td>
<td>.44</td>
</tr>
<tr>
<td>Urinary albumin creatinine ratio Median (range), mg/g</td>
<td>29 (11-40)</td>
<td>45 (3-6700)</td>
<td>.01</td>
</tr>
</tbody>
</table>

Abbreviations: CKD-EPI, chronic kidney disease epidemiology collaboration

*11 donors were on insulin plus (nonmetformin) oral hypoglycemic drugs, 4 donors were on other (nonmetformin) oral hypoglycemic drugs alone, 3 donors only were on insulin alone. No donors were on insulin + metformin.

Table 9. Correlation Between BMI and Urinary Albumin Creatinine Ratio of the Studied Donors

<table>
<thead>
<tr>
<th>Urinary Albumin Creatinine Ratio mg/g</th>
<th>Diabetic Donors Postdonation n = 43</th>
<th>All Studied Group of Donors n = 388</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diabetic Donors Postdonation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMI kg/m²</td>
<td>0.32</td>
<td>.03</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: BMI, body mass index

Discussion

Our results disclosed 43 subjects who developed diabetes mellitus after live kidney donation representing a percentage of 11% of the studied 388 donors the percentage which was significantly lower than the percentage of diabetes among general Egyptian population (0.013 y). In Egypt, the percentage of DM was 15.6% according to International Diabetes Federation Atlas 6th Edition 2013.9 This relatively lower percentage may be explained by the closer follow-ups with earlier identification of the risk factors of diabetes than the general Egyptian population also by the healthy nature of the donor population. The mean BMI of the 43 diabetic donors was 34.3 ± 4.3 kg/m², which is higher than the mean BMI of the general Egyptian diabetic population 31.3 kg/m², according the IDF Atlas 2013.9 Also the highest incidence of diabetes was among the BMI group above 30 kg/m² being 79.1% of the 43 diabetic donors.

Our study also revealed 60 donors with prediabetes representing a percentage of 15.4% of the studied group (impaired fasting glycaemia and fasting blood glucose between 110 and 125 mg/dL and/or impaired glucose tolerance 2-hour postprandial blood glucose of 140 and 199 mg/dL (according to the WHO Classification of 2007).
percentage is significantly higher ($P < .0001$) than the percentage of prediabetes in the Egyptian general population 6.97% according to IDF Atlas 6th Edition 2013.9 We also noticed the mean BMI increased significantly ($P = .03$) after donation (Figure 3); this may explain the high frequency of prediabetic status among the studied donors.

Of the studied 43 diabetic donor 58.1% (25 donors) showed high albuminuria, very high albuminuria, and/or decreased creatinine clearance. All those 25 donors had evidence of different types and stages of diabetic retinopathy. The male percentage of them was 56.3%, the mean interval between kidney donation, and development of high albuminuria, very high albuminuria, and/or decreased creatinine clearance was $10.1 \pm 4.6$ years (range, 4-21 y) and the mean interval between the development of DM and high albuminuria, very high albuminuria, and/or decreased eGFR below 70 mL/min was $6 \pm 3.9$ y (range, 1-14). The percentage of high albuminuria, very high albuminuria, and/or decreased creatinine clearance below 70 mL/min is significantly higher in our study than a UKPDS64 study, which showed 30.8% in a study of 5100 T2DM patients with 2 kidneys enrolled in it.10 Also, the mean interval between both

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**Figure 1.** Correlation Between Body Mass Index and Urinary Albumin Creatinine Ratio of the Diabetic Group

**Figure 2.** Correlation Between Body Mass Index and Urinary Albumin Creatinine Ratio of the Studied Whole Group of Donors

**Figure 3.** Comparison of the Mean Body Mass Index of the Studied Group at the Day of Donation and the Last Follow-Up Postdonation
development of DM and the evidence of nephropathy was significantly lower than the previously mentioned study being 5.7 and 10.1 years. This may be explained by the hyperfiltration theory. The percentage of high albuminuria alone defined as urinary albumin creatinine ratio between 30 to 300 mg/g among the 43 diabetic donors was (32.6%) 17 donors. This percentage was higher than the 20% (1021 patients) in the 5100 T2DM patients with 2 kidneys enrolled in UKPDS64.10 This may be explained by the previously described hyperfiltration theory. The percentage of very high albuminuria alone defined as urinary albumin creatinine ratio among the 43 diabetic donors above 300 mg/g was 8 donors (18.6%). This percentage is higher than the 10% in a 5100 T2DM patients with 2 kidneys enrolled in UKPDS64.10

There is a positive correlation between diabetic donors BMI and urinary albumin creatinine ratio as shown in Figure 1 and Figure 2. So BMI is also considered as a risk factor for the development of high albuminuria, very high albuminuria, and/or decreased eGFR below 70 mL/min among donors. The percentage of high albuminuria among the 60 prediabetic donors was 16 donors (26.7%).

The percentage of diabetic donors with a family history of diabetes was (28 donors out of 43) (64.3%); 18 of them (64.2%) developed high or very high albuminuria the percentage which made our center question the acceptance of donors with positive family history of DM.

In our study, we compared diabetic donors on metformin containing regimens (17/43 diabetic donors) between other (nonmetformin) oral hypoglycemics containing regimens (29/43 diabetic donors). The mean urinary albumin creatinine ratio of the metformin group was significantly lower ($P = .04$), and the estimated creatinine clearance by CKD-EPI equation was significantly higher ($P = .003$) than the other oral hypoglycemic agents group. This may be one of the principle factors explaining the improvement of vascular outcome associated with metformin as in UKPDS 34.11 Patients allocated metformin, compared with the conventional group, had risk reductions of 32% for any diabetes-related endpoint, 42% for diabetes-related death, and 36% for all-cause mortality including vascular complications. Among patients allocated intensive blood-glucose control, metformin showed a greater effect than chlorpropamide, or glibenclamide for any diabetes-related endpoint ($P = .0034$), all-cause mortality ($P = .021$), and stroke ($P = .032$) (UKPDS34).11

In comparing diabetic donors on insulin (14/43 diabetic donors) versus diabetic donors noninsulin containing regimens (29/43 diabetic donors), there was no significant difference between the mean estimated creatinine clearance of both groups. The mean urinary albumin creatinine ratio was significantly lower ($P = .01$) in the insulin group, which may reflect the beneficial effect of insulin on the vascular outcome that may be due to the anti-inflammatory and vasodilator effects of insulin. In the UKPDS 33,12 the insulin group compared with 7.9% in the conventional group an 11% reduction. Compared with the conventional group, the risk in the intensive group was 12% lower for any diabetes-related endpoint; 10% lower for any diabetes-related death; and 6% lower for all-cause mortality. Most of the risk reduction in the any diabetes-related aggregate endpoint was due to a 25% risk reduction ($P = .0099$) in microvascular endpoints, including the need for retinal photocoagulation (UKPDS 33).12

References

Human Leukocyte Antigen-DR Mismatched Pediatric Renal Transplant: Patient and Graft Outcome With Different Kidney Donor Sources

Torki Al-Otaibi,1 Osama Gheith,1,2 Ahmed Mosaad,1 M.R. Naryanan Nampoory,1 Medhat Halim,1 Tarek Said,1 Prasad Nair1

Abstract

Objectives: Kidney transplant is well accepted as the optimal therapy for children with end-stage renal disease, and new trends suggest using human leukocyte antigen-DR mismatched grafts. The aim of work was to assess the effect of human leukocyte antigen-DR mismatch on the outcome of pediatric renal transplant recipients, regardless of the source of kidney graft.

Materials and Methods: According to human leukocyte antigen-DR matching, 104 pediatric patients were categorized into 3 comparable groups. With optimized immunosuppression protocols, long-term graft and patient outcomes were assessed.

Results: We found that posttransplant complications were comparable in the 3 groups, without significant increase in the risk of infections or malignancies, especially in the full human leukocyte antigen-DR-mismatched group. Moreover, we found no significant difference in the 3 groups regarding long-term graft or patient survival.

Conclusions: With optimization of immunosuppression, human leukocyte antigen-DR-mismatched donors can be safely accepted for pediatric kidney transplant with comparable long-term patient and graft survival.

Key words: Children, End-stage renal disease, Renal transplant, Human leukocyte antigen-DR, Outcome

Introduction

Renal transplant is the therapy of choice for children with end-stage renal disease.1 Compared with dialysis, renal transplant provides better survival rates and also a better quality of life. However, renal graft survival is limited because of immunologic and nonimmunologic factors. Ethnicity affects renal transplant survival rates substantially.2 Several papers, mainly from the United States, have reported that adults and African American child recipients had worse patient and transplant survival rates than did white recipients.3-8 Biological factors such as different major histocompatibility complex antigens, and/or socioeconomic factors (eg, the lack of education), may have determined the unfavorable results in these studies.9-11 A novel approach has been recommended to ensure well-matched transplants for younger patients, and human leukocyte antigen (HLA) matching is less important for older patients because revision transplant is less likely to be required.12

The HLA mismatches may correlate with risk of death with a functioning graft because of the requirement for higher immunosuppression doses and more antirejection therapy. The most frequent causes of death with a functioning graft were infection, cardiovascular disease, and malignancy (32.2%, 30.9%, and 3.6% in year 1; 16.4%, 29.6%, and 15.9% in years 2-5).13 In addition, multivariate analyses showed that mismatches for HLA class II were more strongly associated with hospitalization and death with a functioning graft than mismatches for HLA class I.13

From the 1Department of Nephrology, Hamed Al-Essa Organ Transplant Center, Kuwait; 2Urology and Nephrology Center, Mansoura University, Mansoura, Egypt

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Corresponding author: Osama Gheith, Urology and Nephrology Center, Mansoura University, Mansoura, Egypt

Phone: +96 56 664 1967 Fax: +2 462 0963 E-mail: ogheith@yahoo.com

Another study was performed to determine the effect of HLA-DR mismatch on rejection, graft survival, and sensitization in a local allocation system that emphasized donor quality rather than HLA matching for pediatric patients, and to determine the likelihood of finding an appropriate donor that was based on HLA-DR mismatch. The 1- and 5-year graft survival rates were 97% and 82%; HLA-DR mismatching was not a significant risk factor for either failing, or will fail; but a history of rejection was an independent predictor of graft failure. However, although avoiding HLA-DRB1 mismatching is beneficial, it was concluded that the likelihood of finding an HLA-DRB1-matched donor also should be considered in donor selection.

We aimed to assess the effect of HLA-DR mismatch on the outcome of pediatric renal transplant recipients.

**Materials and Methods**

**Patients**

The charts of all children and adolescents who underwent a renal transplant between 1994 and 2011 at the Hamed Al-Essa Organ Transplant Center of Kuwait were reviewed. In these patients, the following data were retrospectively analyzed: age at transplant, sex, underlying renal disease, prior renal replacement therapy, organ donor source, cold ischemia time, and HLA match. According to HLA-DR matching, patients were categorized into 3 groups: group 1, patients with full HLA-DR match; group 2, patients with 1 HLA-DR mismatch; and group 3, patients with full HLA-DR mismatch.

To assess the renal transplant outcome, we analyzed patient and graft survival rates, acute rejection episodes, rejection-free time, allograft function, infection episodes necessitating hospitalization, and number of antihypertensive drugs at transplant and last follow-up. The underlying renal diseases of the examined patients were classified etiologically into 4 groups: glomerulonephritis, chronic tubulointerstitial disease, congenital diseases, and idiopathic disease. Pretransplant complement-dependent cytotoxicity and flow cytometry crossmatches were negative.

**Immunosuppressive regimens**

All patients received triple immunosuppressive regimens consisting of a calcineurin inhibitor, mycophenolate mofetil, and corticosteroids. All induction therapy was based on our protocol guidelines and transplant risk factors. Patients at high risk for acute rejection, including patients having revision transplant, panel reactive antibodies (PRA) > 20%, or deceased-donor transplant, received rabbit antithymocyte globulin (Thymoglobulin, Genzyme Corp., Cambridge, MA, USA) at 1.0 mg/kg daily (total, 5 doses). Other patients received interleukin 2 receptor antagonist (IL-2RA) induction using basiliximab (Simulect, Novartis Pharmaceuticals, New York, NY, USA) at 10 mg/m² intravenously as a bolus within 2 hours of engraftment on day 0 and the second dose of 10 mg/m² on day 4, or daclizumab (Zenapax, Roche Pharmaceuticals, Nutley, NJ, USA) at 1 mg/kg body weight. The first dose was administered within 2 hours before transplant with subsequent doses given at 2, 4, 6, and 8 weeks after transplant. Patients with zero HLA mismatches received neither rabbit antithymocyte globulin nor IL-2RA induction. Corticosteroids were initiated intraoperatively as methylprednisolone 250 to 500 mg according to body weight (1 mg/kg to a maximum of 60 mg/d from day 1) and tapered down to low-dose prednisone (0.1-0.5 mg/kg/d) by 3 months after transplant.

Target 12-hour whole blood trough concentrations for tacrolimus (Prograf, Astellas Pharmaceuticals, Deerfield, IL, USA) were as follows: 1 to 6 weeks, 10 to 15 ng/mL; 7 to 12 weeks, 8 to 12 ng/mL; 3 to 12 months, 6 to 10 ng/mL; > 1 year, > 5 ng/mL, or as clinically indicated. Target 12-hour whole blood trough concentrations for cyclosporine (Neoral, Novartis Pharmaceuticals) were as follows: 1 to 6 weeks, 200 to 275 ng/mL; 7 to 12 weeks, 175 to 225 ng/mL; 3 to 12 months, 125 to 175 ng/mL; > 1 year, > 70 ng/mL, or as clinically indicated. Doses of calcineurin inhibitors were minimized during antithymocyte globulin induction but were returned to full dose 2 days before discontinuation of the induction regimen. All patients received mycophenolate mofetil (CellCept, Roche Pharmaceuticals, Nutley, NJ, USA), with initial doses of 600 mg/m² oral twice daily.

Doses were adjusted for efficacy and toxicity. Graft failure was defined as a situation where any other form of renal replacement therapy had to be started. Death with functioning graft was not considered as graft failure. Deaths that were not primarily associated with renal transplant were censored in the Kaplan-Meier survival analysis.
Meier analysis. The glomerular filtration rate (GFR) was computed using the Schwartz formula (GFR [mL/min/1.73 m²] = 0.55 × body length [cm]/serum creatinine [mg/dL]).

All cytomegalovirus-positive recipients and cytomegalovirus-negative recipients of a kidney from a cytomegalovirus-positive donors were given prophylaxis with valacyclovir for the first 3 months after transplant. All patients received prophylaxis against *Pneumocystis carinii* with sulfamethoxazole and trimethoprim for 6 months. All patients had blood polymerase chain reaction for BK virus at 3 months and 1 year after transplant and every year thereafter.

### Acute rejection

Acute rejection included biopsy-proven and clinically suspected episodes (defined as the use of high-dose steroids or antibody treatment with subsequent improvement of renal function). Acute rejection was biopsy-proven and the diagnosis was made according to Banff classification 2007 and treated with high-dose corticosteroids. Borderline rejection was included in the analysis if treated as acute rejection. Patients were considered to have delayed graft function if they required dialysis within the first week after transplant.

### Statistical analyses

Data were manually collected in a spreadsheet (Excel, Microsoft, Redmond, WA, USA). Statistical analysis was conducted using software (SPSS, version 11.0, SPSS Inc., Chicago, IL, USA). Data were reported as number (%) or mean ± standard deviation. The *t* test was used to compare means and standard deviations of the 3 groups. Categoric data were compared using the chi-square test. Graft and patient survival were computed using the Kaplan-Meier method. Statistical significance was defined by *P* ≤ .05.

### Results

Most of our pediatric transplant patients were Kuwaiti males with mean ages 13.4 ± 5.4 years, 13.9 ± 3.8 years, and 13.6 ± 4.3 years in the 3 studied groups. Male donors were significantly higher among patients with 1 and full HLA-DR-mismatch groups compared with full HLA-DR-matched group (*P* = .01) with mean age 35.7 ± 8.5 years, 34.6 ± 7.4 years, and 30.7 ± 9 years in the same groups (*P* = .075). We found nonsignificant difference between the 3 groups regarding pretransplant comorbidities, especially anemia; patients who received treatment for tuberculosis, hypertension, or diabetes mellitus; viral profile (especially hepatitis C virus, cytomegalovirus, hepatitis B virus, and herpes viruses); and bone disease (*P* > .05) (Table 1). The original kidney disease was comparable in all groups (*P* > .05). Moreover, the number of patients who underwent preemptive renal transplant, and those who were on regular hemodialysis or peritoneal dialysis, were comparable (*P* = .41) (Table 1).

### Table 1. Demographic Characteristics of Study Patients

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Full HLA-DR Match (n = 15)</th>
<th>1 HLA-DR Mismatch (n = 63)</th>
<th>Full HLA-DR Mismatch (n = 26)</th>
<th><em>P</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient age (mean ±SD) (y)</td>
<td>13.4 ± 5.4</td>
<td>13.9 ± 3.8</td>
<td>13.6 ± 4.3</td>
<td>.89</td>
</tr>
<tr>
<td>Patient sex (male/female)</td>
<td>8/7</td>
<td>42/21</td>
<td>16/10</td>
<td>.091</td>
</tr>
<tr>
<td>Donor age (mean ±SD) (y)</td>
<td>35.7 ± 8.5</td>
<td>34.6 ± 7.4</td>
<td>30.7 ± 9</td>
<td>.075</td>
</tr>
<tr>
<td>Donor sex (male/female)</td>
<td>8/7</td>
<td>43/17</td>
<td>26/3</td>
<td>.01</td>
</tr>
<tr>
<td>Original kidney disease</td>
<td>5</td>
<td>17</td>
<td>8</td>
<td>.3</td>
</tr>
<tr>
<td>Glomerulonephritis</td>
<td>2</td>
<td>5</td>
<td>5</td>
<td>.3</td>
</tr>
<tr>
<td>Tubulointerstitial disease</td>
<td>5</td>
<td>29</td>
<td>8</td>
<td>.3</td>
</tr>
<tr>
<td>Congenital</td>
<td>3</td>
<td>12</td>
<td>7</td>
<td>.41</td>
</tr>
<tr>
<td>Idiopathic</td>
<td>3</td>
<td>13</td>
<td>7</td>
<td>.41</td>
</tr>
<tr>
<td>Graft function</td>
<td>10</td>
<td>32</td>
<td>16</td>
<td>.41</td>
</tr>
<tr>
<td>Preemptive</td>
<td>1</td>
<td>16</td>
<td>6</td>
<td>.41</td>
</tr>
<tr>
<td>Hemodialysis</td>
<td>8</td>
<td>24</td>
<td>3</td>
<td>.3</td>
</tr>
<tr>
<td>Deceased</td>
<td>8</td>
<td>19</td>
<td>10</td>
<td>.3</td>
</tr>
<tr>
<td>Donor type</td>
<td>0</td>
<td>14</td>
<td>19</td>
<td>.3</td>
</tr>
<tr>
<td>Living-related</td>
<td>5</td>
<td>19</td>
<td>10</td>
<td>.3</td>
</tr>
<tr>
<td>Living-unrelated</td>
<td>13/2</td>
<td>33/30</td>
<td>14/12</td>
<td>.13</td>
</tr>
<tr>
<td>Deceased</td>
<td>3/12</td>
<td>58/5</td>
<td>23/3</td>
<td>.98</td>
</tr>
<tr>
<td>Non-TB vs TB</td>
<td>3/12</td>
<td>58/5</td>
<td>23/3</td>
<td>.98</td>
</tr>
<tr>
<td>Diabetic vs nondiabetic</td>
<td>4/11</td>
<td>18/45</td>
<td>11/15</td>
<td>.53</td>
</tr>
<tr>
<td>Osteopenia vs healthy</td>
<td>13/2</td>
<td>40/23</td>
<td>20/6</td>
<td>.15</td>
</tr>
</tbody>
</table>

Abbreviations: HLA-DR, human leukocyte antigen-DR; TB, tuberculosis

We observed that the number of patients who received their grafts from living donors (related or unrelated) was significantly higher in groups with ≤ 1 HLA-DR mismatch; the number of patients who received their grafts from deceased donors was significantly higher in the full HLA-DR-mismatched group (*P* ≤ .001) (Table 1). The number of patients who received less-induction therapy was significantly higher in the group with 1 HLA-DR mismatch. The number of patients who received more potent induction (antithymocyte globulin) was significantly higher in the group with ≥ 1 HLA-DR mismatch (*P* ≤ .001). However, the number of patients who were receiving
either type of calcineurin inhibitor was comparable in all groups ($P > .05$) (Table 2). Most studied patients experienced immediate graft function regardless of HLA-DR match ($P = .3$).

**Posttransplant complications and outcome**

Most rejection episodes developed during the first posttransplant year. We found that biopsy-proven acute rejection episodes, with different varieties, were comparable in the study groups ($P = .16$). Regarding nonimmunologic complications, we observed that posttransplant new-onset diabetes, de novo hypertension, and infection episodes that necessitated hospitalization all were comparable in the study groups regardless of the number of HLA-DR mismatches and subsequent induction therapy ($P > .05$) (Table 2).

We did not observe any evidence of malignancy except 1 case of visceral Burkett lymphoma among patients in the full HLA-DR match group. We observed that there was no significant difference between the 3 groups regarding graft survival ($P = .053$) or patient survival ($P = .84$). Graft survival was 95%, 85%, 65%, 72%, and 51.4% in the first group; 93%, 83%, 79%, 70%, and 81% in second group; and 87%, 82%, 82%, 67%, and 24.5% in the third group at 1, 2, 3, 5, and 10 years after transplant ($P = .31$). Patient survival was 93%, 93%, 80%, 80%, and 50.5% in the first group; 95%, 83%, 80%, 70%, and 24% in second group; and 87%, 82%, 78%, 63%, and 24.3% in the third group at 1, 2, 3, 5, and 10 years after transplant ($P = .056$).

**Discussion**

Kidney transplant is well-accepted as the optimal therapy for children with end-stage renal disease. By analyzing large databases, many investigators have found that recipients of HLA-matched kidneys experience superior outcomes, as defined by lower rates of rejection and higher rates of graft and patient survival, than recipients of HLA-mismatched kidneys.15 Furthermore, multifactorial analysis of 1252 pediatric deceased-donor renal transplants performed in the United Kingdom and Ireland between 1986 and 1995 also revealed that HLA matching was 1 of the most important determinants of outcome.16

A previous retrospective analysis was performed with data from the Organ Procurement and Transplantation Network and the Scientific Registry of Transplant Recipients to examine the effect of HLA-DR matching on allograft survival and sensitization rates in children receiving kidneys from deceased donors.17 With the aims of shortening the waiting time on dialysis of pediatric renal transplant candidates and minimizing the morbidity of these individuals, it was suggested to use HLA-DR-mismatched grafts. This should be cautiously analyzed. The increased risks of acute rejection, graft loss, and sensitization should be considered and weighed against the problems associated with longer exposure to dialysis.

They analyzed data from 1585 children who had received kidneys between 1996 and 2004 from deceased donors aged ≤ 35 years. However, they

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**Table 2. Types of Immunosuppression and Posttransplant Complications Between Different Study Groups**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Full HLA-DR Match (n = 15)</th>
<th>I HLA-DR Mismatch (n = 63)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Immunosuppression</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Induction</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>10</td>
<td>25</td>
<td>.16</td>
</tr>
<tr>
<td>IL-2R blocker</td>
<td>3</td>
<td>6</td>
<td>.67</td>
</tr>
<tr>
<td>Thymoglobulin (Humanized)</td>
<td>0</td>
<td>46.2%</td>
<td>.076</td>
</tr>
<tr>
<td>Antithymocyte globulin (Rabbit ATG)</td>
<td>3.7%</td>
<td>44.4%</td>
<td>.91</td>
</tr>
<tr>
<td>Maintenance</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cyclosporine-based</td>
<td>12 (81.8%)</td>
<td>32 (50.9%)</td>
<td>.06</td>
</tr>
<tr>
<td>Tacrolimus-based</td>
<td>3 (18.2%)</td>
<td>31 (49.1%)</td>
<td>.59</td>
</tr>
<tr>
<td><strong>Posttransplant complications:</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Immunologic</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Biopsy-proven rejection episodes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>10</td>
<td>25</td>
<td>15</td>
</tr>
<tr>
<td>ACR</td>
<td>3</td>
<td>6</td>
<td>2</td>
</tr>
<tr>
<td>AAMR</td>
<td>0</td>
<td>7</td>
<td>5</td>
</tr>
<tr>
<td>Mixed</td>
<td>5</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>Total acute rejection</td>
<td>3 (20%)</td>
<td>13 (23.8%)</td>
<td>10</td>
</tr>
<tr>
<td>Nonimmunologic</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypertension</td>
<td>2/13</td>
<td>5/58</td>
<td>3/23</td>
</tr>
<tr>
<td>NODAT</td>
<td>2/11</td>
<td>4/52</td>
<td>3/31</td>
</tr>
<tr>
<td>Infections necessitating hospitalization:</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>None</td>
<td>5</td>
<td>18</td>
<td>15</td>
</tr>
<tr>
<td>Urinary tract infection</td>
<td>8</td>
<td>36</td>
<td>16</td>
</tr>
<tr>
<td>Chest infection</td>
<td>2</td>
<td>7</td>
<td>4</td>
</tr>
<tr>
<td>Others</td>
<td>0</td>
<td>2</td>
<td>1</td>
</tr>
</tbody>
</table>

---

**Table 3. Patient and Graft Outcomes Between the Study Groups**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Full HLA-DR Match (n = 15)</th>
<th>I HLA-DR Mismatch (n = 63)</th>
<th>Full HLA-DR Mismatch (n = 26)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Graft outcome</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Functioning graft</td>
<td>14</td>
<td>51</td>
<td>16</td>
<td>.053</td>
</tr>
<tr>
<td>Failed graft</td>
<td>1</td>
<td>12</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td><strong>Patient outcome</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Living</td>
<td>14</td>
<td>58</td>
<td>23</td>
<td>.84</td>
</tr>
<tr>
<td>Died</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>.72</td>
</tr>
<tr>
<td>Lost to follow-up</td>
<td>1</td>
<td>3</td>
<td>3</td>
<td>.84</td>
</tr>
</tbody>
</table>

**Abbreviations:** AAMR, acute antibody-mediated rejection; ACR, acute cellular rejection; IL-2R, interleukin 2 receptor; NODAT, new-onset diabetes after transplant
reported identical 5-year graft survival rates in the
grafts with zero HLA-DR mismatches and in those
with 2 HLA-DR mismatches (71%). In addition, the
authors showed that the odds of developing PRA
> 30% by the time of a second transplant did not
increase significantly in the presence of HLA
mismatches.

Reduced use of HLA matching in kidney
transplant has been advocated recently. In our
retrospective study, we included 104 pediatric kidney
transplant recipients and compared 3 groups of
patients, including deceased- and living-donor
transplants, that had different HLA-DR mismatches.
The studied groups were matched regarding
demographic and pretransplant comorbidities. We
observed that the number of pediatric patients with
≤ 1 HLA-DR mismatch who received their graft from
living donors (related or unrelated) with their ages
< 36 years was significantly higher compared with
the other group with full HLA-DR mismatches who
received grafts from deceased donors (P ≤ .001).
Patients of these groups had received significantly
less induction therapy, as IL-2 Ras or no induction
at all, compared with the same group (P ≤ .001)
(Table 2). In contrast, most patients who had full
HLA-DR mismatches received their grafts from
deceased donors aged < 35 years (P ≤ .001) (Table 1),
and had received significantly more potent
induction immunosuppression (P ≤ .001) (Table 2).
This was consistent with a previous study that
reported that the potential benefit of HLA-matching
for the reduction protocol for immunosuppressants
may play a role in the withdrawal program.18 It
appears unnecessary to pay attention to HLA
compatibility in donor selection in living-related liver
transplant, except for 1-way HLA matching, or to
adjust immunosuppression according to HLA
compatibility.

In the same direction, this issue previously was
extrapolated but not confirmed by others who stated
in their report that children who received HLA-DR
mismatched kidneys probably would undergo more
intensive immunosuppressive treatment to overcome
the potentially increased risks of acute rejection, graft
failure, and sensitization.19 Ghoneim and Refaie19
criticized a paper by other workers17 when referred
to a previous study by the Ghoneim group20 to
support the author’s contention. They reported that
all the kidneys offered to their pediatric recipients
were retrieved from living-related donors, and they
did not accept any HLA-DR-mismatched grafts.
Moreover, mismatches in HLA-A or HLA-B were a
significant predictor of graft loss in their series. The
frequency of rejection episodes, antirejection
treatment, and the adverse effects of such treatment
to which pediatric patients are particularly
vulnerable) were not reported.17 In our study, we
observed that most acute rejection episodes
developed during the first year after transplant.
Moreover, we found that biopsy-proven acute
rejection episodes (T-cell-mediated or antibody-
mediated) were comparable in the study groups
(P = .16), which could be explained by the potent
immunosuppressant protocol used. This finding was
matched with the report of others21 who found that
mismatching of HLA-A and -B antigens did not affect
frequency of early cellular rejection, but the presence
of 2 HLA-DR locus mismatches increased the risk of
high-grade rejection in pediatric heart transplant
recipients treated with cyclosporine. They added that
the potent effects of tacrolimus-based immuno-
suppression mitigated the effect of HLA-DR
mismatching because patients treated with
tacrolimus who had 2 HLA-DR mismatches had less
rejection than patients treated with cyclosporine who
had 1 HLA-DR mismatch and seemed to be at no
greater risk for rejection than patients treated with
tacrolimus and who had 1 HLA-DR mismatch.
Moreover, there was no evidence that HLA matching
was associated with improved kidney or pancreas
survival. However, a higher rate of acute rejection
was observed with poor HLA match, which may
affect long-term survival.22

We found no significant difference between the 3
groups regarding delayed graft function, because the
majority of patients experienced immediate graft
function regardless of HLA-DR match (P = .3).
However, the notion of comparable success rates has
been challenged by a previous study that examined
the outcomes of 135970 deceased-donor transplants
performed during 2 decades (1985-1994 and 1995-
2004).23 The data were provided by 363 transplant
centers in 41 countries, and 7315 recipients (11%)
were aged < 18 years. In that report, the number of
mismatches correlated significantly with the rate of
graft survival and rejection. Long-term follow up
showed that graft outcome was comparable in the
study groups (P = .053) (Figure1), as was patient
survival (P = .84) (Figure 2). This was matched with
a previous report, despite shorter follow-up, that
suggested that the transplant community, especially pediatric transplant programs, should accept HLA-DR-mismatched kidneys from deceased donors aged ≤ 35 years for transplant into children with end-stage renal disease. A previous study noted that if immunosuppression had improved to a degree that enabled transplant of well- and poorly HLA-matched kidneys with identical success rates, then HLA typing and matching would be abolished from kidney allocation algorithms.

We believe that optimization of induction and maintenance immunosuppression might be responsible for such results among our patients. This observation matched the results of a previous study that included 672 renal transplant recipients with different HLA-DR mismatch status and treated with antithymocyte globulin as induction therapy followed by tacrolimus, prednisone, and mycophenolate mofetil for maintenance immunosuppression. They concluded that the independent effect of HLA-DR mismatches on adverse graft survival was diminished under potent antibody induction and maintenance immunosuppression in African Americans. Patients on tacrolimus with 1 or 2 mismatches at the HLA-B or -DR loci may have increased rates of patient and graft survival compared with patients having no mismatches, with the appearance of a protective effect of tacrolimus.

Some authors have recommended that transplants with 2 HLA-DR mismatches be avoided to reduce the risk of posttransplant non-Hodgkin lymphoma. However, we did not observe any evidence of malignancy except 1 case of visceral Burkett lymphoma among patients with full HLA-DR match.

In conclusion, with optimization of immunosuppression, HLA-DR-mismatched donors can be safely accepted for pediatric kidney transplant with comparable long-term patient and graft survival.

References

Results of Liver Transplant in Elderly Patients: A Single Center Experience

Aydincan Akdur,1 Cihan Fidan,2 Ebru Ayvazoglu Soy,1 Mahir Kirnap,1 Feza Yarbug Karakayali,1 Adnan Torgay,3 Sedat Yildirim,1 Gokhan Moray,1 Mehmet Haberal1

Abstract

Objectives: With the increased life span, the need for liver transplant for elderly patients also increased in the world. In this study, we reviewed our experience to determine the outcomes and problems of patients aged > 60 years who had liver transplants.

Materials and Methods: Data of recipients aged > 60 years were reviewed retrospectively. We analyzed 16 elderly patients who had liver transplant for chronic liver disease between 2001 and 2014 in our center.

Results: In our series, there were 5 women and 11 men between age 60 and 65 years. The mean Child-Pugh score was 7.9 ± 1.7 and Model for End-Stage Liver Disease score was 14.1 ± 5.1. Primary liver disease was hepatitis B in 9 patients (34.5%), most of them with hepatocellular carcinoma. The other causes of liver failure were hepatitis C (n = 4), alcoholic cirrhosis (n = 2), and cryptogenic cirrhosis (n = 2); 1 patient had both hepatitis B and hepatitis C virus, and 1 patient had both hepatitis B virus and alcoholic cirrhosis. There were 9 patients who had hepatocellular carcinoma. Mortality was observed in 4 patients. The reasons for mortality were sepsis (n=3) and hepatocellular carcinoma (n=1).

Conclusions: Liver transplant can be safely performed and has acceptable long-term outcomes in low-risk elderly recipients. Age alone should not be a contraindication for liver transplant in elderly patients.

Key words: Age, Liver failure, Survival, Treatment

Introduction

Health care for older people is becoming increasingly important in industrialized nations. Advanced age is not considered an absolute contraindication for liver transplant. When liver transplant began, the upper limit of age for liver transplant was 50 years.1 Advances in the medical treatment of chronic liver diseases have resulted in an increase in life expectancy.2 Along with increased life span, the need for liver transplant for elderly patients also increased worldwide. Transplant in the elderly patient with comorbid diseases still is a subject of debate because of the high risk of surgery. Promising results with liver transplant in elderly people aged > 60 years have been reported recently.1 We reviewed our experience to determine the outcomes and problems of elderly patients with liver transplant.

Materials and Methods

Data of liver transplant recipients aged > 60 years were reviewed retrospectively. We analyzed 16 elderly patients who had liver transplant for chronic liver disease between 2001 and 2014 in our center. Data included age, sex, cause of liver disease, presence or absence of hepatocellular carcinoma (HCC), Child-Pugh, and Model for End-Stage Liver Disease (MELD) scores, past medical history (alcohol or tobacco abuse, arterial hypertension, diabetes mellitus requiring oral hypoglycemic agents or insulin, and hypercholesterolemia), survival and
cause of death, and incidence of the main post-
transplant complications (early acute rejection,
major infection, cardiovascular and neurologic
complications, postoperative dialysis requirement,
and incidence of de novo neoplasia).

A recipient for liver transplant had to satisfy
the following criteria: a high likelihood of having
a healthy daily life after successful living-donor
liver transplant; liver transplant was the only
treatment option to save the patient’s life; the
patient’s vital organs, other than the liver, showed
well-preserved function; there was no un-
controllable malignancy or active infection in any
organ except the liver; and the patient and
patient’s supporting family members were
expected to show good compliance with medical
treatment.

All of our patients satisfied these criteria. All
patients were examined in detail by the cardiology,
pulmonary, psychiatry, and infectious diseases
departments, and nonhepatic malignancies were
screened preoperatively.

After transplant, all patients were treated with
the same immunosuppressive protocol including
tacrolimus, mycophenolate mofetil, and pred-
nisolone. No protocol liver biopsy specimens were
obtained, and biopsies were performed only for
investigation of biochemical abnormalities such as
elevated serum transaminase or bilirubin levels.

### Results

In our series, there were 5 women and 11 men, aged
60 to 65 years (Table 1). The mean Child score was
7.9 ± 1.7 and MELD score was 14.1 ± 5.1. The most
common indication for liver transplant was hepatitis
B (n = 11; 68.7%) and 8 of these patients had HCC.
The other indications for liver transplant were
hepatitis C virus (n = 2), cryptogenic cirrhosis (n = 2),
and alcoholic cirrhosis (n = 1). Preoperatively, 2
patients had diabetes mellitus that required oral
hypoglycemic agents, and 1 patient had coronary
artery disease that was treated with an endovascular
stent.

After transplant, all patients stayed in the
intensive care unit 1 day, and mean hospitalization
was 18.1 ± 10.6 days. There were 3 patients who
needed hemodialysis because of acute renal failure
early after transplant. During follow-up, creatinine
levels were normal. In 1 patient, hepatic artery
thrombosis was observed on postoperative day 5 and
was treated with an endovascular stent. There were
4 patients who died during follow-up, including 3
patients who died from sepsis; the causative
pathogen was Acinetobacter in 2 patients and
Aspergillus in 1 patient. During follow-up, major
infection was not observed in the other 13 patients.
Only 1 patient had 1 acute rejection episode and this
was treated with pulse steroids. The 5-year survival

<table>
<thead>
<tr>
<th>Age at Liver Transplant (y)</th>
<th>Current Age (y)</th>
<th>Sex</th>
<th>Indication For Liver Transplant</th>
<th>Child Class</th>
<th>MELD Score</th>
<th>Donor Type</th>
<th>Acute Rejection</th>
<th>Outcomes</th>
</tr>
</thead>
<tbody>
<tr>
<td>60</td>
<td>68</td>
<td>M</td>
<td>HBV</td>
<td>C</td>
<td>19</td>
<td>LDLT</td>
<td>-</td>
<td>Alive</td>
</tr>
<tr>
<td>60</td>
<td>66</td>
<td>M</td>
<td>Cryptogenic cirrhosis</td>
<td>C</td>
<td>19</td>
<td>LDLT</td>
<td>-</td>
<td>Alive</td>
</tr>
<tr>
<td>61</td>
<td>70</td>
<td>M</td>
<td>HCC (HBV)</td>
<td>A</td>
<td>10</td>
<td>LDLT</td>
<td>-</td>
<td>Sepsis (40 d after liver transplant)</td>
</tr>
<tr>
<td>61</td>
<td>-</td>
<td>F</td>
<td>HCV</td>
<td>B</td>
<td>25</td>
<td>LDLT</td>
<td>-</td>
<td>Sepsis (26 mo after liver transplant)</td>
</tr>
<tr>
<td>62</td>
<td>-</td>
<td>M</td>
<td>Alcoholic cirrhosis</td>
<td>A</td>
<td>25</td>
<td>LDLT</td>
<td>-</td>
<td>Sepsis (30 d after liver transplant)</td>
</tr>
<tr>
<td>62</td>
<td>69</td>
<td>M</td>
<td>HBV</td>
<td>B</td>
<td>16</td>
<td>LDLT</td>
<td>-</td>
<td>Alive</td>
</tr>
<tr>
<td>63</td>
<td>-</td>
<td>M</td>
<td>HCC (HBV)</td>
<td>A</td>
<td>13</td>
<td>LDLT</td>
<td>-</td>
<td>Alive</td>
</tr>
<tr>
<td>63</td>
<td>74</td>
<td>F</td>
<td>HBV</td>
<td>C</td>
<td>16</td>
<td>LDLT</td>
<td>-</td>
<td>Myocardial infarction (45 d after liver transplant)</td>
</tr>
<tr>
<td>64</td>
<td>72</td>
<td>F</td>
<td>HCC (HBV)</td>
<td>B</td>
<td>19</td>
<td>LDLT</td>
<td>-</td>
<td>Alive</td>
</tr>
<tr>
<td>64</td>
<td>71</td>
<td>M</td>
<td>HCC (HBV)</td>
<td>A</td>
<td>11</td>
<td>LDLT</td>
<td>*</td>
<td>Alive</td>
</tr>
<tr>
<td>65</td>
<td>74</td>
<td>M</td>
<td>HCC (HBV) and alcoholic cirrhosis</td>
<td>A</td>
<td>11</td>
<td>LDLT</td>
<td>-</td>
<td>Alive</td>
</tr>
<tr>
<td>65</td>
<td>73</td>
<td>M</td>
<td>HCC (HBV)</td>
<td>B</td>
<td>17</td>
<td>LDLT</td>
<td>-</td>
<td>HCC (17 mo after liver transplant)</td>
</tr>
<tr>
<td>65</td>
<td>72</td>
<td>M</td>
<td>HCC (HBV)</td>
<td>A</td>
<td>7</td>
<td>LDLT</td>
<td>-</td>
<td>HCC (17 mo after liver transplant)</td>
</tr>
</tbody>
</table>

**Abbreviations:**
- DDLT, deceased-donor liver transplant; F, female; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; HCV, hepatitis C virus;
- LDLT, living-donor liver transplant; M, male; MELD, model for end-stage liver disease
rate was 75%. In our series, de novo neoplasia was not observed in any patient.

Discussion

Numerous studies have confirmed that transplant surgery can be performed safely in elderly patients. Investigators have found no differences in perioperative events, length of hospitalization, postoperative complications, incidence of rejection, and short-term survival in elderly liver recipients compared with cohorts of younger adults.3 Individual centers have shown that there is no significant difference in outcome between recipients aged > 60 years compared with younger recipients.4

Many centers have reported that the most common causes of mortality in elderly liver transplant patients were de novo or recurrent malignancy, but the incidence of these events was low.2,3 In addition to posttransplant lymphoproliferative disorder, solid tumors were observed including tumors of the colon, pancreas, lung, and breast. In a study of > 300 cases of liver transplant, de novo malignancy was the leading cause of death 3 years after liver transplant, and the risks for malignancy were advanced age, smoking, Epstein-Barr virus, and sun exposure.5 In contrast with reports in the literature, in our series de novo neoplasia was not observed in any patient and recurrent malignancy was observed only in 1 patient.

Many centers have reported fewer episodes of acute cellular rejection in older patients.5,6 In some studies, researchers have shown defects in both cell mediated and humoral immune systems of the elderly.7 The humoral defect is thought to be the result of an exaggerated anti-idiotype antibody response to antigens that down-regulates the antibody response to those antigens, thus inhibiting the humoral response.8,9 Depending on this defective immunity, it might be expected that a lower incidence of acute rejection and higher rate of opportunistic infections may occur in elderly liver transplant patients. The most common cause of fatal infection in older patients was fungal sepsis.3 In the present study, similar to the literature, we observed only 1 acute rejection episode and 3 of our patients died from bacterial and fungal infections.

In previous studies, the presence of comorbid disease and high MELD and Child scores were independent risk factors for prognosis.10-13 Although there is not a sufficient number of patients in our series, we could not say that the prognosis for patients with comorbid disease and high MELD score was worse.

In conclusion, liver transplant should not be withheld from older recipients on the basis of age alone. However, comprehensive screening for comorbidities should be performed. Patients aged > 60 years have low rejection rates and excellent graft survival. Careful screening is necessary to detect de novo malignancy and disease recurrence.

References

Efficacy and Safety of Lamivudine or Tenofovir Plus Intramuscular Hepatitis B Immunoglobulin in Prevention of Hepatitis B Virus Reinfection After Liver Transplant

Mohssem Nassiri-Toosi,2 Amir Kasraianfard,3 Zahra Ahmadinejad,1 Habibollah Dashti,3 Majid Moini,3 Atabak Najafi,3 Javad Salimi,3 Ali Jafarian4

Abstract

Objectives: Hepatitis B immunoglobulin prophylaxis in combination with antiviral drugs is recommended for prevention of hepatitis B virus reinfection after liver transplant. However, there is no consensus on a standard prophylactic method, and controversy exists over the duration, dose, and route of administration. We conducted a prospective study to evaluate the safety and effectiveness of intramuscular hepatitis B immunoglobulin in combination with lamivudine and/or tenofovir and discontinuation of hepatitis B immunoglobulin after 1 year for prevention of hepatitis B virus reinfection.

Materials and Methods: Patients with hepatitis B-related liver cirrhosis who had undergone primary liver transplants were enrolled. The prophylactic protocol involved intraoperative intramuscular hepatitis B immunoglobulin at 10 000 IU, tapering to 5000 IU daily for the first 6 days, weekly for a month, every 2 weeks for the next month, and monthly for a year after liver transplant, in combination with antiviral drugs.

Results: From January 2002 until March 2014, two hundred sixty-eight liver transplants were performed. Forty-four patients (16.4%) who underwent liver transplants due to hepatitis B-related liver failure were enrolled. Five patients had hepatocellular carcinoma; 20 had both hepatitis D and hepatitis B virus infection. The median age was 47 years (range, 26-59 y) with a median model for end stage liver disease score of 20. Thirty-three patients were men (76%). Sixty-one percent of patients were negative for hepatitis B virus DNA at the time of transplant. The median follow-up was 13.6 months (range, 0-142 mo). Only 1 patient (2.3%) experienced hepatitis B virus reinfection (at 44.7 months posttransplant), which was successfully treated with tenofovir. Five patients died (11.4%) during the follow-up from nonhepatitis B causes.

Conclusions: Intramuscular hepatitis B immunoglobulin in combination with lamivudine or tenofovir and discontinuation of hepatitis B immunoglobulin after 1 year posttransplant may provide safe and cost-effective protection against posttransplant hepatitis B reinfection.

Key words: HBV reinfection, HBig, Intramuscular HBig

Introduction

Liver transplant is the treatment of choice for hepatitis B virus (HBV)-related liver failure;1 HBV accounts for approximately 5% to 10% of liver transplants in the United States. However, it is the leading indicator for liver transplant in Asia.2,3 In our center, HBV-related liver failure is the third leading cause for liver transplants with a liver transplant rate of 19% after cryptogenic cirrhosis and autoimmune hepatitis (AIH).4 Our center for liver transplants was started in 2002. At that time, chronic hepatitis B disease was the second most common cause of primary liver disease for patients referred to our
center for liver transplants. Since 2002, five hundred forty-three patients with cirrhosis have been registered on the waiting list, and after cryptogenic cirrhosis, hepatitis B-related cirrhosis is the second most common diagnosis as the primary cause of liver disease leading to a need for transplant. Other diagnoses—autoimmune hepatitis (AIH), hepatitis C virus (HCV), primary sclerosing cholangitis (PSC), and Wilson disease—are also linked to a need for liver transplant (Table 1).

Without prophylactic treatment, the rate of posttransplant allograft reinfection is as high as 80% to 100%, often leading to aggressive recurrent hepatitis, subsequent HBV-related liver failure, and death. In the last 2 decades, with the advent of hepatitis B immunoglobulin (HBIg) and antiviral drugs as prophylaxis against HBV recurrence after liver transplants, the HBV reinfection and 5-year patient mortality rates have been reduced to 10% and 20%, respectively.

Hepatitis B immunoglobulin prophylaxis in combination with the nucleoside/nucleotide analogs (NAs) lamivudine or tenofovir is a well-accepted treatment recommended by many centers for prevention of HBV reinfection after liver transplants. However, there is no consensus on a standard prophylactic method, and controversy over the duration, dose, and route of administration of HBIg exists among transplant centers.

We conducted this prospective study to evaluate the safety and effectiveness of intramuscular HBIg in combination with lamivudine or tenofovir and discontinuation of HBIg after 1 year, for prevention of HBV reinfection after liver transplants.

Materials and Methods

Patients with HBV-related liver failure who underwent primary liver transplants in the Imam Khomeini Hospital, Tehran University of Medical Sciences from 2002 to March 2014 were enrolled in the study. Data were recorded prospectively. All protocols, experimental studies, and clinical trials involving human subjects were approved by the ethics committee of the institution before the study began, and protocols conformed to the ethical guidelines of the 1975 Helsinki Declaration. Written informed consent was obtained from patients or their guardians. In a review of 82 medical files of patients with cirrhosis resulting from HBV, 52% had undetectable serum HBV-DNA and 95% were hepatitis B e antigen (HBe Ag) negative and 55% were on one anti-viral drug (tenofovir). Seventeen percent of hepatitis B patients with cirrhosis on the waiting list at our center also had hepatitis D; coinfection with HDV was significantly higher in patients with undetectable virus. In our study, 2% of hepatitis B patients with cirrhosis had HCC as a complication resulting from cirrhosis. Patients with undetectable serum HBV DNA had a history of longer treatment duration with oral NAs. The median HBV viral load in HBV patients awaiting transplant was 10000 copies/mL, and there was no significant difference between viral load in HBV patients with detectable or undetectable serum virus. Thus, the majority of candidate patients on the transplant waiting list had a low viral load and were considered at low risk for hepatitis B after liver transplant (Table 2).

The major concern for patients with hepatitis B is recurrence of HBV infection in the new liver. Hepatitis B immunoglobulin, in combination with NAs, has been the standard of care recommended by many centers for prevention of HBV recurrence after liver transplants. However, there is no consensus on a standard prophylactic method. Controversy over the duration, dose, and route of administration of HBIg exists among transplant centers. Cost and access to intravenous (IV) HBIg are also concerns in some regions. Alternatively, low-dose intramuscular HBIg has been suggested as an equally effective, safe, less expensive, and more available substitution for IV HBIg which, in combination with NAs, can be the most cost-effective and efficient regimen for the prevention of posttransplant HBV recurrence. We used a prophylactic method of intraoperative intramuscular HBIg 10000 IU followed by 5000 IU daily for the first 6 days and weekly for a month. Intramuscular HBIg was administered from the second month until 1 year posttransplant, if the anti-HBs titer was lower than 250 IU/L. After the first year, HBIg was completely discontinued. Lamivudine was administered to 11 patients before and after transplants. Patients who had undergone liver transplants from 2012 (n = 33) received tenofovir...
instead of lamivudine before and after transplants. In our study, the definition of HBV recurrence included detectable levels of HBV DNA and/or the persistence or reappearance of serum HBsAg after its initial loss with or without clinical evidence of recurrent disease.10

Immunosuppressive therapy included treatment with 1000 mg IV methylprednisolone during the anhepatic stage, followed by a 3-drug regimen of corticosteroid, calcineurin inhibitor, and mycophenolate mofetil. Corticosteroid was tapered off after the first month, and mycophenolate mofetil was tapered after year 1.

Results

From January 2002 until March 2014, two hundred sixty-eight liver transplants were performed. Forty-four patients (16.4%) who underwent liver transplants due to HBV-related liver failure were enrolled in the study. Five patients had hepatocellular carcinoma and, in addition to HBV infection, twenty had hepatitis D virus. The median age was 47 years (range, 26-59) with a median model for end stage liver disease (MELD) score of 20. Thirty three patients were males (76%). Sixty-one percent of patients were negative for HBV DNA at the time of transplant. The median follow-up was 13.6 months (range, 0-142) (Table 3). HB Ab titers were recorded at week 1, month 1, month 6, and month 12. Additional doses of intramuscular HBIg were prescribed for certain patients (Figure 1). At 44.7 months post-transplant, 1 patient experienced HBV reinfection, but survived. He had received lamivudine before and after transplant and was successfully treated with the addition of tenofovir to lamivudine (Table 4). Thus, the overall rate of HBV reinfection was 2.3%. Five patients (11.4%) died during the follow-up from non-HBV causes. Deaths occurred at 1, 12, 19, 60, and 150 days posttransplant due to primary nonfunction, cardiac failure, hepatic artery thrombosis-related sepsis, sepsis, and posttransplant lymphoproliferative disorder (Table 5).

Discussion

Controversy exists over the best standard prophylactic method to prevent HBV reinfection after liver transplant; there is also little consensus among transplant centers concerning the duration,
Table 3. Characteristics and Demographics of HBV-Affected Patients With Cirrhosis After Liver Transplant (Tehran University of Medical Sciences), 2002-2014

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Total 82</th>
<th>HBs Ag +ve Detectable</th>
<th>HBs Ag +ve Undetectable</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>HBV DNA level (IU/mL)</td>
<td></td>
<td>(61,000 - 0)</td>
<td>(61,000 - 20)</td>
<td>N/S</td>
</tr>
<tr>
<td>Age (y)</td>
<td>47 (59 - 26)</td>
<td>49 (59 - 26)</td>
<td>46 (59 - 30)</td>
<td>N/S</td>
</tr>
<tr>
<td>Gender, male</td>
<td>76%</td>
<td>70%</td>
<td>80%</td>
<td>N/S</td>
</tr>
<tr>
<td>MELD score</td>
<td>20 (43 - 15)</td>
<td>20 (5-15)</td>
<td>20 (43 -15)</td>
<td>N/S</td>
</tr>
<tr>
<td>Follow up in waiting list, (y)</td>
<td>1 (5 - 1)</td>
<td>1 (5-1)</td>
<td>1 (5-1)</td>
<td>N/S</td>
</tr>
<tr>
<td>HBeAg, negative %</td>
<td>97%</td>
<td>98%</td>
<td>100%</td>
<td>N/S</td>
</tr>
<tr>
<td>Type of pretransplant antiviral therapy</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lamivudine</td>
<td>54.5%</td>
<td>42%</td>
<td>60%</td>
<td>N/S</td>
</tr>
<tr>
<td>Tenofovir</td>
<td>39.4%</td>
<td>46%</td>
<td>35%</td>
<td>N/S</td>
</tr>
<tr>
<td>Adefovir</td>
<td>6.1%</td>
<td>12%</td>
<td>5%</td>
<td>N/S</td>
</tr>
<tr>
<td>Pre Tx antiviral treatment (mo)</td>
<td>8 (72-2)</td>
<td>8 (72-2)</td>
<td>8.5 (36-2)</td>
<td>N/S</td>
</tr>
<tr>
<td>HDV positive, %</td>
<td>45%</td>
<td>25%</td>
<td>60%</td>
<td>N/S</td>
</tr>
<tr>
<td>HCC diagnosis, %</td>
<td>11.3%</td>
<td>17.6%</td>
<td>7.4%</td>
<td>N/S</td>
</tr>
</tbody>
</table>

Abbreviations: HBV DNA, hepatitis B virus deoxyribonucleic acid; HCC, hepatitis C carcinoma; HDV, hepatitis D virus; IU, international units; MELD, model for end-stage liver disease; Tx, treatment

Table 4. Characteristics of Patient With Recurrent Hepatitis B After Liver Transplant For Hepatitis B (Tehran University of Medical Sciences)

<table>
<thead>
<tr>
<th>Age (y)</th>
<th>Gender</th>
<th>HBe Ag</th>
<th>Pretransplant Antiviral</th>
<th>MELD</th>
<th>HCC</th>
<th>HDV</th>
<th>Pre liver Transplant</th>
<th>Follow-up After Liver Transplant</th>
<th>Time of HBeAg+ve</th>
</tr>
</thead>
<tbody>
<tr>
<td>46</td>
<td>Male</td>
<td>Positive</td>
<td>Lamivudine</td>
<td>21</td>
<td>No</td>
<td>No</td>
<td>0</td>
<td>7 y</td>
<td>4 y</td>
</tr>
</tbody>
</table>

Abbreviations: HBe Ag, hepatitis B e antigen; HBV DNA, hepatitis B virus deoxyribonucleic acid; HCC, hepatitis C carcinoma; HDV, hepatitis D virus; MELD, model for end-stage liver disease

Table 5. Characteristics of Patients Who Died After Liver Transplant (Tehran University of Medical Sciences)

<table>
<thead>
<tr>
<th>Follow-up after liver transplant (d)</th>
<th>Patient 1</th>
<th>Patient 2</th>
<th>Patient 3</th>
<th>Patient 4</th>
<th>Patient 5</th>
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<tr>
<td>Cause of death</td>
<td>1 day</td>
<td>12 days</td>
<td>19 days</td>
<td>60 days</td>
<td>150 days</td>
</tr>
<tr>
<td>Age (y)</td>
<td>37</td>
<td>26</td>
<td>43</td>
<td>47</td>
<td>49</td>
</tr>
<tr>
<td>Gender</td>
<td>Male</td>
<td>Male</td>
<td>Male</td>
<td>Male</td>
<td>Male</td>
</tr>
<tr>
<td>HBe Ag</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>Pretransplant antiviral</td>
<td>Lamivudine</td>
<td>Lamivudine</td>
<td>Lamivudine</td>
<td>Lamivudine</td>
<td>Adefovir</td>
</tr>
<tr>
<td>MELD score</td>
<td>24</td>
<td>19</td>
<td>23</td>
<td>15</td>
<td>22</td>
</tr>
<tr>
<td>HCC diagnosis</td>
<td>No</td>
<td>No</td>
<td>no</td>
<td>no</td>
<td>yes</td>
</tr>
<tr>
<td>HDV status</td>
<td>Positive</td>
<td>Negative</td>
<td>Negative</td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>HBV DNA level (IU/mL)</td>
<td>&lt;100</td>
<td>136</td>
<td>0</td>
<td>2296</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: HBe Ag, hepatitis B e antigen; HBV DNA, hepatitis B virus deoxyribonucleic acid; HCC, hepatitis C carcinoma; HDV, hepatitis D virus; MELD, model for end-stage liver disease
dose, and route of administration of HBIG and the type of NAs to be used. Major disadvantages of using HBIG for prevention of recurrent post-transplant HBV infection are its inconvenience administration, limited supply and very expensive costs. Thus, withdrawal from HBIG treatment is highly desirable; however, the safety, efficacy and timing of withdrawal are not well-understood.

Samuel and associates demonstrated that a high-dose IV HBIG protocol, including 10 000 IU intraoperatively daily for the first posttransplant week followed by 10 000 IU monthly thereafter, resulted in 29% HBV reinfection rate after 2 years of transplant. However, major disadvantages of high-dose IV HBIG for prevention of posttransplant HBV reinfection include its high cost, decreased efficacy in patients who are HBVDNA/HBeAg positive at time of transplant, and development of resistance due to genetic HBV mutants.

To reduce the amount of HBIG needed, individualized doses of HBIG are based on anti-HB titers; many transplant centers adjust the dose of HBIG accordingly, to maintain the anti-HB titer at a protective level. Additionally, intramuscular administration of HBIG reduces the amount of HBIG needed. In some studies, intramuscular HBIG during the posttransplant period is equally effective to IV HBIG; it has resulted in reduction of anti-HB titers to the same levels as IV HBIG, to a 0% to 24% HBV reinfection rate after liver transplant. The great advantage of intramuscular administration of HBIG, compared to the IV route, is a substantial reduction in HBIG dose needed to achieve adequate titers, as well as cost reduction. However, intramuscular administration involves a painful injection. The combination of HBIG and NAs is more effective against HBV reinfection after liver transplant than HBIG alone, because of synergistic activity; the combination results in a greater reduction in the needed time course and doses of HBIG.

A recent systematic review by Cholongitas and associates, showed that HBIG could safely be replaced with potent NAs, however the timing of HBIG withdrawal is not clear and further studies are required. In a randomized trial by Buti and associates, HBIG was discontinued in 20 patients soon after liver transplant and lamivudine was continued indefinitely; in 9 other patients, HBIG with lamivudine were continued. At 91 months of follow-up, 15% of patients for whom HBIG was discontinued showed signs of HBV reinfection, a rate similar to the 11% of patients receiving HBIG with lamivudine. In another randomized study, patients received HBIG with lamivudine, but HBIG was replaced with adefovir at the 1 year posttransplant. These patients were compared to 18 patients who received HBIG in combination with lamivudine indefinitely. After 24 months, HBV reinfection rates were similar in both groups (1/18 vs 0/18). In a prospective study of 58 patients, a prophylactic method included lamivudine in combination with IV HBIG 10 000 IU intraoperatively, 2000 IU daily for a week, 2000 IU (when anti-HB titers were less than 100 IU/L), and discontinuation of HBIG at the 1 year posttransplant timepoint. After 4 years of follow-up, 6.8% of patients were positive for HBV reinfection. In 2011, Stravitz and associates showed that HBIG withdrawal and continuing tenofovir with emtricitabine is a safe and effective method against HBV reinfection. In our study, after approximately 2 years of HBIG withdrawal and prophylactic treatment with lamivudine or tenofovir, only 1 patient experienced HBV reinfection.

Hepatitis B virus antiviral therapy combined with potent NAs before transplant decreases both the viral load and the risk of reinfection; it achieves this by lowering the amount of circulating virus at the time of transplant and by prolonging the half-life of HBIG. Low dose intramuscular HBIG combined with NAs has been successful in preventing reinfection in patients with low detectable HBV DNA (particularly those with a viral load less than 10 000 copies/mL) and negative HBe Ag pretransplant. Antiviral therapy with tenofovir, a potent drug with a very low risk for resistance, could result in complete viral suppression after liver transplants. With increased availability of tenofovir and other potent NAs, the
role of HBIG is evolving. Potent NAs alone without HBIG might suffice in preventing recurrent hepatitis B, but longer follow ups and precautions are needed in patients with HDV, HIV, and HCC hepatitis B.

This current prospective study suggests that the use of intramuscular HBIG in combination with lamivudine or tenofovir, and discontinuation of HBIG after 1 year posttransplant, may be a safe and cost-effective method to prevent HBV reinfection in patients who are HBV DNA negative at the time of transplant. These findings require follow-up to confirm their clinical significance.

References

Abstract

Objectives: Hepatitis B and D virus coinfection or superinfection lead to chronic liver disease and have poor treatment results and poor prognosis. After transplant, these patients have difficult problems. We aimed to report long-term data of liver transplant recipients who had hepatitis B and D virus-related chronic liver disease.

Materials and Methods: This retrospective, longitudinal study included 25 consecutive hepatitis B surface antigen-positive patients with anti-hepatitis D virus antibodies. Patient data (age, sex, antiviral treatment, posttransplant use of hepatitis B hyperimmunoglobulin and/or nucleoside/nucleotide analogues, the presence of hepatocellular carcinoma, age at transplant, follow-up) were extracted from patient records.

Results: Females comprised 32% patients. The median age was 44 years (range, 23-63 y). The serum Hepatitis B envelope antigen level was negative in all patients. At the time of transplant, 4 patients were positive for hepatitis B virus DNA and 11 patients also had hepatocellular carcinoma. Posttransplant follow-up was 59 months (range, 3-120 mo). During follow-up, 4 patients died, 4 patients were lost to follow-up, and 17 patients were alive. Posttransplant survival of patients with hepatocellular carcinoma was 50.45 months (range, 3-84 mo) and without hepatocellular carcinoma was 65.8 months (range, 4-120 mo). There were 3 patients who had acute rejection and were treated successfully with pulse doses of prednisolone. Hyperimmunoglobulin therapy was used in conjunction with oral nucleotide/nucleoside analogues for 12 months (range, 3-24 mo) and then stopped. After transplant, 4 patients had antiviral medicine changed to adefovir or entecavir because of drug resistance, and otherwise all patients remained negative for hepatitis B virus DNA during follow-up.

Conclusions: Patients transplanted for hepatitis B and D virus cirrhosis, even with hepatocellular carcinoma, had favorable prognosis and good long-term results. Close follow-up of patients and effective viral suppression with suitable drugs were key factors for efficient patient care.

Key words: Antiviral, Cirrhosis, Coinfection, Hepatocellular carcinoma, Therapy

Introduction

Rizzetto and colleagues discovered hepatitis delta virus (HDV) in the mid-1970s while investigating a group of patients with hepatitis B virus (HBV) who had severe hepatitis. The HDV is a single-stranded RNA virus similar to viroids of plants that is coated in hepatitis B surface antigen (HBsAg), and presence of HBV is crucial for the completion of its life cycle. The HDV is thought to be entirely dependent on HBV for its replication and expression. Infection by HDV can be either a coinfection (simultaneous transmission with HBV) or superinfection (new infection in people who already are chronic HBV carriers). It has been estimated that 15 to 20 million people worldwide have chronic HDV infection, and there are substantial
geographic differences. Approximately 5% HBsAg-positive patients also are infected with HDV. The HDV infection has a worldwide distribution and is endemic in the Middle East, Mediterranean area, Amazon region, and many African countries.

Chronic HDV occurs as superinfection in 90% and coinfection in 10% patients. Chronic HDV infection frequently is associated with active chronic hepatitis that leads to cirrhosis in 70% patients in 5 to 10 years. Patients with chronic HDV infections have more rapidly progressive liver damage than patients infected with HBV alone. The incidence of cirrhosis is 3-fold higher in patients with HBV/HDV chronic coinfection than chronic HBV monoinfection, with a higher risk of early decompensation and development of hepatocellular carcinoma (HCC). Therefore, HDV infection in the world is an important health burden. The only established treatment for chronic HDV is interferon at high doses, but interferon therapy is associated with therapeutic success in only 25% to 30% treatments.

Chronic infection with HDV is a risk factor for cirrhosis and HCC. However, a high proportion of patients are lost due to cirrhosis and its complications or HCC unless they receive orthotopic liver transplant. The HBV DNA integrates into the host DNA and shows a series of potentially oncogenic properties, but HBV is not an acutely transforming virus because HCC develops decades after infection. Other factors such as cirrhosis, inflammation, alcohol intake, and viral superinfection could promote the oncogenic process induced by HBV DNA integration.

After transplant, these patients have different and difficult problems. The aim of our study was to report the long-term data of our liver transplant recipients who had HBV/HDV-related chronic liver disease. In this retrospective cohort, we examined posttransplant follow-up data that extended up to 10 years.

**Materials and Methods**

**Patients and hepatitis assays**

This study was a retrospective, longitudinal study that included 25 consecutive HBsAg-positive patients with anti-HDV antibodies who were referred to the Department of Gastroenterology and Hepatology, Baskent University Medical Faculty between 2003 and 2014. The HBV and HDV infections were diagnosed by commercially available enzyme-linked immunosorbent assays for HBsAg and anti-HDV antibody. The data about patients were extracted from patient records including age, sex, pretransplant treatment, pretransplant liver imaging, explant liver pathology, antiviral treatment, posttransplant use of hepatitis B hyperimmunoglobulin (HB Ig) and/or nucleoside/nucleotide analogues, presence of HCC, follow-up, and tumor recurrence.

**Other serum assays**

Pretransplant serologic profiles were analyzed thoroughly including HBsAg, AntiHBs, hepatitis B e antigen (HBeAg), hepatitis B core immunoglobulin G (HBc-IgG), antihepatitis C virus (anti-HCV), anti-HDV, HBV DNA, and HDV RNA. Lamivudine-resistant mutations were evaluated in patients who had virologic (HBV DNA) or biochemical (liver enzyme) nonresponse or HBV reactivation, which was defined by reappearance or elevation of HBV DNA in patients who were inactive previously.

**Liver imaging**

The imaging of the liver was performed by both ultrasonography and dynamic liver computerized tomography before and after transplant when needed.

**Liver biopsy**

The histopathologic examination of liver tissue samples was performed before and after liver transplant. The pathologic examination focused mainly on general evaluation before transplant, presence of posttransplant rejection, and evaluation of possible recurrence of HBV or HDV hepatitis. Explanted livers of all patients were examined.

**Statistical analyses**

Statistical analyses were performed with software (IBM SPSS Statistics for Windows, Version 21.0, IBM Corp., Armonk, NY, USA). The chi-square test and Fisher exact test were used to compare nominal data between groups. Comparison of numeric variables was performed with Mann-Whitney U test. The level of significance was considered to be $P \leq .05$.

**Results**

The data of 25 patients with chronic HBV/HDV and HBeAg negative who underwent liver transplant
were evaluated. Females comprised 32% patients (17 male and 8 female patients). The median age was 44 years (range, 23–63 y) at transplant. The mean pretransplant waiting period after listing was 19 months (range, 1–54 mo) (Table 1). After liver transplant, the patients were treated with one or with multiple immunosuppressive therapy such as mycophenolate mofetil, tacrolimus, sirolimus, and everolimus.

<table>
<thead>
<tr>
<th>Table 1. Basic Characteristics of Patients*</th>
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<tbody>
<tr>
<td>Characteristics</td>
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<tr>
<td>Sex (male/female)</td>
</tr>
<tr>
<td>Age at transplant (y)</td>
</tr>
<tr>
<td>Donor (living/deceased)</td>
</tr>
<tr>
<td>Pretransplant follow-up (mo)</td>
</tr>
<tr>
<td>Interferon treatment before transplant</td>
</tr>
<tr>
<td>Acute rejection</td>
</tr>
<tr>
<td>Follow-up after transplant (mo)</td>
</tr>
<tr>
<td>Patients with hepatocellular carcinoma</td>
</tr>
<tr>
<td>Patients matching Milan criteria</td>
</tr>
</tbody>
</table>

*Data reported as number or mean (range, minimum-maximum).

At transplant in all patients, serum HBsAg levels were positive but AntiHBs and HBeAg levels were negative. Interferon treatment was used prior to transplant in 6 patients; 2 of these patients received interferon for 2 months and 7 months, and 4 patients completed 12 months interferon treatment.

At transplant, 4 patients tested positive for HBV-DNA and these patients were started on entecavir or lamivudine therapy. The other 2 patients tested positive under lamivudine treatment; 1 of these patients was changed to adefovir and the other patient had adefovir add-on treatment. There were 20 patients who used lamivudine treatment and 1 patient received adefovir treatment.

All patients had liver cirrhosis before transplant according to imaging techniques, liver biopsies, and examination of explanted livers. The examination of explanted livers showed HCC in 11 patients, including 2 patients in whom the tumors were not detected by imaging techniques. The application of Milan criteria revealed that only 7 of 11 tumors matched Milan criteria and 4 patients did not match Milan criteria. There were 24 patients who had living-donor transplant and 1 patient who had deceased-donor transplant.

Hyperimmunoglobulin therapy was used in conjunction with oral nucleotide/nucleoside analogues for 12 months (range, 3–24 mo) and then stopped. After transplant, 4 patients had antiviral medicine changed to either entecavir or adefovir because of drug resistance, and otherwise all patients remained HBV-DNA-negative during follow-up. After stopping hyperimmunoglobulin therapy, 6 patients were HBsAg-positive and 8 patients remained anti-HBs-positive.

Recurrence of HCC was observed in 2 patients (not matching Milan criteria) at 2 years after transplant. There was 1 patient who was lost to follow-up, and the other patient was successfully treated with radiofrequency ablation, transarterial chemoembolization, and sorafenib treatment.

Posttransplant survival of patients with HCC was 50.45 months (range, 3–84 mo) and without HCC was 65.8 months (range, 4–120 mo). Survival plots in patients with or without hepatocellular carcinoma were shown (Figure 1). The 1-, 3-, and 5-year survival after liver transplant in our patients with or without hepatocellular carcinoma was shown (Figure 2).

Posttransplant follow-up was 59 months (range, 3–120 mo). During follow-up, 4 patients died, 4 patients were lost to follow-up, and 17 patients were alive. The timing of death was within 5 months after
transplant in 3 patients, and the fourth patient died at 72 months posttransplant. Cause of death was pulmonary aspergillosis in 1 patient and sepsis in 3 patients. In 4 patients lost to follow-up, mean follow-up was 48.5 months (range, 24-84 mo). There were 3 patients who experienced acute rejection and were treated successfully with pulse doses of prednisolone.

Discussion

Persistent HBV/HDV coinfection results in end-stage liver disease and 15% mortality.17 The range of clinical presentation is wide, varying from mild disease to fulminant liver failure, end-stage liver disease, and HCC. Although HDV infection is less commonly observed, chronic HDV infection leads to more severe liver disease than HBV monoinfection. This accelerated phase is characterized by accelerated fibrosis progression, earlier hepatic decompensation, and an increased risk for the development of HCC.18,19 The incidence of HDV infection has decreased in endemic countries as a result of effective immunoprophylaxis against HBV and improvement in socioeconomic and hygienic conditions.20-22 The only definitive therapy for patients with end-stage liver disease, HCC, or fulminant hepatitis due to HDV is liver transplant.23 The HBV/HDV coinfection was noted in 2% patients who had liver transplant in 2009 across Europe.24

Chronic liver disease related to HBV/HDV coinfection has 7% to 9% annual mortality rate. The 2- to 5-year follow-up of these patients in developing cirrhosis is 20% to 50%. In a study by Gheorghe and associates, HDV-related cirrhosis had a median time to decompensation < 2 years and median survival < 5 years, and almost all deaths were liver-related.25 In another study, a total of 299 HBV/HDV patients had been followed for a mean 223 months. At the time of first evaluation, 113 patients had chronic hepatitis and 186 patients had cirrhosis. In the chronic hepatitis subgroup, 75 patients were alive, 9 patients were dead, and 29 patients were lost to follow-up with neither decompensation nor HCC. In the cirrhosis subgroup, 46 patients had developed HCC and 54 patients had progressed to decompensated cirrhosis.17 Romeo and associates reported that among the 105 patients with chronic hepatitis at baseline, 31 patients (29%) developed cirrhosis, 10 patients (9%) developed HCC, 7 patients (6%) developed liver decompensation, and 7 patients (6%) died of liver-related events.14

Chronic HDV has been considered primarily an HBeAg-negative disease. Biochemical activity of liver disease is more severe in patients who have HDV with HBeAg-positive than HBeAg-negative serology. The HBeAg-positive patients have significantly higher HBsAg levels than HBeAg-negative patients.26 Although severe HDV occurs in patients who are HBeAg-positive,27,28 the percentage of liver transplant recipients is higher in HBeAg-negative patients. In our patients, the anti-HBe antibody positive rate was 100%. This comparison is not available in our study because all patients were anti-HDV-positive/HBeAg-negative.

The survival of patients depends on the prevention of allograft reinfection, acute or chronic rejection, and progression in patients who have recurrent disease. Data from different centers reveal a range of 77% to 85% 5-year posttransplant survival in patients who have HDV-related chronic liver disease.29-31 The cumulative 1-, 3- and 5-year surveillance in our patients was 88%, 79%, and 74%.

The overall outcomes following liver transplant are better in patients with HDV than patients transplanted for HBV alone.29,31,32 The presence of HDV infection appears to provide a protective effect against HBV reinfection, possibly via suppression of HBV replication.33 In contrary to the well-known belief that the presence of HDV suppressed HBV replication, a recent study showed a higher degree of replicative activity of either or both viruses.34 In patients with HBV/HDV coinfection after transplant, recurrence of HBV or HDV can occur. In the 1990s, several studies on patients with liver transplant for HDV-related liver disease showed that patients who had no or short-term HBIg prophylaxis showed a high rate of HDV reinfection (70%-82%) with a lower rate of hepatitis recurrence (40%) in the graft recipients and a milder course of hepatitis than patients who had HBV.29,35 The clinical course after transplant can be improved further by long-term administration of HBIg with recurrence of HBsAg in only 9% patients.31 The use of low-dose intramuscular HBIg and antiviral prophylaxis in combination results in < 5% rate of recurrent HBV-HDV infection.

There are various strategies for using HBIg in the posttransplant setting, and in our institution we have a definite period of HBIg use which is limited to a
maximum 24 months posttransplant. Before routine prophylaxis against HBV, recurrence was almost universal in the 1990s. Although there are several regimens available against viral recurrence after liver transplant, HBIG is given in our patients. In our center, the dosing of HBIG is 10000 IU in the anhepatic phase, 2000 IU daily during the first week, and subsequent doses to maintain anti-HBs levels > 100 IU/L for mean 12 months (3-24 mo). The oral nucleoside/nucleotide analogues started before liver transplant were continued after liver transplant. Only 4 patients required modification because the viral breakthrough on lamivudine (3 patients) or deterioration of renal function on adefovir (1 patient). At last follow-up, HBsAg was positive in only 3 of 17 patients, with 2 patients at 6 months after transplant, but we do not know the long-term results. In all other 14 patients that were negative for HBsAg and HBV DNA are provided. In all patients, HBV suppression (defined by HBV DNA negativity) was achieved by oral antiviral prophylaxis by lamivudine (12 patients), entecavir (3 patients), and adefovir (2 patients).

In recent years, much published evidence suggests that there is an increased risk of malignant transformation in the presence of HDV/HBV coinfection than HBV infection alone. Cells infected with HDV have altered gene expression and cellular responses, which is also evident from augmented expression of proinflammatory, growth, and antiapoptotic factors. In an Italian study, 9% patients were coinfected with HBV and HDV, and 13% of those patients that had cirrhosis developed HCC during a 10-year follow-up. Romeo and associates diagnosed HCC in 46 of 299 patients (15%) and reported that the risk of developing HCC was affected by previous treatment with interferon and persistent HBV replication. Fattovich and associates reported that HCC developed in 5 patients (13%) that were anti-HDV-positive/HBeAg-negative and 19 patients (16%) that were anti-HDV negative/HBeAg-negative with cirrhosis; concomitant HDV infection was associated with a 3-fold increased risk of HCC and 2-fold increased risk of mortality. In a retrospective study with HBV/HDV, 29 of 299 patients diagnosed with HBV/HDV had liver transplant; in these 29 patients, 10 patients (34%) with HCC were available. After transplant, 5 patients died (3 with primary graft failure, 1 with tumor recurrence, and 1 with nonliver–cancer-related reasons).

Patients with HCC matching Milan criteria are most likely to benefit from liver transplant, with 5-year survival rates of 70% and a recurrence rate < 10%. The application of Milan criteria revealed that only 7 of 11 tumors were matching with Milan criteria and 4 patients were not matching Milan criteria (in whom 2 patients had HCC recurrence after 2 years). The recurrent tumors were treated by ablation techniques and this resulted in excellent 5-year survival.

In conclusion, we presented our liver transplant experience in patients with HBV/HDV. Our data suggest that in patients with liver transplant who have HBV/HDV-positive hepatitis, anti-HBs immunoglobulin, and antiviral drugs may be useful by reducing HBV and HDV reinfection. In our patient cohort, recurrent HCC was successfully treated by radiologic interventions such as transcatheter arterial chemoembolization, radiofrequency ablation, and sorafenib. Viral prophylaxis changes the natural history of liver disease after liver transplant. Definite use of HBIG is an original treatment strategy in these patients.

References


Predictors of Tumor-Free Survival After Liver Transplant in Patient With Hepatocellular Carcinoma

Alireza Shamsaeefar,1 Saman Nikeghbalian,1 Kourosh Kazemi,1 Siavash Gholami,1 Nasrin Motazedian,2 Nadia Motazedian,2 Mohammad Ebrahim Fallahzadeh,1 Maryam Moini,1 Bita Gramizadeh,3 Seyed Ali Malekhosseini1

Abstract

Objectives: To identify the predictors of overall survival and tumor-free survival of 88 hepatocellular carcinoma patients who were treated with orthotopic liver transplant at Shiraz Organ Transplant Center.

Materials and Methods: We performed this retrospective study after reviewing the transplant database of all patients who underwent orthotopic liver transplant secondary to hepatocellular carcinoma and liver cirrhosis. Hepatocellular carcinoma was diagnosed in 70 patients before liver transplant and 18 patients on histologic examination of the explanted livers. Cox regression identified independent factors that affected post-transplant survival.

Results: The overall survival rate was 83% and the tumor-free survival rate was 79.5%. Independent factors for tumor recurrence were Milan criteria, alpha-fetoprotein level before operation ≥ 400 ng/mL, tumor grade, vascular invasion, and age. Vascular invasion (odds ratio, 5; 95% confidence interval, 1.1 to 25.496; \( P = .049 \)) and tumor grade (odds ratio, 14.42; 95% confidence interval, 3.652 to 56.95; \( P < .001 \)) were statistically significant.

Conclusions: Vascular invasion and tumor grade were predictive factors for tumor-free survival.

Key words: Cancer, End-stage liver disease, Outcome, Vascular

Introduction

Hepatocellular carcinoma (HCC), with an annual incidence of more than half a million cases, is the most common primary hepatic carcinoma and the fifth most common cause of cancer-related mortality globally. This neoplasm almost invariably arises in the setting of cirrhosis induced by hepatitis B virus (HBV) or hepatitis C virus (HCV) at a rate of 2.5% per year.1,2

The incidence of HCC has doubled in the past 20 years, with an estimated 8500 to 11 000 new cases annually in the United States. The global burden of HCC is expected to double in the next few decades HCV accounted for this increase from the 1970s and 1980s. Overall, HCC is a lethal malignancy with poor outcomes regardless of treatment, with overall 5-year survival rate 20% to 40%. Moreover, survival without transplant in patients with HCC and end-stage cirrhosis is estimated at < 1 year. Chronic HCV hepatitis, chronic HBV infection, exposure to aflatoxins, tyrosinemia, exogenous hormone intake, and heavy alcohol use are important risk factors associated with the development of HCC.3,4

Orthotopic liver transplant (OLT) remains the preferred curative modality for patients with cirrhosis and HCC because it removes the tumor and premalignant cirrhotic liver, which is the main risk factor for recurrence. Nonetheless, recurrence after OLT is occurring with growing evidence, and many reports suggest that the tumor biology of HCC (perhaps the most important predictor of HCC recurrence) can be highly variable. The HCV, as the
most important risk factor for HCC, typically affects patients who are older, have more advanced liver disease, and have higher numbers of neoplastic lesions at diagnosis, and HCV leads to higher risk of HCC recurrence after resection.5

Various factors (such as size and number of lesions) should be identified at the time of patient selection to reduce recurrence of HCC after liver transplant. Based on this fact, several criteria have been introduced such as Milan criteria (single tumor diameter ≤ 5 cm or 3 tumors with diameter ≤ 3 cm) and University of California, San Francisco (UCSF) criteria (single tumor < 6.5 cm; maximum of 3 tumors with the largest < 4.5 cm; and cumulative tumor size < 8 cm).2 A study conducted by Yao et al. showed several important predictors of survival in univariate analysis such as α-fetoprotein (AFP) level > 1000 ng/mL, total tumor diameter > 8 cm, age ≥ 55 years, and poorly differentiated histologic grade. Only tumor stage and total tumor diameter remained statistically significant in multivariate analysis.6

Some studies showed that liver transplant offers better outcomes than other strategies, with expected 5-year survival 70% to 90%. Despite these good results, 10% patients experience posttransplant HCC recurrence, many ending with death.7

Although the true prevalence of HCC in Iran is unknown, it is not an uncommon malignancy; 80% HCC cases in Iran are positive for at least 1 of the markers of HBV, and this virus appears to be the most common cause of HCC in Iran.8

The primary aim of our study was to identify the potential predictors of survival and tumor-free survival in a cohort of 88 HCC patients who were treated with OLT at Shiraz Organ Transplant Center between 2008 and 2013.

Materials and Methods

Patients

We performed this retrospective study after reviewing the transplant database of all patients who underwent OLT secondary to HCC and liver cirrhosis at Nemazee Hospital, Shiraz, Iran, a referral organ transplant center. Between January 2008 and December 2013, approximately 1000 liver transplants were performed at Nemazee Hospital. To ensure adequate follow-up, only patients who received liver transplant before December 31, 2013 were enrolled in the study. We selected the patients according to Milan criteria. With some exceptions, patients meeting UCSF criteria by preoperative imaging were included in the study. If OLT was not feasible according to UCSF criteria, patients were considered for percutaneous ethanol injection, transarterial chemoembolization, and/or radiofrequency ablation. Patients who had successful down-staging and came within UCSF criteria were enrolled in the transplant list. In the 5 patients (5.7%) who had successful down-staging, 3 patients received transarterial chemoembolization and 2 patients received radiofrequency ablation.

Tumor-related criteria for exclusion were gross hilar involvement and extrahepatic spread based on computed tomography (CT) or ultrasonography (US). Vascular involvement also was recognized after transplant by the pathologist.

Study design

According to the Child-Turcotte-Pugh classification of cirrhosis, patients with Child class A or B who had HCC as the primary indication for OLT were diagnosed based on histologic findings or imaging evidence of tumor formation in the liver (with arterial hypervascularization) on at least 2 imaging techniques, CT and US or magnetic resonance imaging. In patients with Child class C, the diagnosis of HCC was based on imaging evidence of a focal liver lesion on at least 2 imaging techniques. Vascular invasion was assessed by Doppler US and/or contrast-enhanced CT scan. To exclude extrahepatic metastasis before OLT, bone scintigraphy, brain CT, and thoracic CT were performed. Serum AFP levels were repeatedly measured in all cases before and after transplant. The explanted livers were fixed in formalin, cut into slices (thickness, 1 cm), and examined by an experienced pathologist. Parameters recorded included the number, site, and maximum diameter of all tumor nodules, capsule formation, presence of vascular invasion, and degree of differentiation (well, moderately, or poorly differentiated). Incidental (or undetected) HCCs were defined as carcinomas identified only on pathologic evaluation of the explanted liver in patients with negative pretransplant evaluation for focal liver lesions.
Macroscopic vascular invasion was defined by gross involvement of the lobar or segmental branches of the portal or hepatic veins. Microscopic vascular invasion was defined by the presence of tumor emboli within the central hepatic vein, portal vein, or large capsular vessels.

Follow-up
The OLT recipients were screened for tumor recurrence every 3 months by US and measurement of AFP level and every 12 months with abdominal CT scan and chest radiography. Thoracic or cerebral CT scans and radionuclide bone scans were performed only when there was a suspicion or evidence of extrahepatic neoplastic disease.

Immunosuppression and antiviral protocols
Immunosuppressive therapy after OLT consisted of a triple drug regimen with calcineurin inhibitors (tacrolimus or cyclosporine), mycophenolate mofetil (MMF), and prednisolone which were administered to all patients. Prednisolone was administered as methylprednisolone (1 g/d for 3 days; maintenance dose, 20 mg per day for 1 week, then gradually tapered and discontinued in most patients during the next 6 months). Cyclosporine dose adjustments were based on blood concentrations, aiming at drug levels from 200 to 350 ng/mL during the first month and 150 to 250 ng/mL thereafter. For tacrolimus, the drug blood level was adjusted to 10 to 15 ng/mL in the first month and <10 ng/mL thereafter. The MMF was started with 2 to 3 g/d and reduced according to the patient’s laboratory data. After the first post-transplant month, sirolimus (whole blood trough level aimed at 6 to 10 ng/mL) was added and the doses of prednisolone and calcineurin inhibitors were decreased. Acute rejection episodes were treated with 3 to 5 episodes of steroid pulses.

Hepatitis B immunoglobulin was administered to all HBV-infected patients. Antiviral drugs such as lamivudine and/or tenofovir were added to the treatment regimen of patients who were positive for hepatitis B surface antigen.

We assessed demographics, laboratory status, tumor characteristics, and transplant technique (piggy-back or standard technique) applied for 88 recipients in our study (Table 1). Patient survival from time of transplant to the time of HCC recurrence and death were selected as end points in our study.

Statistical analyses
Cox proportional hazards regression model was performed to identify independent factors that affected posttransplant survival. All analyses were performed using statistical software (SPSS, version 16.0, SPSS Inc., Chicago, IL, USA). Differences were considered statistically significant at \( P \leq 0.05 \).

Results
Average age was 46.6 ± 18 years; 70 patients (79.5%) were men and 18 patients (20.5%) were women. Underlying liver disease was present in all patients and most commonly was caused by HBV. The presence of tumor was confirmed before transplant in 70 patients (79.5%) and incidentally at OLT in 18 patients (20.5%) on histologic examination of the

<table>
<thead>
<tr>
<th>Table 1. Baseline Characteristics of 88 Patients With Hepatocellular Carcinoma Who Underwent Liver Transplant in Shiraz (2008 to 2013)*</th>
<th>Patients Without Recurrent HCC</th>
<th>Patients With Recurrent HCC</th>
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<tr>
<td>Age (y)</td>
<td>46.8 ± 18.2</td>
<td>46.1 ± 17.6</td>
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<tr>
<td>Sex</td>
<td></td>
<td></td>
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<tr>
<td>Male</td>
<td>57 (81.4)</td>
<td>13 (72.2)</td>
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<tr>
<td>Female</td>
<td>13 (18.6)</td>
<td>5 (27.8)</td>
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<tr>
<td>Etiology of liver disease</td>
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<td></td>
<td></td>
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<td>Hepatitis B</td>
<td>44 (62.9)</td>
<td>9 (52.9)</td>
<td>.765†</td>
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<tr>
<td>Hepatitis C</td>
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<tr>
<td>Tyrosinemia</td>
<td>6 (8.6)</td>
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<td></td>
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<tr>
<td>Cryptogenic</td>
<td>4 (5.7)</td>
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<tr>
<td>Other‡</td>
<td>11 (15.7)</td>
<td>3 (17.6)</td>
<td></td>
</tr>
<tr>
<td>Diagnosis of HCC</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Preoperative</td>
<td>53 (75.7)</td>
<td>17 (94.4)</td>
<td>.079</td>
</tr>
<tr>
<td>During OLT</td>
<td>17 (24.3)</td>
<td>1 (5.6)</td>
<td></td>
</tr>
<tr>
<td>Liver allograft</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DDLT</td>
<td>64 (91.4)</td>
<td>18 (100)</td>
<td>.339†</td>
</tr>
<tr>
<td>Living</td>
<td>6 (8.6)</td>
<td>0 (0)</td>
<td></td>
</tr>
<tr>
<td>Vascular invasion</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Present</td>
<td>6 (8.6)</td>
<td>5 (27.8)</td>
<td>.028</td>
</tr>
<tr>
<td>Absent</td>
<td>64 (91.4)</td>
<td>13 (72.2)</td>
<td></td>
</tr>
<tr>
<td>Site of tumor</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Right lobe</td>
<td>55 (78.6)</td>
<td>13 (72.2)</td>
<td>.719</td>
</tr>
<tr>
<td>Left lobe</td>
<td>9 (12.9)</td>
<td>3 (16.7)</td>
<td></td>
</tr>
<tr>
<td>Bilobar</td>
<td>6 (8.6)</td>
<td>2 (11.1)</td>
<td></td>
</tr>
<tr>
<td>Technique of operation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Piggy-back</td>
<td>48 (68.6)</td>
<td>11 (61.1)</td>
<td>.548</td>
</tr>
<tr>
<td>Standard</td>
<td>22 (31.4)</td>
<td>7 (38.9)</td>
<td></td>
</tr>
<tr>
<td>Grade</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Poorly differentiated</td>
<td>0 (0)</td>
<td>3 (16.7)</td>
<td>.007†</td>
</tr>
<tr>
<td>Well differentiated</td>
<td>70 (100)</td>
<td>15 (83.3)</td>
<td></td>
</tr>
<tr>
<td>Size of tumor</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤ 50 mm</td>
<td>59 (90.8)</td>
<td>13 (72.2)</td>
<td>.04</td>
</tr>
<tr>
<td>&gt; 50 mm</td>
<td>6 (9.2)</td>
<td>5 (27.8)</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: DDLT, deceased-donor liver transplant; HCC, hepatocellular carcinoma; OLT, orthotopic liver transplant
†Fisher exact test.
‡Other: nonalcoholic steatohepatitis, neonatal hepatitis, autoimmune hepatitis, primary sclerosing cholangitis.
*Data reported as mean ± SD or number (%).
explanted livers (incidental HCC) (Table 1). In the latter group of patients, imaging studies performed before OLT did not reveal the presence of HCC. In this study, 62 patients (70.5%) were within Milan criteria and 26 patients (29.5%) were beyond Milan criteria; 74 patients (84.1%) were within UCSF criteria and 14 patients (15.9%) exceeded UCSF criteria (Table 2). The median Model for End-Stage Liver Disease (MELD) score was 22 (mean, 20.8 ± 3.4). Serum AFP levels were obtained before transplant, with median value 124 ng/mL; 23 patients (32%) had AFP ≥ 400 ng/mL. The overall survival rate was 83% and tumor-free survival rate was 79.5%.

Recurrence characteristics
To understand the cause of recurrence, the profile of each patient with recurrence was assessed independently. In our entire cohort of 88 patients who underwent liver transplant, tumor recurrence was detected in 18 patients (20.5%). Recurrence appeared from 3 to 35 months after transplant (mean, 11.3 ± 9 mo).

In 18 patients with HCC recurrence, sites of recurrence were the liver (8 patients), pancreas (2 patients), adrenal (1 patient), pelvis (1 patient), scalp (1 patient), lung and liver (2 patients), skin and liver (1 patient), and liver and extrahepatic lymph nodes (2 patients). There were 8 deaths (44.4%) in 18 patients with HCC recurrence during follow-up.

There was no significant association between liver transplant technique (piggy-back vs standard technique), site of HCC in the liver (right, left, or bilobar), type of transplant (deceased- vs living donor liver transplant) and tumor recurrence (Table 1).

The overall survival rates of patients with incidental and nonincidental HCC were 94.4% and 80%. Tumor-free survival rates of patients with incidental and nonincidental HCC were 94.4% and 75.7%. Recurrence of HCC occurred in 8 patients beyond Milan criteria (30.8%) and 5 patients beyond UCSF criteria (35.7%), but there was no significant difference (Table 2).

Multivariate Cox regression analysis was performed with the following variables: Milan criteria, AFP levels before operation ≥ 400 ng/mL, tumor grade, age, and vascular invasion to predict tumor-free survival and overall survival.

### Table 2. Follow-Up Data For 88 Patients Who Were Within or Beyond Milan and UCSF Criteria and Who Had Transplant for Hepatocellular Carcinoma in Shiraz (2008 to 2013)*

<table>
<thead>
<tr>
<th>Variables</th>
<th>Within Selection Criteria</th>
<th>Beyond Selection Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Milan Criteria</td>
<td>UCSF</td>
</tr>
<tr>
<td>No. of patients</td>
<td>62</td>
<td>74</td>
</tr>
<tr>
<td>HCC recurrence</td>
<td>10 (16.1)</td>
<td>13 (17.6)</td>
</tr>
<tr>
<td>Postoperative death</td>
<td>11 (17.7)</td>
<td>13 (17.6)</td>
</tr>
</tbody>
</table>

*Data reported as number (%).

Multivariate analysis showed that vascular invasion (odds ratio [OR], 5; 95% confidence interval [CI]: 1.1 - 25.496; P = .049) and tumor grade (OR, 14.42; 95% CI: 3.652 - 56.95; P < .001) were independent predictors of tumor-free survival. Multivariate analysis also showed that vascular invasion (OR, 7.14; 95% CI: 1.781 - 28.623; P = .006) and age ≥ 60 years (OR, 3.25; 95% CI: 1.078 - 9.811; P = .036) were independent predictors of overall survival.

**Discussion**

Liver transplant is the best available option for the treatment of HCC, with > 70%, 5-year survival and low recurrence rates (< 10%). However, despite various patient selection criteria available, recurrence following transplant is inevitable and a major challenge. Moreover, there are few treatment strategies available for HCC recurrence and no absolute adjuvant therapy. Following the introduction of the Milan criteria in 1996, application of OLT in HCC patients has been limited by restrictive selection criteria. Proposals to expand the selection criteria from transplant centers include UCSF criteria, Hangzhou criteria, and up to 7 criteria, and these have made patients with larger tumors eligible for liver transplant.9,10 The OLT also is limited by a shortage of donors, which facilitates tumor progression while waiting for transplant and might lead to poor patient survival.2

Although the expanded criteria (UCSF criteria) for liver transplant have provided satisfactory results in several consensus studies, several unclear issues remain for discussion. Tumor size is one of the most important parameters, and there is no clear boundary for tumor diameter with the lowest recurrence rate. Radiographic imaging underestimates tumor size in 27% to 49% cases. However,
there are other important factors in tumor pathology that could aid patient selection, such as DNA heterogeneity, tumor differentiation degree, and vascular invasion, but pretransplant biopsy is required for detection of these factors and as such risks tumor spread.11

In the present study, the overall survival (83%) and tumor-free survival (79.5%) were within the expected range. However, we observed a higher rate of HCC recurrence (20.5%), with most (70.5%) within the Milan criteria at transplant time. This result is higher than reported in comparable studies from Switzerland (12.4%), Canada (13.1%), and the United States (18.3%).7,12 A study from Korea reported a higher rate of recurrence than our study (22.4%).13

Currently, HCC is one of the major indications for OLT, with expected recurrence rates between 15% to 20% and less than 10% in patients who meet the Milan criteria, and overall survival rate of patients within Milan criteria is over than 70% after liver transplant.10,14 In patients in our study, the tumor-free survival rate for tumors within Milan criteria was 83.9% and beyond Milan criteria was 69.2%. However, in our study there was no significant difference in survival of patients who were within or beyond Milan criteria; this finding could be explained by small sample size. The successful outcome of OLT with HCC patients and also presence of several consensus studies demonstrated that patient survival was not worse following OLT in tumors exceeding the Milan criteria.15

When comparing risk factors for posttransplant recurrence, a study by Lai et al. identified 4 independent factors: microvascular invasion, poor tumor grading, diameter of the largest tumor, and previous liver resection.16 Another study using Cox multiple regression analysis revealed that only histologic grading could negatively affect recipient survival.17

Based on our analysis, histologic grade of differentiation and vascular invasion were strong independent predictors of tumor-free survival. Moreover, we observed that age and vascular invasion were predictive factors for overall survival. A meta-analysis of tumor recurrence of HCC after liver transplant reported that patients who had tumors within the Milan criteria had 8% recurrence and patients with tumor pathology outside these criteria had a much higher incidence of recurrence (50%). These results differed from our findings, suggesting that characteristics of the pathologic evaluation of the resected specimen should be used to stratify screening.18

There were 2 main limitations of our study, including the small number of cases of OLT due to HCC in our center and short follow-up because the liver transplant program for HCC patients was started only 5 years ago at Shiraz University Hospital.

References

Liver and Kidney Transplant in Primary Hyperoxaluria: A Single Center Experience

Gökhan Moray,¹ Tugan Tezcaner,¹ Figen Özçay,² Esra Baskın,² Aydincan Akdur,¹ Mahir Kırmak,¹ Sedat Yıldırım,¹ Gülnaz Arslan,³ Mehmet Haberal¹

Abstract

Objectives: Primary hyperoxaluria, especially type 1, is a severe disease with multisystem morbidity and high mortality. We present 3 primary hyperoxaluria type 1 patients who underwent liver transplant, including living-donor liver transplant or combined liver and kidney transplant in our institution.

Case Reports: Patients who underwent liver transplant or combined liver/kidney transplant at our institution were evaluated, retrospectively. Between January 2002 and 2013, there were 3 patients who underwent transplant for primary hyperoxaluria. All 3 patients had disease onset in childhood, and the definitive diagnosis was established at age < 1, 6, and 8 years. Although early diagnosis was made, primary hyperoxaluria resulted in end-stage renal disease in 2 patients, and hemodialysis was introduced before liver transplant. All 3 patients underwent living-donor liver transplant. Case 1 was a 10-year-old girl who had an uneventful course after living-donor liver transplant, and she received a living-donor kidney transplant from the same donor 4 months after living-donor liver transplant. Case 2 was a 7-year-old boy who was the younger brother of the first patient; he did not have end-stage renal disease or any renal disorder after successful living-donor liver transplant. Case 3 was a 3-year-old boy who was diagnosed at age 2 months with renal disorders; although he was discharged from the hospital after living-donor liver transplant, he was readmitted because of unconsciousness that developed 1 day after discharge, and he died because of intracranial hemorrhage 2 months after liver transplant, unable to receive a kidney transplant.

Conclusions: Primary hyperoxaluria is a rare disorder that is difficult to diagnose until end-organ damage is severe. Outcomes may be improved with early and accurate diagnosis, aggressive supportive treatment, and correction of the enzyme defect by liver transplant before systemic oxalosis develops. However, kidney transplant or combined liver and kidney transplant is required in many primary hyperoxaluria type 1 patients because of the delayed diagnosis or long organ waiting time.

Key words: Oxalate, Oxalosis, End-stage liver disease, End-stage kidney disease, Solid-organ transplant

Introduction

Primary hyperoxaluria type 1 (PH-1) is a rare metabolic disorder that is transmitted in an autosomal recessive manner.¹ The PH-1 is caused by a deficiency of the liver-specific peroxisomal enzyme, alanine-glyoxylate aminotransferase (AGT), leading to excessive oxalate production, deposition of calcium oxalate crystals in the kidney, nephrocalcinosis, progressive renal failure, and systemic deposition of oxalate (oxalosis).¹ ² Neither dialysis nor isolated kidney transplant may remove calcium oxalate efficiently.¹ Isolated kidney transplant is followed by recurrence of nephrocalcinosis because of the overproduction of oxalate by the liver, leading to a high rate of graft loss.³ The only definitive treatment is combined liver and kidney transplant,
which has improved patient and graft survival for patients with PH-1. We present 3 PH-1 patients who underwent liver transplant including living-donor liver transplant or combined liver and kidney transplant in our institution.

Case Reports

Case 1
The patient was a 10-year-old girl born to consanguineous parents in June 1992. She had 2 siblings, including 1 younger brother (case 2) who had the same disease, and there was no family history of any renal disease. She was diagnosed with PH-1 at age 8 years, developed renal failure, and was started on peritoneal dialysis. After 2 years, her family elected transplant, and she was admitted to our center.

On admission, her body weight was 25 kg (10th-25th percentile) and height was 132 cm (50th percentile). She had end-stage renal disease but good liver function. Abdominal tomography revealed medullary nephrocalcinosis and renal atrophy bilaterally. She underwent a living-donor left lobe liver transplant from her mother in June 2012. The biliary anastomosis was performed with duct-to-duct technique. The operation was completed without any complications. She began taking triple immunosuppression with steroids, mycophenolate mofetil, and tacrolimus.

On postoperative day 5, she underwent repeat laparotomy because of hemorrhage. Bleeding from the hepatic arterial anastomosis was identified and repaired. The subsequent postoperative course was uneventful and she had excellent function of the hepatic graft.

At 4 months after liver transplant, she received a kidney transplant from her mother. After an uneventful postoperative period, she was discharged from the hospital with excellent liver and kidney graft function.

During the 26-month follow-up, no rejection episode had occurred.

Case 2
This 7-year-old boy had been born to consanguineous parents in October 2005. He had 2 siblings, including 1 older sister (case 1) who had the same disease, and there was no family history of any renal disease. He was diagnosed with PH-1 at age 6 years. His renal function was normal, but he had nephrolithiasis. After his sister’s transplant, his family elected transplant for him, and he was admitted to our center. His kidneys were not affected during the 2 years since the diagnosis was made.

One admission, his body weight was 20 kg (25th-50th percentile) and height was 113 cm (25th-50th percentile). He had high oxalate level and normal calcium level in a 24-hour urine test, and he had good liver and renal function. Abdominal tomography showed renal parenchymal thinning and bilateral nephrolithiasis. He underwent living-donor left lobe liver transplant from his aunt in March 2013. The biliary anastomosis was performed with duct-to-duct technique. The operation was performed without any complications. He began taking triple immunosuppression with steroids, mycophenolate mofetil, and tacrolimus.

Later on the day of transplant, he underwent repeat laparotomy because of stenosis at the hepatic artery anastomosis. The anastomosis was identified and reconstructed. After this incident, the postoperative period was uneventful and he had excellent function of the hepatic graft.

Most recent follow-up at 18 months after surgery showed that he had normal hepatic graft function (normal levels of liver enzymes, serum bilirubin, and serum albumin) for 18 months after the operation. No rejection episode occurred during follow-up.

Case 3
A 3-year-old boy had been born to consanguineous parents (cousins) in December 2008. He had no brother or sister (his mother previously had an abortion) and there was no family history of renal disease. He was diagnosed with PH-1 at age 2.5 months, developed severe renal failure, and was started on peritoneal dialysis. After 3 years, his family elected transplant, and he was admitted to our center.

On admission, his body weight was 9 kg (< 3rd percentile) and height was 82 cm (< 3rd percentile).
He had end-stage renal disease but good liver function. Abdominal tomography revealed bilateral renal parenchymal oxalate accumulation and renal atrophy. He underwent a living-donor left lobe liver transplant from his father in May 2012. Biliary anastomosis was performed with Roux-en-Y hepaticojejunostomy. The operation was performed without any complications, and he started taking triple immunosuppression with steroids, mycophenolate mofetil, and tacrolimus.

At 2 months after transplant, he was discharged from the hospital with normal liver graft function. However, he was readmitted to the hospital because of unconsciousness and convulsion 1 day after discharge. Cranial computed tomography revealed brain edema and intraparenchymal hematoma. He died because of intracranial hemorrhage 2 months after liver transplant, and he was unable to receive a kidney transplant.

Discussion

In patients who have primary hyperoxaluria, the need for organ transplant should be recognized before the onset of systemic oxalosis and end-stage renal failure. There are several options for patients who have PH-1: (1) isolated renal transplant to correct end-stage renal disease, (2) isolated liver transplant to correct the metabolic defect before the occurrence of major renal damage, and (3) combined liver and kidney transplant to correct both problems simultaneously.5

The liver is the only organ responsible for glyoxylate detoxification by the enzyme AGT, and PH-1 can be cured only by replacing the deficient host liver with an unaffected liver. For these children, liver transplant could be considered an effective functional gene therapy and enzyme replacement therapy.6 Isolated liver transplant is a good treatment option in selected patients before advanced chronic renal failure develops. The timing of preemptive liver transplant is controversial because the procedure is invasive, it has risks, and the decision to remove the native liver can be difficult when the course of the disease is difficult to predict.7

In conclusion, patients who have PH-1 with systemic oxalosis may be difficult to treat. Outcomes may be improved with early and accurate diagnosis, aggressive supportive treatment, and correction of the enzyme defect by liver transplant before systemic oxalosis develops. Combined liver and kidney transplant, either sequential or simultaneous, has been accepted as the treatment of choice for children who have PH-1. Aggressive dialysis therapy may be required to avoid progressive oxalate deposition in patients who have established end-stage renal disease, and minimizing the duration on dialysis may improve quality of life and patient survival.

References

Abstract

Objectives: Training on organ donation and transplantation is relevant for transplantation improvement. This study aimed at investigating the perceived benefits of Transplant Procurement Management training programs on professional competence development and career evolutions of health care workers in organ donation and transplantation.

Materials and Methods: An online survey was developed in 5 languages (Spanish, English, Italian, French, and Portuguese) and its link was emailed to 6839 individuals. They were asked to forward it to other professionals in organ donation and transplantation. The link was also shared on Facebook and at relevant congresses. Two research questions on the perceived influence of specialized training programs were identified.

Results: A total of 1102 participants (16.1%) took the survey; 87% reported participating in Transplant Procurement Management training programs, of which 95% selected Transplant Procurement Management courses as the most influential training they had participated in. For research question one, 98% reported influence on knowledge (score 4.5 [out of 5]), 93% on technical (4.2) and communication skills (4.1), 89% on attitude toward organ donation and transplantation (4.1), 92% on motivation to work (4.2), 91% on desire to innovate (4.0), 87% and 79% on ability to change organ donation and transplantation practices (3.9) and policies (3.5). For research question 2, main and interaction effects for position at the time of training and type of training were reported.

Conclusions: Transplant Procurement Management training programs had positive perceived effects.

Key words: International, Education, Survey

Introduction

Organ and tissue donor shortages have been analyzed in the search for solutions to overcome the gap between demand and supply. Different approaches, focusing on specific factors (attitudes toward organ donation, presumed consent, financial incentives, and education)\(^1\)-\(^6\) or multilevel factors,\(^7\) and different campaigns, organizational models, and national initiatives have been assessed\(^8\)-\(^13\) to analyze organ, and tissue donor shortages, and propose potential solutions to optimize donation processes, and outcomes. Guidelines\(^14\) and resolutions\(^15\) adopted by the World Health Organization, as well as conventions, directives, and action plans issued by the Council of Europe and the European Union, have called for balanced and regulated donation and transplantation activities across the European Union\(^16\) and the world.

One pivotal element identified as a key to success is the training of health care professionals involved in
the donation process to improve skills, competencies, and awareness. The European Training Program on Organ Donation (ETPOD) achieved a significant increase in organ donation rates. The Council of Europe provided guidelines and recommendations to governments of member states about the role, functions, responsibilities, and training of transplant donor coordinators. Moreover, through its Action Plan on Organ Donation and Transplantation (2009-2015), the European Commission supported the implementation of effective training programs for transplant donor coordinators.

Several educational programs have been designed to support the European Commission’s action plan. Over the years, Transplant Procurement Management (TPM) was developed and grew to become one of the largest and most international training programs. Designed on a model of the continuous improvement program (CIP) method, TPM was launched in 1991 under the auspices of the University of Barcelona in Barcelona, Spain, with the support of the Spanish National Transplant Organization. It gained the recognition of the European Committee on Organ Transplantation of the Council of Europe in 1994 and was awarded the TTS-Genzyme Award for Education and Training in Transplantation by The Transplantation Society in 2008. TPM is supported through the academic endorsement of the University of Barcelona and offers face-to-face and online courses as well as a master’s degree in organ, tissue, and cell donation for transplantation.

Face-to-face training programs are delivered at 5 levels following a progression of expertise, aims, and length: new vital cycle (awareness, 8 hours); introductory (motivation, 16 hours); intermediate (collaboration, 24 hours); advanced (specialization, 40 hours); master’s degree (experts, 300 hours). They build on participants’ knowledge based on their previous training and experience in the field with proactive involvement in the learning environment. As proven by Knowles, adult learning is most effective when information is presented in the context of a real-life situation. Thus, TPM uses the Kolb learning cycle to engage and immerse learners in the learning situation in a constructivist approach. Through Web-based learning programs, participants further fine-tune their skills and competencies, which optimize direct application of skills and knowledge in their own professional environment. To assess training needs and develop and refine the training programs according to the audience, TPM applies the ADDIE instructional design model. Since 1991, about 10,000 professionals from 101 countries throughout the world have been trained through the different educational models of TPM as following: 87.5% in face-to-face courses and 12.5% in online courses.

With such a large number of professionals participating in these courses, the effect of these TPM training programs must be evaluated. The effect of the TPM continuous improvement methodology on the donation process could underscore the importance of further development and fine-tuning of such training programs.

The objective of this study was to investigate the perceived benefits of TPM specialized training programs on professional competence development and career evolutions of health care workers in organ donation and transplantation.

**Materials and Methods**

A Web-based questionnaire including 49 multiple-choice, open-ended, rating-scale, and agreement-scale questions was developed in 5 languages (English, Spanish, Italian, French and Portuguese). This study reports on a subset of questions from this survey dealing with the objective of the study (Figure 1). The time required to complete the survey was approximately 15 to 20 minutes. A pilot test was taken by 10 participants. No survey pitfalls were reported. The study was approved by the Ethical Committee institutional review boards at the University of Barcelona, Spain, and Purdue University, West Lafayette, Indiana, United States. All of the protocols conformed with the ethical guidelines of the 1975 Helsinki Declaration. The survey initiated with the research participant consent form. By continuing, participants indicated their consent to participate.

Participants were recruited in May 2012 from the TPM database and other methods. A cover letter and link to the online survey were e-mailed to 6839 people who had participated in TPM or related training courses. They were also asked to forward the link to other individuals active in organ donation and transplantation. Two reminder e-mails were sent during the following month. Additionally, links were posted on Facebook (www.facebook.com/transplantationprocurementmanagement) and
handed out at organ donation meetings and congresses.

Participation in the study was voluntary. Participants received no direct benefits or compensation. All data were collected by means of Qualtrics (Provo, UT, USA) Web-based survey software and kept confidential through personal password control. Additionally, all responses were anonymous and no identifiable information was collected.

Two main research questions were identified for the current study. Research question 1 was “What is the perceived influence of specialized training programs on career, collaboration, and skills and ability in organ donation and transplantation?” Research question 2 was “Do the different types of training programs (online, face-to-face, local/national/international, etc) and individual characteristics (sex, position at time of training) have different perceived influences on competences (career, collaboration, skills, and ability) in organ donation and transplantation?”

Participants were asked to select the training they believed was most influential in considering their responses to the remaining survey items. Participants who selected TPM were asked to specify which courses they had attended. The types of courses were grouped in terms of similarity and were ranked from 1 to 7 on the basis of how advanced and intensive the training was, with a ranking of 1 being the most advanced, as follows: the master’s course in donation and transplantation was ranked as 1, the introductory face-to-face course was ranked as 2, the intermediate face-to-face course was ranked as 3, the advanced face-to-face course was ranked as 4, the essentials in donation course was ranked as 5, the blended [online and face-to-face] courses were ranked as 6, and online-only courses were ranked as 7. When a participant identified multiple courses, the more advanced training category was used in our analyses.

Respondents were asked to rate the influence of trainings on 12 different items (on a scale from 1 to 5: 1- no influence, 5-great deal of influence), including respect from peers, advantages in promotions, technical skills, knowledge, networking ability, motivation to work in transplantation, collaborative opportunities, ability to change policies, ability to change practices, desire to innovate, and communication skills related to organ donation and transplantation (Figure 1). For most questions, the analysis focused on professionals who are still active in the field.

**Statistical analyses**

Research question 1 was subject to descriptive data analysis, plotting frequencies, percentages, and means, and referred to all participants who answered the survey items, regardless of which training they selected as being most influential. For research question 2, a series of additional analyses were performed using general linear model univariate analysis run on types of TPM trainings, sex, and position at time of training on the dependent variables presented above. A value of $P < .05$ was considered to be statistically significant. Statistical analyses were performed with SPSS software (SPSS: An IBM Company, version 21.0, IBM Corporation, Armonk, NY, USA).

**Figure 1.** Subset of Survey Questions Dealing With the Objective of the Study

1. By clicking the link to the survey I am indicating my consent to participate.
   Answer
   Yes, I agree to take the survey
   No thanks
2. What organ donation/transplant related training courses have you participated in? (Check all that apply)
   TPM (TPM) trainings
   European Training Program on Organ Donation (ETPOD)/ETPOD
   Dissemination
   United Network for Organ Sharing (UNOS) trainings
   National Association of Transplant Coordinators (NATCO) trainings
   European Transplant Coordinators Organization (ETCO) workshops
   European Society for Organ Transplantation (ESOT) courses
   The Transplantation Society scholarship/training
   Organización Nacional de Transplantes (ONT) courses
   None
   Other (please specify)
3. Please select the course that you feel has been the most influential. (Please use the course selected below as the basis for the rest of the survey).
   TPM (TPM) trainings
   European Training Program on Organ Donation (ETPOD)/ETPOD
   Dissemination
   United Network for Organ Sharing (UNOS) trainings
   National Association of Transplant Coordinators (NATCO) trainings
   European Transplant Coordinators Organization (ETCO) workshops
   European Society for Organ Transplantation (ESOT) courses
   The Transplantation Society scholarship/training
   Organización Nacional de Transplantes (ONT) courses
   None
   Other (please specify)
4. What specific TPM course(s) did you participate in?
   Face-to-Face Introductory
   Face-to-Face Intermediate
   Face-to-Face Advanced
   Essentials in Organ Donation Seminars
   Blended Professionals in Organ Donation
   Blended Training for Trainers
   Blended Organ Donation Quality Management
   Online Donor Detection System
   Online Brain Death Diagnosis
   Online Donor Management
   Online Family Approach
   Online Organ Retrieval
   Online International Tissue banking Course
   TPM Masters/ International Master in Donation of Organs, Tissues and Cells for Transplantation
Panama (n = 10), Brazil (n = 38), Turkey (n = 19), Lebanon (n = 10), and Spain (n = 173), France (n = 132), Portugal (n = 47), most participants responding from Italy (n = 349), France (n = 132), Portugal (n = 47), many professionals.

Respondents reported participating in 1498 training courses in 46 countries, with many respondents reporting participating in multiple courses. Participants were from 46 countries, with the most participants responding from Italy (n = 349), Spain (n = 173), France (n = 132), Portugal (n = 47), Brazil (n = 38), Turkey (n = 19), Lebanon (n = 10), and Panama (n = 10).

Eighty-seven percent of respondents (910/1102 = 87%) reported participating in a TPM course (472 [45%] reported attending TPM training programs only, whereas 438 [42%] reported participating in TPM and other training programs, and 102 [9%] reported attending non-TPM courses). Forty-seven respondents (4%) indicated they had not participated in any training courses and were not included in further analyses.

Eight hundred four participants answered this question, of which 669 (83%) selected TPM as most influential and 135 (17%) selected other training programs. Based on 87% having taken a TPM course, and 83% selecting TPM as best, we concluded that 95.4% (83/87) of TPM attendants found them as most influential.

The perceived influence of specialized training programs on career, collaboration, and skills and ability in organ donation and transplantation (research question 1) is shown in Table 1. Because of the small number of respondents who selected training programs other than TPM as being most influential, only TPM training programs were selected for analysis of research question 2.

Men reported greater influence of trainings than women on respect from peers (men: 3.4 ± 1.5; women: 3.0 ± 1.4; P = .025) and networking ability (men: 3.8 ± 1.2; women: 3.4 ± 1.1; P = .033) across all TPM training programs. No effect of sex was found in analyses of the other items.

Position at time of training had significant effects on technical skills for organ donation and transplantation (P = .001), knowledge of organ donation and transplantation (P = .029), attitude toward donation (P = .002), motivation to work in organ donation and transplantation (P < .001), “collaborative opportunities” (P < .001), ability to change practice (P < .001), ability to change policy (P = .004), desire to innovate (P = .006), and communication skills (P = .001) (Table 2).

Physicians report the highest influence of training on most of the items listed, such as attitude toward donation, motivation to work in organ donation and transplantation, ability to change practice, and ability to change policy. Nurses and social workers perceived trainings to have the most influence on ability to change policy and motivation to work in organ donation and transplantation. Social workers reported the most collaborative opportunities. However, laboratory technicians and biologists
reported the lowest levels of perceived influence of training on all of the items (Table 2).

Type of training showed a significant effect on advantages in promotions ($P = .033$), with online, blended, and TPM master courses offering the most perceived benefit and essentials in organ donation and introductory face-to-face offering the least perceived benefit (Table 3).

A significant interaction effect between position at time of training and type of training on respect from peers ($P = .022$) and advantages in promotions ($P = .011$) was reported (Table 4). Physicians perceived more effect on advantages in promotion from TPM masters/international courses than nurses did. Nevertheless, physicians and nurses found the advanced training less beneficial than respondents in the “other” category did.

A significant interaction effect between position at time of training and type of training was also reported on networking ability ($P = .017$). Physicians and nonmedical doctors (PhDs) reported higher levels of networking ability in TPM masters/international courses but slightly lower levels than nurses and social workers reported in the advanced courses.

Finally, significant interaction effects between position at time of training and type of training were further reported on collaborative opportunities ($P = .033$), with physicians reporting the most collaborative opportunities in the TPM masters/international courses, and nurses and social workers reporting the most collaborative opportunities in the advanced face-to-face training (Table 4).

**Discussion**

TPM specialized training programs in organ donation and transplantation had positive effects for a significant percentage of health care workers in the field on professional competence development and career evolution. This may be explained by the ongoing effort of TPM to improve its products and services in compliance with professional requirements and provide increased efficiency and quality over time. To achieve a continuous improvement program, TPM has been applying the plan-do-check-act cycle, which allows it to constantly evaluate, improve, and refine its training programs. TPM training programs have many advantages beyond the traditional measures of increasing knowledge of a specific practice. Well-designed programs provide certifications and prestige that are likely to result in increased respect from peers and advantages in promotions. These programs result in improvement in technical skills and knowledge, as well as the ability to communicate effectively about organ donation and transplantation. Additionally,

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**Table 1. Influence of Specialized Donation/Transplantation Programs Reported by All Survey Respondents Regardless of Which Training Program They Selected as Being Most Influential**

<table>
<thead>
<tr>
<th>No. of Respondents</th>
<th>Respondents Who Reported Some to a Great Deal of Influence, %</th>
<th>Score†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Respect from peers</td>
<td>674 69</td>
<td>3.22 ± 0.45</td>
</tr>
<tr>
<td>Advantages in promotions</td>
<td>677 46</td>
<td>2.46 ± 0.15</td>
</tr>
<tr>
<td>Technical skills for donation/transplantation</td>
<td>690 93</td>
<td>4.15 ± 0.96</td>
</tr>
<tr>
<td>Knowledge of donation/transplantation</td>
<td>698 98</td>
<td>4.45 ± 1.24</td>
</tr>
<tr>
<td>Networking ability</td>
<td>679 84</td>
<td>3.63 ± 0.60</td>
</tr>
<tr>
<td>Attitude toward donation/transplantation</td>
<td>690 89</td>
<td>4.08 ± 0.97</td>
</tr>
<tr>
<td>Motivation to work in donation/transplantation</td>
<td>695 92</td>
<td>4.23 ± 1.14</td>
</tr>
<tr>
<td>Collaborative opportunities for donation/transplantation</td>
<td>688 83</td>
<td>3.75 ± 0.71</td>
</tr>
<tr>
<td>Ability to change practices for donation/transplantation</td>
<td>686 87</td>
<td>3.85 ± 0.74</td>
</tr>
<tr>
<td>Ability to change policies for donation/transplantation</td>
<td>671 79</td>
<td>3.51 ± 0.54</td>
</tr>
<tr>
<td>Desire to innovate for donation/transplantation</td>
<td>687 91</td>
<td>3.98 ± 0.82</td>
</tr>
<tr>
<td>Communication skills for donation/transplantation</td>
<td>694 78</td>
<td>4.14 ± 0.96</td>
</tr>
</tbody>
</table>

†Scored on a scale from 1 to 5: 1 = no influence, 5 = great deal of influence; data expressed as mean ± SD.

**Table 2. Effect of Position at Time of Training on 9 Items**

<table>
<thead>
<tr>
<th>Position at Time of Training</th>
<th>Technical Skills for D&amp;T</th>
<th>Knowledge of D&amp;T</th>
<th>Attitude Toward D&amp;T</th>
<th>Motivation to Work in D&amp;T</th>
<th>Collaborative Opportunities</th>
<th>Ability to Change</th>
<th>Ability to Change Practice</th>
<th>Desire to Innovate Policy</th>
<th>Communication Skills</th>
</tr>
</thead>
<tbody>
<tr>
<td>MD</td>
<td>4.4 ± 0.2</td>
<td>4.6 ± 0.1</td>
<td>4.4 ± 0.2</td>
<td>4.4 ± 0.2</td>
<td>3.9 ± 0.2</td>
<td>4.3 ± 0.2</td>
<td>3.9 ± 0.2</td>
<td>4.1 ± 0.2</td>
<td>4.6 ± 0.2</td>
</tr>
<tr>
<td>RN</td>
<td>4.4 ± 0.2</td>
<td>4.6 ± 0.1</td>
<td>4.3 ± 0.2</td>
<td>4.5 ± 0.2</td>
<td>3.9 ± 0.2</td>
<td>4.1 ± 0.2</td>
<td>3.7 ± 0.2</td>
<td>4.2 ± 0.2</td>
<td>4.3 ± 0.2</td>
</tr>
<tr>
<td>Laboratory technician</td>
<td>3.0 ± 0.7</td>
<td>3.0 ± 0.5</td>
<td>2.0 ± 0.8</td>
<td>3.0 ± 0.7</td>
<td>2.0 ± 0.8</td>
<td>1.5 ± 0.7</td>
<td>1.5 ± 0.8</td>
<td>2.5 ± 0.7</td>
<td>2.5 ± 0.7</td>
</tr>
<tr>
<td>Biologist</td>
<td>2.8 ± 0.3</td>
<td>3.8 ± 0.3</td>
<td>2.8 ± 0.4</td>
<td>2.7 ± 0.4</td>
<td>1.8 ± 0.4</td>
<td>2.1 ± 0.4</td>
<td>2.1 ± 0.4</td>
<td>2.8 ± 0.4</td>
<td>2.6 ± 0.3</td>
</tr>
<tr>
<td>Social worker</td>
<td>4.8 ± 0.6</td>
<td>4.5 ± 0.4</td>
<td>4.3 ± 0.6</td>
<td>4.8 ± 0.6</td>
<td>4.5 ± 0.7</td>
<td>4.0 ± 0.6</td>
<td>3.8 ± 0.7</td>
<td>4.0 ± 0.6</td>
<td>4.5 ± 0.6</td>
</tr>
<tr>
<td>Nonmedical PhD</td>
<td>4.2 ± 0.3</td>
<td>4.4 ± 0.2</td>
<td>3.8 ± 0.3</td>
<td>4.1 ± 0.3</td>
<td>3.5 ± 0.4</td>
<td>3.8 ± 0.3</td>
<td>3.4 ± 0.4</td>
<td>3.5 ± 0.3</td>
<td>4.2 ± 0.3</td>
</tr>
<tr>
<td>Other</td>
<td>4.4 ± 0.2</td>
<td>4.7 ± 0.2</td>
<td>4.0 ± 0.3</td>
<td>4.2 ± 0.2</td>
<td>3.9 ± 0.3</td>
<td>4.0 ± 0.3</td>
<td>3.9 ± 0.3</td>
<td>4.2 ± 0.2</td>
<td>4.3 ± 0.2</td>
</tr>
<tr>
<td>$P$ value</td>
<td>.001</td>
<td>.029</td>
<td>.002</td>
<td>&lt;.001</td>
<td>&lt;.001</td>
<td>&lt;.001</td>
<td>&lt;.001</td>
<td>.004</td>
<td>.006</td>
</tr>
</tbody>
</table>

Abbreviation: D&T, donation and transplantations

Scored on a scale from 1 to 5: 1 = no influence, 5 = great deal of influence; data expressed as mean ± SE.
they bring together people who have similar interests who are likely to become influential in their fields and thus increase networking ability and collaborative opportunities. Furthermore, having well-designed programs taught by passionate faculty using innovative approaches increases motivation to work in transplantation and the desire to innovate in organ donation and transplantation. Many participants act on these motivations and report that the training programs are influential on their ability to change policy and practice related to organ donation and transplantation, collaborative opportunities, desire to innovate, and communication skills related to organ donation and transplantation.

Literature research reveals that similar studies have been conducted in different medical fields. In Toronto, Ontario, Canada, a study was performed to examine how interprofessional education clinical placement influences health care students’ perceptions of interprofessional collaboration. Findings suggest that structured interprofessional education clinical placements may provide students with valuable collaborative learning opportunities, enhanced respect for other professionals, and insight into the value of interprofessional collaboration in health care delivery. The results of another study, conducted by the American Medical Association to evaluate changes in practice behaviors, suggest that a well-designed education intervention can enhance health professionals’ confidence and clinical practice.

However, not all types of trainings have the same outcomes for all participants, although most training still received high evaluations. These differences are important to note in terms of evaluating overall success and for consideration of who is likely to benefit most from a certain type of training. It appears that overall, organ donation and transplantation is still a male-dominated field, and female participants were less likely to feel the same influence of training programs on respect from peers. Additionally, it is a bit surprising that blended and online training programs were reported to have more of an influence on promotions than face-to-face courses. There are a couple of possible explanations for this. First, the overall numbers of participants in these categories was significantly lower than for the TPM masters/international and the advanced face-to-face courses. Moreover, in the masters and
advanced courses, the majority of respondents were nurses and physicians. Thus, it may be less common for a physician or a nurse to report a specific type of promotion.

Overall, this report provides a new type of evaluation of training programs that goes beyond rating the quality of the course or instructors and focuses specifically on how different groups perceive the benefits of training programs in their ongoing work life. Generally, physicians reported the greatest influence of the trainings on improving their attitudes toward organ donation and transplantation. Physicians also reported more influence of the trainings on their ability to change policies and practices related to organ donation and transplantation. However, in many categories, nurses and social workers also reported high levels of influence of the trainings on their ability to change policy and practice as well. Laboratory technicians and biologists seemed to perceive less benefit from the training than did medical professionals, social workers, and other professionals.

The survey was developed together with experts from University of Barcelona and the Brian Lamb School of Communication, Purdue University, who helped to ensure the accuracy and consistency of our measurements. It was further revised, readjusted, translated, and validated by experts in organ donation and transplantation. Piloting and pretesting was also performed to increase both validity and reliability of the survey, and finally, the survey was approved by the institutional review boards of the University of Barcelona and Purdue University, which conferred validity and reliability to the survey results.

However, we also should consider some limitations of the study. One of the identified limitations is that only 16.1% of the total potential participants contacted agreed to take the survey. While the response rate is not as high as we would like, it is in line with response rates for online and mail surveys, especially because no incentives to participate were provided. While we have no direct knowledge of the reasons the rest of those contacted did not complete the survey, there are several possible explanations. Some contact details could have been erroneous, contacted individuals may not have been interested in taking the survey, or they did not find training programs beneficial. So, we cannot rule out that participants most influenced by training had the greatest motivation to take the survey. As such, the results may be positively skewed. However, given the success of training programs in increasing rates of transplantation, we think the results are likely to be fairly representative.

Additionally, a degree of caution should be taken in interpreting the data for biologists, laboratory technicians, and social workers, especially when broken out by type of training, as the number of participants in a given course for each category might be very low. Moreover, while online courses seem to be more influential to physicians and nurses, there is a low sample size for these categories, making interpretation more difficult.

Last, the study focuses on the perceived benefits from the trainings on career, collaboration, and skills and ability in organ donation and transplantation and not on the actual impact of the trainings on the different items. However, previous findings show that the educational initiatives undertaken by TPM along with the consortium partners and the support of the European Commission within ETPOD project (DGSANCO–EAHC2005205) were successful and achieved significant increase in organ donation figures.

In conclusion, TPM specialized training programs in organ donation and transplantation are influential and have positive effects for a significant percentage of health care workers in the field on professional competence development and career evolutions.

This study lends support to the importance of TPM specialized training programs, not just for development of technical skills but also development of career conditions that will keep health professionals active in organ donation and transplantation. This, combined with an increase in knowledge, skills and abilities to communicate should help to improve organ donation and transplantation around the world. The data collected allow future evaluations focusing on issues such as networks and collaboration, success in changing policy and practice, and career advancement and committees.

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Management of BK Virus Nephropathy in Kidney Transplant Recipients at the Royal Hospital - Clinical Audit - Oman

Fatma Al-Raisi, Nabil Mohsin, Pramod Kamble

Abstract

Objectives: Nephropathy from BK virus (BKV) infection is a growing challenge in kidney transplant recipients globally. It is the result of contemporary potent immunosuppressives aimed at reducing acute rejection and improving allograft survival. Untreated BK virus infections lead to kidney allograft dysfunction or loss. Decreased immunosuppression is the principle treatment but predisposes to acute and chronic rejection. Screening for early detection and prevention of symptomatic BK virus nephropathy may improve outcomes. Although no approved antiviral drug is available, leflunomide, cidofovir, quinolones, and intravenous immunoglobulin have been used.

Since the introduction of the new immunosuppressive agents in the transplant regimen at the Royal Hospital, few cases of BK virus have been detected, and the challenge was to decide upon the best treatment option.

Materials and Methods: The audit was carried out at the Royal Hospital-Oman between January 2010 and December 2012. The nephrology consultant and the clinical pharmacist reviewed all the BK cases and the Royal Hospital. Extensive literature review carried out by the pharmacist to look into the prevalence, prognosis and treatment of BK nephropathy.

A treatment protocol was prepared by the clinical pharmacist through guidance of the consultant and was peer reviewed by team of clinical pharmacists and nephrology doctors and approved by the consultant.

Results: The audit included 19 patients with positive BK virus polyomavirus nephropathy. The treatment options were applied stepwise in all the patients with BK virus nephropathy with success rate more than 70%.

Conclusions: BK virus nephropathy is emerging at an alarming rate and requires increasing awareness. The uses of current treatment options are still questionable. Our audit confirms that reducing immunosuppression appears to be the criterion standard for the treatment of BK nephropathy.

Key words: BK, Nephropathy, Immunosuppressive, Kidney, Transplantation

Introduction

BK virus (BKV) was first detected in early 1970s. The name was originated from patients initials “BK.” Soon after, there were series of cases where BK virus (BKV) nephropathy (BKVN) was reported in kidney transplant recipients.

BK virus-induced nephritis is increasing in alarming numbers worldwide, which may be the result of newer, more potent immunosuppressive agents in kidney transplant recipients. Although these drugs are meant to prevent or reduce the incidence of acute rejections, and prolong the survival of the transplanted graft, the harm these drugs are causing is debatable.

Much research has shown that BKVN is self-limiting in many cases; however, some cases might need strategic interventions to eliminate the virus.
while preserving the transplanted kidney functions.\textsuperscript{1,2} Starting with reduction of immunosuppressive agents; which is the criterion standard—to using other agents (eg, quinolone antibiotics, intravenous immunoglobulins, and leflunomide).\textsuperscript{4-8} Additionally some centers also use cidofovir as an antiviral.\textsuperscript{3} It is important to consider treating BKVN, as untreated BKV infections lead to graft dysfunction or loss. The challenge that faces most centers with patients having BKVN is that there is no standard antiviral agent, and all the above-mentioned agents are used off-label.

The prevalence of positive BKV in Omani transplant population is approximately 6.3%. Newer immunosuppressive agents in the transplant regimen were introduced almost 10 years back at the Royal Hospital; and since 2008, many cases of BKV have been detected, and the dilemma was to select the best treatment option for eradicating BKV in the transplanted kidney.

A treatment protocol was prepared by the clinical pharmacist through guidance of the nephrology consultant and based on the literature review. The protocol was then peer-reviewed by a team of clinical pharmacists and nephrology doctors.

The main aim of this audit was to monitor the effectiveness of the treatment strategy applied to BKVN patients.

**Materials and Methods**

An extensive literature review was performed by the team to examine the prevalence, prognosis, and treatment of BK nephropathy worldwide. The protocol was then developed, based on the literature findings.

The audit was carried out at the Royal Hospital, the largest tertiary hospital in Oman between January 2010 and December 2012. From 2008, in our center, we prospectively screened all kidney transplant recipients for BKV. Almost 300 patients were screened for BKV using a polymerase chain reaction to quantify the viral load. Almost 700 tests were performed to quantify the viral load in the 300 patients. All the cases with positive polymerase chain reaction were candidates for kidney biopsy to confirm the diagnosis.

The nephrology team, including the renal clinical pharmacist, reviewed all the BKVN cases from January 2008 until December 2012 at the Royal Hospital. The systematic approaches in the prepared protocol were applied to all patients who were diagnosed with BKVN. The biographic data of all patients were filled in a designed spreadsheet. All the results were filled in the Excel data sheet and simple analytic method was used to generate the results.

**Results**

Nineteen patients (6.3%) out of 300 kidney transplant recipients were diagnosed with BKV. All 19 patients were transplanted between 2005 and 2012. Of the 19 patients 11 were male and their mean age was 26.7 y (range, 6-52 y). All recipients were on mycophenolate mofetil-based immunosuppressant regimen among which 15, 3, and 1 were on tacrolimus, ciclosporin, and sirolimus (Figure 1). Only 1 patient was on a steroid-free regimen, while all others were on prednisolone as part of their immunosuppressants.

Most patients (90%) were induced by basiliximab, and only a few (10%) were given antithymoglobulin for induction.

Treatment of BKVN was step-wise as per the protocol. All patients were started with immunosuppressive dose reduction (n = 19). Five patients (26.3%) responded well after reducing their doses of tacrolimus and mycophenolate mofetil, and BKV was eradicated successfully without compromising the renal functions. In 9 patients (47.4%), tacrolimus was switched to sirolimus and ciporofloxacin was added to their regimen. Three patients (15.8%) received leflunamide as a substitution for mycophenolate mofetil and 6 patients (31.6%) received intravenous immunoglobulin along with the previous treatment options. Cidofovir was the last treatment given to 4 patients (21%).

**Figure 1.** Immunosuppressive Regimens of Patients

**Abbreviations:** FK, tacrolimus; MMF, mycophenolate mofetil
Treatment options followed by the nephrologists were in accordance with the protocol, and the success rate of BKV eradication was high. Unfortunately, 2 (10.5%) patients experienced complete graft lost, despite all measures, to save the transplanted kidney from failing. On the other hand, all 17 patients (89.5%) were completely treated from BKVN with good renal function except four patients (21%), who were having their creatinine clearance less than 50 mL/min/1.73m².

**Discussion**

Approximately 60% of renal grafts with BKVN develop progressive graft loss.1 The incidence of BKVN in our patients was more than 6%, which is quiet high; fortunately, the graft survival rate was favorable, only 2 patients (10.5%) lost their graft and went back to renal replacement therapy, and 4 patients had their glomerular filtration rate < 50 mL/min/1.73m².

Existing treatment of BKVN is scarce as there are no specifically effective antiviral agents currently available.2 The best strategy to eliminate BKV seems to be the prevention of BKVN. Brennan and associates concluded that potential monitoring of urine and blood for BKV and preemptive withdrawal of the antimetabolite upon development of BKV resulted in clearance of viremia and this appeared to decrease the risk of BKVN without increasing the risk of acute rejection.9

In many set-ups, primary approaches include minimizing or stopping the antimetabolite such as mycophenolate mofetil while concurrently keeping the calcineurin inhibitor trough levels at the lower side. Other approaches were to switch from calcineurin to another or switching from a calcineurin to sirolimus. The latter has been reported to decrease risk of BKVN. However, care must be taken because rapid reduction in immunosuppression may keep the graft at risk of acute rejection.

The current strategies of treatment as per the protocol developed appear to be effective, and the outcomes of our patients were satisfactory. Yet further validation of the protocol is warranted.

**Conclusions**

BK virus nephropathy is an emerging complication of kidney transplant. The prevalence is alarming and requires increasing awareness. The use of cidofovir, leflunomide, ciprofloxacin, and immunoglobulins remain questionable.3-8 Our audit confirms that reducing immunosuppression appears to be the best approach for treating BKVN until a safe antiviral agent becomes available to treat this condition.

**References**

Progression of Hepatic Histopathology in Kidney Transplant Recipients With Chronic Hepatitis C Virus Infection and Effect of Immunosuppression on the Course of Hepatitis C Virus Infection

Murat Korkmaz,1 Sevgül Fakı,2 Serkan Öcal,1 Özgür Harmancı,1 Haldun Selçuk,1 Mehmet Haberal3

Abstract

Objectives: There is no correlation between alanine aminotransferase levels, viral load, and histologic findings at dialysis in patients with chronic hepatitis C virus infection. Identification of the severity of hepatitis C-related liver disease before transplant could provide valuable data about the risk for liver-related mortality after transplant. In this study, we aimed to identify the severity of liver disease in end-stage renal disease patients with chronic hepatitis C virus infection, the progression of hepatic histopathology after kidney transplant, and whether immunosuppressive therapy affected post-transplant viral replication and hepatic histology.

Materials and Methods: Antihepatitis C virus-positive kidney transplant recipients (45 patients) enrolled in the study. Liver biopsy was performed in 45 patients before and 16 patients after kidney transplant. Interferon was given to 28 of 45 patients before kidney transplant. Biopsy before and after kidney transplant was performed in 5 of 14 patients.

Results: Patients had higher viral load, with genotype 1 predominancy (91%). Sustained viral response was achieved in 14 of 28 patients (50%). The histopathologic features of 45 patients who had pretransplant liver biopsy were as follows: 22 patients had mild hepatocellular injury, 17 patients had mild chronic hepatitis, 5 patients had moderate chronic hepatitis, and 1 patient had serious hepatitis. Follow-up biopsy after kidney transplant (mean, 2 y) in 16 of 45 patients showed that 3 of 16 patients had mild hepatocellular injury, 4 of 16 patients had mild hepatitis, 6 of 16 patients had moderate hepatitis, 2 of 16 patients had serious hepatitis, and 1 patient had cirrhosis. Patients showed neither progression, regression, nor stable liver histology.

Conclusions: Even with worse genotype profiles, chronic hepatitis C virus infection has an indolent progression in patients with end-stage renal disease and kidney transplant. Follow-up biopsies of kidney transplant recipients show reasonable progression during the first 2 years.

Key words: End-stage renal disease, Hepatitis, Liver biopsy, Pathology

Introduction

Prevalence studies indicate that between 3.4% and 49% patients who are on maintenance hemodialysis are positive for antihepatitis C virus (HCV) in different regions of the world.1,2 The prevalence of HCV infection is higher in kidney transplant recipients (11%-49%) than hemodialysis patients.3 Although previous studies with short-term follow-up have shown similar outcomes between HCV-positive and HCV-negative kidney transplant recipients, several studies have shown worse graft and patient survival in HCV-positive patients after transplant (follow-up, 10 and 20 y).4,5 However, the survival advantage associated with transplant still is
present in comparison with HCV-infected patients who have end-stage renal disease (ESRD) and are on hemodialysis.\(^6\)

In dialysis patients with chronic HCV infection, serum aminotransferase levels are not reliable in determining disease activity and fibrosis severity.\(^7\) There is no correlation between alanine aminotransferase levels, viral load, and histologic findings in dialysis patients with chronic HCV infection.\(^7\) It is not routine in most transplant centers to perform liver biopsy and determine severity of liver disease in HCV-positive patients who are candidates for kidney transplant. However, histopathologic evaluation of the liver in HCV-positive kidney transplant candidates might increase accuracy of staging liver disease and improve patient selection. Patients with HCV infection are at increased risk for progressive liver disease, and HCV infection is an independent risk factor for death and graft loss.\(^9\)

The progression of liver disease is slow and does not occur in all patients. Immunosuppressive protocols and other comorbid conditions could contribute to liver disease progression. Most mortality in HCV-positive kidney recipients is associated with cardiovascular disease and nonliver-related sepsis. Therefore, identification of the severity of HCV-related liver disease before transplant could provide valuable data about the risk of liver-related mortality after transplant.\(^10,11\)

In this study, we aimed to identify the severity of liver disease in ESRD patients who had chronic HCV infection, the progression of hepatic histopathology after kidney transplant, and whether immunosuppressive therapy affected posttransplant viral replication and hepatic histology.

Materials and Methods

Patients

Anti-HCV-positive patients (83 patients) who were diagnosed as having chronic HCV infection, either by serology or histopathology, and had kidney transplant at Baskent University Ankara Hospital from 1982 to 2013 enrolled in the study. There were 45 of 83 patients (45\%) who had liver biopsy before kidney transplant and 16 of 45 patients who had follow-up liver biopsies after kidney transplant. Liver histopathology was assessed according to modified Knodell classification. Patients were classified as having mild hepatocellular injury or mild chronic hepatitis with Histology Activity Index (HAI) from 0 to 6 and fibrosis 0 to 1; moderate chronic hepatitis with HAI 7 to 12 and fibrosis 2 to 4; or serious chronic hepatitis with HAI 13 to 18, fibrosis 5, and presence of cirrhosis. All data were obtained retrospectively from the patient medical files.

Inclusion criteria were patients having regular evaluation at our center and diagnosed as anti-HCV-positive \(\geq 6\) months before kidney transplant, serologically diagnosed patients who were positive for both anti-HCV and HCV RNA, or anti-HCV-positive and HCV RNA negative patients who had histopathologically proven chronic HCV infection (age, 20-70 years).

Exclusion criteria were patients with irregular studies, HCV RNA-negative patients with biopsy findings that were incompatible with chronic HCV infection, and patients who had alcohol abuse or drug addiction.

Assays

Serum anti-HCV-positive status was diagnosed with a microparticle enzyme assay (AxSYM HCV version, MEIA, Abbott, Abbott Park, IL, USA) and chemiluminescent microparticle assay (The Architect System, CMIA, Abbott). Serum HCV RNA was diagnosed with nested polymerase chain reaction (PCR) from 1994 to 2003, newer technology (LightCycler) from 2003 to 2005, and real-time PCR assay since 2005 (Serum HCV RNA 10-time PCR, Cobas Tachman 48 HCV, Roche Diagnostics).

Statistical analyses

Data evaluation was performed with statistical software (SPSS, Version 16.0, SPSS Inc., Chicago, IL, USA). Numeric data were reported as median (minimum-maximum) or mean \(\pm\) standard deviation. Comparisons between groups were evaluated with Mann-Whitney test, Fisher exact test, chi-square test, Cox proportional hazards regression model, and Kaplan-Meier method. Statistical significance was defined by \(P \leq .05\).

Results

There were 45 kidney transplant patients with chronic HCV infection enrolled in the study, including 30 male (66.6\%) and 15 female patients (33.3\%) with median age 46 years (range, 26-69 y). All
45 patients had pretransplant liver biopsy, and 16 of 45 patients had liver biopsies before and after transplant. Posttransplant biopsies were performed 2 years after kidney transplant. There were 41 patients (91.1%) on hemodialysis, 1 patient had peritoneal dialysis, and 3 patients had both hemodialysis and peritoneal dialysis at different times (median time, 48 mo; range, 1-276 mo). The leading causes of ESRD were glomerulonephritis (20%), hypertension (8.8%), pyelonephritis (8.8%), and unknown (40%). There were 39 patients (86.6%) who had living-related kidney transplant and 6 of 45 patients who had deceased-donor kidney transplant. Patients were on combined immunosuppressive treatment with cyclosporine (86.7%), azathioprine (32.5%), mycophenolate mofetil (45.8%), tacrolimus (30.1%), sirolimus (21.7%), and mycophenolic acid (30.1%).

Genotype profile of the patients included 30 patients (66.6%) who were genotype 1b, 11 patients (24.4%) who were genotype 1a, and 4 patients who were genotype 4.

Median viral load before kidney transplant was 55,000,000 IU/mL (range, 0-98,000,000 IU/mL). There were 21 of 45 patients (46.6%) who were HCV RNA-negative during kidney transplant and 7 of 21 patients (30%) who remained HCV RNA-negative after kidney transplant; 14 of 21 patients (70%) became HCV RNA-positive after kidney transplant. Seroconversion to HCV RNA-positive after kidney transplant was significantly lower in patients who were on tacrolimus than other immunosuppressive regimens ($P \leq .01$). However, higher viral load was associated with higher mortality caused by sepsis ($P \leq .008$).

There were 30 patients who received interferon (IFN) monotherapy before kidney transplant, and 28 of 30 patients had long-term follow-up data. There were 10 patients who received treatment for median 6 months, and 20 patients received treatment > 12 months (median, 12 mo; range, 12-19 mo). No patients had antiviral treatment after kidney transplant. Sustained viral response (SVR) rates were calculated with 28 patients because 2 patients were lost to follow-up; 14 of 28 patients (50%) achieved SVR. During long-term follow-up, only 1 patient had relapsed. Graft survival was 7.8 ± 2.5 years.

The histopathologic evaluation in 45 patients who had pretransplant liver biopsy included 22 patients (48.8%) who had mild hepatocellular injury, 17 patients (37.7%) who had mild chronic hepatitis, 5 patients (11.1%) who had moderate chronic hepatitis, and 1 patient (2.2%) who had serious hepatitis. No patients were cirrhotic. In 16 of 45 patients who had follow-up biopsies after kidney transplant (mean, 2 y), 3 of 16 patients (18.7%) had mild hepatocellular injury, 4 of 16 patients (25%) had mild hepatitis, 6 of 16 patients (37.5%) had moderate hepatitis, 2 of 16 patients (12.5%) had serious hepatitis, and 1 patient (6.2%) had cirrhosis on the follow-up biopsies. The comparison of pretransplant and posttransplant biopsy results of 7 patients showed that moderate hepatitis in these patients progressed to serious hepatitis in 2 patients, remained stable in 3 patients, and regressed to mild hepatitis in 2 patients.

There were 26 of 28 patients who had IFN treatment and had pretransplant liver biopsies, and 13 of 26 patients also had posttransplant biopsies. In 5 of 26 patients who had mild hepatitis, follow-up biopsies showed that 3 of 5 patients progressed to moderate hepatitis, 2 patients remained stable, and 1 patient progressed to cirrhosis from serious hepatitis (Table 1).

<table>
<thead>
<tr>
<th>Biopsy Before Transplant</th>
<th>No. of biopsies</th>
<th>Total</th>
<th>Interferon-Positive Biopsy Before Transplant</th>
<th>Interferon-Positive Biopsy After Transplant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mild hepatocellular injury</td>
<td>22 (48.8%)</td>
<td>26</td>
<td>Mild hepatitis: 5</td>
<td>Mild hepatitis: 2</td>
</tr>
<tr>
<td>Moderate hepatitis</td>
<td>17 (37.7%)</td>
<td>13</td>
<td>Moderate hepatitis: 7</td>
<td>Moderate hepatitis: 3</td>
</tr>
<tr>
<td>Serious hepatitis</td>
<td>5 (11.1%)</td>
<td></td>
<td>Moderate hepatitis: 3</td>
<td></td>
</tr>
</tbody>
</table>
| SVR rates were calculated with 28 patients because 2 patients were lost to follow-up; 14 of 28 patients (50%) achieved SVR. During long-term follow-up, only 1 patient had relapsed. Graft survival was 7.8 ± 2.5 years.

There were 14 patients who achieved SVR after IFN treatment, including 6 patients who had pretransplant and 5 of 14 patients who had posttransplant liver biopsies. In 3 patients who were diagnosed as having mild hepatitis before kidney transplant and after kidney transplant, 1 patient regressed to mild hepatocellular injury and 2 patients progressed to moderate hepatitis. There were 3 patients who were diagnosed with moderate hepatitis before kidney transplant; 1 of these patients remained stable and 1 patient regressed to mild hepatitis after kidney transplant, and 1 patient had no follow-up biopsy (Table 2).

<table>
<thead>
<tr>
<th>Biopsy After Transplant</th>
<th>Total</th>
<th>Interferon-Positive Biopsy After Transplant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mild hepatitis</td>
<td>5</td>
<td>Moderate hepatitis: 2</td>
</tr>
<tr>
<td>Moderate hepatitis</td>
<td>1</td>
<td>Serious hepatitis: 2</td>
</tr>
<tr>
<td>Cirrhosis</td>
<td>1</td>
<td></td>
</tr>
</tbody>
</table>

*Data reported as number (%) or number.
Patients with HCV infection are generally considered as having increased risk for progressive liver disease. The HCV-infected ESRD patients have milder hepatic necroinflammation and fibrosis than nonuremic HCV patients. The reason for this difference is not fully understood, but several theories have been proposed to explain this generally more indolent course. These include the altered immunologic state and relatively low HCV viral load as a result of clearance of HCV RNA by dialysate and/or possible viral clearance by dialyzer surface membranes; in addition, cytokines such as IFN-α and hepatocyte growth factor and antiviral activity may play a role. Long-term patient and graft survival rates are lower in anti-HCV-positive than anti-HCV-negative kidney transplant recipients. However, data are contradictory about whether or not the severity of underlying liver disease in patients with HCV has an effect on outcomes after kidney transplant. After kidney transplant, few HCV infected patients (4.3%) show progression to end-stage liver disease, and liver disease had no effect on overall patient and graft survival after mean follow-up 7.4 years. In a study that compared an immunocompetent control group with HCV-infected kidney transplant recipients, more rapid progression of liver fibrosis was observed in kidney transplant recipients. The rate of progression of liver fibrosis was similar between patients with HCV infection without kidney disease and HCV infected kidney transplant recipients. Another study examined the effects of liver fibrosis in pretransplant liver biopsy on patient survival in 58 patients who had chronic HCV and ESRD, including 10 patients with advanced fibrosis (METAVIR stages 3 or 4) who had kidney transplant; at 52 months after transplant, mortality was similar between patients who had advanced fibrosis (stages 3 and 4) and less-advanced fibrosis (stages 0, 1, and 2). However, some authors suggest that chronic liver disease increases mortality in patients with than without HCV after kidney transplant.

The course of liver histopathology after kidney transplant is not clear or easily predicted. In sequential biopsies every 3 to 4 years in 51 kidney transplant recipients, variable outcomes were reported including progression, stability, and improvement in liver fibrosis. The severity of pretransplant liver fibrosis and longer duration of posttransplant follow-up were associated with progression of liver fibrosis.

In a large study with 207 patients who had liver biopsy before transplant, 44 patients had 51 follow-up liver biopsies at 5-year intervals, either while on the waiting list for a kidney or after kidney transplant. Despite many years of immunosuppression, liver histology remained stable or improved in most rebiopsied transplant patients, but liver injury progressed more commonly in patients who remained on the waiting list; kidney transplant was associated with a survival advantage in HCV-infected kidney transplant recipients compared with patients who remained on dialysis.

In the present patients, the interval between 2 biopsies was short (2 y) and patient numbers were small, but we observed an indolent course and good prognosis for liver histology without major variations between the 2 biopsies. Therefore, immunosuppressive therapy did not have a detrimental effect on liver histology in HCV-infected kidney transplant recipients.

The question about the effect of HCV RNA load or genotype on progression of liver fibrosis after kidney transplant remains unanswered. However, some authors did not observe such a correlation between these parameters. The present patient group mainly consisted of genotype 1 patients (91%) who had high viral load (median viral load before kidney transplant, 55,000,000 IU/mL). There were 21 of 45 patients (46.6%) who were HCV RNA-negative during kidney transplant; 7 of 21 patients (30%) remained HCV RNA-negative after kidney transplant, and 14 of 21 patients (70%) became HCV RNA-positive after kidney transplant. Although viremia increases after transplant, the progression of liver fibrosis may decrease.

The primary causes of mortality for kidney transplant recipients, either HCV-positive or negative, were sepsis, cardiovascular disease, and...
malignancy. In the present patients, higher viral load was associated with higher mortality that was caused by sepsis (P ≤ .008).

Successful IFN treatment has favorable outcomes on liver histology in ESRD patients with HCV, and patients with an SVR have decreased liver histology progression after kidney transplant. The present patient group had 50% SVR (14 of 28 patients) but pre- and posttransplant biopsy results were available for only 5 patients. These patients demonstrated either progression, regression, or stable liver histology. The patient number was not large enough for further analysis of the effect of successful IFN treatment on posttransplant liver histology. However, after median follow-up 2 years in HCV infected kidney transplant recipients, these patients had favorable outcomes, and this suggests that HCV infection is not a contraindication for performing kidney transplant.

Seroconversion to HCV RNA-positive after kidney transplant was significantly less frequent in patients who were on tacrolimus therapy than other immunosuppressive regimens (P ≤ .01), and we did not observe such an effect on patients who were on cyclosporine treatment. This finding was inconsistent with previous studies. Cyclosporine has inhibitory effects on HCV replication in vitro. Although it was believed that tacrolimus did not have this property, a prospective study of 253 HCV-positive patients who underwent transplant showed that patients receiving cyclosporine or tacrolimus showed no significant differences in virologic and clinical outcomes. In 71 HCV infected kidney transplant recipients who were on cyclosporine or tacrolimus, analysis of viral kinetics and liver fibrosis showed that HCV viral load was lower in patients treated with tacrolimus than cyclosporine, and this effect became negligible 3 months after transplant. The extent of liver fibrosis was similar in both groups of HCV infected patients and a control group of non-HCV-infected patients after kidney transplant.

In conclusion, even with worse genotype profiles, we believe that chronic HCV infection has an indolent progression in patients with ESRD and kidney transplant. Follow-up biopsies of kidney transplant recipients show reasonable progression during the first 2 years after transplant. Tacrolimus may have favorable effect on outcome in patients with HCV infection compared with other common immunosuppressive drugs. Kidney transplant is a better option than hemodialysis for ESRD patients who have chronic HCV infection.

References

Efficacy and Safety of Tandem Hemodialysis and Immunoadsorption to Desensitize Kidney Transplant Candidates

Lionel Rostaing,1,2,3 Sébastien Maggioni,1 Corinne Hecht,1 Martine Hermelin,1 Eric Faudel,1 Nassim Kamar,1,2,4 Federico Sallusto,2 Nicolas Doumerc,2 Asma Allal1

Abstract

Objectives: We conducted a desensitization program in our center in patients undergoing kidney transplant for end-stage renal disease. These patients had a living-donor either ABO incompatible and/or human-leukocyte antigen-incompatible. The safety and efficacy of this program were evaluated.

Materials and Methods: A pretransplant desensitization program relies on immunosuppressants and apheresis to remove detrimental antibodies. We chose immunoadsorption as the apheresis technique, and coupled this with hemodialysis in a tandem procedure.

Results: We report on the efficacy of this new method in 120 procedures performed in 20 patients (14 ABO incompatible, 6 ABO incompatible/human leukocyte antigen-incompatible). The tandem procedure was well tolerated, and saved time compared with conducting sequential immunoadsorption and hemodialysis (6 h vs 10 h). The tandem procedure was associated with significantly decreased isoagglutinin titers and donor-specific alloantibodies (assessed by mean fluorescence intensity). Dialysance was effective (183, 102-264). The biochemical and hematologic parameters were similar to those observed after a conventional hemodialysis session, with the exception of protidemia; this might be related to some degree of albumin loss during the immunoadsorption procedure. The posttransplant events included 1) one ABO incompatible / human leukocyte antigen-incompatible patient with vein thrombosis and ultimate kidney loss; 2) two patients with steroid-sensitive cellular acute rejection; and 3) two patients with acute antibody-mediated rejection, which was successfully treated with apheresis and steroid pulses, plus rituximab in one and eculizumab in the other.

Conclusions: We conclude that the tandem immunoadsorption-hemodialysis procedure is efficient at desensitizing patients with end-stage renal disease who are candidates for a living ABO incompatible and/or human leukocyte antigen-incompatible donor-kidney transplant.

Key words: Pretransplant desensitization, Tandem procedure, Dialysance, ABO incompatible kidney transplantation, Nursing time

Introduction

In our kidney transplant center at Toulouse University Hospital, France, we have a large number of patients awaiting kidney transplants (> 500); we can only perform around 180 kidney transplants per year. Three years ago, we implemented a living kidney transplant program, which now accounts for about a third of our kidney transplants a year. However, we often face the problem of ABO incompatibility (ABOi) and / or HLA incompatibility (HLAi). In the latter case, this is due to the presence of preformed donor-specific alloantibodies (DSAs) in the recipient. In our center, DSAs are detected by the Luminex technique.
In 2011, we implemented a pretransplant desensitization program to increase the ability to use ABOi and/or HLAi living kidney donors. Desensitization relies on pretransplant immunosuppression in association with apheresis to remove culprit antibodies: isoagglutinins in the setting of ABOi kidney transplants, and DSAs in the setting of HLAi kidney transplants. The goal of these procedures is to obtain isoagglutinin titers of ≤ 1/8, and DSA titers (expressed as mean fluorescence intensity) of below 3000 at the time of kidney transplant. Although in some cases these goals were not met, we have conducted kidney transplants with the assumption that the pretransplant desensitization would nonetheless show benefit posttransplant.

Because immunoadsorption, which is normally performed on its own, is cumbersome both for the patients and medical staff, we coupled immunoadsorption with hemodialysis in a tandem session. This saves time for the medical and nurse staff and avoids the need for a return visit to the hospital the following day for hemodialysis. Herein, we report on the efficacy and safety of 120 tandem immunoadsorption/hemodialysis sessions.

Materials and Methods

This single-center study included 20 consecutive hemodialysis patients who were referred to our department for ABOi and/or HLAi kidney transplant from a living donor. There were 14 ABOi patients and 6 ABOi/HLAi patients. An ABOi kidney transplant was only considered when the pre-desensitization titer of the specific isoagglutinins was ≤ 1/256. In such cases, the patient could begin the pretransplant desensitization procedure (Figure 1).

In the setting of ABOi kidney transplant, we used specific immunoadsorption (IA) with non-reusable columns able to remove either anti-A isoagglutinins or anti-B isoagglutinins (Glycosorb ABO, Glycorex Transplantation, Lund, Sweden). For HLAi kidney transplants, we used immunoadsorption with reusable semi-specific columns (Immunosorba, Fresenius, Bad Homburg, Germany).

This protocol included a single injection of rituximab (375 mg/m²) at 30 days pretransplant. On the basis of the initial titer of the specific isoagglutinins, specific IA sessions were started at pretransplant days 8 to 10. We scheduled 3 specific IA sessions on alternating days, with titration of the isoagglutinin levels before every IA session. The goal was to achieve a pretransplant titer of ≤ 1/8. In cases when isoagglutinin titers were not decreasing rapidly, we conducted a plasmapheresis session between 2 IA sessions. In some cases, we also performed a few plasmapheresis sessions between pretransplant days 5 to 2; plasmapheresis sessions were never conducted the day before transplant. Other immunosuppressants (tacrolimus [0.2 mg/kg/d], mycophenolate mofetil [1 g b.i.d.], and prednisolone [0.5 mg/kg/d]) were started at 12 days pretransplant. At the same time, we also started *Pneumocystis jiroveci* prophylaxis using sulfamethoxazole (400 mg) and trimethoprim (80 mg), scheduled for 1 year. During this period, the dosage of recombinant erythropoietin was increased to avoid the need for blood transfusions at pretransplant.

We used a different protocol in the setting of ABOi + HLAi kidney transplant (Figure 2). Intravenous immunoglobulin was given at 1 g/kg during the hemodialysis sessions once, 40 days pretransplant. Patients received 2 doses of rituximab (375 mg/m²) on pretransplant days 30 and 15. Semi-specific IA was started on pretransplant day 17. The number of IA sessions was determined on the basis of the intensity of the pre-sensitization DSA titer; if > 10 000 units of mean fluorescence intensity was measured, we planned at least 9 sessions. If the DSA mean fluorescence intensity was < 10 000, we planned 6 sessions (Figure 2). After every 3 sessions we assessed the DSA mean fluorescence intensity to enable us to plan the next block of IA sessions.

Immunoadsorption was performed in tandem, as previously described. This allowed us to perform the two procedures within half a day of each other. The tandem procedure also allowed us to stop one or the other procedure if necessary. All patients were first connected to the hemodialysis generator and

Figure 1. Pretransplant Desensitization Protocol in the Setting of ABO Incompatible Kidney Transplant

Abbreviations: d, day; IA, immunoadsorption; MMF, mycophenolate mofetil
then to the immunoadsorption generator. Patients were treated via a large vascular access, either an arteriovenous fistula or a jugular central venous catheter. Before and after each tandem procedure, we assessed the patient’s weight (using a single balance for the whole apheresis unit) and drew blood to enable analyses of the following parameters: Na+ (mmol/L), K+ (mmol/L), bicarbonates (mmol/L), protidemia (g/L), calcemia (mmol/L), creatinine (µmol/L), hemoglobin (g/dL), platelet count (×10^3/mm^3), fibrinogen (g/L), and isoagglutinin titers (anti-A and/or anti-B, according to the incompatibility).

**Results**

We have analyzed 120 procedures performed over an 18-month period. There were no technical problems during the procedures; all sessions were completed satisfactorily. We did not observe any allergic reactions. The tandem procedure saved time compared to conducting sequential immunoadsorption and hemodialysis (6h vs 10 h). The average weight loss during these procedures was 3 kg (range, 0.7–4.3). The dialysance was 183 units (range, 102–264) in the hemodialysis sessions.

Table 1 presents the biochemical and hematologic parameters following the tandem procedure. We observed no change at all with regard to natremia; conversely kalemia decreased from 4.4 (3.4–6.0) to 3.9 (3.1–5.2) mmol/L, and bicarbonates and calcemia increased from 28 (16–37) to 31 (25–35) mmol/L and from 2.0 (1.6–2.45) to 2.23 (1.85–3.0) mmol/L, respectively. We observed an increase in hemoglobin levels from 9.9 (7.8–13) to 10.8 (8.8–14.3), whereas protidemia decreased from 51 (36–79) to 47 (30–67) g/L. There was no change with regard to platelets counts and fibrinogen levels.

At the final follow-up, the patient-survival rate was 100% and graft-survival rate was 90%. One patient presented at day 1 posttransplant with a renal-vein thrombosis, which led to a transplantectomy. A second patient presented on day 2 posttransplant with a renal-vein thrombosis, but this was successfully treated by surgery. Nonetheless, over the following days he presented with delayed graft function, which had hidden an acute humoral rejection. By day 10 posttransplant, he had features of thrombotic microangiopathy (thrombopenia, schistocytic anemia); because of this, he underwent a kidney-allograft biopsy, which indicated typical features of acute humoral/vascular rejection despite the fact that he had an isoagglutinin titer of ≤ 1/4. He was treated with pulses of methylprednisolone ([10 mg/kg] on 3 consecutive days) and daily plasmapheresis (up to 9 sessions). Because these therapies did not improve his condition, he was treated successfully with eculizumab (1200 mg a week for 2 weeks, then 900 mg every 2 weeks for up to 3 months). He progressively regained renal function and serum-creatinine levels stabilized at ~300 µmol/L. However, within the next few months, his renal function slowly deteriorated and hemodialysis had to be resumed at 15 months posttransplant.

Two patients presented with acute T-cell mediated rejection, which occurred on days 10 and 15 posttransplant; they were successfully treated with 3 pulses of methylprednisolone (10 mg/kg each). Their last serum creatinine levels were 160 and 170 µmol/L, respectively. Another patient presented with acute humoral/vascular rejection on day 10 posttransplant, which was treated by daily plasmapheresis (6 sessions in total) plus one injection of rituximab (375 mg/m^2) and 3 pulses of methylprednisolone (10 mg/kg each). The last serum creatinine measurement was 160 µmol/L.

**Table 1. Biochemical and Hematologic Parameters Before and After Tandem Procedures (n = 120)**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Preprocedure</th>
<th>Postprocedure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na(^+) (mmol/L)</td>
<td>141 (129–144)</td>
<td>141 (135–144)</td>
</tr>
<tr>
<td>K(^+) (mmol/L)</td>
<td>4.4 (3.4–6.0)</td>
<td>3.9 (3.1–5.2)</td>
</tr>
<tr>
<td>Bicarbonates (mmol/L)</td>
<td>28 (16–37)</td>
<td>31 (25–35)</td>
</tr>
<tr>
<td>Protidemia (g/L)</td>
<td>51 (36–79)</td>
<td>47 (30–67)</td>
</tr>
<tr>
<td>Calcemia (mmol/L)</td>
<td>2.0 (1.6–2.45)</td>
<td>2.23 (1.85–3.0)</td>
</tr>
<tr>
<td>Urea (mmol/L)</td>
<td>18.8 (6.4–46)</td>
<td>6.4 (1.4–30.6)</td>
</tr>
<tr>
<td>Creatinine (µmol/L)</td>
<td>797 (350–1515)</td>
<td>350 (97–786)</td>
</tr>
<tr>
<td>Hb (g/dL)</td>
<td>9.9 (7.8–13)</td>
<td>10.8 (8.8–14.3)</td>
</tr>
<tr>
<td>Platelets (×10^3/mm(^3))</td>
<td>171 (67–300)</td>
<td>161 (66–258)</td>
</tr>
<tr>
<td>Fibrogen (g/L)</td>
<td>2.4 (1.4–8.7)</td>
<td>2.8 (1.3–6.0)</td>
</tr>
</tbody>
</table>

Values are expressed as medians (ranges).
Discussion

In this study we have demonstrated that IA and hemodialysis, performed in tandem, are safe and efficient. With regard to safety, all tandem procedures were completed satisfactorily; none needed to be interrupted for any reason. No adverse events were observed. The tandem procedure was much more convenient than the serial procedure: we could stop either the immunoadsorption or hemodialysis without affecting the other, if needed. This gave the medical staff more flexibility. These results are consistent with the findings from other studies which have also reported on the safety and efficiency of plasmapheresis performed in tandem with hemodialysis. 2,3,4

Weight loss per tandem session averaged 3 kg, although the IA procedure was associated with a mean weight gain of ~1 kg. 1 Thus, before each tandem procedure, we anticipated the weight loss by taking the patient's dry weight into account as well as the weight delivered via the IA procedure.

The mean dialysance was efficient and was within the recommended values, an average of 183. In the setting of tandem procedure, with regard to electrolyte levels, natremia was stable at 141 mmol/L after the procedure and there was a decrease in kalemia from 4.4 to 3.9 mmol/L. We observed a slight increase in bicarbonates and calcemia; this is usually observed in conventional hemodialysis sessions. Because of the good dialysance we obtained, there was a large decrease in both urea (from 18.8 to 6.4 mmol/L) as well as serum creatinine (from 797 to 350 mmol/L). There was an increase in hemoglobin level, from 9.9 to 10.8 g/dL, which reflects the hemo-concentration. There was also a slight decrease in protidemia from 51 to 47 g/L, which may have been related to the loss of some albumin during the IA procedures. However, we cannot be certain of this explanation as albumin levels were not monitored during this study.

Coagulation was not impaired as assessed by fibrinogen levels or platelets counts.

In performing an ABOi kidney transplant, the goal is to have an isoagglutinin titer of ≤ 1/8 immediately before transplant. 5 Most recipients had only 1 ABO incompatibility, although 2 had both incompatibilities (an AB donor into an O-group recipient).

Isoagglutinin titers (anti-A and anti-B) were assessed before every tandem procedure. The tandem procedure reduced both isoagglutinin titers by 1 dilution; isoagglutinin anti-A was decreased, on average, from 1/32 to 1/16, and isoagglutinin anti-B was decreased, on average, from 1/8 to 1/4. These are values typically seen following an IA session. Because measuring DSA levels (as assessed by Luminex) is very expensive, we did not assess DSAs before and after every procedure.

This desensitization program enabled us to give 20 patients a living-donor kidney transplant. Patients’ survival rates at the last follow-up (> 2 years) was excellent, at 100%. There were 2 renal-vein thromboses within the first day posttransplant; 1 led to transplantectomy, whereas, in the second case, the thrombus was retrieved. However, the second patient subsequently developed oliguria and delayed graft function. This condition had initially masked the clinical signs of acute rejection. He eventually developed thrombotic microangiopathy 10 days posttransplant which was related to humoral/vascular rejection. This acute rejection episode responded poorly to pulses of methyl-prednisolone and daily plasmapheresis. Thus, we had to implement eculizumab therapy. There have been a few reports on humoral/vascular rejection after ABOi kidney transplant that have been resistant to conventional therapy but were responsive to eculizumab therapy. 6,7 Our patient, similarly, had a good clinical and biological response with eculizumab. However, because of sequelae, he continued to have poor renal function and eventually lost his allograft at 15 months posttransplant.

Two other patients (10%) presented with mild, reversible acute-rejection episodes; these were episodes of acute T-cell–mediated rejection. The reported acute cellular rejection rates after ABOi kidney transplant range between 5% to 12%. 8,9 Our series is therefore within the expected rates.

From our study, we conclude that a tandem desensitization procedure of immunoadsorption...
plus hemodialysis is safe and effective in the treatment of patients undergoing ABOi and/or HLAi kidney transplant for end-stage renal disease. Moreover, hemodialysis parameters, decreased isoagglutinin titers and DSAs indicate that this tandem procedure of immunoadsorption and hemodialysis is more efficient than its sequential counterpart.

References

T-Regulatory Cells in Chronic Rejection Versus Stable Grafts

Fatima Al-Wedaie,1,2 Eman Farid,1,2 Khaled Tabbara,1 Amgad E. El-Agroudy,3 Sumaya M. Al-Ghareeb3

Abstract

Objectives: Studying regulatory T cells in kidney allograft acceptance versus chronic rejection may help in the understanding of more mechanisms of immune tolerance and, in the future, may enable clinicians to induce immune tolerance and decrease the use of immunosuppressive drugs. The aim of the current study was to evaluate regulatory T cells in kidney transplant patients with stable graft versus transplant with biopsy-proven chronic rejection.

Materials and Methods: The 3 groups that were studied included: kidney transplanted patients with no rejection episodes (n = 43); transplanted patients with biopsy-proven renal rejection (n = 27); and healthy age-matched nontransplanted individuals as controls (n = 42). The percentage of regulatory T cells (CD4+CD25+Foxp3+) in blood was determined by flow cytometry.

Results: The regulatory T cell percentage was significantly lower in chronic rejection patients than control or stable graft groups. No significant difference was observed in regulatory T cell percentage between the stable graft and control groups. In the stable graft group, patients on rapamycin had a significantly higher regulatory T cell percentage than patients on cyclosporine. No effect of donor type, infection, or duration after transplant was observed on regulatory T cell percentage.

Conclusions: The results of the current study are consistent with previous studies addressing the function of regulatory T cells in inducing immunotolerance after kidney transplant. Considering the established role of regulatory T cells in graft maintenance and our observation of high regulatory T cell percentage in patients receiving rapamycin than cyclosporine, we recommend including rapamycin when possible in immunosuppressive protocols. The findings from the current study on the chronic rejection group support ongoing research of having treatment with regulatory T cells, which may constitute a novel, efficient antirejection therapy in the future.

Key words: Cyclosporine, End-stage renal disease, Rapamycin, Tacrolimus, Transplant

Introduction

Many studies show that T cells have an important function in peripheral and central tolerance. Naturally arising CD4+ T cells that express interleukin 2 receptor (IL-2R) chain (CD25) are involved in the regulation of immune responses and maintenance of natural self tolerance. Regulatory T (Treg) cells (CD4+CD25+) are required to prevent immunologic rejection of the fetus and graft transplant.1 The Treg cells perform primary regulatory mechanisms that are used to maintain immune homeostasis and prevent autoimmunity and have regulatory functions in pregnancy and allograft tolerance.2-4

An important key transcription factor, forkhead box P3 (Foxp3), is required for Treg development, maintenance, and function. Lacking Foxp3 leads to the development of autoimmune like lymphoproliferative disease.5 The Tregs that represent 5% to 10% peripheral circulating CD4+ T cells in humans and rodents are
observed in lymphoid tissue and at the graft site and can be isolated from the peripheral blood of recipients. Interleukin 10 (IL-10) is required for the generation and suppressor functions of Tregs. It was reported by van den Boogaardt and colleagues that more IL-10-producing cells were observed in kidney recipients with stable graft function than rejection. The Treg cells are present in 2 general categories: induced and naturally-occurring Tregs. There are 2 origins have been described for Foxp3+ cells. The first is the thymus, where Foxp3+ cells are generated approximately with positive selection of conventional CD4+ T cells. The second is the periphery, where a number of triggers induce the expression of Foxp3 in induced Treg cells. A 2-step Treg cell differentiation process occurs in which a Foxp3-CD25+ population, already enriched in a T-cell-receptor sequence found in mature Treg cells, is the first intermediate. Exposure to interleukin 2 (IL-2) can convert these intermediates into fully differentiated CD25+Foxp3+ cells.

However, some studies show that CD4+CD25+ Tregs also are found within tolerated allografts. The Treg cells suppress the function of the effector CD4+ T cells, CD8+ cytotoxic T cells, antigen-presenting cells, natural killer cells, and B cells. These are modes of action by which the Treg cells exert their regulatory effect in the induction and maintenance of transplant tolerance, anthropogenically. These include physical cell-to-cell contact with potential target cells, autocrine properties, and paracrine properties.

Calcineurin inhibitors are widely used such as cyclosporine, tacrolimus, and rapamycin, which down-regulate IL-2. Mycophenolate mofetil has a marked effect on interleukin 4 (IL-4) expression, alloantibody deposition, and expression of other cytokines. Both mycophenolate mofetil and mammalian target of rapamycin inhibitor (mTORi) inhibit proliferation of T and B lymphocytes, which is a key mechanism thought to cause their immunosuppressive effects. Rapamycin (RAPA) (also known as sirolimus) is a macrolide antibiotic produced by Streptomyces hygroscopicus. The RAPA binds to FK506-binding protein-12, a highly conserved cytoplasmic receptor. The FK506-binding protein-12-RAPA complex binds to and inhibits the activities of the serine/threonine protein kinase mammalian target of RAPA (mTOR), the activation of which is essential for protein translation and cell cycle initiation in T cells. The RAPA can convert peripheral CD4+CD25- naive T cells to CD4+Foxp3+ Treg cells using B cells as antigen-presenting cells, and this subtype of Tregs can potently suppress T-cell effector (Teff) proliferation and maintain antigenic specificity. The CD25-specific monoclonal antibodies such as daclizumab and basiliximab have positive effects on the ratio of Tregs to Teff cells. In addition, Bloom and coworkers showed that alemtuzumab promotes an increase in peripheral Tregs and may act as an intrinsic generator of Tregs in vivo. Immunosuppressive agents used also have an effect on Tregs, to generate Treg transcription factor Foxp3 and expand or induce alloantigen-reactive Tregs in vivo and in vitro. This maintains and induces specific immune tolerance for long graft survival.

The hypothesis of this study was that there is an increase in the percentage of CD4+CD25+Foxp3+ Tregs with successful renal allograft. In contrast, in patients with frequent episodes of immune-mediated (biopsy-proven) rejection, Treg cells are reduced. Studying immune tolerance induced by Treg cells (CD4+CD25+) in allograft acceptance may improve the understanding of the mechanisms involved. Understanding these immunosuppressive mechanisms might allow us to manipulate them to boost immunosuppression and decrease the dose of immunosuppressive drugs and their well-known serious adverse events.

Materials and Methods

Study design
This prospective study was done on kidney transplanted patients in the Nephrology Transplant Unit, Salmaniya Medical Complex, Ministry of Health, Kingdom of Bahrain. This study was a population-based case-control study that included selected kidney transplanted subjects, based on their graft stability status (either stable or having graft rejection), in which we tested the function of Treg cells in graft tolerance.

Study subjects
Individuals having the following diseases or conditions were excluded from the study: autoimmune disease, malignancy, pregnancy, or allergy. In addition,
pediatric (age < 15 y) transplanted cases were excluded from this study. The subjects were studied in 3 groups: (1) group 1, kidney transplanted patients with no rejection episodes (n = 56); (2) group 2, transplanted patients with biopsy-proven renal rejection (n = 27); 3 patients had acute rejection and the other patients had chronic rejection; and (3) group 3, healthy age-matched nontransplanted individuals as controls (n = 43). Group 1 and 2 were further divided according to duration to transplant (< 5 y, 5 - 10 y, and > 10 years) and immunosuppressive drug used (cyclosporine, RAPA, or tacrolimus).

Flow cytometry
Whole blood (2 mL) was collected from the patients and controls in tubes that contained ethylenediaminetetraacetic acid. Peripheral blood mononuclear cells were isolated by density gradient centrifugation (Ficoll-Paque, Pharmacia, Uppsala, Sweden). The cells were used directly after isolation (control group) or frozen (-80°C). Frozen cells were first treated with 10% dimethylsulfoxide in fetal calf serum and frozen at -23°C for 30 minutes and then at -80°C until processed.

Flow cytometry analysis with 3 colors was used to detect CD4+CD25+Foxp3+ cells. A commercial kit was used that was composed of fluorochrome-conjugated antihuman CD4, CD25, and Foxp3 antibodies and the needed buffers (Human Treg Flow Kit FOXP3 Alexa Fluor 488/CD4 PE-Cy5/CD25PE, Biolegend, San Diego, CA, USA), and that had been designed and formulated specifically for flow cytometry analysis of human Treg (CD25+CD4+) cells in a mixed lymphocyte population. The test procedure was performed according to instructions from the manufacturer. Samples were read with the flow cytometer (FC500 Flow cytometer Beckman Coulter, Brea, CA, USA).

Statistical analyses
Descriptive statistics were performed to compare the various parameters between the different groups. Statistical analysis was performed using a spreadsheet (Excel 2007, Microsoft, Redmond, WA, USA) and statistical software (SPSS, Version 15.0, SPSS Inc., Chicago, IL, USA). Data were expressed as mean ± standard deviation (SD), percentage, range, and median. Differences were analyzed for statistical significance with the chi-square test. Significant differences were defined by $P \leq 0.05$.

Ethical research approval
Transplanted patients and healthy controls recruited for the study were asked to complete and sign an informed consent form to participate in the study. After explaining to them the purpose of the study and its implications, they were asked to complete a standardized questionnaire form. Approval of the Salmaniya Medical Complex and Ministry of Health research committees was obtained, and all of the protocols conformed to the ethical guidelines of the 1975 Helsinki Declaration.

Results
The study population included 3 groups: transplanted patients with a stable graft, transplanted patients with graft rejection, and a healthy control group with no history of transplant. There were 112 samples that were tested for percentage of Treg cells. The age of subjects ranged from 17 to 70 years. Further subgrouping was done according to sex, immunosuppressive medication (cyclosporine, RAPA, or tacrolimus), donor type (living-related, living-nonrelated, or deceased), posttransplant period (< 5 y, 5 - 10 y, or > 10 y) and presence of infection (urinary tract infection, cytomegalovirus, hepatitis B virus, or hepatitis C virus) (Table 1). Percentage of Tregs was calculated from total

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Rejection (n = 27)</th>
<th>Stable (n = 43)</th>
<th>Control (n = 42)</th>
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<tr>
<td>Sex</td>
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<tr>
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<tr>
<td>Hepatitis C virus</td>
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</table>
peripheral blood lymphocytes. Flow cytometry dot plots were generated (Figure 1).

To investigate the relation between the percentage of Tregs and graft outcome (stable or chronic rejection), we compared the percentages in the 3 groups; our results showed that percentage of Tregs was significantly lower in chronic rejection patients (0.442% ± 0.321%) than control subjects (1.576% ± 0.607%; \( P \leq .001 \)) or patients with a stable graft (1.814% ± 0.775%; \( P \leq .001 \)) (Figure 2). Comparing 2 groups simultaneously showed that the total rejection group (\( n = 27 \)) had a significantly lower mean Treg percentage (0.534% ± 0.534%) than the control group (\( n = 42 \)) (1.576% ± 0.607%; \( P \leq .001 \)) (Similarly, the chronic rejection group [\( n = 24 \)] had a significantly lower mean Treg percentage [0.442% ± 0.321%] than the stable graft group [\( n = 43 \)] [1.814% ± 0.775%; \( P \leq .001 \)]. However, no significant difference was observed in the percentage of Tregs between the stable graft group (\( n = 43 \)) (1.814% ± 0.775%) and control group (\( n = 42 \)) (1.576% ± 0.607%; \( P = .119 \)).

To examine the effect of the immunosuppressive drug (cyclosporine, RAPA, or tacrolimus) on the percentage of Tregs, we compared the percentage of Tregs in the stable graft group according to the immunosuppressive medication. Patients on RAPA had the highest mean Treg percentage compared with the other 2 drugs and the control group (Figure 3); transplanted patients with stable graft on RAPA (\( n = 12 \)) had a significantly higher Treg percentage (2.475% ± 0.638%) than patients on cyclosporine (\( n = 18 \)) (1.550% ± 0.734%; \( P = .001 \)); transplanted patients with stable graft on RAPA (\( n = 12 \)) had a significantly higher Treg percentage (2.475% ± 0.638%) than patients on tacrolimus (\( n = 13 \)) (1.569% ± 0.598%; \( P = .001 \)); and transplanted patients with stable graft on RAPA (\( n = 12 \)) had a significantly higher Treg percentage (2.475% ± 0.638%) than the control group (\( n = 42 \)) (1.576% ± 0.607%; \( P < .001 \)).

No significant difference was observed in the percentage of Tregs according to the type of donor, whether living-nonrelated (\( n = 39 \)) (1.280% ± 0.860%) or living-related donor (\( n = 28 \)) (1.280% ± 0.860%; \( P = .636 \)) in all examined transplant recipients (\( n = 67 \)). Similar findings were observed in the stable graft group (\( n = 43 \)), in which there was no significant...
difference in the percentage of Tregs between the living-nonrelated (n = 23) (1.80% ± 0.659%) and living-related donors (n = 19) (1.744% ± 0.845%; P = .821).

No significant difference was observed in the percentage of Tregs according to presence of infection in the stable graft (n = 43) group when comparing patients with no infection (n = 39) (1.826% ± 0.754%) and patients with infection (n = 4) (1.450% ± 0.191%; P = .027). Similar findings were observed in the graft rejection group (n = 27), in which no significant difference was observed in the percentage of Tregs between patients with no infection (n = 21) (0.527% ± 0.453%) and patients with infection (n = 6) (0.560% ± 0.397%; P = .875). The small number of patients with infection affected the validity of the statistical comparison in both groups.

No significant difference was observed in the percentage of Tregs according to sex in the stable graft group (n = 43) when comparing males (n = 32) (1.745% ± 0.711%) with females (n = 11) (2.036% ± 0.682%; P = .244). Similar findings were observed in the graft rejection group (n = 27), in which no significant difference was found in the percentage of Tregs between males (n = 13) (0.418% ± 0.253%) and females (n = 14) (0.485% ± 0.372%; P = .605).

No significant difference in percentage of Tregs was observed when comparing the studied 3 periods; < 5 years (n = 24) (1.416% ± 0.817%) versus 5 to 10 years (n = 29) (1.430% ± 0.952%; P = .953); or 5 to 10 years (n = 29) (1.430% ± 0.952%) versus > 10 years (n = 17) (1.089% ± 0.871%; P = .239).

In summary, the results of the current study did not show any significant effect of donor type, infection, or duration posttransplant on the percentage of Tregs. However, the percentage of Tregs was affected by the immunosuppressive medication and was significantly lower in patients with graft rejection.

Discussion

The current study explored the role of Tregs in kidney transplant as an immunotolerance tool of the immune system. There were 3 different groups studied: patients with stable grafts, patients with graft rejection, and healthy controls with no history of kidney transplant. The Tregs were identified as CD4+CD25+Foxp3+.

To evaluate whether clinical parameters affected the frequency of Tregs, we evaluated some transplant factors such as donor origin (whether the kidney was from related [living-related donor] vs nonrelated donor [living-nonrelated or deceased donor]) and infectious factors (BK virus, cytomegalovirus, hepatitis B virus, and hepatitis C virus). The results of our study revealed that neither the type of donor nor the presence of infection had an effect on circulating Tregs; this also was reported in other studies.6,13,23

Regarding the effect of sex, we found no significant difference in Treg percentage between male and female patients. To study the effect of duration posttransplant on Tregs, we divided the patients according to the period posttransplant into 3 subgroups: < 5 years; 5 to 10 years; and > 10 years. No significant difference was found between the 3 groups.

In this study, we showed that there was a significantly lower Treg population in patients with chronic graft rejection than stable graft. The lower percentage of Treg cells with graft rejection reflects the role of Tregs in graft acceptance. The Tregs play a central role in the induction and maintenance of transplant tolerance.24 In addition, a significant difference was found between healthy control and chronic graft rejection patients in the current study, which agrees with findings of Karczewski and coworkers.13 However, there was no significant

Abbreviations: CSA, cyclosporine; Prog, prograf (tacrolimus); RAPA, rapamycin; stab, stable
difference between healthy controls and patients with graft acceptance, in agreement with findings of Kim and associates, but not with findings of other studies that found an increase in patients with graft acceptance or a significant decrease of Tregs in transplant patients compared with healthy controls. The variation between the different studies could be explained by the heavy immunosuppressive regimen posttransplant that may differ from one center to another and that affects Tregs.

We were able to measure the percentage of Tregs in 2 patients, at 2 different times: the first during the acute rejection phase and later when reaching the chronic rejection state. We observed that the level of Tregs decreased to half, which supports the role played by Tregs in maintaining immunotolerance and emphasizes the prominent role Tregs have in reversing acute rejection and preventing patients from reaching chronic rejection state. This hypothesis was supported by Zheng and coworkers, who suggested that Tregs generated ex vivo can act like a vaccine that generates host suppressor cells, with the potential to protect major histocompatibility complex-mismatched organ grafts from rejection. They showed this in an animal model in which they injected nontransplanted mice with a single dose of CD4+ and CD8+ Tregs, transferred donor cells every 2 weeks to mimic the continuous stimulation of a transplant, and observed increased splenic Tregs that were of recipient origin. This is further supported by another group that reported that levels of urinary mRNA for Foxp3 were correlated with the reversal of acute rejection in renal transplant patients receiving conventional immunosuppressive therapy.

The CD4+CD25+Foxp3+ Tregs are the most important subpopulation involved in immunoregulation. These cells have been suggested to prevent acute and chronic graft rejection. The absence of CD4+CD25+Foxp3+ T cells within the grafted kidney appears to be associated with irreversible acute rejection, indicating their role in local immunoregulation. Chauhan and associates showed that the frequencies of Tregs remain the same in allograft-rejecter and allograft-acceptor patients. However, the frequency of Tregs isolated from the lymph nodes of the allograft-acceptor patients is significantly higher than those isolated from allograft rejecters. In addition, those highly expressed Foxp3+ Tregs are functionally highly effective in preventing rejection. Indeed, Foxp3 is the major transcription factor associated with Treg development and function. Mutations within the Foxp3 gene locus can lead to immune dysregulation, polyendocrinopathy, enteropathy, X-linked (IPEX) disease, type 1 diabetes, thyroiditis, hemolytic anemia, and thrombocytopenia.

According to Kim and associates, who studied patients before and after kidney transplant and healthy matched individuals, the median frequency of Tregs in the recipients was 4.2% (range, 2.5% to 9.7%) and healthy controls was 2.7% (range, 1.6% to 5.7%). However, there was no significant difference between the recipients and healthy controls before transplant. They further compared the median frequencies of Tregs in patients with different underlying diseases and found no significant differences based on the underlying disease in kidney transplanted patients. According to their study, the frequency of Tregs decreased significantly after transplant. They reported the median frequency of circulating Tregs as 2% (1% to 3.6%) at 1 week, 1.6% (0.5% to 3.3%) at 2 weeks, and 2.5% (1.7% to 3.7%) at 8 weeks.

To study the effect of immunosuppressive drugs on Tregs, we divided our patients into 3 subgroups according to the therapy taken: RAPA, cyclosporine, or tacrolimus. Patients with stable graft on RAPA had a significantly higher level of Tregs than control subjects, and patients on cyclosporine had significantly lower levels. The RAPA had the significant effect of increasing the production of Tregs, unlike cyclosporine which had an inhibitory effect. This finding is supported by several other studies. In contrast, other studies reported that everolimus is best, and RAPA is second best, at increasing Tregs; we had no study patients on everolimus.

In conclusion, the results of the current study are consistent with previous studies addressing the role of Tregs in inducing immunotolerance after kidney transplant.

In summary, the following results were observed:

- Treg percentage was significantly decreased in chronic rejection patients than control subjects or stable graft patients.
- There was no significant difference between the
percentage of Tregs in the stable graft and control groups.

- Patients on RAPA in the stable graft group maintained a significantly higher Treg percentage than transplanted patients on cyclosporine.
- There was no effect of donor type, infection, or duration posttransplant on Tregs.

Considering the established role of Tregs in graft maintenance, and our observation of higher Treg percentage in patients receiving RAPA than cyclosporine, we recommend that clinicians include RAPA when possible in their immunosuppressive protocols. The findings from the current study on the chronic rejection group support ongoing research about treatment with Tregs that, in the future, may constitute a novel, efficient antirejection therapy and a way to monitor progress of transplant patients.

References

Spectrum of Histopathologic Diagnosis of Lymph Node Biopsies After Liver and Kidney Transplant

Eylem Akar Özkan,1 B. Handan Özdemir,1 E. Şebnem Ayva,1 Funda Gerçeker,1 Fatih Boyvat,2 Mehmet Haberal3

Abstract

Objectives: Our aim was to review our single center experience regarding histopathologic features arising from enlarged lymph nodes following solid-organ transplant.

Materials and Methods: In 2148 people who had solid-organ transplant from 1985 to 2013, there were 34 patients (1.58%) who developed lymphadenopathy. A retrospective review was performed to evaluate demographic, clinical, and histopathologic features of medical and pathologic records.

Results: Nonneoplastic lesions were more common, comprising 70.5% (n = 24) all cases which included nonspecific reactive lymphoid hyperplasia in 8 patients (33.3%), tuberculous lymphadenitis in 6 patients (25%), amyloid lymphadenopathy in 4 patients (16%), dermatopathic lymphadenopathy in 2 patients (8.3%), Kikuchi-Fujimoto disease in 1 patient (4.16%), hemangioma in 1 patient (4.16%), plasmacytic form of Castleman disease and amyloid lymphadenopathy in 1 patient (4.16%), and sea blue histiocytosis in 1 patient (4.16%). Neoplastic lesions comprised 29.41% (n = 10) cases which included posttransplant lymphoproliferative disorder in 6 patients (60%), Kaposi sarcoma in 2 patients (20%), posttransplant lymphoproliferative disorder and Kaposi sarcoma in 1 patient (10%), and metastatic carcinoma in 1 patient (10%).

Conclusions: Detecting enlarged lymph nodes in solid-organ transplant recipients is an infrequent occurrence. Infectious diseases, posttransplant lymphoproliferative disorder, and malignancies related to transplant should be considered in the differential diagnosis when enlarged lymph nodes in solid-organ transplant recipients are encountered.

Key words: Biopsy, Kaposi sarcoma, Lymphadenopathy, Posttransplant lymphoproliferative disorder, Tuberculosis

Introduction

Organ transplant is a life saving option for individuals with end-stage organ disease, and more than 28,000 solid-organ transplants are performed yearly in the United States.1 However, solid-organ transplant patients must receive intensive long-term immunosuppressive therapy to prevent rejection of the transplant, putting them at high risk of developing de novo malignancies and opportunistic infections. In addition, recurrent diseases are some of the main entities leading to late graft loss.

Clinically, lymphadenopathy may be peripheral or visceral. Peripheral lymphadenopathies are detected easily by routine physical examination and biopsied often because they are accessible easily for lymphadenectomy, which is a minor surgical procedure. In contrast, visceral lymphadenopathy requires laparotomy or sophisticated imaging techniques for detection. Among the peripheral nodes, those in the upper part of the body (cervical, supraclavicular, or axillary) are biopsied preferentially than lower limb nodes (popliteal, inguinal, or femoral) because the former are more likely to yield definitive diagnosis, whereas the latter often are characterized by
nonspecific reactive or chronic inflammatory and fibrotic changes. However, there is a paucity of information about the spectrum of diseases affecting lymph nodes from this region.

Enlarged lymph nodes often are biopsied in transplant recipients to determine whether the adenopathy is due to reactive lymphoid hyperplasia, infection, lymphoma, or Kaposi sarcoma. It is important to distinguish between these entities because treatments differ and adverse events may arise from delayed diagnosis of infectious or malignant etiologies. However, it often is challenging to determine the cause of enlarged peripheral lymph nodes on clinical examination, and clinicians often are faced with the decision to biopsy enlarged lymph nodes and the urgency of this procedure.

The aim of this study was to highlight some of the nonneoplastic and neoplastic conditions that may be seen more commonly in lymph node biopsies from transplant recipients, bearing in mind that some of these conditions may occur concurrently. In addition, lymphoproliferative disorders are mentioned even though these are predominantly extranodal in transplant recipients. We examined the etiology of lymphadenopathy in these biopsies and tried to determine clinical factors that may serve as predictive markers for diagnosis.

Materials and Methods

All 2148 solid-organ transplant (1740 kidney, 408 liver) recipients who underwent transplant between 1985 and 2013 were included in the study. In these patients, there were 34 patients (1.58%) who had lymph node biopsy at the same center.

Basic demographic data, age, sex, Epstein-Barr virus status, immunosuppressive therapy, time from transplant to lymph node biopsy, B symptoms (fever, weight loss, and night sweats), purified protein derivative (PPD) status, medical history, and location of lymphadenopathy were abstracted from the electronic medical records. Follow-up information was obtained from medical records or by direct communication with patients or families.

In this study, we included lymph node biopsies which were taken from the head and neck, axilla, thorax, abdomen, pelvis, and inguinal region. In some patients, multiple lymphadenopathies were observed at different locations. The size of lymph nodes was determined based on either radiographic or pathologic measurements.

Clinical symptoms were recorded such as painful lymphadenopathy, palpable lymphadenopathy, documented fever > 37.7°C, and unintentional weight loss ≥ 10% total body weight within 1 year. Type of biopsy (fine needle aspiration biopsy [FNAB], core needle (Tru-Cut) biopsy, or surgical excision) and pathologic and microbiologic diagnoses were documented.

The study was approved by the Ethical Review Committee of the institute.

Data analysis was performed with statistical software (SPSS for Windows, Version 16.0, SPSS Inc., Chicago, IL, USA). Average data were reported as mean ± SD. Comparisons between groups were made with Mann-Whitney and Kruskal-Wallis tests. Categorical data were compared with Fisher exact test and chi-square test. Statistical significance was defined by \( P \leq .05 \).

Results

The frequency of lymphadenopathy was greater in kidney recipients (30 of 1740 patients [1.72%]) than liver recipients (4 of 408 patients [0.98%]). Of 34 patients, 23 patients (67.6%) were male and 11 patients (32.3%) were female.

Mean age at transplant was 31 ± 13 years (range, 0.5 to 55 y). Mean age at the time of lymph node biopsy was 36.64 ± 14.15 years (range, 1 to 59 y) and the mean interval to lymph node biopsy after transplant was 70.14 ± 86.3 months (range, 1 to 274 mo). There were 3 patients (8.8%) who were younger than 19 years at the time of lymph node biopsy.

There were 23 patients (67.6%) who had living-related-donor transplant and 11 patients (32.35%) who had deceased-donor transplant. Indications for transplant varied (Table 1).

There were 15 patients who had progressive dysfunction of the renal allograft at a mean 31.6 ± 55.5 months (range, 1 to 195 mo) after renal transplant, and 5 of these 15 patients had kidney retransplant. A patient with oxalosis also underwent kidney transplant at 4 months after liver transplant.
All liver transplant and 4 kidney transplant recipients received tacrolimus and 24 kidney transplant recipients received cyclosporine as immunosuppressive drugs. Additionally, all aforementioned 34 patients received corticosteroids. The remaining 2 patients received only corticosteroids.

At lymph node biopsy, all patients had immunosuppressive therapy for a mean 5.8 ± 7.19 years (range, 0.5 - 22.8 y).

Lymphadenopathy was detected incidentally during transplant (n = 7) or graft nephrectomy (n = 5) in 12 of 34 patients. In the remaining 22 patients, lymphadenopathy was detected by physical (n = 12) and radiographic examination (n = 10). The examination of 7 patients who had lymphadenopathy that was detected during kidney transplant showed amyloidosis in 3 patients, hemangioma in 1 patient, sea blue histiocytosis in 1 patient, Castleman disease and amyloidosis in 1 patient, and granulomatous inflammation in 1 patient. Patients with a diagnosis of tuberculosis did not have a history of tuberculosis and/or positive PPD before the recent diagnosis. Patient demographics and characteristics were summarized (Table 2).

The distribution of lymphadenopathy that was biopsied showed that the most common region of lymphadenopathy was the pelvic area (Table 3). In this study, 41.7% patients (n = 14) had generalized lymphadenopathy. Excisional biopsy was performed in 76.4% patients (n = 26), Tru-Cut biopsy in 20.5% patients (n = 7), and FNAB in 2.9% patients (n = 1).

During follow-up, 22 of these 34 patients were alive and 12 patients were dead at a mean 41.25 ± 41 months after lymph nodes biopsy (range, 1 to 140 months).

Histopathologic examination showed that 24 of 34 cases were nonneoplastic and 10 cases were neoplastic. Histopathologic features of the 34 cases were shown (Table 4).

The PTLD was the most common malignancy, occurring in 7 patients and accounting for 70% of all malignancies in our study. Histologic diagnosis was

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Table 1. Indications for Transplant in Patients Who Developed Lymphadenopathy After Liver and Kidney Transplant

<table>
<thead>
<tr>
<th>Transplant</th>
<th>Indication</th>
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</tbody>
</table>

---

Table 2. Patient Demographics and Characteristics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>PTLD</th>
<th>Kaposis</th>
<th>Metastatic</th>
<th>Reactive</th>
<th>Infectious</th>
<th>Amyloid</th>
<th>Dermatopathic</th>
<th>Castleman</th>
<th>Kikuchi</th>
<th>Sea Blue Histiocytosis</th>
<th>Hemangioma</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patients</td>
<td>7</td>
<td>2</td>
<td>1</td>
<td>8</td>
<td>6</td>
<td>4</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
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<tr>
<td>Mean age at first transplant (y)</td>
<td>26.21</td>
<td>24.5</td>
<td>23</td>
<td>30.8</td>
<td>38.5</td>
<td>30</td>
<td>20</td>
<td>48</td>
<td>35</td>
<td>50</td>
<td>28</td>
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<tr>
<td>Mean age at biopsy (y)</td>
<td>32.5</td>
<td>26.5</td>
<td>42</td>
<td>38.6</td>
<td>42.1</td>
<td>35.2</td>
<td>20</td>
<td>48</td>
<td>53</td>
<td>50</td>
<td>28</td>
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<tr>
<td>Sex (male/female)</td>
<td>4/3</td>
<td>1/1</td>
<td>0/1</td>
<td>8/0</td>
<td>2/4</td>
<td>3/1</td>
<td>1/1</td>
<td>1/0</td>
<td>1/0</td>
<td>1/0</td>
<td>1/0</td>
</tr>
<tr>
<td>Transplanted organ</td>
<td>Liver</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<td></td>
<td>Kidney</td>
<td>4</td>
<td>2</td>
<td>1</td>
<td>8</td>
<td>6</td>
<td>4</td>
<td>1</td>
<td>1</td>
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<td>1</td>
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<tr>
<td>Immunosuppressive therapy (%)</td>
<td>Tacrolimus</td>
<td>57</td>
<td>0</td>
<td>0</td>
<td>25</td>
<td>17</td>
<td>0</td>
<td>50</td>
<td>0</td>
<td>100</td>
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<td></td>
<td>Cyclosporine</td>
<td>43</td>
<td>100</td>
<td>100</td>
<td>62.5</td>
<td>83</td>
<td>75</td>
<td>50</td>
<td>0</td>
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<td></td>
<td>Steroid</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>12.5</td>
<td>0</td>
<td>25</td>
<td>0</td>
<td>100</td>
<td>0</td>
<td>0</td>
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<td>Average duration (mo)</td>
<td>75.2</td>
<td>26.5</td>
<td>102</td>
<td>234</td>
<td>102</td>
<td>42</td>
<td>68.5</td>
<td>3</td>
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<td>Fever (%)</td>
<td>72</td>
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<td>0</td>
<td>37.5</td>
<td>83</td>
<td>25</td>
<td>100</td>
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<td>Night sweats (%)</td>
<td>14</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>33</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>100</td>
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<tr>
<td>Weight loss (%)</td>
<td>43</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>17</td>
<td>25</td>
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<td>0</td>
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<tr>
<td>Lymphadenopathy detection (%)</td>
<td>Palpable</td>
<td>86</td>
<td>100</td>
<td>0</td>
<td>12.5</td>
<td>50</td>
<td>0</td>
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<tr>
<td>Radiographic</td>
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<td>0</td>
<td>100</td>
<td>37.5</td>
<td>33</td>
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<td>50</td>
<td>0</td>
<td>100</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Incidental</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>50</td>
<td>17</td>
<td>100</td>
<td>50</td>
<td>100</td>
<td>0</td>
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<tr>
<td>Localization type (%)</td>
<td>Localized</td>
<td>57</td>
<td>0</td>
<td>100</td>
<td>100</td>
<td>67</td>
<td>50</td>
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<td>0</td>
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</tr>
<tr>
<td>Generalized</td>
<td>43</td>
<td>100</td>
<td>0</td>
<td>0</td>
<td>33</td>
<td>50</td>
<td>50</td>
<td>100</td>
<td>100</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Mean lymph node size (cm)</td>
<td>2.37</td>
<td>3.75</td>
<td>1.8</td>
<td>2.67</td>
<td>2.53</td>
<td>2.5</td>
<td>2</td>
<td>6</td>
<td>1.4</td>
<td>2.2</td>
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<tr>
<td>Diagnostic technique (%)</td>
<td>Excisional</td>
<td>86</td>
<td>0</td>
<td>0</td>
<td>87.5</td>
<td>83</td>
<td>100</td>
<td>50</td>
<td>100</td>
<td>0</td>
<td></td>
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<tr>
<td>Core needle (trucut) biopsy</td>
<td>14</td>
<td>100</td>
<td>0</td>
<td>12.5</td>
<td>17</td>
<td>0</td>
<td>50</td>
<td>0</td>
<td>100</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Fine needle aspiration biopsy</td>
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<td>0</td>
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<td>0</td>
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<td>0</td>
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</tbody>
</table>

Abbreviations: PTLD, posttransplant lymphoproliferative disorder
monomorphic B-cell PTLD (diffuse large B-cell lymphoma) in 4 patients (57.4%), early lesion PTLD in 2 patients (28.5%), and monomorphic T-cell PTLD in 1 patient (14.2%). Epstein-Barr virus was positive in 4 histologic specimens.

For patients diagnosed with PTLD, mean age at transplant was 26.21 ± 15.56 years (range, 0.5 to 51 y). Mean age at lymph node biopsy was 32.5 ± 19.54 years (range, 1 - 52 y) and the mean interval to lymph node biopsy after transplant was 75.2 ± 101.54 months (range, 5 - 250 mo). The frequency of nodal PTLD was greater in liver transplant patients (3 of 408 patients [0.73%]) than kidney transplant patients (4 of 1740 patients [0.22%]). There were 4 patients who had deceased-donor transplant and 3 patients who had living-related-donor transplant. Graft loss was experienced by only 1 renal transplant patient. The mean short-axis lymph node size was 2.37 ± 0.77 cm (range, 1.5 - 3.5 cm) in all cases with PTLD.

In 3 of the 34 lymph node biopsies, Kaposi sarcoma was observed. In 3 patients who had Kaposi sarcoma (8.8%), immunosuppressive therapy was based on cyclosporine. Most Kaposi sarcoma cases were seen in male patients. Mean age at lymph node biopsy was 25 ± 3.6 years (range, 22 - 29 y) and the mean interval to lymph node biopsy after transplant was 23.6 ± 5.1 months (range, 18 - 28 mo) for these 3 cases. Furthermore, gastrointestinal system involvement was observed in 2 of these patients who were diagnosed with Kaposi sarcoma. All of the patients died at a mean 12.6 ± 13.8 months after the diagnosis of Kaposi sarcoma (range, 1 - 28 mo). Additionally, as a result of the postmortem examination, which was performed only in 1 of these 3 patients, early-lesion PTLD (plasmacytic hyperplasia) was observed in a different lymph node.

In 1 of the 4 liver transplant recipients, thyroid papillary carcinoma was detected in the right supraclavicular lymph node 5 years after transplant. She was treated with total thyroidectomy and radioactive iodine therapy and she is alive during follow-up.

Among the nonneoplastic etiologies, nonspecific reactive hyperplasia was most common and tuberculous lymphadenitis was second most common. There were 6 patients who were diagnosed with tuberculosis; 4 of the patients had living-related-donor transplant and 2 patients had deceased-donor transplant. In 2 patients who were diagnosed with tuberculous lymphadenitis, graft loss occurred and 1 patient was retransplanted. There were 2 patients who had tuberculosis history; the PPD values of these patients were 13 mm and anergic. Hepatitis C virus (HCV) seropositivity was observed in 2 patients who were diagnosed with tuberculous lymphadenitis. Enlarged lymph nodes were located in the head and neck and intrathoracic regions. In 2 cases, lymphadenopathy was observed in multiple locations. There were 4 patients who were diagnosed with tuberculous lymphadenitis who were alive, and 2 patients died at 2 and 140 months after diagnosis.

<table>
<thead>
<tr>
<th>Table 3. Distribution of Lymph Node Biopsy Localization</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diagnosis</td>
</tr>
<tr>
<td>-------------------------------</td>
</tr>
<tr>
<td>PTLD (n = 7)</td>
</tr>
<tr>
<td>Kaposi sarcoma (n = 2)</td>
</tr>
<tr>
<td>Metastatic cancer (n = 1)</td>
</tr>
<tr>
<td>Reactive (n = 8)</td>
</tr>
<tr>
<td>Infectious (n = 6)</td>
</tr>
<tr>
<td>Amyloid (n = 5)</td>
</tr>
<tr>
<td>Dermatopathic (n = 2)</td>
</tr>
<tr>
<td>Kikuchi-Fujimoto (n = 1)</td>
</tr>
<tr>
<td>Sea blue histiocytosis (n = 1)</td>
</tr>
<tr>
<td>Hemangioma (n = 1)</td>
</tr>
<tr>
<td>Total (n = 34) (%)</td>
</tr>
</tbody>
</table>

**Abbreviations:** PTLD, posttransplant lymphoproliferative disorder

<table>
<thead>
<tr>
<th>Table 4. Histopathologic Diagnosis of Lymphadenopathy in 34 Solid-Organ Transplant Patients Undergoing Lymph Node Biopsy</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Histopathologic Diagnosis</strong></td>
</tr>
<tr>
<td>------------------------------------------------------------------</td>
</tr>
<tr>
<td>Total</td>
</tr>
<tr>
<td>Malignant lymph node group</td>
</tr>
<tr>
<td>PTLD</td>
</tr>
<tr>
<td>Kaposi sarcoma</td>
</tr>
<tr>
<td>Papillary thyroid carcinoma</td>
</tr>
<tr>
<td>PTLD and Kaposi sarcoma</td>
</tr>
<tr>
<td>Benign lymph node group</td>
</tr>
<tr>
<td>Reactive</td>
</tr>
<tr>
<td>Tuberculous lymphadenitis</td>
</tr>
<tr>
<td>Amyloid lymphadenopath</td>
</tr>
<tr>
<td>Dermatopathic lymphadenopathy</td>
</tr>
<tr>
<td>Castleman's Disease and amyloid lymphadenopathy</td>
</tr>
<tr>
<td>Kikuchi-Fujimoto disease</td>
</tr>
<tr>
<td>Sea blue histiocytosis</td>
</tr>
<tr>
<td>Hemangioma</td>
</tr>
<tr>
<td><strong>Total</strong></td>
</tr>
</tbody>
</table>

**Abbreviations:** PTLD, posttransplant lymphoproliferative disorder
In this nonneoplastic group, there were 5 amyloidosis cases, including 1 patient who had associated Castleman disease. Of these 5 patients with amyloidosis, 2 patients lost their grafts. There were 4 of 5 patients who had tuberculosis history, and 3 of these 4 patients had negative PPD values measured. In 2 patients with amyloid A(AA), the cause of end-stage renal disease was renal amyloidosis secondary to familial Mediterranean fever in 1 patient and juvenile rheumatoid arthritis in 1 patient. The cause of renal amyloidosis was unknown in 2 patients; 1 patient had a history of tuberculosis (PPD was negative) and the other 1 patient had Castleman disease and nephrolithiasis, which leads us to think that these diseases could be linked with AA type amyloidosis. At follow-up, 1 patient who was diagnosed with amyloidosis was alive and 4 patients were deceased.

Dermatopathic lymphadenopathy was observed in 2 patients, including 1 patient who had liver and kidney transplant due to oxalosis and 1 patient who had kidney transplant due to nephrolithiasis. Itchy skin lesions were observed in both patients.

Castleman disease, Kikuchi-Fujimoto disease, sea blue histiocytosis, and nodal hemangioma were observed incidentally in lymph node biopsies.

In statistical analysis, fever, weight loss, night sweats, generalized lymphadenopathy, graft loss, sex, age, and lymph node size were not associated with lymphadenopathy etiology.

**Discussion**

We reviewed the records of 34 solid-organ transplant recipients with lymphadenopathy over a 28-year period to determine the prevalence of various etiologies of lymphadenopathy.

We found that readily available clinical characteristics could help distinguish patients at high risk for malignant or infectious etiologies from those with reactive lymphadenopathy. These results may aid clinicians in deciding the need and urgency for lymph node biopsy in renal and liver transplant recipients.

The development of neoplastic pathologies in lymphadenopathy could be explained by intense immunosuppressive therapy during acute rejection episodes in patients with graft loss.

The non-Hodgkin lymphoma is one of the most common malignancies diagnosed after transplant. Risk of non-Hodgkin lymphoma in transplant recipients is 3- to 21-fold higher than the general population and 120-fold higher in children who receive transplants. In our series, most malignancies were categorized as PTLD, which typically occurs with advanced disease and frequent extranodal involvement. Our previous study showed that 33% patients were diagnosed with nodal PTLD.

The risk of Kaposi sarcoma in transplant recipients parallels Human herpesvirus 8 (HHV-8) seroprevalence in different countries and regions. The reported incidence of Kaposi sarcoma in solid-organ transplant recipients varies from 0.5% in transplant recipients from North America, Asia, and northern Europe to 28% in HHV-8-seropositive transplant recipients from the Middle East. In 25% cases, Kaposi sarcoma has visceral involvement and may involve the lymph nodes, lungs, gastrointestinal tract, and liver. In our study, most Kaposi sarcoma cases were diagnosed in the first 2 years after receiving a renal allograft, which is compatible with previous studies.

A study that evaluated radiographic images of enlarged lymph nodes in the abdominal region in liver transplant patients showed that 26% lymphadenopathies were reactive. Nonspecific reactive lymphoid hyperplasia accounted for 23.5% biopsies in our study, similar to the previous study.

Posttransplant tuberculosis is a life-threatening opportunistic infection that is encountered, but the diagnosis often is delayed. Mycobacterial infection occurred approximately 20 to 74 times more frequently in renal transplant patients than the general population. In Turkey, tuberculosis is observed in 26.2 per 100,000 population. Posttransplant tuberculosis is observed in 3.5% of our transplant population. In the current study, 17.6% lymphadenopathy patients had tuberculous lymphadenitis. There were 6 cases of tuberculous lymphadenitis in this study, including positive PPD documented in 33.6% patients (n = 2), most of whom were treated for latent tuberculosis infections after this diagnosis was made. In an earlier population-based study, chronic obstructive pulmonary disease, HCV infection, and cyclosporine-based immunosuppressants were independent risk factors for subsequent tuberculosis in renal transplant recipients.
recipients. In the current study, 2 patients had tuberculosis history; the PPD value in 1 of the patients was 13 mm, and the other patient was anergic to the PPD test. The HCV seropositivity was observed in 2 patients who were diagnosed with tuberculous lymphadenitis. Most of our patients (5 of 6 patients) who were diagnosed with tuberculous lymphadenopathy were using cyclosporine as an immunosuppressive therapy, which is consistent with the literature. In 2 of our patients who were diagnosed with tuberculous lymphadenitis, graft loss occurred, and 1 of them was retransplanted. There were 2 patients (33%) who developed tuberculosis in the first year after transplant. Optimal immunosuppressive agent use, monitoring of individuals at high risk for tuberculosis, and appropriate treatment are required to treat tuberculosis infection in solid-organ transplant patients.

Previously, we showed that the incidence of amyloid lymphadenopathy was 22% in uremic patients, but in the current study, this incidence was 14.7%.

Symptoms such as night sweats, weight loss, and generalized lymphadenopathy, which may be indicators of malignancy in immunocompetent individuals, were not different for immunosuppressed patients. These symptoms may be observed in some benign cases such as tuberculosis and amyloidosis (familial Mediterranean fever). This similarity may cause difficulties in evaluating lymphadenopathy cases at transplant.

There is no comprehensive study about the etiology of lymphadenopathy in solid-organ transplant patients. In the literature, multivariate analysis in individuals whose immune systems were suppressed, such as HIV-infected persons, showed an association between nonreactive etiology and factors such as increasing age, antiretroviral use, presence of fever, weight loss, and lymph node size. However, in our study on solid-organ transplant patients, we could not identify any association between nonreactive etiology of lymphadenopathy and these factors.

A limitation of this study was that the study included retrospective data which probably disregarded clinical features of lymphadenopathy such as duration and consistency. These features may have contributed valuable data to a prediction tool, but they could not be documented reliably from charts.

In summary, lymphadenopathy in solid-organ transplant recipients in areas endemic with tuberculosis is likely reactive or malignant. Readily available clinical factors such as immunosuppressive drug use, larger size, presence of fever, or weight loss may direct clinicians to decide about the urgency of lymph node biopsy in this setting.

References
Bone Marrow Involvement by Lymphoproliferative Disorders After Solid-Organ Transplant

Eylem Akar Özkăn,1 B. Handan Özdemir,1 Eda Yılmaz Akçay,1 Ayşen Terzi,1 Sema Karakuş,2 Mehmet Haberal3

Abstract

Objectives: Posttransplant lymphoproliferative disorders are classified as monomorphic, polymorphic, early lesions, or Hodgkin lymphoma. Bone marrow staging examination is recommended in posttransplant lymphoproliferative disorder patients. However, information about bone marrow involvement in these disorders is scarce. We evaluated 19 transplant patients with posttransplant lymphoproliferative disorder to investigate incidence of bone marrow involvement, associated morphologic changes, and prognosis.

Materials and Methods: We retrospectively assessed bone marrow findings of 19 transplant patients with posttransplant lymphoproliferative disorder who underwent bone marrow staging at Baskent University from 1985 to 2013. Clinical and pathologic data were reviewed from the medical records. Follow-up information was obtained from medical records or communication with patients or families. Data collected including age, sex, Epstein-Barr virus status, immunosuppressive therapy, elapsed time from transplant to diagnosis of posttransplant lymphoproliferative disorder, B symptoms, number of extranodal sites, involvement of different organs, Ann Arbor clinical staging, hematologic parameters, and serum lactate dehydrogenase levels.

Results: There were 5 of 19 patients (26.3%) who had bone marrow involvement with posttransplant lymphoproliferative disorder, including 2 patients diagnosed with posttransplant lymphoproliferative disorder by lymph node biopsy and 1 patient each diagnosed by native liver biopsy, nasopharyngeal biopsy, or allograft liver biopsy. In 4 patients, there was monomorphic posttransplant lymphoproliferative disorder subtype and 1 patient had early lesion posttransplant lymphoproliferative disorder subtype. In 10 of 19 patients (52.6%), Epstein-Barr virus was detected with in situ hybridization, including 3 patients with bone marrow involvement who were diagnosed with Burkitt lymphoma (n = 1), diffuse large B-cell lymphoma (n = 1), or early lesion (n = 1).

Conclusions: Patients with posttransplant lymphoproliferative disorder have high incidence of bone marrow involvement and high mortality rates. Therefore, bone marrow examination may be important in the diagnosis and staging evaluation of posttransplant lymphoproliferative disorder.

Key words: Epstein-Barr virus, Posttransplant lymphoproliferative disorder, Staging

Introduction

Posttransplant lymphoproliferative disorder (PTLD) is a heterogeneous group of abnormal lymphoid proliferations that occur after solid-organ transplant or hematopoietic transplant. Most PTLDs are B-cell proliferations that are positive for Epstein-Barr virus (EBV). However, PTLDs of T- and/or natural-killer-cell lineage have been documented. According to the present World Health Organization classification...
system, PTLDs are classified as early lesions, polymorphic, monomorphic, or Hodgkin lymphoma-type. The monomorphic PTLDs have been subclassified, based on morphologic, immunophenotypic, genetic, and clinical features, into diffuse large B-cell lymphoma, Burkitt lymphoma, plasma cell myeloma, plasmacytoma-like lesions, and various subtypes of T- and natural-killer-cell lymphomas.1,2

Recommendations for staging PTLD currently are based on Ann Arbor clinical staging criteria. Bone marrow examination is an integral part of the Ann Arbor staging system for all lymphoproliferative malignancies. In non-Hodgkin lymphoma, Ann Arbor stage III to IV disease is an adverse prognostic feature in the International Prognostic Index. Bone marrow involvement by PTLD defines stage IV disease, and similar to immunocompetent patients with non-Hodgkin lymphoma, patients with advanced-stage PTLD have worse prognosis and shorter overall survival. Although bone marrow involvement by monomorphic PTLD is uncommon, there have been few studies documenting the frequency of bone marrow involvement in this patient population. Although some studies have shown that 40% of patients with PTLD have bone marrow involvement at staging, most studies have not addressed this issue in the context of the different subtypes of PTLD.3

Elimination of routine bone marrow biopsies in newly diagnosed patients would require a sensitive surrogate predictor of bone marrow infiltration because of the potential prognostic significance of bone marrow involvement.

In this study, we analyzed the clinicopathologic characteristics of patients with PTLD who were evaluated and treated at our institute, focusing on the frequency of bone marrow involvement by PTLD. In addition, we assessed the use of hematologic parameters (hemoglobin level and platelet count) and lactate dehydrogenase level as predictors of bone marrow involvement in patients with PTLD.

Materials and Methods

Case selection and clinical characteristics
We retrospectively analyzed results of 19 patients who were diagnosed with PTLD after solid-organ transplant and who underwent bone marrow examination during staging evaluation at Baskent University from 1985 to 2013. The study was approved by the Ethical Review Committee of the institute.

Clinical and pathologic data were reviewed from the medical records of patients who had PTLD. Follow-up information was obtained from medical records or by direct communication with patients or families. Data were collected including age, sex, EBV status, immunosuppressive therapy, time from transplant to diagnosis of PTLD, B symptoms, number of extranodal sites, involvement of different organs, Ann Arbor clinical stage, hematologic parameters, and serum lactate dehydrogenase level.

A clinical and radiographic staging approach was used in conjunction with bone marrow assessment, in accordance with the American Joint Committee on Cancer non-Hodgkin lymphoma staging system (AJCC Staging Manual, Sixth Edition).4 Morphologic features were assessed using sections of formalin-fixed, paraffin-embedded bone marrow biopsies stained with hematoxylin-eosin. All staging bone marrow biopsies considered positive for lymphomatous involvement showed morphologic evidence of disease on sections stained with hematoxylin-eosin and/or immunohistochemical staining.

Immunohistochemistry and in situ hybridization
Immunohistochemical stains performed on bone marrow biopsies included primary antibodies CD20, CD79a, PAX5, MUM1, CD138, BCL2, BCL6, CD30, CD3, CD2, CD5, CD7, CD4, and CD8 after heat-induced antigen retrieval and visualization (Envision plus, DAKO, Carpinteria, CA, USA) and diamino benzidine. The EBV-encoded small RNA in situ hybridization (EBER-ISH) was performed on paraffin sections using the supplied protocol from the manufacturer (Bond-max system, Leica Microsystems, Buffalo Grove, IL, USA).

Statistical analyses
Data analyses was performed with statistical software (SPSS for Windows, version 16.0, SPSS Inc, Chicago, IL, USA). Statistical comparisons between patient subgroups were performed using chi-square and Fisher exact tests for proportions. The Mann-Whitney test and 1-way analysis of variance were used to
analyze differences in hemoglobin level, platelet count, and lactate dehydrogenase level between patients with and without bone marrow involvement. Statistical significance was defined by $P < .05$.

Results

Posttransplant lymphoproliferative disorder case characteristics and staging bone marrow results

The incidence of PTLD in our transplant population was 1.2% in our previous study. The mean age of the 19 patients (14 men and 5 women) at PTLD diagnosis was $28.3 \pm 18.5$ years (range, 1 - 52 y), with 5 patients (26.3%) being younger than 18 years. Organs transplanted included kidney in 11 patients (57.8%) and liver in 8 patients (42.1%). All pediatric patients had liver transplant. The mean time from transplant to diagnosis of PTLD was $61.9 \pm 71.8$ months (range, 4 - 250 mo) (Table 1).

Based on the World Health Organization classification of PTLD, the subtypes of PTLD were monomorphic in 18 patients (94.7%) and early lesion in 1 patient (5.2%). In the 18 patients with monomorphic subtype, 14 patients had DLBCL and 1 patient each had Burkitt lymphoma, primary cutaneous CD30-positive T-cell lymphoma, peripheral T-cell lymphoma (not otherwise specified), and T-cell acute lymphoblastic leukemia. There was 1 patient who showed early lesion PTLD in 2 different lymph nodes with plasmacytic hyperplasia and infectious mononucleosis like (IM-like) features; in this patient, we diagnosed early lesion PTLD with IM-like features in bone marrow 3 years after detecting early lesion PTLD in lymph nodes.

Bone marrow involvement was detected in 5 of 19 PTLD cases (26.3%): 4 of 5 monomorphic PTLDs (80%) (3 DLBCLs and 1 Burkitt lymphoma) and 1 of 5 early lesion PTLDs (20%) (Table 2). None of the patients who had T-cell monomorphic PTLD ($n = 3$) had bone marrow involvement.

All patients ($n = 19$) underwent a single organ transplant before developing PTLD. In 1 patient, primary cutaneous CD30-positive T-cell lymphoproliferative disorder recurred and was restaged with bone marrow examination.

The EBV status of the PTLD analyzed by EBER-ISH was determined in 19 cases. In the EBV-negative cases, 2 of 9 patients (22.2%) exhibited bone marrow involvement, compared with 3 of 10 EBV-positive cases (30%), and this difference was not statistically significant.

In 14 of 19 patients who had PTLD (73.6%), there was extranodal disease involving the gastrointestinal tract (6 patients), lung (3 patients), skin (2 patients), native liver (1 patient), allograft liver (1 patient), mediastinum (1 patient), nasopharynx (1 patient), spleen (1 patient), breast (1 patient), omentum (1 patient) and soft tissue (1 patient). Disseminated PTLD was observed in 2 of 19 PTLD patients, and 4

### Table 1. Clinical Characteristics of the Patients*

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Bone Marrow Positive ($n = 5$)</th>
<th>Bone Marrow Negative ($n = 14$)</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>16 ± 11.4</td>
<td>32.7 ± 18.8</td>
<td>&gt; .05</td>
</tr>
<tr>
<td>Pediatric (age &lt; 19 y)</td>
<td>2 (40)</td>
<td>3 (21.4)</td>
<td>&gt; .05</td>
</tr>
<tr>
<td>Sex (male/female)</td>
<td>4/1</td>
<td>10/4</td>
<td>&gt; .05</td>
</tr>
<tr>
<td>Organ</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kidney</td>
<td>1 (20)</td>
<td>10 (71.4)</td>
<td>&gt; .05</td>
</tr>
<tr>
<td>Liver</td>
<td>4 (80)</td>
<td>4 (28.5)</td>
<td></td>
</tr>
<tr>
<td>Immunosuppressive therapy</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tacrolimus</td>
<td>4 (80)</td>
<td>3 (21.4)</td>
<td>&gt; .05</td>
</tr>
<tr>
<td>Cyclosporine</td>
<td>1 (20)</td>
<td>11 (78.5)</td>
<td>&gt; .05</td>
</tr>
<tr>
<td>Use of induction therapy</td>
<td>3 (60)</td>
<td>2 (14.2)</td>
<td>&gt; .05</td>
</tr>
<tr>
<td>Time from transplant to PTLD (mo)</td>
<td>14.8 ± 10.8</td>
<td>78.7 ± 77.1</td>
<td>&lt; .05</td>
</tr>
<tr>
<td>Extranodal sites</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Involved</td>
<td>3 (60)</td>
<td>11 (78.5)</td>
<td>&gt; .05</td>
</tr>
<tr>
<td>Not involved</td>
<td>2 (40)</td>
<td>3 (21.4)</td>
<td></td>
</tr>
<tr>
<td>B symptoms</td>
<td>2 (33.3)</td>
<td>6 (42.8)</td>
<td>&gt; .05</td>
</tr>
<tr>
<td>Follow-up (mo)</td>
<td>74.4 ± 50.7</td>
<td>60.5 ± 57.6</td>
<td>&gt; .05</td>
</tr>
</tbody>
</table>

*Data reported as mean ± SD, number (%), or number.

### Table 2. Characteristics of 5 Patients With Bone Marrow Involvement With Posttransplant Lymphoproliferative Disorders*

<table>
<thead>
<tr>
<th>Case</th>
<th>Age (y)/Sex</th>
<th>Transplant Organ</th>
<th>PTLD site</th>
<th>Tm EBER</th>
<th>Bone Marrow Finding</th>
<th>Duration (mo)</th>
<th>ACR</th>
<th>Immunosuppressive Therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3/male</td>
<td>Liver</td>
<td>Allograft liver</td>
<td>Positive</td>
<td>Monomorphic, B cell (Burkitt lymphoma)</td>
<td>17</td>
<td>2</td>
<td>Tacrolimus</td>
</tr>
<tr>
<td>2</td>
<td>29/female</td>
<td>Liver</td>
<td>Lymph node</td>
<td>Positive</td>
<td>Early lesions (IM-like and PH)</td>
<td>13</td>
<td>0</td>
<td>Tacrolimus</td>
</tr>
<tr>
<td>3</td>
<td>22/male</td>
<td>Liver</td>
<td>Lymph node</td>
<td>Negative</td>
<td>Monomorphic, B cell (DLBCL, T-cell rich)</td>
<td>32</td>
<td>1</td>
<td>Tacrolimus</td>
</tr>
<tr>
<td>4</td>
<td>5/male</td>
<td>Liver</td>
<td>Nasopharynx</td>
<td>Positive</td>
<td>Monomorphic, B cell, DLBCL</td>
<td>4</td>
<td>0</td>
<td>Tacrolimus</td>
</tr>
<tr>
<td>5</td>
<td>21/male</td>
<td>Kidney</td>
<td>Lymph node, spleen</td>
<td>Negative</td>
<td>Monomorphic, B cell, DLBCL</td>
<td>17</td>
<td>1</td>
<td>Cyclosporine</td>
</tr>
</tbody>
</table>

*Duration from kidney transplant to first diagnosis of PTLD.

**Abbreviations:** PTLD, posttransplant lymphoproliferative disorder; EBER, Epstein-Barr virus-encoded small RNA; IM-like, infectious mononucleosis like; PH, plasmacytic hyperplasia; PTLD, posttransplant lymphoproliferative disorder; Tm, Tumor
patients had multiorgan involvement. Involvement of extranodal sites was more common in cases without bone marrow involvement (11 of 14 patients [78.5%]) compared with cases with bone marrow involvement (3 of 5 patients [60%]), but this difference was not statistically significant.

Time from transplant to PTLD diagnosis in patients with bone marrow infiltration was 14.8 ± 10.8 months (range, 4 - 32 mo) and in patients without bone marrow involvement was 78.7 ± 77.1 months (range, 5 - 250 mo). Time from transplant to PTLD diagnosis was shorter in patients with bone marrow involvement compared with patients without bone marrow involvement, and this difference was statistically significant ($P < .05$) (Table 1).

Bone marrow infiltration was observed in 3 patients (60%) who underwent induction therapy. This difference was not statistically significant when compared with patients who did not receive induction therapy.

Tacrolimus was the most common immunosuppressive medicine in patients who had bone marrow involvement compared with other agents. A statistically significant difference was observed between tacrolimus and other immunosuppressive agents between patients who had or did not have bone marrow involvement ($P < .05$) (Table 1).

There were 9 of 19 PTLD cases (47.3%) who presented with a high clinical stage (III or IV) before bone marrow staging. The staging of bone marrow biopsy affected the final stage in only 2 of 19 cases (10.5%). In the 4 monomorphic PTLDs (21%) with bone marrow involvement at staging, bone marrow involvement changed the Ann Arbor stage in 2 cases, from stage II to IV (1 case) and from stage I to IV (1 case). Hemoglobin and lactate dehydrogenase levels were not significantly different between PTLD cases with or without bone marrow involvement (Table 3).

Survival in patients with bone marrow involvement (74.4 ± 50.7 mo [range, 28 - 155 mo]) was longer than in patients without bone marrow involvement (60.57 ± 57.6 mo [range, 1 - 180 mo]), but this difference was not statistically significant.

All patients with bone marrow involvement were alive at mean follow-up 74.4 ± 50.7 months (range, 28 - 155 mo). In contrast, 42.8% patients without bone marrow involvement had died at mean 49.8 ± 52.4 months (range, 1 - 131 mo). This result can be due to death of the patients not from PTLD; all patients died because of complications of liver or kidney transplant.

Bone marrow involvement in PTLD was not related to the subtype of PTLD, patient age, sex, EBV status of the PTLD, type of organ transplanted, B symptoms, or donor type.

**Discussion**

The incidence of PTLD in our transplant population was 1.2% in our previous study. Bone marrow involvement in PTLD patients was observed in 26.3% patients in the current study, and 22% cases had monomorphic PTLD. Montanari and coworkers reported a 23.5% incidence of bone marrow involvement at PTLD diagnosis, and this was confirmed in our study. When we compared the patients with or without bone marrow involvement, we found that the mean age of the former group was lower, and this finding is consistent with results in the literature.

In pediatric PTLD patients, Maeker and associates reported 15% incidence of bone marrow involvement at diagnosis. In the current study, 2 of 5 pediatric patients (40%) had bone marrow PTLD involvement.

Male predominance was observed in patients with and without bone marrow involvement in our study. A study performed with liver transplant patients showed that bone marrow PTLD was significantly more likely to present in male patients.

The time from transplant to diagnosis of PTLD in our series varied, ranging from < 4 months to > 20 years. Time from transplant to PTLD was shorter in patients with bone marrow involvement. A study performed with renal transplant patients showed findings similar to our study, but another study in solid-organ transplant patients did not detect a statistically significant relation between time from transplant to PTLD and bone marrow involvement.

### Table 3. Hematologic Parameters in Posttransplant Lymphoproliferative Disorder Patients With Respect To Bone Marrow Status

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Bone Marrow Positive ($n = 5$)</th>
<th>Bone Marrow Negative ($n = 14$)</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemoglobin (g/dL)</td>
<td>9.2 ± 1.38</td>
<td>11.1 ± 3.04</td>
<td>&gt; .05</td>
</tr>
<tr>
<td>Platelet count ($\times 10^9$/L)</td>
<td>238 ± 175</td>
<td>245 ± 503</td>
<td>&gt; .05</td>
</tr>
<tr>
<td>Lactate dehydrogenase (U/L)</td>
<td>559 ± 404</td>
<td>348 ± 201</td>
<td>&gt; .05</td>
</tr>
</tbody>
</table>
The PTLDs are believed to have a tendency for extranodal organ involvements. However, factors that affect the spread of the disease are not well defined. Another finding in the current study was the lower incidence of extranodal involvement in patients with than without bone marrow involvement. Similar to our study, Montanari and coworkers showed less bone marrow involvement at patients with extranodal PTLD compared with patients with nodal PTLD. The underlying pathophysiologic mechanisms responsible for this inverse relation between extranodal and bone marrow involvement are unclear and require further investigation.

In 14 patients who had extranodal PTLD, only 1 patient had allograft involvement (liver) coincident with bone marrow involvement, which is consistent with a previous study. In addition, consistent with the literature, we observed bone marrow involvement coincident with involvement of the native liver and spleen in 1 patient.

Survival was significantly longer in patients with than without bone marrow involvement. This finding can be explained by the observation that patients who did not have bone marrow involvement had PTLD diagnosis later than patients with bone marrow involvement. Thus, there is more likelihood to have other risk factors causing shorter survival such as infection and rejection during the longer period.

The high incidence of bone marrow involvement in patients with PTLD and high mortality rate of PTLD patients suggest that bone marrow examination may be important in the diagnosis and staging evaluation of PTLD. Thus, more detailed and larger studies are needed to clarify prognostic factors in these patients and understand the pathophysiology of bone marrow involvement in patients with PTLD.

References

The Effect of Pretransplant Chronic Hepatitis C Virus Infection Treatment on Graft and Patient Survival in Renal Transplant Recipients

Murat Korkmaz,1 Sevgül Fakı,2 Serkan Öcal,1 Özgür Harmancı,1 Fatih Ensaroğlu,1 Haldun Selçuk,1 Mehmet Haberal3

Abstract

Objectives: Studies have demonstrated worse graft and patient survival among hepatitis C virus-positive patients following kidney transplant. Eradication of hepatitis C virus infection before renal transplant with interferon should be considered in hepatitis C virus-infected patients undergoing dialysis who are on the waiting list for transplant. We investigated whether pretransplant hepatitis C virus infection treatment affected graft and patient survival, and we evaluated other contributing factors to these outcomes.

Materials and Methods: We enrolled 83 antihepatitis C virus-positive patients who were diagnosed with chronic hepatitis C virus infection by serology or histopathology and had renal transplant at Baskent University Ankara Hospital from 1982 to 2013. Data were obtained from patient medical files retrospectively. Patients were divided into 2 groups that had or did not have interferon treatment.

Results: In 83 renal transplant patients with chronic hepatitis C virus infection (57 male [69%] and 26 female [31%]), median age was 46 years (range, 26 - 69 y), and most patients were genotype 1-dominant (92%). Interferon monotherapy was received by 30 patients before renal transplant and 28 of 30 patients had long-term follow-up data. There were 14 of 28 patients (50%) who achieved sustained virologic response, and only 1 patient had relapse. Graft survival was significantly lower in patients who had treatment (6 y vs 9 y; \( P \leq .003 \)). However, patient survival rates were similar between groups. Patients who had interferon were younger and had longer hemodialysis duration before renal transplant than patients without treatment. Higher viral load was associated with higher mortality which was caused by sepsis.

Conclusions: Pretransplant hepatitis C virus infection treatment, although recommended before renal transplant, does not always have good outcomes. Pretransplant dialysis treatment period, age of recipient, and posttransplant higher viral replication rates may be important contributing factors related to graft and patient survival.

Key words: End-stage renal disease, Interferon, Outcomes

Introduction

Studies in developed countries indicate that 3.4% to 32.1% patients who are on maintenance hemodialysis are positive for antibodies against hepatitis C virus (HCV), and HCV positivity in less developed countries is 49%.1,2 The diversity of these rates results from differences between centers, countries, and geographic areas.3 The prevalence of HCV infection is higher in renal transplant recipients (11% to 49%) than the hemodialysis population.4,5

There is controversy regarding the long-term outcomes following kidney transplant between patients who have or do not have HCV. Although previous studies with relatively short-term follow-
up have found similar outcomes, several studies have demonstrated worse graft and patient survival among HCV-positive patients following kidney transplant after follow-up for 10 and 20 years.6,7

The main causes of mortality for renal transplant recipients, either HCV-positive or negative, were sepsis, cardiovascular disease, and malignancy.8 However, survival advantage associated with transplant still is present in HCV-infected patients who have end-stage renal disease (ESRD).9 It was shown that the relative risk of mortality was 0.36 for kidney transplant recipients, a 64% lower risk of death than individuals on the waiting list.10

The primary goal of HCV treatment is to achieve sustained virologic response (SVR) which is defined as undetectable serum HCV RNA (< 50 IU/mL) at 6 months after stopping treatment. The SVR is associated with normalization of alanine aminotransferase (ALT) levels and improved histology in most treated patients.11 Gordon and coworkers published long-term HCV RNA outcomes in a systematic review and stated that probability of remaining HCV RNA-negative test was 86% for patients followed on hemodialysis or 95% for renal transplant recipients at 48 months after achieving SVR.12 Thus, eradication of HCV infection before renal transplant is rational, and treatment with interferon (IFN) should be considered in HCV-infected patients undergoing dialysis who are on the waiting list for transplant.

In this study, we investigated whether pre-transplant HCV infection treatment had an effect on graft and patient survival, and we searched for the effect of other contributing factors to these outcomes.

**Materials and Methods**

**Patients**

In this study, we enrolled 83 anti-HCV-positive patients who were diagnosed with chronic HCV infection by serology or histopathology and had renal transplant at Baskent University Ankara Hospital from 1982 to 2013. Data were obtained from patient medical files retrospectively. Inclusion criteria were regular evaluation at our center; diagnosis as anti-HCV-positive ≥ 6 months before renal transplant; serologically diagnosed patients with positive anti-HCV and HCV RNA tests, or anti-HCV positive and HCV RNA negative tests; patients with histopathologically proven chronic HCV infection; and age from 20 to 70 years.

Exclusion criteria were patients with irregular evaluation, HCV RNA-negative patients with biopsy findings incompatible with chronic HCV infection, or alcohol or drug addiction.

Patients were divided into 2 groups as having or not having IFN treatment. Response to treatment was classified as: (1) early viral response (EVR) (≥ 2 logarithm decrease in basal HCV RNA or negative HCV RNA at week 12 of treatment); (2) SVR (serum HCV RNA negative at 6 months after stopping treatment); and (3) end-of-treatment response (EOTR) (serum HCV RNA negative and normal ALT levels at the end of treatment); and (4) graft survival (time between renal transplant and cessation of immunosuppressive drugs and beginning hemodialysis); or (5) relapse after renal transplant (reappearance of HCV RNA after renal transplant which was negative before renal transplant).

**Assays**

Serum anti-HCV positivity was diagnosed with a microparticle enzyme assay (AxSYM HCV version, MEIA, Abbott Diagnostics, Chicago, IL, USA) and chemiluminescent microparticle assay (The Architect System CMIA, Abbott). Serum HCV RNA was diagnosed with nested polymerase chain reaction from 1994 to 2003, new technology (LightCycler, Roche Diagnostics, Basel, Switzerland) from 2003 to 2005, and real-time polymerase chain reaction assays (Serum HCV RNA 10-time PCR, COBAS TaqMan 48 HCV, Roche).

**Statistical analyses**

Data analysis was performed with statistical software (SPSS, Version 16.0, SPSS Inc., Chicago, IL, USA). Numerical data were reported as median (range, minimum to maximum) or mean ± standard deviation. Comparison between groups was performed with Mann-Whitney test, Fisher exact test, chi-square test, Cox proportional hazards regression model, and Kaplan-Meier method. Statistical significance was defined by P ≤ .05.

**Results**

There were 83 renal transplant patients with chronic HCV infection enrolled in the study (57 male [69%] and 26 female patients [31%]; median age, 46 years [26 to 69 y]). There were 76 patients (92%) who were
on hemodialysis, 1 patient who had peritoneal dialysis, and 6 patients who used both methods at different times. Median was 48 months (range, 1 to 276 mo) for all patients. The most frequent causes of ESRD were glomerulonephritis (19% patients), hypertension (9%), pyelonephritis (9%), and unknown (36%). Living-related transplant was performed in 68 patients (81.9%) and deceased-donor renal transplant in 15 patients (18.1%).

Patients were on combined immunosuppressive treatment with cyclosporine (86.7%), azathioprine (32.5%), mycophenolate mofetil (45.8%), tacrolimus (30.1%), and other drugs.

Genotypes of the patients were genotype 1b in 56 patients (68%), genotype 1a in 19 patients (24.6%), and genotype 4 in 8 patients.

There were 30 patients who received IFN monotherapy before renal transplant, and 28 of 30 patients had long-term follow-up data. Median treatment was 6 months in 10 patients and > 12 months in 20 patients (median, 12 mo; range, 12 to 19 mo). No patient had antiviral treatment after renal transplant. There was EVR in 22 of 30 patients (75.8%) and EOTR in 20 of 30 patients (68.9%). There were 2 patients who were lost to follow-up; in the other 28 patients, SVR was achieved in 14 of 28 patients (50%). During long-term follow-up, only 1 patient had relapsed.

Graft survival was significantly lower in patients who had treatment (6 y vs 9 y; \( P \leq .003 \)). However, patient survival rates were similar between groups. The duration of treatment (< 12 mo or > 12 mo) had no effect on EVR, EOTR, or SVR rates \( (P > .05) \). Patients who had IFN treatment were younger than patients without treatment \( (P \leq .01) \), and hemodialysis duration before renal transplant was significantly longer in patients who had IFN treatment \( (P \leq .001) \). Other variables such as ESRD etiology, sex, and immunosuppressive protocols were similar between patients who had or did not have treatment.

Median viral load before renal transplant was 55000000 IU/mL (range, 0 to 98000000 IU/mL) and viral load was not statistically associated with EVR, EOTR, and SVR rates. However, higher viral load was associated with higher mortality due to sepsis \( (P \leq .008) \).

There were 39 patients who were HCV RNA-negative before renal transplant and 28 of 39 patients (72%) became HCV RNA-positive after renal transplant. Tacrolimus use was associated with less probability of reappearance of HCV RNA after renal transplant \( (P \leq .01) \).

Discussion

The HCV infection remains a major health problem in ESRD patients and renal transplant recipients. The HCV in patients with ESRD on hemodialysis ranges between 10% and 30%, which is > 5 times the frequency in the general population.13 A large cohort study which included 73 707 kidney transplant recipients, including 535 patients with HCV, showed no difference in patient and graft survival at 8 years following transplant between patients with and without HCV.14 Previous studies with short-term follow-up (< 10 y) have shown patient and graft outcomes similar after transplant between HCV-positive and negative groups.15-17 However, after long-term follow-up study results (> 10 y) were published, data about the negative effect of HCV infection on patient and graft survival began to accumulate.18-20 The reported relative risk of mortality in patients with HCV was 1.59 to 1.93, and relative risk of graft loss was 1.5. Liver-related mortality accounted for at least some of the excess mortality, and most deaths were from sepsis and cardiovascular causes. Although viremia increases after transplant, the progression of liver fibrosis possibly slows.21,22 This might explain the delayed adverse effect of HCV on survival. Fabrizi and coworkers showed that the presence of anti-HCV antibodies was an independent risk factor for graft failure (relative risk, 1.56).23 In a systematic review of 18 studies, the combined hazard ratio in HCV-infected recipients was 1.56-fold greater than in HCV-negative recipients. Potential explanations for the different study results include heterogeneous patient populations, small numbers of groups with short-term follow-up, differences in the stage of liver disease at transplant, prevalence of associated comorbidities such as diabetes and cardiovascular disease in the transplant recipients, and differences in immunosuppressive regimens.

Although there is controversy about the effect of HCV infection on the course of ESRD and graft and patient survival after renal transplant, it is generally accepted that HCV has a negative effect on these outcomes. Therefore, all hemodialysis patients with confirmed detectable HCV RNA who have not
previously received treatment for HCV infection should be considered as candidates for antiviral therapy based on IFN-α. American Gastroenterological Association and American Association for the Study of Liver Diseases guidelines recommend reduced doses of pegylated IFN-α (PEG-IFN) as monotherapy and consider ribavirin as contraindicated in this setting.²⁴,²⁵

Studies about HCV treatment in ESRD patients with IFNs reveal that response rates are acceptable and better than response in patients who have normal renal function, but there is high cost and sometimes intolerable adverse effects. The data of 482 patients derived from 25 published studies indicated that patients who received IFN monotherapy had SVR rates of 33% to 39%.²⁶,²⁷ The subanalysis results showed SVR rates 26% to 30.6% in patients with genotype 1. These SVR rates were higher than those reported in nondialysis patients who received non-PEG-IFN monotherapy (6% to 19%).²⁸ Possible explanations could be increased IFN exposure in dialysis patients as a result of the lower clearance of IFN or possible viral clearance with hemodialyse membranes.²⁹ Alavian and coworkers compared standard and PEG-IFN therapy results of 21 studies conducted with 491 hemodialysis patients who used standard IFNα and 12 studies conducted with 279 hemodialysis patients who used PEG-IFNα.³⁰ The pooled SVR rates for standard IFN monotherapy was 39.1% and PEG-IFNα monotherapy was 39.3%. Dropout rates were high (IFNα, 22.6%; PEG-IFNα, 29.7%). Only age < 40 years was significantly associated with SVR. The HCV RNA level, HCV genotype, ALT pattern, female sex, duration of infection, liver fibrosis stage, and treatment duration were not associated with SVR.³⁰ However, 1 head-to-head, randomized trial showed that the overall efficacy and safety were better in patients treated with PEG-IFN than conventional IFN.³¹

In our study 30 patients treated with either PEG-IFN or standard IFN before transplant and 14 of 28 patients (50%) who had long-term follow-up data had SVR rates. Our patient group mainly included genotype 1 patients, all patients used only IFN monotherapy, no patients used additional ribavirin, and we believe that our results are compatible with results in the literature.

Only 1 patient with SVR had relapse after transplant. In our study, graft and patient survival rates and factors which could affect these parameters were investigated. Graft survival rates were significantly lower in patients who were treated with IFN than patients who did not have HCV treatment (P ≤ .003). Although our patients had 50% SVR rates after treatment, graft survival rates were poorer than expected. Therefore, in addition to high SVR rates, we believe that other factors should be taken into consideration. Treated patients were younger (P ≤ .01), and average hemodialysis duration before transplant was longer in these patients than in nontreated patients (P ≤ .001). Our follow-up was 48 months and patient number was too small in comparison with large meta-analysis results. These factors could explain our results. However, these results could point out the importance of hemodialysis duration before renal transplant and younger age as negative contributing factors for graft survival.

Rostami and associates analyzed the results of 18 observational studies including 8348 HCV-infected patients from 123 228 living- and deceased-donor renal transplant recipients.³² They found that the combined hazard ratio for patient mortality in HCV-infected recipients was 1.69-fold (P = .0001) greater than in HCV-negative recipients. Their results were consistent with previous surveys, and all causes of mortality were significantly higher in HCV-infected patients.

In our study, overall patient survival was similar between patients who had or did not have treatment (P = .053), with a tendency toward better results in untreated patients. Mortality of patients with higher viral replication rates was higher (P ≤ .008), and sepsis was the main risk factor for mortality in highly viremic patients.

In conclusion, we suggest that pretransplant HCV infection treatment, although recommended before renal transplant, does not always have good outcomes. Pretransplant dialysis treatment duration, age of recipient, and higher posttransplant viral replication rates may be important contributing factors related to graft and patient survival.

References


Panel Reactive Antibodies in Predicting Hepatitis C Virus Treatment Outcome in Kidney Transplant Candidates

Serkan Öcal,¹ Özgür Harmancı,¹ Murat Korkmaz,¹ Fatih Ensaroğlu,¹ Turan Çolak,² Haldun Selçuk,¹ Gökhan Moray,³ Mehmet Haberal³

Abstract

Objectives: Chronic hepatitis C virus infection compromises hemodialysis patients and increases liver-related mortality. Interferon treatment is associated with improved sustained virological response rates and increased risk of graft loss after kidney transplant. This may be related to the development of antihuman leukocyte antigen antibodies, which may be a surrogate marker of potent immune response. We evaluated panel reactive antibody 1 and 2 levels for prediction of sustained viral response in patients with kidney transplant.

Materials and Methods: In this retrospective cohort study, we reviewed data from hepatitis C virus-infected hemodialysis patients who received interferon treatment before kidney transplant. Panel reactive antibody > 20% was considered positive. Sustained viral response rates for interferon treatment were obtained and compared with panel reactive antibody 1 and 2 values.

Results: There were 40 patients (16 female and 24 male patients; mean age, 41.5 y; range, 18-65 y). Sustained viral response rate was 18/40 (45%). Panel reactive antibody 1 was negative in 31 patients and positive in 9 patients. Sustained viral response ratio was not correlated with panel reactive antibody 1 positivity. Panel reactive antibody 2 was negative in 31 patients (sustained viral response: present, 11 patients; absent, 20 patients) and positive in 9 patients (sustained viral response: present, 7 patients; absent, 2 patients). Sustained viral response ratio was significantly correlated with panel reactive antibody 2 positivity.

Conclusions: We showed a correlation between panel reactive antibody 2 positivity and sustained viral response rates that may be a predictive tool for hepatitis C virus treatment response. In patients with other complications that compromise hepatitis C virus treatment, panel reactive antibody 2 may be a surrogate marker for sustained viral response prediction. The induction of cellular immunity may cause clearance of hepatitis C virus infection and formation of high panel reactive antibody 2 levels.

Key words: End-stage renal disease, Human leukocyte antigens, Interferon, Sustained viral response

Introduction

Chronic liver disease secondary to hepatitis C virus (HCV) is an important cause of morbidity and mortality in patients who receive hemodialysis treatment and kidney transplant.¹ The prevalence of HCV infection ranges from 5% to 15% in kidney transplant recipients.² The natural course of HCV in hemodialysis patients has not been fully elucidated. Monotherapy with nonpegylated interferon (IFN) (3 to 5 MU, 3 times weekly) or pegylated (PEG) IFN preparations (PEG IFN-α2a or PEG IFN-α2b) once weekly have shown efficacy defined by sustained viral response (SVR) rate from 30% to 60%.³-⁶ Although IFN-based treatment regimens have been linked to low levels of tolerance, higher adverse effects, and marginal results,⁷ use of IFN in these patients is required because there are no evidence-based alternative treatment options.⁸ However, in kidney transplant recipients, numerous studies have
reported an increased risk of acute rejection after IFN treatment for chronic active hepatitis C.9,10

Human leukocyte antigens (HLAs) conduct the development of a xenophobic immune response against the transplanted organ.11 Panel reactive antibody (PRA) is produced against HLA and induced by transfusions, pregnancies, infections, autoimmune diseases, and prior transplants for HLA alloimmunization.12,13 Presence of PRA is a major risk factor for increased incidence of hyperacute or acute graft rejection and graft dysfunction.12,14 The class, avidity, and affinity of PRAs to their HLA counterparts show variability according to the degree of immune response of the host, which may be affected by concurrent IFN treatment.

In contrast, HLA class I and II molecules play a central role in regulating host immune responses against microbial infections because they present foreign antigens to CD8+ (class I) and CD4+ (class II) T lymphocytes.15-17 Several cytokines such as IFN up-regulate HLA class I and II gene expression.16 The IFN exhibits a wide spectrum of biological activities in target cells including antiviral, immunomodulatory, antiangiogenic, and growth inhibitory effects.18 The IFN may cause increased cell surface expression of HLA antigens and induction of cytokine gene expression with subsequent stimulation of antibody production.18,19 However, recent studies investigating the effect of HCV in HLA class I response have shown that IFN-stimulated HLA class I expression is reduced by HCV infection.20 This gives rise to inhibition of antiviral response mediated by CD8+ lymphocytes. Therefore, the relation between IFN-stimulated HLA expressions in HCV infection is complex and may be affected by multiple factors. The levels of PRAs are increased by IFN treatment,21 and this increases risk of graft loss after kidney transplant. This may be related to the development of anti-HLA antibodies.

There are well known predictive factors related to patient and viral characteristics for HCV treatment including HCV genotypes 2 or 3, lower basal HCV viral load, rapid viral response (HCV RNA levels are undetectable at treatment week 4), IL-28B rs12979860 C/C genotype, IFNL3 genotypes, a lower degree of liver inflammation, and fibrosis. We hypothesized that the development of anti-HLA antibodies may be used as a surrogate marker of the development of a potent immune response against HCV. We evaluated the possibility of using PRA levels (PRA 1 for HLA class I and PRA 2 for class II) to predict SVR in patients after kidney transplant.

Materials and Methods

Study design and data collection
In this retrospective cohort study, we reviewed the data from IFN-treated HCV-infected patients who were evaluated for kidney transplant. We collected data of the patients who were followed in the nephrology and gastroenterology clinics between January 2000 and March 2013. The demographic data, serologic profile (including anti-HCV, HCV RNA, PRA 1, and PRA 2 levels), characteristics of IFN treatment (dose and duration), liver biopsies, and changes during follow-up were recorded and analyzed.

Definitions
To investigate a possible correlation, we compared PRA 1 and PRA 2 levels and presence of SVR in patients treated with conventional IFN or PEG IFN. Any patient receiving treatment for ≥ 12 weeks was accepted as having an adequate minimum duration of treatment for inclusion. Exclusion criteria included a history of immunosuppressive drug treatment, patients who underwent transplant (because of modification of PRA values by immunosuppressive treatment), treatment duration < 12 weeks, and patients with highly variable PRA levels.

Measurement of exposure
Qualitative detection of antibodies against HCV was performed using a microparticle enzyme immunoassay (AxSYM HCV, Abbott, Abbott Park, IL, USA) and chemiluminescent microparticle immunoassay (The Architect System, Abbott). The HCV RNA was detected with a real-time polymerase chain reaction (PCR) from 2003 to 2005 (LightCycler, Roche, Basel, Switzerland) and another real-time PCR within a linear interval (serum HCV RNA between 10 and 20 IU/mL) from 2005 to 2013 (CobasTaqman 48 HCV, Roche). Serum samples were tested for immunoglobulin G anti-HLA class I- and II-specific antibodies (PRA 1 and PRA 2) with a commercial enzyme-linked immunosorbent assay kit according to the instructions from the manufacturer (LATM20x5, One Lambda, Canoga Park, CA, USA). A PRA value > 20% was considered positive. The SVR rates were calculated and compared with PRA 1 and PRA 2 values.
Statistical analyses
Statistical analyses were performed using software (SPSS version 11.0, SPSS Inc., Chicago, IL, USA). The percentages of anti-HLA antibodies, demographic characteristics, and HCV treatment results were compared with chi-square test) and Fisher exact test, and numeric variables were compared with Mann-Whitney U test. The level of significance was defined by \( P \leq .05 \).

Results
After evaluating 112 patients, a total of 40 patients (16 female and 24 male patients) were included after application of exclusion criteria. The mean age was 41.5 years (range, 18-65 y). The average duration of dialysis therapy was 11.2 years (range, 4-21 y), and the average duration of IFN treatment was 37.5 months (range, 12-52 mo). The SVR rate was 18 of 40 patients (45%). The duration of renal failure and duration of IFN treatment were comparable between PRA 1 and PRA 2 groups (Table 1).

There were 31 patients with negative PRA 1 (13 with SVR and 18 with no SVR) and 9 patients with positive PRA 1 (5 with SVR and 4 with no SVR). The SVR ratio was not correlated with PRA 1 positivity (Fisher exact test, \( P > .05 \)). There were 31 patients with negative PRA 2 (11 with SVR and 20 with no SVR) and 9 patients with positive PRA 2 (7 with SVR and 2 with no SVR). The SVR ratio was significantly correlated with PRA 2 positivity (Fisher exact test, \( P \leq .05 \)) (Table 1).

Discussion
The clinical importance of HLAs in transplant has been known for 40 years. The antibodies against HLA, when they have been formed before transplant, are important in allograft survival and they form a significant barrier in kidney transplant. Pretransplant positive PRAs are related to increased incidence of hyperacute/acute rejection, chronic rejection, and early/latent graft loss.\(^{22,23}\) Kidney transplant recipients with high PRA have worse outcomes than those with lower PRA.

Genetic factors of the host determine the clinical outcome of viral infections and CD4+ T-cell-mediated immune response. The HLA class I and II genes also modify cellular and humoral immune responses. Class I and class II genes are expressed on the surfaces of activated T lymphocytes, B lymphocytes, Langerhans cells, and dendritic cells. These molecules function during presentation of internalized antigenic proteins to the CD4+ T-helper cells.

Previous evidence indicated that positive anti-HCV serology is inversely related to graft and overall survival.\(^{2,24,25}\) This finding is partially explained by elevated PRA percentages (secondary to increased antigenic stimuli by HCV) which eventually results in graft rejection.

The IFN-\(\alpha\) may activate an inflammatory response by a bystander mechanism, which may develop into a cellular or humoral response, depending on the genetic susceptibility of the individual. Although IFNs exert strong immunomodulatory effects in several ways, the mechanisms by which they trigger anti-HLA antibody production are incompletely understood. The up-regulation of both HLA and cytokine genes and induction of long-lived antibody production and immunologic memory are possible explanations for this phenomenon.\(^{18}\)

In a previous case report, IFN-\(\alpha\) treatment resulted in increased percentage PRA levels and development of new specific PRAs, indicating that IFN may mediate changes in immune system responses.\(^{26}\) The IFN-\(\alpha\)-based therapy induces expression of various genes, which include genes involved in antigen presentation and T-cell activation.\(^{27,28}\) The IFN-stimulated genes related to T-cell activation also are induced during treatment of HCV with IFN-\(\alpha\).\(^{29}\)

In the present study, we hypothesized that PRA change can be used as a surrogate marker of SVR

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>PRA 1 Negative</th>
<th>PRA 1 Positive</th>
<th>PRA 2 Negative</th>
<th>PRA 2 Positive</th>
<th>Overall</th>
<th>( P ) Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>42</td>
<td>40</td>
<td>43</td>
<td>37</td>
<td>41.5 (18-65)</td>
<td>.570</td>
</tr>
<tr>
<td>Sex (F/M)</td>
<td>11/20</td>
<td>5/4</td>
<td>11/20</td>
<td>5/4</td>
<td>16/24</td>
<td>.335</td>
</tr>
<tr>
<td>Duration of hemodialysis (y)</td>
<td>11</td>
<td>12</td>
<td>11</td>
<td>12</td>
<td>11.2 (4-21)</td>
<td>.485</td>
</tr>
<tr>
<td>Duration of IFN treatment (mo)</td>
<td>38</td>
<td>36</td>
<td>38</td>
<td>35.5</td>
<td>37.5 (12-52)</td>
<td>.844</td>
</tr>
<tr>
<td>SVR (absent/present)</td>
<td>18/13</td>
<td>4/5</td>
<td>20/11</td>
<td>2/7</td>
<td>22/18</td>
<td>PRA 1: .364</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>PRA 2: .031</td>
</tr>
</tbody>
</table>

*Abbreviations:* F, female; IFN, interferon; M, male; PRA, panel reactive antibody; SVR, sustained viral response
prediction. Our preliminary findings might support such a novel use of PRAs. In contrast, IFN treatment itself might have resulted in an increase in PRA levels. Various secondary antibodies and autoimmune conditions have been related to chronic HCV infection. Therefore, development of high PRAs may be explained by development of HCV-related autoimmunity. However, in a previous study in end-stage renal disease patients, PRA levels did not differ between anti-HCV-positive (PRA 1 [ABC], 28.9%; PRA 2 [DR], 21.8%) or negative subgroups (PRA 1 [ABC], 19.4%; PRA 2 [DR], 20.9%) (P > .05), indicating that there was no correlation between HCV-related autoimnunity and PRA levels.

In our study, we observed that persistent PRA elevation in patients receiving IFN treatment had higher SVR rates. We believe that future studies can be performed to identify this specific risk by identifying anti-HLA antibodies and comparing them before and after IFN treatment. In addition, prospective studies are required to delineate the natural history of increased PRA levels induced after IFN treatment.

References
Polymorphism of the CYP3A5 Gene and Its Effect on Tacrolimus Blood Level

Sreeja S. Nair, Sreeja Sarasamma, Noble Gracious, Jacob George, Thekkumkara Surendran Nair Anish, Reghunathan Radhakrishnan

Abstract

Objectives: Tacrolimus is the cornerstone for immunosuppression in renal transplant and is metabolized by the cytochrome P 450 3A (CYP3A) subfamily of enzymes in the liver and small intestine. A polymorphism in intron 3 of the CYP3A5 gene affects the expression of this enzyme and tacrolimus trough blood levels. The purpose of this study was to identify the proportion of CYP3A5 gene polymorphisms in South Indian renal transplant patients and determine the effect of CYP3A5 gene polymorphisms on tacrolimus trough blood levels in patients with and without CYP3A5 expression.

Materials and Methods: We included 25 adult patients who underwent renal transplant at Government Medical College, Trivandrum. All patients received tacrolimus (dose, 0.1 mg/kg/body weight, in 2 divided doses). Tacrolimus trough blood levels were determined on postoperative day 6. The CYP3A5 genotype analysis was performed by polymerase chain reaction amplification of target and detection by restriction fragment length polymorphism analysis.

Results: The CYP3A5*1/*1 genotype was detected in 5 recipients (20%), *1/*3 genotype in 5 recipients (20%), and *3/*3 genotypes in 15 recipients (60%) of the total 25 graft recipients. Mean tacrolimus level in the CYP3A5*1/*1 group was 5.154 ng/mL (range, 4.42 to 6.5 ng/mL), CYP3A5*1/*3 group was 5.348 ng/mL (range, 3.1 to 9.87 ng/mL), and CYP3A5*3/*3 group was 9.483 ng/mL (range, 4.5 to14.1 ng/mL). Acute rejection episodes were significantly more frequent for CYP3A5*1/*1 homozygous patients (40%) than patients with CYP3A5*1/*3 (20%) or CYP3A5*3/*3 (13%) genotypes.

Conclusions: Most patients carried the mutant allele CYP3A5*3 (A6986G). Tacrolimus drug level correlated well with presence or absence of CYP3A5 polymorphisms. Acute rejection episodes were more frequent in expressors, and they may require higher doses of tacrolimus. Similarly, tacrolimus nephrotoxicity was more frequent in non-expressors. Therefore, CYP3A5 polymorphism analysis before renal transplant may help determine the optimal dose of tacrolimus in this population and prevent acute rejection episodes or tacrolimus toxicity.

Key words: Cytochrome P450, End-stage renal disease, Genetics, Immunosuppression

Introduction

Tacrolimus is the corner stone of immunosuppression in renal transplant. It is a macrolide antibiotic compound that acts by inhibiting the calcineurin pathway by binding to FK binding protein. However, it has a narrow therapeutic index and requires therapeutic drug monitoring to prevent graft rejection as a result of inadequate immunosuppression with low drug levels or toxicity due to high drug levels.

Tacrolimus is metabolized by the CYP3A subfamily of enzymes in the liver and small intestine.
Although both CYP3A4 and CYP3A5 are involved in the metabolism of tacrolimus, previous studies have shown that polymorphisms in CYP3A5 genes are responsible for interindividual variations in bioavailability of tacrolimus.2

A polymorphism in intron 3 of the CYP3A5 gene affects the expression of this enzyme. The CYP3A5*3 allele (guanine at position 6986) produces a cryptic splice site and encodes an abnormal spliced mRNA with a premature stop codon; thus, individuals who are homozygous for this allele (CYP3A5*3/*3) are called nonexpressors. Presence of CYP3A5*1 allele (adenine at position 6986) produces normal mRNA, resulting in a high expression of this enzyme in the intestine and in the liver; individuals expressing at least one CYP3A5*1 allele are called expressors. Therefore, expressors can be either homozygous (CYP3A5*1/*1) or heterozygous (CYP3A5*1/*3).3,4

Previous studies showed that expressors achieved 2-fold lower tacrolimus concentration-to-dose ratio compared with nonexpressors.5-9 Therefore, we aimed to find the proportion of nonexpressors and expressors and clarify the role of CYP3A5 polymorphism on tacrolimus drug levels in our renal transplant population.

Materials and Methods

Patients
We included 25 adult patients who underwent renal transplant at Government Medical College, Trivandrum, Kerala, India. The study was approved by the ethics committee of the institution before the study began, and the protocol conformed to the ethical guidelines of the 1975 Helsinki Declaration. Written informed consent was obtained from all patients. All patients received tacrolimus (dose, 0.1 mg/kg bodyweight) with prednisolone and mycophenolate mofetil. Tacrolimus 12-hour trough blood level was determined on postoperative day 6 using a chromatographic method (liquid chromatography-tandem mass spectrometry assay). The lower limit of quantification of the assay was 0.003 ng/mL.

Genotype analyses
Genotype analyses of all patients were performed to identify the CYP3A5 allele. Nucleic acid isolation was performed using a standard magnetic bead-based extraction protocol (MagJET DNA and RNA Purification Kits, Thermo Fisher Scientific Corporation, MA, USA) according to the manufacturer’s instructions. Custom-designed primers synthesized from human cytochrome P450 PCN3 mRNA (cytochrome P450, family 3, subfamily A, polypeptide 5) complete cDNAs were used for polymerase chain reaction amplification of the target. The polymerase chain reaction protocol was a standardized procedure for the selected primer sequence done using thermal cycler (Applied Biosystem, Thermo Fisher Scientific Corporation. MA, USA). Postamplification detection was performed by restriction fragment length polymorphism analysis using SspI endonuclease (New England Biolabs Inc, MA, USA).

Statistical analyses
Data were reported as mean ± standard deviation (SD) or mean (range, minimum to maximum) for all quantitative estimates. Tacrolimus trough blood levels between 2 groups were compared with independent t test. Statistical analysis was done using software (SPSS for Windows, Version 16.0, SPSS Inc., Armonk, NY, USA).

Results

Characteristics of study population
In the 25 renal graft recipients who were included in the study, there were 22 males and 3 females (Table 1). The mean age was 32 ± 20 years and body weight was 58 ± 11 kg. There were 3 patients who received induction therapy (2 patients received antithymocyte globulin and 1 patient received basiliximab). Mean donor age was 44 ± 12 years. At 1 month after transplant, the incidence of biopsy-proven acute rejection was 20% (all T-cell-mediated rejection). There were 8 graft biopsies obtained from the study population in the first month after transplant, and the incidence of tacrolimus nephrotoxicity in this subgroup was 8%.

Frequency of CYP3A5 genotypes and relation to tacrolimus level
The CYP3A5*1/*1, *1/*3, and *3/*3 genotypes were detected in 5 (20%), 5 (20%), and 15 (60%) of the 25 graft recipients (Table 1). Mean tacrolimus trough level in the CYP3A5*1/*1 group was 5.154 ng/mL (range, 4.42 to 6.5 ng/mL), CYP3A5*1/*3 group was 5.348 ng/mL (range, 3.1 to 9.87 ng/mL) and...
CYP3A5*3/*3 group was 9.483 ng/mL (range, 4.5 to 14.1 ng/mL). Tacrolimus level difference between expressors and nonexpressors was significant when compared with independent $t$ test. ($t$, -4.28; degrees of freedom, 23; $P \leq .001$).

Effect of CYP3A5 genetic polymorphisms on acute rejection episodes and tacrolimus nephrotoxicity

Biopsy-proven acute renal graft rejection on biopsies obtained at 1 month after transplant was compared between the 3 CYP3A5 genotype groups. Acute rejection episodes were significantly more frequent for CYP3A5*1/*1 homozygotes (2 out of 5, 40%) than patients with CYP3A5*1/*3 (1 out of 5, 20%) or CYP3A5*3/*3 genotypes (2 out of 15, 13%). We examined the relation between CYP3A5 genetic polymorphism and biopsy-proven nephrotoxicity due to tacrolimus use; 8 renal biopsies were obtained during 1 month, and 2 biopsies (both in nonexpressors) had evidence of calcineurin-induced toxicity.

Discussion

Tacrolimus is a potent immunosuppressive drug used in solid-organ transplant. However, it has a narrow therapeutic range, which is further complicated by wide variation in intraindividual and interindividual bioavailability of the drug. Tacrolimus is metabolized by CYP3A4 and CYP3A5 in the liver and small intestine. Genetic polymorphisms in CYP3A5 affect the intraindividual variability in tacrolimus trough blood levels.

In our study, we evaluated the effect of CYP3A5 genetic polymorphisms on tacrolimus daily dose requirements in a cohort of kidney transplant recipients. Our results showed that carriers of at least 1 active allele (CYP3A5*1) needed significantly higher doses of tacrolimus than patients homozygous for CYP3A5*3 (CYP3A5 nonexpressors). This result relied on the fact that carriers of CYP3A5*1 allele exhibit high levels of CYP3A5 expression and enzymatic activity, leading to higher daily dose requirement to achieve sufficient trough levels of tacrolimus. Such results have been reported previously in the literature concerning this polymorphism.

A previous study by Patel and associates studied the effect of CYP3A5 polymorphism on tacrolimus drug dosing in North Indian renal allograft recipients. To our knowledge, the present study is the first study to show the association between CYP3A5 genetic polymorphism and tacrolimus drug level in a South Indian population.

We evaluated the risk of biopsy-proven acute rejection during the first month after transplant. We observed that patients with CYP3A5*1/*1 genotype had a higher risk of developing acute graft rejection episodes than CYP3A5*3 homozygotes. This observation is in agreement with the fact that carriers of the wild-type allele (CYP3A5*1) have higher levels of CYP3A5 expression, higher metabolic clearance of tacrolimus, and low trough concentrations resulting in acute rejection.

A previous study by Quteineh and coworkers showed that CYP3A5*1 homozygotes had increased risk of acute rejection episodes (38%) than patients with CYP3A5*1/*3 (10%) or CYP3A5*3/*3 (9%) genotypes ($P = .01$). They also reported that few rejection episodes occurred after the first month after transplant, and overall rejection episodes were more important during the first month after transplant. This showed the importance of performing tacrolimus daily doses early posttransplant, when there is a greater risk of developing acute rejection episodes.

We studied the relation between CYP3A5 genotype and biopsy-proven tacrolimus nephrotoxicity. We observed increased occurrence of nephrotoxicity in CYP3A5 nonexpressors. This was expected because of high trough blood levels in these patients. However, we were limited by the small number of biopsies to substantiate this finding. The previous study by Quteineh and associates showed no relation between the development of tacrolimus-related nephrotoxicity and CYP3A5 genetic polymorphism.

In conclusion, our results confirmed that CYP3A5 genetic polymorphism is an important factor in

| Table 1. Comparison of the Clinical Characteristics of the Study Population* |
|------------------|------------------|------------------|
| Characteristic   | CYP3A5*1/*1      | CYP3A5*1/*3      | CYP3A5*3/*3      |
| No. of patients  | 5 (20%)          | 5 (20%)          | 15 (60%)         |
| Age at transplant (y) | 16(12 to 22)   | 33 (22 to 47)   | 36 (20 to 50)   |
| Sex (male/female) | 4/1             | 5/0             | 13/2             |
| Age of donor (y)  | 44 (32 to 52)    | 46 (37 to 52)   | 43 (33 to 58)   |
| Hemoglobin (g/dL) | 10.2 (8.8 to 11.8) | 10.0 (8.4 to 11.0) | 9.8 (8.6 to 10.8) |
| Blood urea (mg/dL) | 18 (16 to 26)  | 20 (15 to 28)   | 21 (16 to 27)   |
| Creatinine (mg/dL) | 1.6 (0.9 to 1.8) | 1.5 (1.0 to 1.7) | 1.6 (0.9 to 2.4) |
| Potassium (mmol/L) | 4.2 (4.0 to 4.4) | 4.4 (4.0 to 5.6) | 4.4 (3.8 to 5.6) |
| Total bilirubin (mg/dL) | 1.0 (0.8 to 1.1) | 0.8 (0.6 to 1.0) | 0.9 (0.6 to 1.1) |
| Alanine aminotransferase (U/L) | 32 (26 to 38) | 34 (26 to 36) | 36 (26 to 40) |
| Aspartate aminotransferase (U/L) | 28 (26 to 30) | 27 (24 to 32) | 30 (25 to 38) |
| Albumin (g/dL)     | 3.5 (3.0 to 3.8) | 3.6 (2.8 to 4.0) | 3.5 (2.8 to 4.2) |

*Data reported as number (%), mean (range, minimum to maximum), or number.
determining tacrolimus daily requirements and adjusting tacrolimus trough concentrations. Furthermore, it was shown in our study that genetic polymorphism is a risk factor for developing acute rejection episodes. Screening for this polymorphism in patients waiting for solid-organ transplant could be helpful to predict the best individualized tacrolimus oral dose and may prevent early acute rejection related to insufficient immunosuppression.

References

Efficacy of Immunoadsorption To Reduce Donor-Specific Alloantibodies in Kidney-Transplant Candidates

Lionel Rostaing,1,2,4 Nicolas Congy,2,4 Alice Aarnink,2,4 Sébastien Maggioni,1 Asma Allal,1 Federico Sallusto,5 Xavier Game,2,4,5 Nassim Kamar1,2,4

Abstract

Objectives: We implemented a desensitization program at our center to enable transplant in kidney-transplant candidates who have a living human-leukocyte antigen-incompatible (HLAi) donor. We report on the efficacy of semispecific immunoabsorption to allow HLAi kidney transplant in 6 highly sensitized patients.

Materials and Methods: We chose immunoabsorption as the apheresis technique coupled to hemodialysis as a means to decrease donor-specific alloantibodies in kidney transplant candidates submitted to a pretransplant desensitization program to remove detrimental antibodies.

Results: Six highly sensitized kidney-transplant patients (5 females), awaiting their first (n = 1) or second (n = 5) kidney transplant from a living donor, were enrolled in this desensitization program. They had 1 (n = 2), 2 (n = 1), 3 (n = 2), or 4 (n = 1) donor-specific alloantibodies; their mean fluorescent intensities at predesensitization ranged from 1200 to 19 000. Each patient underwent between 10 and 16 immunoabsorption sessions. At the time of transplant, donor-specific alloantibodies were undetectable in 2 patients (A24, DR3); donor-specific alloantibodies decreased by > 50% in 8 patients (A11, B44, DR3, DR11, DQ3 thrice, DQ5); donor-specific alloantibodies remained unchanged in 2 patients (B50, DR13); and mean fluorescent intensities were slightly increased in 2 patients (Cw6, DQ8). In the analysis of final outcomes, 2 patients experienced no rejection (1 experienced donor-specific alloantibody elimination, and 1 experienced a > 50% decrease in donor-specific alloantibodies). One patient presented with acute antibody-mediated rejection, which required immunoabsorption sessions and eculizumab therapy (donor-specific alloantibodies between 5000 and 19 000). Two patients presented with subacute antibody-mediated rejection; 1 was treated by plasmapheresis/rituximab therapy, and the other was treated with plasmapheresis/ methylprednisolone pulses. Another patient presented with chronic antibody-mediated rejection, which was treated unsuccessfully with plasmapheresis/rituximab; a tentative rescue therapy with eculizumab was attempted without success.

Conclusions: Desensitization of the human-leukocyte antigen using this immunoabsorption procedure effectively reduced or eliminated donor-specific alloantibodies in 71% of patients undergoing kidney transplant, at the time of transplant.

Key words: Eculizumab, Apheresis, Antibody-mediated, Rejection, HLA sensitized patients

Introduction

The number of end-stage renal disease patients is increasing rapidly in the Western world and is associated with major costs.1 Its treatment relies on dialysis and for some patient we can offer kidney transplantation. Although patient treatment in the first year following a kidney transplant is as expensive as hemodialysis treatment for a year, the cost and quality-of-life is much improved thereafter.2 Because of the shortage of organs from deceased...
donors, one means to cope with the huge numbers of patients with end-stage renal disease treated with dialysis and awaiting a kidney transplant is to implement living-donor kidney transplant. However, in some cases, the potential recipient has antibodies against the donor, which can render kidney transplant problematic. These antibodies can be directed against human-leukocyte antigens [donor-specific alloantibodies (DSAs)] and/or against ABO antigens [anti-A or/and anti-B isoagglutinins].

If kidney donation is to be undertaken in the setting of incompatibility to human-leukocyte antigens (HLAi) and/or ABO incompatibility (ABOi), medical personnel must implement pretransplant desensitization protocols. These rely on (1) immunomodulatory agents (eg, intravenous immunoglobulin [IV-Ig]) and/or immunosuppressants (eg, rituximab, tacrolimus, mycophenolate mofetil, steroids), and (2) apheresis. Apheresis is used to remove deleterious antibodies from the blood, which could trigger acute antibody-mediated rejection. Immunomodulatory and immunosuppressant agents are then given to prevent resynthesis of these deleterious antibodies. Apheresis techniques include plasmapheresis, double-filtration plasmapheresis, and immunoadsorption. We chose to use immunoadsorption because, relative to plasmapheresis or double-filtration plasmapheresis, it removes only immunoglobulins and has no effect on clotting factors or other components in the plasma. In the setting of HLAi kidney transplant, we use semispecific immunoadsorption using protein-A Immunosorba® absorber columns (Fresenius Medical Care, Bad Homburg, Germany), as previously described. The number of pretransplant immunoadsorption sessions needed is guided by the levels of DSAs expressed in mean intensity fluorescent (MFI) units. In cases where HLAi is associated with ABOi, semispecific immunoadsorption also can be used to decrease isoagglutinin titers.

Materials and Methods

We prospectively included 6 hemodialysis kidney-transplant candidates. There were 5 females; the median age was 52 years (range, 32-62 y). One patient was a liver-transplant recipient with calcineurin-inhibitor-induced end-stage renal disease; the 5 other patients have had a previous kidney transplant that had failed. Because they were highly sensitized to human-leukocyte antigens, they had been waiting for a second kidney transplant for many years. All patients had a potential living-related donor (see Table 1). However, every patient had at least 1 DSA (up to 4 in 1 patient). The study was approved by the Ethical Review Committee of our Institute. All of the protocols conformed to the ethical guidelines of the 1975 Helsinki Declaration. Written informed consent was obtained from all subjects.

To be included in our desensitization program, we required a negative T-cell crossmatch between the donor and recipient, as assessed by microlymphocytotoxicity. Two patients (Nos. 1 and 2) had positive B-cell crossmatches (as assessed by microlymphocytotoxicity). In the other 4 patients, both T- and B-cell crossmatches were negative (as assessed by microlymphocytotoxicity). We did not perform crossmatches by flow cytometry. All crossmatches were performed before any rituximab infusion to avoid false positive results on B cells.

With regards to the desensitization protocol, all patients followed the same protocol (as shown in Figure 1). At 40 days pretransplant and during an hemodialysis session, the patients were perfused with IV-Ig at 1 g/kg. At 30 and 15 days pretransplant, all patients received rituximab at 375 mg/m², paracetamol (1 g), plus methylprednisolone (1 mg/kg).

At 10 days pretransplant, immunosuppression was started with tacrolimus (0.1 mg/kg bid), mycophenolate mofetil (1 g bid), and prednisone (0.5 mg/kg/d). At the same time, we implemented prophylaxis against Pneumocystis jirovecii using sulfamethoxazole (400 mg)/trimethoprim (80 mg) every other day for a period of 1 year.

Immunoadsorption sessions were started at 17 days pretransplant and were then scheduled on pretransplant days 16, 15, 12, 11, and 10. Subsequently, they were performed according to the DSA levels assessed at days 10 and 5 pretransplant, i.e IA sessions were done if DSA(s) were > 3000.

The immunoadsorption procedure was performed as previously described. In all cases, the immunoadsorption sessions were performed as a tandem procedure with hemodialysis.

Kidney transplant was performed on the day scheduled, regardless of the results of the DSA MFI at
5 days pretransplant. Induction therapy used antithymocyte globulin at 1 mg/kg, which was given IV just before transplant and then postoperatively on days 2 and 4 at the same dosage.

Donor specific alloantibodies (DSA) were detected by Luminex assays using the LABScreen Single antigen kit (One Lambda, Canoga Park, CA, USA) according to the manufacturer’s instructions. The presence and specificity of antibodies were then detected using a LABScan TM 100 (One Lambda) and the mean fluorescence (baseline value) for each sample in each bead was evaluated. The baseline value was calculated as follows: (raw sample MFI – raw negative MFI) – (negative-bead raw MFI with sample – negative-bead raw MFI with negative serum control). A baseline value of > 1000 was considered positive.

**Results**

Patients underwent from 10 to 16 sessions of immunoadsorption before receiving a kidney transplant (see Table 1). At the time of transplant, this resulted in (1) elimination of 2 DSAs; (2) the reduction of 8 DSAs by > 50%; (3) two stable DSAs; but (4), an increase in 2 DSAs (Cw6 and DQ8). Overall, DSAs were eliminated or reduced by more than 50% in 71% of patients by the time of transplant. At the last follow-up, DSAs remain negative in 5 settings; they remain reduced > 50% in 6 settings; they are stable in 1 setting, but have increased in 2 settings.

The median posttransplant follow-up was 11 months (range, 7-15 mo). Patient- and graft-survival rates were 100% and 84%. Three of the 6 patients had a (sub)acute humoral-rejection episode and 1 had a chronic antibody-mediated rejection. None of the patients presented with an acute cellular rejection (Table 2). Two of the patients (Nos. 4 and 5) did not have an acute-rejection episode, and both had significantly decreased DSA levels after the immunoadsorption sessions (Table 3).

Patient 1 presented with an acute antibody-mediated rejection episode on postoperative day 4. She recovered renal function by postoperative day 4, but then suddenly became oligo-anuric, was subfebrile (38°C), and had features of thrombotic microangiopathy, which included schistosotic hemolytic anemia and thrombopenia. At this time, tacrolimus trough levels were 8 ng/mL. Because a diagnosis of acute antibody-mediated rejection was apparent, she was given pulses of methylprednisolone (10 mg/kg/d) and daily plasmapheresis with fresh frozen plasma as a replacement fluid. After 4 days of this therapy, there was no improvement. We then performed a kidney-allograft biopsy, which confirmed acute antibody-mediated rejection.

**Figure 1.** Desensitization Protocol Used at Toulouse University Hospital in the Setting of HLA-Incompatible Kidney-Transplant Recipients

**Table 1.** Demographic Data of Patients Within the Study Group

<table>
<thead>
<tr>
<th>Patients No.</th>
<th>Gender</th>
<th>Age at the Previous Time on HD (y)</th>
<th>Time on HD (mo)</th>
<th>IA (PP) Before KTx</th>
<th>Posttransplant Immunosuppression</th>
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<tr>
<td>2</td>
<td>M</td>
<td>52</td>
<td>56</td>
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<tr>
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<td>F</td>
<td>55</td>
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<td>Tac-MMF Cs</td>
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<tr>
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<td>F</td>
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<td>NA</td>
<td>10</td>
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<td>5</td>
<td>F</td>
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<td>57</td>
<td>10</td>
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<tr>
<td>6</td>
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<td>39</td>
<td>48</td>
<td>16</td>
<td>Tac-MMF Cs</td>
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*Abbreviations:* Cs, corticosteroids; DSA, donor-specific alloantibodies; HD, hemodialysis; IA, immunoadsorption; KTx, kidney transplant; MMF, mycophenolate mofetil; MPA, mycophenolic acid; NA, not applicable; PP, plasmapheresis; Tac, tacrolimus
rejection with diffuse C4d deposits. Assessment of DSAs showed that there was rebound of anti-DQ5 (MFI = 18 000 vs 8000 at the time of transplant). Facing a severe episode of acute antibody-mediated rejection, we gave the patient eculizumab (1200 mg) on days 1 and 8, and then 900 mg every 2 weeks for 3 months. At 1 week after the first dose, urine output was present, renal function had recovered quite quickly, and the thrombotic microangiopathy had disappeared. By 2 weeks after starting eculizumab therapy, the patient was discharged; serum creatinine was 130 μmol/L. At 3 months after transplant, serum creatinine was 100 μmol/L. At this point, a surveillance kidney transplant biopsy was performed: this was almost normal. Thus, we decided to stop eculizumab therapy. At 1 year after transplant, serum creatinine was 110 μmol/L.

Although DSA were nil, because there was an increase in isoagglutinine titers were from 1/2 to 1/16, in this patient who was also an ABO incompatible kidney transplant patient we assumed that she had an acute antibody-mediated rejection. This was treated with 6 plasmapheresis sessions and a large increase in prednisone (1 mg/kg/d for 10 days). Serum-creatinine levels improved within 5 days and have returned to baseline values.

Patient 3 presented at 4 months posttransplant with a slight increase in serum creatinine, from 90 to 130 μmol/L. DSAs were stable. We performed a kidney biopsy, which showed capillaritis, glomerulitis, and diffuse C4d staining. We treated this patient with pulses of methylprednisolone (10 mg/kg/d, 3 days), 6 plasmapheresis sessions, and 2 doses of rituximab (375 mg/m² each). Thereafter, her renal function stabilized at ~100 μmol/L of serum creatinine. One month later, she developed severe hypertension, which was resistant to quadruple therapies. An echo-Doppler echogram of the kidney revealed severe stenosis of the renal-artery anastomosis. We performed a percutaneous angioplasty, which was successful but was complicated with a dissection of the renal artery. In the subsequent days, serum creatinine rose to 300 μmol/L, but then stabilized at ~250 μmol/L. Since then, her blood pressure has been controlled and, although renal function is impaired, it is stable.

Patient 2 had a serum creatinine level of 180 μmol/L at the time of discharge. At that time he had no proteinuria. At 5 months posttransplant, he developed overt proteinuria of ~2 g/d, slightly increased serum creatinine levels at (220 μmol/L), but his DSA levels were stable. Because he was receiving anticoagulant therapy for an aortic mechanical valve, we did not perform a kidney biopsy. With the assumption that he had a subacute antibody-mediated rejection, we performed 6 plasmapheresis sessions and gave 2 injections of rituximab (375 mg/m² each, at 1 week apart).
Subsequently, there was no change in his renal parameters. At 8 months posttransplant, because serum creatinine had risen to 300 μmol/L and because proteinuria was 6 g/d, we performed a percutaneous kidney biopsy despite the anticoagulant therapy. This primarily showed features of a chronic antibody-mediated rejection. Thus, we decided to implement eculizumab therapy (1200 mg/week, for 2 weeks, and then 900 mg every 2 weeks). The renal parameters stabilized for 2 months but subsequently deteriorated and he returned to hemodialysis by 11 months post-transplant.

Discussion

Herein, we report on the effect of pretransplant desensitization, using immunoadsorption with conventional immunosuppression, in the setting of incompatibility to human-leukocyte antigens with a living-kidney transplant. We demonstrate that this therapy is very effective at reducing pretransplant DSA levels; despite that, two-thirds of patients presented with antibody-mediated rejection post-transplant.

We included 6 patients (5 women and 1 man) who were highly sensitized to human-leukocyte antigens; of these, 5 patients had received a previous kidney transplant. All had been waiting for a deceased donor for > 40 months. When their serums were tested against a potential living donor we found that the recipients had at least 1 DSA against the donor; 12 of the 14 DSAs had MFI values > 3000 units. However, 2 of the patients had a positive crossmatch (assessed by microlymphocytotoxicity) with B lymphocytes; conversely, crossmatches on T lymphocytes were always negative in the 6 patients.

Recently, Montgomery and associates have shown that, in the setting of live-donor kidney transplant, desensitization using plasmapheresis and low doses of IV-Ig provides a significant survival benefit for patients with sensitization to human-leukocyte antigens when compared to those awaiting a compatible organ from a deceased donor. By 8 years, this survival advantage, compared with those that remained on dialysis and on the kidney-waiting list, had more than doubled. In previous studies, desensitization has been conducted without apheresis, or with plasmapheresis, or with plasmapheresis and specific immunoadsorption. To the best of our knowledge, our study is the first to report on the efficacy of semispecific immunoadsorption using Immunosorba® columns in the setting of incompatibility to human-leukocyte antigens in living-kidney transplants.

It was necessary to perform between 10 and 16 immunoadsorption sessions to decrease DSAs to a sufficient level. The 2 patients needing in addition plasmapheresis pretransplant were those with a positive B-cell crossmatch.

Our procedure, which was combined with pre-transplant immunosuppression, resulted in either the disappearance or a decrease of > 50% of DSAs (according to MFI) in 71% of the patients. These results were sustained: at the last follow-up, 5 DSAs were no longer detectable, and MFI values were < 50% of those at predesensitization for 2 DSAs.
Altogether, at last follow-up, as compared to pre-desensitization, 80% of DSAs had decreased. However, despite these good results, only 2 of the 6 patients did not experience an antibody-mediated rejection. For the 4 patients who experienced antibody-mediated rejection, this was very acute in 1 patient and required eculizumab therapy; rejection was subacute in the other 3 patients. One of these 3 patients subsequently developed a chronic antibody-mediated rejection, which required eculizumab. Other recent case studies also report that eculizumab, a humanized anti-C5a antibody, saved the kidneys of patients with acute humoral rejection and who were resistant to all other therapies.9,10

In the setting of living kidney donation with DSAs, the use of eculizumab can be valuable in prevention of acute antibody-mediated acute rejection. Hence, Stegall and associates have reported on the efficacy of terminal complement inhibition with the humanized anti-C5 antibody, eculizumab, in the prevention of antibody-mediated rejection (AMR) in renal transplant recipients with a positive crossmatch against their living donor. The incidence of biopsy-proven AMR in the first 3 months posttransplant in 26 highly sensitized recipients of living-donor renal transplants who received eculizumab posttransplant was compared to a historical control group of 51 sensitized patients treated with a similar plasma exchange (PE)-based protocol without eculizumab. The incidence of AMR was 7.7% (2/26) in the eculizumab group compared to 41.2% (21/51) in the control group (P = .0031). Eculizumab also decreased AMR in patients who developed high levels of DSAs early after transplant that resulted in proximal complement activation. With eculizumab, AMR episodes were easily treated with PE, reducing the need for splenectomy. On 1-year protocol biopsy, transplant glomerulopathy was found to be present in 6.7% (1/15) eculizumab-treated recipients and in 35.7% (15/42) of control patients (P = .044). These researchers concluded that inhibition of terminal complement activation with eculizumab decreases the incidence of early AMR in sensitized renal transplant recipients.11 Randomized clinical trials are currently underway evaluating the benefit of adding eculizumab to prevent acute AMR in de novo kidney transplant patients.

We conclude that semispecific immuno-adsorption can decrease DSAs at pretransplant in more than two-thirds of cases when it is combined with immunosuppression, thus allowing kidney transplant from a live donor. Despite the associated high rate of posttransplant antibody-mediated rejection, our findings indicate that such rejection may be treated by treatment with plasmapheresis, rituximab or eculizumab if needed.

References

Oxidative Stress in Kidney Transplant Biopsies

Avneesh Kumar,1 Abdul Hammad,1 Ajay K. Sharma,1 Frank Mc-Cardle,2 Rana Rustom,2 Steve E. Christmas3

Abstract

Objectives: Kidney allograft biopsies are performed after kidney transplant to determine graft dysfunction. We aimed to define and measure the oxidative stress occurring in these biopsies and compared these biopsies with donor pretransplant biopsies.

Materials and Methods: The biopsy procedure was done according to the unit protocol. A core of tissue was taken for research purposes only when it was safe enough to proceed for an extra core. Common indications for biopsy were acute or chronic graft dysfunction, delayed graft function, acute cellular rejection, and calcineurin toxicity. There were 17 pretransplant biopsies taken from deceased-donor kidneys. Biopsy specimens were snap frozen immediately in liquid nitrogen and stored at -70°C. Samples were processed for Western blot and tested for markers of oxidative stress.

Results: There were 61 biopsies analyzed. Oxidative stress enzymes were evaluated by Western blot including catalase, manganese superoxide dismutase, copper zinc superoxide dismutase, thioredoxin reductase, and thioredoxin. Up-regulation of most antioxidant enzymes was observed in pretransplant biopsies. Increased expression of manganese superoxide dismutase was observed in donor kidneys and kidneys with acute cellular rejection and calcineurin toxicity. Copper zinc superoxide dismutase and catalase were elevated in donor and acute cellular rejection biopsies. Thioredoxin was elevated in donor biopsies and thioredoxin reductases were elevated in donor biopsies and biopsies with acute cellular rejection and calcineurin toxicity.

Conclusions: The kidney allograft biopsies showed that oxidative stress levels were elevated during allograft dysfunction in all biopsies regardless of diagnosis, but not significantly. The levels also were elevated in pretransplant biopsies. The study showed that oxidative stress is involved in various acute injuries occurring within the allograft.

Key words: Catalase, Copper zinc superoxide dismutase, Manganese superoxide dismutase, Oxidation, Thioredoxin, Thioredoxin reductase

Introduction

Kidney transplant offers patients with end-stage renal failure improved survival and quality of life compared with dialysis. Overall long-term graft and patient survival have remained unchanged since 1995. This lack of improvement has been observed despite reduced early and late acute rejection rates1 resulting in 5-year graft survival of 70% and 10-year graft survival of 50%.2

“Oxidative stress” was a term first described in 1985 as a disturbance in the pro-oxidant-antioxidant balance in favor of the former.3 However, it is important that reactions involving free radicals are not necessarily deleterious; on the contrary, they are of fundamental importance for life because free radicals take part in key biochemical reactions in all living organisms. A more useful contemporary definition is a disruption of redox signaling and control.4 Reactive oxygen intermediates can affect the signaling of a wide variety of kinase pathways such as the mitogen-activated protein kinases/extracellular...
signal-regulated kinases and nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) pathways. These kinase pathways can have both antiapoptotic and apoptotic roles. Reactive oxygen species can participate in neutrophil recruitment by up-regulation of adhesion molecules and chemotactic factors. Oxidative stress also is increased in patients with chronic allograft nephropathy (CAN). We aimed to define and measure the oxidative stress occurring in kidney allograft biopsies and to compare these biopsies with donor pretransplant biopsies.

Materials and Methods

Biopsies

Kidney transplant graft biopsies were collected from patients who were undergoing the biopsy procedure because of graft dysfunction. The common indications for biopsy were delayed graft function (DGF), acute cellular rejection (ACR), calcineurin toxicity, and CAN. The procedure was done under local anesthesia according to the unit protocol with ultrasonography guidance. An automatic biopsy gun was used with a 16-F Tru-Cut needle. There was 1 core of tissue taken for research purposes only when it was safe to proceed for an extra core and the patient was comfortable and gave verbal consent. Some biopsy samples were obtained from deceased-donor kidneys before transplant only when the family gave consent for the organ to be used for research purposes. The biopsy specimens were snap frozen immediately in liquid nitrogen and stored at -70°C until further analysis. Samples were later processed for Western blot and tested for markers of oxidative stress. Biopsies were taken from the patients undergoing kidney transplant biopsies for any clinical indication after signing an informed consent form.

Informed written consent was obtained from all patients. Ethical approval was granted by the Liverpool adult local research ethics committee before the study. All of the protocols conformed with the ethical guidelines of the 1975 Helsinki Declaration.

Analyses

Biopsy specimens were taken from the -70°C freezer and ground mechanically in liquid nitrogen. Homogenization buffer was added and the mixture was centrifuged at 16000 × g at 4°C for 10 minutes; the supernatants were stored at -70°C. The protein concentration of the sample was determined using the bicinchoninic acid assay. Aliquots of samples containing 40 μg total protein were heated for 5 minutes at 95°C in denaturing loading buffer. The proteins were resolved on 4% and 12.5% sodium dodecyl sulfate polyacrylamide gel electrophoresis mini gels and blotted onto nitrocellulose membranes. The membranes were blocked for 1 hour at room temperature in 5% skim milk in phosphate-buffered saline (PBS) (Blotto, Santa Cruz Biotechnology, Dallas, TX, USA) supplemented with polysorbate surfactant (0.05% Tween 20, Promega Corporation, Madison, WI, USA) (PBST) and exposed at room temperature to primary antibodies using antibody dilutions and times recommended by the manufacturer. The membranes were repeatedly washed in PBST and exposed at room temperature to secondary antibodies as recommended by the manufacturer. Protein bands were visualized using chemiluminescent substrate (Super Signal West Dura Extended Duration, Pierce Perbio Science, Rockford, IL, USA) and recorded using an imaging system and software (Chemidoc XRS and Quantity One, Bio-Rad Laboratories, Hercules, CA, USA). The intensity of the signal from each band was corrected for background signal and expressed as a percentage of the content of control cells. Western blot analysis was done on several gels because of the large number of samples. On each gel, samples were loaded from different clinical conditions. Beta-actin was used as loading control. Quantification graphs were produced as a percent change from a CAN sample from each individual gel by performing densitometry. Data were analyzed using software (Prism 5, GraphPad Software, San Diego, CA, USA).

Results

Biopsies

There were 61 kidney allograft biopsies collected from kidney transplant recipients who were undergoing this procedure for various clinical reasons; 47 of these biopsies were analyzed and their data were presented. There were 17 pretransplant biopsies from deceased-donor kidneys that were included in this study.

Demographic characteristics of kidney transplant recipients

There were 20 females (42.6%) and 27 males (57.4%). Median age of the recipients was 44.6 ± 2.13 years (range, 19.4-76.9 y). There were 35 deceased-donor
kidney transplants (74.5%) and 12 living-donor kidney transplants (25.5%). There were 11 patients who had a first kidney transplant from a living donor, and 1 patient who had a third kidney transplant from a living donor; there were 29 patients who had their first kidney transplant from a deceased donor, 5 patients who had a second kidney transplant from a deceased donor, and 1 patient had a third kidney transplant from a deceased donor.

Clinical indications for biopsy
The most common indication for undergoing kidney biopsy was renal dysfunction which was usually indicated by a rise in serum creatinine ≥ 10%. There were 34 patients (72.3%) who had renal dysfunction, 8 patients (17%) who had DGF, 4 patients (8.5%) who had renal dysfunction associated with proteinuria, and 1 patient who underwent biopsy because of proteinuria without associated renal dysfunction. Median time between the date of transplant and biopsy was 157 ± 275.7 days (range, 6-8878 d).

Histologic diagnosis was available for all 47 allograft biopsies. Diagnosis was CAN in 14 biopsies (29.8%), acute tubular necrosis (ATN) in 5 biopsies (10.6%), ACR in 19 biopsies (40.4%) (ACR: borderline, 7 biopsies; grade 1A, 9 biopsies; grade 2A, 3 biopsies). Cyclosporine toxicity was observed in 4 biopsies (8.5%) and other diagnoses were noted (Table 1). Banff 97 classification was used to score the grade of ACR and chronic changes in all allograft biopsies.

Analysis of graft biopsies for oxidative stress
There were 109 biopsy samples analyzed. The following antioxidant proteins were detected: catalase (60 kilodaltons), manganese superoxide dismutase (MnSoD) (25 kilodaltons), copper zinc superoxide dismutase (CuZnSoD) (23 kilodaltons), thioredoxin reductase (55 kilodaltons), thioredoxin (2b1) (12 kilodaltons), and thioredoxin (62 kilodaltons).

For quantification, each gel had 1 sample from a kidney with CAN that was used as a control because there was no adequate control biopsy (Figure 1). For quantification, 1 biopsy that was reported as normal, 1 biopsy with immunoglobulin A nephropathy, and 2 biopsies with CAN were grouped together and results were expressed as a percentage change compared with this group. There were 2 biopsies from the ACR and donor group. The CuZnSoD density was higher in biopsies taken from ACR and donor kidneys than CAN samples. However, only donor biopsy samples showed a significant increase (P ≤ .05) (Figure 2). The MnSoD also was increased in both donors and ACR kidneys but did not reach statistical significance. Catalase and thioredoxin reductase were similar between ACR and donor biopsies (not significant). Thioredoxin reductase was elevated in ACR biopsies compared with donor biopsies.

<table>
<thead>
<tr>
<th>Table 1. Histologic Diagnosis of Kidney Allograft Biopsies</th>
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<tr>
<td>Diagnosis on Biopsy</td>
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<tr>
<td>Acute tubular necrosis</td>
</tr>
<tr>
<td>Borderline rejection</td>
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<tr>
<td>Chronic allograft nephropathy</td>
</tr>
<tr>
<td>Cyclosporine toxicity</td>
</tr>
<tr>
<td>Acute rejection (grade 1A)</td>
</tr>
<tr>
<td>Acute rejection (grade 2A)</td>
</tr>
<tr>
<td>Hemolytic uremic syndrome</td>
</tr>
<tr>
<td>Immunoglobulin A nephropathy</td>
</tr>
<tr>
<td>Normal</td>
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<tr>
<td>Posttransplant lymphoproliferative disease</td>
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<td>Total</td>
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Figure 1. Representative Western Blot

Abbreviations: CAN, chronic allograft nephropathy; N, normal
Increased expression of manganese superoxide dismutase (MnSoD), copper zinc superoxide dismutase (CuZnSoD), and catalase in donor biopsies (D) and kidneys with acute cellular rejection (ACR).

Figure 2. Quantification of Gel 1 Showing Copper Zinc Superoxide Dismutase (CuZnSoD), Manganese Superoxide Dismutase (MnSoD), Catalase, and Thioredoxin 55

Abbreviations: ACR, acute cellular rejection; CAN, chronic allograft nephropathy; D, donor (*P ≤ .05).

In another representative gel, catalase, MnSoD, CuZnSoD, and thioredoxin reductase were detected. There were 3 biopsies from the donor group, 3 biopsies from the CAN group, and 2 biopsies each from the ATN and ACR groups. There was 1 biopsy
from the ATN group that also showed ACR; for quantification, this was pooled with the ATN results. Catalase was significantly higher in the donor group ($P \leq .05$). The CuZnSoD also was significantly higher in the donor group than ACR or ATN groups but not higher than the CAN group. Thioredoxin reductase also was significantly higher in the donor group than ATN and ACR groups but not the CAN group, similar to the pattern observed with CuZnSoD. Donor MnSoD had a significant increase compared with all other groups ($P \leq .05$) (Figure 3).

The results of all Western blots were combined and plotted together. The MnSoD, CuZnSoD, and thioredoxin reductase 55 were increased in deceased-donor, ACR, and cyclosporine toxicity groups but this did not reach statistical significance. All enzymes tested showed reduced expression in CAN biopsies (Figure 4).

### Figure 3. Quantification of Gel 7 Showing Copper Zinc Superoxide Dismutase (CuZnSoD), Manganese Superoxide Dismutase (MnSoD), Catalase, and Thioredoxin Reductase 55

#### Abbreviations:
- ACR, acute cellular rejection
- ATN, acute tubular necrosis
- CAN, chronic allograft nephropathy
- D, donor

From the ATN group that also showed ACR; for quantification, this was pooled with the ATN results. Catalase was significantly higher in the donor group ($P \leq .05$). The CuZnSoD also was significantly higher in the donor group than ACR or ATN groups but not higher than the CAN group. Thioredoxin reductase also was significantly higher in the donor group than ATN and ACR groups but not the CAN group, similar to the pattern observed with CuZnSoD. Donor MnSoD had a significant increase compared with all other groups ($P \leq .05$) (Figure 3).

The results of all Western blots were combined and plotted together. The MnSoD, CuZnSoD, and thioredoxin reductase 55 were increased in deceased-donor, ACR, and cyclosporine toxicity groups but this did not reach statistical significance. All enzymes tested showed reduced expression in CAN biopsies (Figure 4).

### Figure 4. Combined Quantitative Data of All Biopsies

#### Abbreviations:
- ACR, acute cellular rejection
- ATN, acute tubular necrosis
- CAN, chronic allograft nephropathy
- D, donor
- CYA, cyclosporine toxicity
- MnSoD, manganese superoxide dismutase
- Trx12, thioredoxin 12 kilodaltons
- Trx62, thioredoxin 62 kilodaltons
- Trx55, thioredoxin reductase 55 kilodaltons

### Discussion

This experimental work on kidney allograft biopsies was aimed to determine the oxidative stress occurring after transplant. Donor pretransplant biopsies provided a unique opportunity to detect oxidative stress occurring because of hypoxia and cold storage. Kidney allograft biopsies are performed routinely to evaluate renal dysfunction. The procedure generally has a low complication rate. The following complications have been reported: gross hematuria, 3.5%; perirenal hematoma, 2.5%; arteriovenous fistula, 7.3%; and vasovagal reaction, 0.5%. Major complications requiring invasive procedures such as blood transfusions or urinary catheterization are observed in 1% cases. Some authors have advocated kidney biopsies for prognostic purposes. Morphologic changes of CAN expressed as tubular atrophy and interstitial fibrosis precede the decline of kidney function. Other authors have suggested the use of biopsies to detect subclinical rejection.

A total of 61 biopsies were analyzed and the following oxidative stress enzymes were detected by Western blot: catalase (60 kilodaltons), MnSoD (25 kilodaltons), CuZnSoD (23 kilodaltons), thioredoxin reductase (55 kilodaltons), thioredoxin (2b1) (12 kilodaltons), and thioredoxin (62 kilodaltons).

Up-regulation of most antioxidant enzymes was detected in biopsies obtained from deceased-donor kidneys before transplant. The MnSoD was expressed more in donor kidneys and kidneys with ACR and cyclosporine toxicity. The CuZnSoD was also elevated in donor and ACR biopsies. Catalase was elevated in donor and ACR biopsy samples. Thioredoxin was elevated in donor biopsies, and thioredoxin reductases were elevated in donor, ACR, and cyclosporine toxicity biopsies. Ischemic injury and related oxidative stress possibly are reasons for the elevation in these enzymes. However, because of lack of adequate control biopsies, the results were difficult to interpret.

Catalase has been investigated by several authors in biopsy tissue. In a proteome analysis of rat kidney allografts, the expression of catalase was down-regulated. Polymorphism in the catalase gene is associated with DGF in kidney allograft recipients. Antioxidative defense was higher in the tubulo-interstitial compartment than glomerular cells, and a reduction was observed in glomerular enzymes catalase and glutathione peroxidase 3 and 4 in nephritic kidneys; tubular gene expression was down-regulated for catalase, glutathione peroxidase 3, and thioredoxin reductase 1 and 2.
Compared with living-donor kidneys, deceased-donor kidneys have a distinctly different set of antioxidant genes originating from the tubulo-interstitial compartment. The deceased-donor kidneys have long periods of cold storage resulting in hypoxic damage and oxidative stress. Our results from such kidneys confirm this phenomenon because biopsies taken from these kidneys showed an increase in oxidative stress enzymes such as MnSoD, CuZnSoD, catalase, and thioredoxin enzymes. Donor kidneys from recipients with impaired allograft function also showed activation of genes mainly belonging to the functional classes of immunity, signal transduction, and oxidative stress response.

The CuZnSoD comprises 90% total superoxide dismutase activity in eukaryotic cells. An increase in CuZnSoD has been reported from cattle liver biopsies after inducing oxidative stress. Genetic expression of mitochondrial MnSoD, but not cytosolic CuZnSoD or glutathione peroxidase, increased with cold exposure, suggesting mitochondria as a cellular source of free radicals and activation under cold injury.

The MnSoD overexpression in renal tubular cells protects against high-glucose-induced oxidative stress. Increased mRNA levels of CuZnSoD and MnSoD enzymes have been observed in the cortex and medulla of rats subjected to hypoxia. In a study of the role of MnSoD and mitochondrial injury in cold preservation, short-term cold ischemia-reperfusion resulted in inactivation of MnSoD; this suggested that compounds designed to prevent early mitochondrial injury in kidneys that undergo cold preservation would improve kidney function and graft survival after transplant. Loss of mRNA of catalase and glutathione peroxidase may be the first markers of alterations in cellular redox status in ischemia-reperfusion injury. The mRNA for MnSoD was up-regulated at all times with ischemia-reperfusion injury, suggesting that antioxidant genes are not coordinate expressed during ischemia-reperfusion and that the differential loss of antioxidant enzymes may contribute to the heterogeneous kidney tissue damage as a result of ischemia-reperfusion induced oxidative stress.

Some authors also have suggested that mitochondrial dysfunction is an early event in a rat model of allotransplant and may cause the development of CAN.

Thioredoxin reductase is prominently expressed in the proximal tubules of rodent kidneys. Thioredoxin is secreted from proximal tubules into urine during renal ischemia-reperfusion and may have a protective effect against renal ischemia-reperfusion injury. Elevated thioredoxin reductase activity has been observed after exposure to lead acetate in rat kidneys along with other antioxidant enzymes such as catalase and superoxide dismutase. In proximal tubules, nuclear and luminal localization was detected for thioredoxin 1 after ischemia-reperfusion injury. The cytosolic thioredoxin R1 was detected in all tubular segments, especially strongly in distal convoluted tubules and thin segments of the inner medulla. Thioredoxin 2 was diffusely expressed in all regions of the kidney. After ischemia-reperfusion injury, thioredoxin 2 immunoreactivity increased in the mTAL segments and in the lumen of thin segments in the inner medulla. Thioredoxin R2, which was detected primarily in connective tissue in the sham group, showed a segment-specific increase after ischemia-reperfusion injury, most notably in the lumina and epithelial cells of distal convoluted tubules. Strong immunoreactivity for thioredoxin R2 in luminal compartments also was observed in the inner medulla.

Several biopsy specimens studied were taken from patients with cyclosporine nephrotoxicity, and the histology was characterized by tubular vacuolation and an ATN-like appearance. Results from Western blot assay revealed that all antioxidant enzymes tested were elevated in cyclosporine nephrotoxicity. Thioredoxin reductase showed the maximum elevation but other enzymes were elevated including MnSoD, CuZnSoD, catalase, and thioredoxin (12 kilodaltons). Cyclosporine nephrotoxicity is manifested by renal insufficiency due to glomerular disease and abnormalities in tubular function. Several mechanisms have been proposed for cyclosporine-induced nephrotoxicity, such as sodium retention, renal vasoconstriction, renal hypoxia as a consequence of renal vasoconstriction, stimulation of the renin-angiotensin system, activation of the sympathetic nervous system, impaired synthesis of nitric oxide, and increased growth factor B1.

Oxidative stress has been implicated in cyclosporine nephrotoxicity. Increase in reactive oxygen species generation and lipid peroxidation may affect renal function and interstitial fibrosis.
A previous report showed reduction of both oxidative stress and increased inducible nitric oxide synthase (iNOS) and NF-κB expression induced by cyclosporine with use of red wine polyphenol. Treatment with N-acetyl cysteine significantly protected animals against cyclosporine-induced structural and functional impairment of kidneys, implicating the role of oxidative stress in the pathogenesis of cyclosporine-induced nephrotoxicity. Cyclosporine nephrotoxicity is mediated by increased expression of iNOS, NF-κB, and matrix metalloproteinase 2, which was altered by the antioxidant S-allylcysteine, rendering protection to the kidney cells. Histologic evaluation before transplant may enable the identification of organs unsuitable for single transplant, but such evaluation of oxidative stress enzymes is not performed in routine practice.

In summary, it is generally believed that reactive oxygen species are involved in a wide variety of diseases including ischemia-reperfusion injury, cancer, and various types of inflammation. Acute injuries such as cold ischemia in donor biopsies were associated with oxidative stress. Kidney allograft biopsies showed that oxidative stress levels were generally elevated in all biopsies regardless of diagnosis, but not significantly. The levels also were elevated in pretransplant biopsies. This study showed that oxidative stress is involved during acute allograft dysfunction. However, because of a lack of control biopsies, the results could not be interpreted effectively.

Future studies involving kidney allograft biopsies must include a control sample from a normal kidney.

References


A 10-Year Experience of Tuberculosis in Solid-Organ Transplant Recipients

Gaye Ulubay,1 Elif Kupeli,1 Ozlem Duvenci Birben,1 Emine Pinar Seyfettin,1 Mustafa Ilgaz Dogrul,1 Aylin Ozsancak Ugurlu,2 Fusun Oner Eyuboglu,1 Mehmet Haberal3

Abstract

Objectives: Tuberculosis remains an important problem in solid-organ transplant patients due to their immunocompromised state. The objective of the present study was to report the incidence, demographic characteristics, and various presentations of tuberculosis in solid-organ transplant recipients.

Materials and Methods: We evaluated a total of 999 patients (male/female = 665/334, 661 renal and 338 liver transplants) who underwent solid-organ transplant between 2003 and 2013. The medical records of all patients were retrospectively reviewed. Patients' demographics, transplant type, primary site of tuberculosis specimen culture and pathology results, chest radiograph, and thoracic computed tomography findings, total blood count and chemistry were all recorded.

Results: Among the 999 subjects, 19 patients (1.9%) (male/female: 15/4, mean ± SD age, 42 ± 18.5 y) were diagnosed with tuberculosis. The majority of patients (85%) were diagnosed with tuberculosis within 6 months after transplant, and 15% were diagnosed within 3 months. Most diagnoses of tuberculosis were based on histopathologic examination of biopsy material. Of these patients, 9 were diagnosed with pulmonary tuberculosis, 8 had extrapulmonary tuberculosis, and 2 had both. Nontuberculosis mycobacteria infections were detected in 3 patients.

Conclusions: Even with a negative exposure history, tuberculosis can manifest as different clinic presentations in solid-organ transplant patients on immunosuppressive drugs, particularly in the first 6 months after transplant. Therefore, clinicians should always consider tuberculosis as the potential cause of an infectious disease with unknown cause to successfully diagnose and manage solid-organ transplant recipients.

Key words: Pulmonary complication, Infection, Transplant

Introduction

Tuberculosis (TB) is an opportunistic infection with high morbidity and mortality, and persistent diagnostic difficulties often result in delayed treatment.1 According to the World Health Organization, more than one-third of the world’s population is infected with Mycobacterium tuberculosis.2 Among those who carry the bacterium, 10% have active (primary or reactivated) TB, with a considerably higher rate among immunosuppressed individuals.

The reported incidence of TB disease is 0.2% to 6.4% in developed countries and is predicted to be as high as 15% in highly endemic countries.3-6 The prevalence of active TB disease among solid-organ transplant (SOT) recipients was estimated to be 20 to 74 times higher than that of the general population.7-9 Although rare, TB infection presents with various clinical manifestations with increased frequency and severity in transplant recipients.10 Consequently, TB mortality is also higher (up to 31%) in SOT patients than in immunocompetent individuals (0-24 cases per 100 000 for the USA and Western Europe, and >1% in South Africa).3,5,11,12

From the 1Department of Pulmonary Diseases, Baskent University Ankara; the 2Department of Pulmonary Diseases, Baskent University Istanbul; and the 3Department of General Surgery, Baskent University Ankara, Turkey

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Corresponding author: Gaye Ulubay, MD, Baskent University, Department of Pulmonary Diseases, Feriz Çalımkır Cd. 10. Sk. No: 45, Bahçelievler, Ankara, Turkey

Phone: +90 312 212 6868 Fax: +90 312 223 7333 E-mail: gayeulubay@yahoo.com


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Tuberculosis clinical presentation is often atypical, with more than half of SOT recipients presenting with extrapulmonary or disseminated disease. Therefore, a high degree of suspicion for *M. tuberculosis* infection based on clinical experience remains the cornerstone of TB diagnosis. Furthermore, TB treatment is highly problematic in these patients because they are at high risk for multiple adverse effects of first-line anti-TB drugs, drug-drug interactions, and acute allograft rejection due to the increased clearance rate of immunosuppressant drugs.

Tuberculosis management in SOT recipients remains complex and challenging. In this study, we report the frequency, clinical features, risk factors, and primary outcomes of TB in SOT patients.

### Materials and Methods

This study was conducted in Baskent University Hospital, and was approved by the Ethical Review Committee of the Institute. All of the protocols conformed with the ethical guidelines of the 1975 Helsinki Declaration. Written informed consent was not required due to the retrospective nature of the study. All SOT recipients who underwent transplant between January 1, 2003, and December 31, 2013, were enrolled, and their medical records were reviewed to determine whether or not the patients had TB after SOT. The retrospectively collected data included patient demographics and characteristics, comorbidities, type of organ transplant, immunosuppressive regimen, prior TB contact, and/or infection history, primary site of TB, clinical presentation, laboratory (biochemistry, microbiology, and histopathology) findings, radiographic features, diagnostic procedures, clinical outcomes, and mortality.

### Results

A total of 999 SOT recipients (male/female: 665/334) were enrolled in this study. Sixty-six percent had received kidney transplants, whereas 34% had received liver transplants. Tuberculosis was diagnosed in 19 of these patients (1.9%). The prevalence rates of TB among renal and liver SOT recipients were 2.1% and 1.5%. The demographic features and laboratory findings of SOT recipients with TB are listed in Table 1.

The majority of patients (57.9%) were ex-smokers with a history of renal transplant (73.7%) and from a living donor (73.7%). There were various comorbidities in 13 patients (68.4%). Four had multiple comorbidities (1 patient had diabetes mellitus and hypertension; 1 patient had chronic obstructive pulmonary disease, coronary artery disease, hypertension, and congestive heart failure; and 2 patients had coronary artery disease and hypertension).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>42 ± 18.5</td>
</tr>
<tr>
<td>Sex (female/male)</td>
<td>4 (21.1%)/15 (78.9%)</td>
</tr>
<tr>
<td>Smoking status (never smoker/ex-smoker)</td>
<td>8 (42.1%)/11 (57.9%)</td>
</tr>
<tr>
<td>Smoking history (pack-y)</td>
<td>22.5 ± 12.3</td>
</tr>
<tr>
<td>Laboratory findings</td>
<td></td>
</tr>
<tr>
<td>Hemoglobin</td>
<td>10.7 ± 2.4</td>
</tr>
<tr>
<td>Hematocrit</td>
<td>31.6 ± 6.9</td>
</tr>
<tr>
<td>Leukocytes</td>
<td>7.43 ± 2.90</td>
</tr>
<tr>
<td>Platelets</td>
<td>193.0 ± 74.0</td>
</tr>
<tr>
<td>Comorbidities</td>
<td></td>
</tr>
<tr>
<td>HTN</td>
<td>13 (68.4%)</td>
</tr>
<tr>
<td>COPD</td>
<td>1 (5.3%)</td>
</tr>
<tr>
<td>DM</td>
<td>1 (5.3%)</td>
</tr>
<tr>
<td>CAD</td>
<td>4 (21.1%)</td>
</tr>
<tr>
<td>CHF</td>
<td>1 (5.3%)</td>
</tr>
<tr>
<td>Transplant type</td>
<td></td>
</tr>
<tr>
<td>Kidney</td>
<td>14 (73.7%)</td>
</tr>
<tr>
<td>Liver</td>
<td>5 (26.3%)</td>
</tr>
<tr>
<td>Donor type</td>
<td></td>
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<tr>
<td>Deceased-donor</td>
<td>5 (26.3%)</td>
</tr>
<tr>
<td>Living</td>
<td>14 (73.7%)</td>
</tr>
</tbody>
</table>

**Table 1. Demographics and Laboratory Findings of 19 SOT Recipients with TB**

**Abbreviations:** CAD, coronary artery disease; CHF, congestive heart failure; COPD, chronic obstructive pulmonary disease; DM, diabetes mellitus; HTN, hypertension; SOT, solid-organ transplant; TB, tuberculosis

Various combinations of immunosuppressive drugs were administered posttransplant. The most commonly used agent was prednisolone (84.2% of SOT recipients with TB), but mycophenolate mofetil (73.7%), sirolimus (52.6%), tacrolimus (47.4%), and cyclosporine (10.5%) also were administered.

None of the patients had any known prior history of TB exposure and/or infection (Table 2). Tuberculosis prophylaxis with isoniazid was given pre- (1 patient) or postoperatively (2 patients). Upon radiologic examination, sequel lesions were found on computed tomography scans and/or chest radiograph in 10 patients (1 patient had sequel lesions on both imaging modalities). Tuberculosis involvement was pulmonary in 9 patients (47.4%), extrapulmonary in 8 (42.1%), and both in 2 SOT recipients with TB (10.5%).

The most commonly used diagnostic tool was histopathologic examination (n = 10, 52.6%).
followed by specimen culture for TB (n = 4, 21.1%), and clinical suspicion (n = 5, 26.3%). Most patients (n = 17, 89.5%) were diagnosed with TB more than 6 months after transplant. None of the M. tuberculosis isolates were resistant to first-line anti-TB drugs. All patients were given combination therapy including isoniazid, rifampicin, pyrazinamide, and ethambutol. None of the patients developed hepatotoxicity due to these medications. Non-TB mycobacterial infections were found in 3 patients (0.3%). Neither death nor organ rejection was recorded during the management of TB disease in our study population. Three of the patients died because of other causes (independent of TB) 4 to 14 years after transplant. Five rejections were recorded 6 to 20 years after transplant.

Discussion

Patients undergoing SOT require potent and generally prolonged immunosuppressive treatment after surgery, which increases their risk several states opportunistic infections.15,16 Tuberculosis is one of the most serious diseases that they can develop as it is associated with high morbidity and mortality in SOT recipients.3,4 Many studies have described TB as a factor that facilitates organ rejection due to drug-drug interactions between anti-TB and immunosuppressive agents.17-19 Therefore, TB remains an important topic for both clinicians and patients in the posttransplant period.

The literature reports that the prevalence for TB in SOT recipients in low-endemicity areas is between 0.2% and 6.4%.6 In the present study, TB frequency was 1.9% in 999 SOT patients. This high prevalence could be attributed to our strict preoperative pulmonary evaluation strategies for excluding the risk of reactivation TB in these patients.9 Another factor could be The National Stop Tuberculosis Strategy, which led to decreased TB rates in Turkey within the last 10 years (the annual change in the rate of new TB cases were 28.5 and 22.5 in 2005 and 2010).20

Most TB occurs within 12 months after transplant.15 The median time interval between transplant and TB diagnosis has been reported as 9 to 31 months in the literature.1,9,15,21 In our study group, 16 patients (85%) developed TB 6 months after transplant; TB was detected within the first 3 months after transplant in just 3 patients. Interestingly, all 3 of these patients received pulse steroid therapy within 1 month after transplant. All of these earlier cases also had radiographic evidence of latent TB. This leads us to speculate that, patients with a history of prior M. tuberculosis exposure develop TB earlier than patients without an exposure history, which is in accordance with the literature.22 In addition, none of the SOT recipients who developed TB had a prior known TB history, even though they had sequel lesions on either chest radiographs or computed tomography scans. Interferon-γ release assays are valuable for diagnosing latent TB infection in this population. The purified protein derivative skin test is not useful in Turkey because Bacillus Calmette–Guérin vaccination is routine. In our center, we began to perform interferon-γ release assays for all transplant candidates and initiate preventive therapy in those with positive reactions.

Because up to one-third of the world’s population has latent TB infection, the main mechanism of TB development in SOT recipients is the reactivation of dormant bacilli in other parts of the body.1 Other sources of TB are primary infection from post-transplant exposure and acquired infection via an infected donor organ.5,11 Donor-derived disease transmission is rare, complicating less than 1% of transplants.23-25 Based on their radiologic findings, the basic mechanism in our 10 SOT recipients with TB could be reactivation of latent TB. However, the

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>No. (%)</th>
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<tbody>
<tr>
<td>History of TB</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>BCG scar</td>
<td>1 (5.3)</td>
</tr>
<tr>
<td>TB prophylaxis</td>
<td></td>
</tr>
<tr>
<td>Preoperatively</td>
<td>1 (5.3)</td>
</tr>
<tr>
<td>Postoperatively</td>
<td>2 (10.5)</td>
</tr>
<tr>
<td>Tuberculosis sequel</td>
<td></td>
</tr>
<tr>
<td>On chest radiograph</td>
<td>3 (15.8)</td>
</tr>
<tr>
<td>On CT scan</td>
<td>11 (58.9)</td>
</tr>
<tr>
<td>Type of mycobacterium species</td>
<td></td>
</tr>
<tr>
<td>Tuberculosis</td>
<td>19 (1.9)</td>
</tr>
<tr>
<td>Nontuberculosis</td>
<td>3 (0.3)</td>
</tr>
<tr>
<td>TB diagnosis period</td>
<td></td>
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<tr>
<td>0-3 months after transplant</td>
<td>2 (10.5)</td>
</tr>
<tr>
<td>&gt; 6 months after transplant</td>
<td>17 (89.5)</td>
</tr>
<tr>
<td>Primary TB site</td>
<td></td>
</tr>
<tr>
<td>Pulmonary</td>
<td>9 (47.4)</td>
</tr>
<tr>
<td>Extrapulmonary</td>
<td>8 (42.1)</td>
</tr>
<tr>
<td>Both</td>
<td>2 (10.5)</td>
</tr>
<tr>
<td>Extrapulmonary involve site</td>
<td></td>
</tr>
<tr>
<td>Lymph nodes</td>
<td>3 (15.8)</td>
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<tr>
<td>Bone</td>
<td>3 (15.8)</td>
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<tr>
<td>Kidney</td>
<td>1 (5.3)</td>
</tr>
<tr>
<td>Bladder</td>
<td>1 (5.3)</td>
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<tr>
<td>Central nervous system</td>
<td>1 (5.3)</td>
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<tr>
<td>Abdominal</td>
<td>1 (5.3)</td>
</tr>
</tbody>
</table>

Abbreviations: BCG, Bacillus Calmette–Guérin; CT, computed tomography; TB, tuberculosis
cause was not clear for the rest of the patients because they had neither prior history of TB nor radiologic findings. The source of TB in this group could be exposure to TB during the posttransplant phase.21,26

Transplant type was found to be related with TB occurrence. In the present study, most TB patients were renal recipients (73.7%), which is in accordance with the literature.5,21,22 This could be partially attributed to the predominance of renal recipients in our center (renal/liver: 661/338). Another explanation could be that renal recipients are at high risk of TB exposure from dialysis units.27 Additionally, reactivation of latent TB might occur more frequently in patients with end-stage renal disease before transplant who have weakened immune systems due to renal failure. Collectively, our findings suggest that renal transplant recipients should be evaluated with more attention paid to TB risk compared with liver transplant recipients.

The most commonly used diagnostic method was histopathologic examination in our SOT recipients with TB, all of whom were diagnosed with extrapulmonary TB (typically in the lymph nodes and bones). Therefore, while searching for the cause of an extrapulmonary infection, we recommend that all histopathologic specimens from SOT recipients should be evaluated for TB. In 4 patients, TB was treated based on clinical suspicion resulting in clinical, radiologic, and laboratory evidence. This supports the general opinion that empirical anti-TB treatment should be immediately administered to SOT recipients with high clinical suspicion of TB to reduce related complications and mortality.

Extrapulmonary TB occurs in approximately 20% of TB patients in a normal population.28 The clinical presentation of extrapulmonary TB in SOT patients is highly variable; they may have no symptoms and may be accidentally diagnosed by routine control testing. Hence, extrapulmonary TB presents more of a diagnostic and therapeutic problem than pulmonary TB, emphasizing the importance of clinical experience in this field.28 Although there are some controversial results in our immunocompromised study population, the frequencies of pulmonary and extrapulmonary TB were similar (47.4% and 42.1%).29-35 Therefore, extrapulmonary TB should be considered in the differential diagnosis of all extrapulmonary infections in SOT recipients.

The mortality rates due to TB in SOT recipients were reported between 13% and 40%.1,36 In our study, this rate was 0%. Similarly, transplanted organ rejection did not occur during TB management. The Health Ministry of Turkey reported the national TB treatment success rate as 91%.20 Thus, these satisfactory outcomes for SOT recipients with TB could be attributable to the government’s successful national treatment strategy, which has made Turkey one of the leading countries in global TB control.36

Atypical mycobacterial infections may occur more often in immunocompromised hosts. The characteristics of a significant non-TB mycobacterial infection include positive culture of the pathogen from a normally sterile site or repeated isolation of potential mycobacterial pathogens from nonsterile sites.15,37 The current frequency of the disease because of various non-TB mycobacteria species is unknown in immune-compromised patients.26,38 Determining the incidence and prevalence of non-TB mycobacterial lung disease is actually quite difficult because it is not required to be reported in many countries. In our study, microbiologic test results revealed that 3 of the 999 SOT recipients (0.3%) had non-TB mycobacterial lung disease. Correctly differentiating between non-TB and TB infections is very important because some non-TB mycobacteria species do not respond well to anti-TB therapies.15,28,38

In conclusion, TB is an important complication in SOT recipients and can increase mortality and morbidity rates. Reactivation TB can develop any time during the posttransplant period but is more common in the first few months after transplant. Additional immunosuppressive treatments against organ rejection may facilitate TB occurrence. Histopathologic analyses play an important role in diagnosing TB in patients with extrapulmonary involvement.

References

Abstract

Objectives: Solid-organ transplant recipients are at an increased risk of developing cancer including cervical cancer compared with woman in the general population, mostly due to long-term immunosuppressive therapy. The Papanicolaou smear remains the primary method of screening cervical pathology including preinvasive and invasive lesions. The objective of this study was to evaluate Pap smear findings in solid-organ transplant recipients, determine the prevalence of abnormal smears, and compare these patients with the general population.

Materials and Methods: We retrospectively examined 111 women patients who received liver or kidney transplant between January 1990 to December 2012 at Başkent University Ankara Hospital. Pap smear findings were compared with normal control patients matched for same age and technical procedure of cervical cytology. To selection of control patients, propensity score matching program was performed. All Pap smears were re-examined according to Bethesda 2001 criteria.

Results: In 111 transplant patients, 2 patients (1.8%) had atypical squamous cells of undetermined significance, 8 patients (7.2%) had low-grade squamous intraepithelial lesion, 15 patients (13.5%) had Candida infection, 2 patients (1.8%) had Trichomonas vaginalis, 1 patient (0.9%) had herpes simplex infection, 13 patients (11.7%) had bacterial vaginosis, 15 patients (13.5%) had reactive changes due to inflammation, and 18 patients (16.2%) had atrophy. When we compared our results with the control group, there were statistically significant differences ($P \leq .05$) between the 2 groups in epithelial cell abnormalities (low-grade squamous intraepithelial lesion), Candida infection, bacterial vaginosis, and atrophy.

Conclusions: Pap smear screening potentially may help recognize cervical preinvasive and invasive lesions. The risk of developing cervical intraepithelial neoplasia is greater in transplant recipients because of immunosuppressive therapy. The incidence of low-grade squamous intraepithelial lesion was significantly greater in transplant recipients than the general population. Intensive follow-up with Pap smear in transplant recipients is important in the early detection of these lesions.

Key words: Cervical cancer, Immunosuppression, Kidney transplant, Liver transplant, Pap smear

Introduction

Solid-organ transplant recipients are at an increased risk of infectious diseases, autoimmune diseases, and malignancy including uterine cervical cancer compared with woman in the general population, mostly due to long-term immunosuppressive therapy. The mechanisms by which immunosuppressive treatment increases malignancy include DNA damage due to immunosuppressive drugs, altered DNA repair, reduced immunologic tolerance.
of neoplastic cells, and the increased changes of infection with oncogenic viruses such as the human papillomavirus (HPV).\textsuperscript{1,2}

The Papanicolaou (Pap) smear remains the primary method of screening cervical pathology including preinvasive and invasive lesions and is associated with a reduction in the incidence and mortality from cervical cancer. The Bethesda system is used for reporting cervical or vaginal cytologic diagnoses and was re-evaluated in 2001.\textsuperscript{3} Abnormal results include atypical squamous cells of undetermined significance (ASC-US), low-grade squamous intraepithelial lesion (LGSIL or LSIL), high-grade squamous intraepithelial lesion (HGSIL or HSIL), atypical squamous cells but cannot exclude HSIL (ASC-H), squamous cell carcinoma, atypical glandular cells not otherwise specified (AGC-NOS), adenocarcinoma in situ (AIS), or atypical glandular cells suspicious for AIS or cancer (AGC-neoplastic).\textsuperscript{3} Progression of cellular abnormalities and preinvasive disease can lead to the development of cervical cancer.

The objective of this study was to evaluate Pap smear findings in solid-organ transplant recipients, determine the prevalence of abnormal smears, and compare results between these patients and the general population according to the Bethesda 2001 criteria. In addition, features such as atrophy, inflammation, and the presence of organisms were compared between solid-organ transplant recipients and the general population.

Materials and Methods

Patients
A retrospective analysis was made of 603 women patients including children and adolescents who received liver or kidney transplant between January 1990 to December 2012 at Başkent University, Ankara Hospital, Ankara, Turkey. Donor treatment and kidney and liver transplant surgery were performed according to standardized procedures. The study was approved by the Ethical Review Committee of the institute. All protocols conformed to the ethical guidelines of the 1975 Declaration of Helsinki.

Cervical Pap smears that were performed in 111 women after transplant were included in this study to examine the effect of normal control subjects matched for age and technical procedure of cervical cytology who were selected randomly with the propensity score matching program. All Pap smears were re-examined according to Bethesda 2001 criteria (Figure 1).

Figure 1. Papanicolaou (Pap) Smear Findings of Transplant Recipients

(A) Atypical Squamous Cells of Undetermined Significance (ASC-US). Nuclear enlargement and hyperchromasia are noted in a superficial cell (Pap stain, original magnification ×200).
(B) Low-Grade Squamous Intraepithelial Lesion (LSIL). Binucleation and koilocytosis are noted (Pap stain, original magnification ×200).
(C) Candida albicans. Fungal organisms with pseudohyphae are noted between squamous cells (Pap stain, original magnification ×200).
(D) Trichomonas vaginalis. Pear-shaped organisms with eccentrically located nucleus and eosinophilic cytoplasmic granules are shown (Pap stain, original magnification ×400).

Statistical analyses
Data analyses were performed with statistical software (SPSS, version 15.0, IBM Corporation, SPSS Inc., Armonk, NY, USA). The chi-square test was used to compare appropriate variables in patients. Results were considered statistically significant when P ≤ .05.

Results
The mean age of the patients who had solid-organ transplant and control subjects was 36.71 years (range, 18-59 y). In the solid-organ transplant recipients, 89 patients received kidney transplant and 22 received liver transplant. The mean interval to Pap smear after transplant was 52.14 ± 43.35 months (range, 1-192 mo).

*Candida* infection was detected in 20 subjects, including 15 solid-organ transplant patients (13.5%) and 5 control subjects (2.3%) (Table 1). There were 3 *Trichomonas vaginalis* infections including 2 solid-
organ transplant patients (1.8%) and 1 control subject (0.5%). Only 1 patient who had solid-organ transplant (0.9%) had herpes simplex infection. Only 2 control subjects (0.9%) had Actinomyces infections. Solid-organ transplant patients had a significantly higher incidence of Candida infection than control subjects; there was no statistically significant differences between solid-organ transplant patients and control subjects regarding other microorganisms (Table 1). A shift in flora suggestive of bacterial vaginosis was detected in 22 Pap smears, including 13 solid-organ transplant patients (11.7%) and 9 control subjects (4.1%); this difference was statistically significant (P ≤ .05). Chlamydia microorganisms, reactive changes due to intrauterine devices, and reactive changes due to radiation were not detected in either group.

### Table 1. Pap Smear Results of the Study Patients

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Transplant Patients (n = 111) (%)</th>
<th>Control Subjects (n = 222) (%)</th>
<th>P ≤ *</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean age (y)</td>
<td>36.7</td>
<td>36.7</td>
<td></td>
</tr>
<tr>
<td>Organisms</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Trichomonas vaginalis</td>
<td>2 (1.8)</td>
<td>1 (0.5)</td>
<td>NS</td>
</tr>
<tr>
<td>Candida</td>
<td>15 (13.5)</td>
<td>5 (2.3)</td>
<td>.0001</td>
</tr>
<tr>
<td>Actinomyces</td>
<td>0 (0)</td>
<td>2 (0.9)</td>
<td>NS</td>
</tr>
<tr>
<td>Herpes simplex virus</td>
<td>1 (0.9)</td>
<td>0 (0)</td>
<td>NS</td>
</tr>
<tr>
<td>Bacterial vaginosis</td>
<td>13 (11.7)</td>
<td>9 (4.1)</td>
<td>.05</td>
</tr>
<tr>
<td>Reactive changes (inflammation)</td>
<td>15 (13.5)</td>
<td>19 (8.6)</td>
<td>NS</td>
</tr>
<tr>
<td>Atrophy</td>
<td>18 (16.5)</td>
<td>7 (3.2)</td>
<td>.0001</td>
</tr>
<tr>
<td>Epithelial cell abnormalities</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ASC-US</td>
<td>2 (1.8)</td>
<td>3 (1.4)</td>
<td>NS</td>
</tr>
<tr>
<td>LSIL</td>
<td>8 (7.2)</td>
<td>2 (0.9)</td>
<td>.05</td>
</tr>
</tbody>
</table>

**Abbreviations:** ASC-US, atypical squamous cells of undetermined significance; LSIL, low-grade squamous intraepithelial lesion

Reactive changes secondary to inflammation were detected in 34 patients, including 15 patients in the solid-organ transplant group (13.5%) and 19 patients in the control group (8.6%); there was no statistically significant differences between groups. Atrophy was detected in 25 patients, including 18 solid-organ transplant patients (16.5%) and 7 control subjects (3.2%); this difference was statistically significant (P ≤ .0001).

In the 333 subjects, 15 subjects had epithelial cell abnormalities (10 patients in the solid-organ transplant group and 5 control subjects) (Table 1). The ASC-US was detected in 5 patients, including 2 solid-organ transplant patients (1.8%) and 3 control subjects (1.4%); this difference was not statistically significant (P > .05). The LSIL was detected in 10 patients, including 8 solid-organ transplant patients (7.2%) and 2 control subjects (0.9%); the difference between the groups was statistically significant (P ≤ .05). The HSIL, squamous cell carcinoma, or glandular cell abnormalities were not detected.

**Discussion**

Solid-organ transplant recipients have a 3- to 4-fold increased risk of developing malignancy compared with the general population. The risk of cervical cancer also is increased. The incidence of cervical neoplasia in these patients is 11%. In organ transplant recipients, invasive cervical cancer may occur in 1% and in situ cervical cancer in 3.3% patients, and transplant recipients had a 3-fold higher incidence of in situ cancer than the general population. In contrast with other invasive cancer types, cervical cancer had no increased incidence in transplant patients. In our study, there was no cervical cancer in our patients. Routine use of Pap smears showed the absence of an increased incidence of invasive cervical cancer. The Pap test is easy to perform, has low cost, and is highly sensitive and specific. This cytologic screening test is recommended for all women.

Long-term immunosuppressive therapy in solid-organ transplant recipients is an important risk factor for developing cervical cancer. Calcineurin inhibitors (cyclosporine and tacrolimus) promote carcinogenesis, potentially through production of cytokines that regulate transforming growth factor β, metastasis, and angiogenesis. All immunosuppressive agents affect the immunologic system, reduce immunologic tolerance of neoplastic cells, and increase infections with oncogenic viruses (such as HPV) that cause DNA damage. Infections with HPV are frequent in solid-organ transplant patients and can lead to cervical cancer. Oncogenic HPV types such as HPV-16 are less common in LSIL (cervical intraepithelial neoplasia [CIN] 1) than HSIL (CIN 3), and nononcogenic HPV types are commonly observed in CIN 1 lesions. Mean time to progression from ASC-US to LSIL or worse, and from LSIL to HSIL or worse, was shorter in women with oncogenic HPV types than women with no HPV infection. In addition, Moscicki and coworkers showed that only HPV status at the current visit was associated with rate of regression, but they did not show an association between LSIL regression and HPV status at baseline in univariate analysis.
A limitation of our study was that the HPV status of transplanted woman could not be determined. Paternoster and associates performed HPV tests and noted that there was an association between high-risk HPV infections and CIN lesions but no association between low-risk HPV infections and CIN lesions.\(^4\) In contrast, Origoni and associates reported no significant differences in the course of HPV infection between transplanted woman and control subjects.\(^6\) We believe that HPV vaccine before transplant and an HPV test are mandatory for solid-organ transplant patients to prevent HPV-related malignancy. In addition, it was reported previously that various antilymphoproliferative induction therapies were associated with reduced incidence of invasive cervical cancer.\(^5\)

The Pap test screening for cervical cancer is useful to identify precancerous lesions of the cervix. In the current study, LSIL was observed in 8 solid-organ transplant patients and 2 control subjects. The statistically significant risk increase of LSIL was observed with solid-organ transplant in our study. Origoni and associates observed a significant difference between groups only for LSIL cytology, similar to the present results.\(^6\) In another study, Paternoster and coworkers reported on 151 transplant patients; 5 patients had HSIL and 5 patients had LSIL. They found that the incidence of intraepithelial lesion was greater in transplant patients than the general population.\(^4\)

Another important effect of immunosuppression on cervical cytology appears to be on fungal infections. In our study, 15 of 20 Candida infections were in solid-organ transplant patients. A statistically significant difference in Candida infection was observed between the 2 groups. However, there were no significant differences between the 2 groups for Trichomonas vaginalis, Actinomyces, and herpes simplex virus infections. Another finding we observed in the Pap smear cytology was a shift in flora suggestive of bacterial vaginosis. In our study, we found significant differences between the 2 groups regarding bacterial vaginosis. It was possible to see the relation between atrophy and immunosuppression for solid-organ transplant. Thinning of the squamous epithelium and reduced mucus production leads to atrophy because of using long-term immunosuppressive drugs. We observed significant differences between the 2 groups for atrophy. This issue and the presence of a shift in flora suggestive of bacterial vaginosis may have been due to disturbances in the estrous cycle; this was mentioned before in another study performed at our institution about Pap smear findings in chronic renal failure patients, in which we compared Pap smear findings between chronic renal failure patients and healthy control subjects according to the Bethesda 2001 criteria to determine whether there was increased risk of epithelial cell abnormalities in dialysis patients.\(^9\)

In conclusion, we suggest that solid-organ transplant recipients are at greater risk of developing cervical precancerous lesions and cervical cancer because of immunosuppressive therapy. Our study also showed that the incidence of LSIL was increased significantly compared with the normal population. Therefore, Pap test screening and HPV vaccination should be performed before solid-organ transplant. Pap test and HPV test should be repeated at regular intervals to detect or prevent precancerous lesions and cervical cancer.

References

Long-Term Risk of Pulmonary Embolism in Solid-Organ Transplant Recipients

Elif Küpeli,1 Gaye Ulubay,1 Ilgaz Doğrul,1 Özlem Birben,1 Pınar Seyfettin,1 Aylin Özsancak Uğurlu,2 Füsun Öner Eyüboğlu,1 Mehmet Haberal3

Abstract

Objectives: Solid-organ transplant recipients can develop chronic hypercoagulation that increases the incidence of pulmonary embolism. Here, we evaluate the frequency of pulmonary embolism in solid-organ transplant recipients during the first 10 years after transplantation and evaluate the risk factors for its development.

Materials and Methods: The medical records of solid-organ transplant recipients who were treated between 2003 and 2013 were retrospectively reviewed. The reviewed data included demographics, type of transplant, comorbidities, procoagulation factors, thromboembolism prophylaxis, and the timing and extent of pulmonary embolism.

Results: In total, 999 solid-organ transplant recipients are included in this study (661 renal and 338 liver transplant recipients) (male: female ratio = 665:334). Twelve renal (1.2%) and 1 liver transplant recipient (0.3%) were diagnosed with pulmonary embolism. Pulmonary embolism developed 1 year after transplantation in 10 patients: 1 patient developed pulmonary embolism < 3 months after transplantation, and the other 9 patients developed pulmonary embolism within 3 to 6 months. No patients had a prior history of deep venous thrombosis or pulmonary embolism. Pulmonary embolism developed 1 year after transplantation in 10 patients: 1 patient developed pulmonary embolism < 3 months after transplantation, and the other 9 patients developed pulmonary embolism within 3 to 6 months. No patients had a prior history of deep venous thrombosis or pulmonary embolism. Five patients received tacrolimus, 7 patients received sirolimus, and 1 patient received cyclosporine. Ten patients received prednisolone, and 8 patients received mycophenolate mofetil.

All patients were homozygous normal for factor V Leiden and prothrombin genes. One patient was homozygous abnormal, and 1 patient had a heterozygous mutation in the methylenetetrahydrofolate reductase gene. Two patients were treated with low-molecular-weight heparin, while the remaining patients received warfarin. Eight patients were treated for 6 months, and the remainder received longer treatments.

Conclusions: Here, the incidence of pulmonary embolism in solid-organ transplant recipients is 1.2%. Renal transplant recipients are at higher risk of developing pulmonary embolism than liver transplant recipients. The factors that increase the risk of pulmonary embolism in solid-organ transplant recipients appear to be multifactorial and include genetic predisposition.

Key words: Hypercoagulation

Introduction

Solid-organ transplant recipients can develop chronic hypercoagulation, which increases the incidence of thromboembolic complications. Prothrombotic changes may present without other comorbidities, especially in renal transplant recipients, thereby suggesting its development as the primary cause of hypercoagulability.1 Hypercoagulation appears to be a lifelong condition; however, the risk is greatest during the first 6 months after transplant.2 Here, our aim is to evaluate the frequency of pulmonary embolism in solid-organ transplant recipients during the first 10 years after transplantation and evaluate the effect of venous thromboembolism prophylaxis and immunosuppressive therapies on its development.
Materials and Methods

Study population
The medical records of patients who underwent solid-organ transplantation at our institution between 2003 and 2013 were retrospectively reviewed. Data included patient demographics, type of transplant, comorbidities, immunosuppressive therapies, degree and onset time of pulmonary embolism, deep venous thrombosis, factor V Leiden mutations, prothrombin (PT G20210A) and methylenetetrahydrofolate reductase (MTHFR C677T) gene mutations, other procoagulation factors (eg, protein C or S deficiency, hyperhomocysteinemia), and venous thromboembolism prophylaxis.

In the present study, pulmonary embolism was suspected based on the patient’s clinical presentation, laboratory data (eg, D-dimer, chest radiograph, arterial blood gas analysis) and objectively confirmed using computed tomography pulmonary angiography. The extent of pulmonary embolism was established using echocardiography and categorized as massive, submassive, or nonmassive. Deep venous thrombosis was diagnosed using bilateral deep venous compression and Doppler ultrasonography.

Data were analyzed using commercially available software (SPSS version 15.0; SPSS Inc., Armonk, NY, USA). Data are presented as the mean ± standard deviation (SD). All of the protocols conformed with the ethical guidelines of the 1975 Helsinki Declaration. Written informed consent was obtained from all subjects.

Results
In total, 999 patients underwent solid-organ transplant during the study period; 661 renal and 338 liver transplants were performed, and 665 patients were male.

A total of 12 patients (1.2%) (9 males; mean age, 49.5 ± 7 y) were diagnosed with pulmonary embolism. Eleven patients underwent renal transplantation, while the remaining patients underwent liver transplantation. Nine, 2, and 1 patients were diagnosed with nonmassive, massive and submassive pulmonary embolism.

Table 1. Characteristics of Our Renal Transplant Recipients With Pulmonary Embolism

<table>
<thead>
<tr>
<th>Patient</th>
<th>Tacrolimus</th>
<th>Sirolimus</th>
<th>Prednisolone</th>
<th>MMF</th>
<th>Cause of Renal Failure</th>
<th>Type of Dialysis Before Tx</th>
<th>Erythrocytosis</th>
<th>CMV Infection</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>HT</td>
<td>HD</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>Idiopathic</td>
<td>HD</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>Idiopathic</td>
<td>HD</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>Idiopathic</td>
<td>HD</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>Fabry's disease</td>
<td>HD</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>HT</td>
<td>HD</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>7</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>Idiopathic</td>
<td>HD</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>8</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>Idiopathic</td>
<td>HD</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>9</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>Nephrolithiasis</td>
<td>HD</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>10</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>Idiopathic</td>
<td>HD</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>11</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>HT</td>
<td>HD</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Abbreviations: CMV, cytomegalovirus; HD, hemodialysis; HT, hypertension; MMF, mycophenolate mofetil; Tx, transplant
pulmonary embolism following the initially administered intravenous heparin therapy. Eight patients were treated for 6 months, and 4 patients were treated > 6 months because of genetic mutations or persistent residual thrombosis. No pulmonary embolism-associated deaths occurred during the 10-year follow-up.

Discussion

Hypercoagulation in solid-organ transplant recipients is a multifactorial pathologic condition that develops in association with various risk factors. Renal transplant recipients are at higher risk of developing hypercoagulation than other solid-organ transplant recipients, especially during the immediate and early postoperative period.1

Previously reported series on renal transplant recipients report an incidence rate of 2% to 14% for pulmonary embolism and a mortality rate of 13.4%.3 A retrospective study that enrolled 480 renal transplant recipients over a 10-year period reported that 8.3% of the patients developed venous thrombosis.4 In the present study, the incidence of pulmonary embolism was 1.6% among 661 renal transplant recipients, but no instances of mortality occurred. In a study by Allen and associates, the combined incidence of deep venous thrombosis and pulmonary embolism was highest within the first 4 months after surgery. Here, only 1 patient developed pulmonary embolism within the first 4 months, and the other patients developed pulmonary embolism within 1 year after transplantation.

The increased risk of thromboembolic events in renal transplant recipients appears to be multifactorial. While these patients can present with the known risk factors for thromboembolic complications at similar rates as the general population, they can also present with other conditions such as primary nephropathy that can also lead to transplant, dialysis, immunosuppression and infection.

Cyclosporine, a calcineurin inhibitor, is believed to increase the risk of thromboembolic complications in renal transplant recipients; however, data remain controversial.5-7 The role of tacrolimus, a relatively new calcineurin inhibitor, in the development of thromboembolic is not fully understood. It has been reported that the antithrombolytic effects of tacrolimus result from inhibiting platelet activity.8 In renal transplant recipients, long-term steroid treatment results in hypercoagulation and hypofibrinolysis (similar to patients with Cushing disease) and leads to thrombotic complications.9 The role of sirolimus in the development of thromboembolic events has not been evaluated in renal transplant recipients. It has been reported that the addition of sirolimus to cyclosporine-steroid regimens does not increase the incidence of thromboembolic events in renal transplant recipients.10 The relation between mycophenolate mofetil and thromboembolic events has not been established either. A monograph on mycophenolate mofetil reported that thrombosis is an adverse effect in renal transplant recipients, but further investigations are needed to confirm this relation.11,12 Here, tacrolimus, sirolimus, prednisolone, and mycophenolate mofetil were the most common drugs administered to renal transplant recipients. We suggest that combining these drugs might have played a role in the development of pulmonary embolism in our patient population.

Original nephropathy, which might lead to end-stage renal disease, is also associated with a high risk of thromboembolism and could contribute a similar risk even after transplantation. Lupus nephropathy, Fabry disease, and nephritic syndrome also reportedly contribute to thrombotic events.13-15 Here, 1 patient had Fabry disease, which may have led to the development of pulmonary embolism (Table 1). We suggest screening for Fabry disease before renal transplantation and especially resistance to protein C activation, and patients should receive venous thromboembolism prophylaxis if coexistence is diagnosed.

Hypercoagulation also reportedly develops in patients receiving continuous ambulatory peritoneal dialysis because of transperitoneal protein loss.16 Peritoneal dialysis patients also demonstrate significantly higher levels of blood coagulation factors in comparison with healthy controls. Therefore, peritoneal dialysis patients may demonstrate clinical thromboembolic patterns during the early posttransplant period. Incidentally, in the present study, no patients were receiving peritoneal dialysis before transplantation.

The incidence of erythrocytosis (> 52% hematocrit in men; > 49% in women) in renal transplant recipients reportedly varies between 8% and 22%.17 Long-term hemodialysis, polycystic kidney disease,
graft artery stenosis, diabetes, smoking, and hypertension may contribute to the development of posttransplantation erythrocytosis.\(^8\) In this study, 1 patient with Fabry disease also had been diagnosed with posttransplantation erythrocytosis, which might have contributed to the development of pulmonary embolism.

It has also been reported that acute cytomegalovirus infection may contribute to thromboembolic events in renal transplant recipients.\(^9\) Cytomegalovirus demonstrates tropism for endothelial cells, and its effects on vascular biology have been reported under different circumstances. Using various mechanisms, active cytomegalovirus is capable of modifying the endothelial phenotype from anticoagulant into procoagulant.\(^10\) Here, no patients had acute cytomegalovirus infection at the time of pulmonary embolism.

This study has several weaknesses. First, this study is retrospective, and therefore we could have underestimated the incidence of thromboembolism. On the other hand, despite the large number of included solid-organ transplant recipients, only a few patients developed thromboembolic events. Additional solid-organ transplant recipients are needed to perform a prospective study, which is currently in development. Second, only 1 of 12 patients underwent liver transplant, and hence we are unable to further study this combination. Because of the retrospective nature of the study, we were also unable to study the effects of acute rejection and cytomegalovirus infection on the occurrence of deep venous thrombosis or pulmonary embolism.

In conclusion, hypercoagulation in renal transplant recipients is a multifactorial pathologic condition that develops due to risk factors that are, in part, specific to this population. Renal transplantation is a major surgery, which may predispose kidney recipients to thromboembolic complications during the immediate and early postoperative period. Surgical trauma, which inevitably occurs when performing arterial and venous anastomosis, may increase the risk of thromboembolic events. However, the risk of pulmonary embolism remains high after this period because of several immunologic and metabolic factors. Immunosuppression seems to play a major role in the development of pulmonary embolism. In our renal transplant recipients, the incidence of pulmonary embolism was 1.6%. Immunosuppressive drugs, original nephropathy, and surgery might also play a role in the development of pulmonary embolism. As a well-known factor, being bedridden always plays a critical role in transplant recipients. At our transplant department, early mobilization is needed to prevent venous thromboembolism during the postoperative period, and we believe mobilization prevented thromboembolism in our renal transplant recipients.

Transplant physicians must remain vigilant about determining all of the factors associated with venous thromboembolism during both the pre- and posttransplant period. Associated comorbidities, such as diabetes, cancer, and inherited thrombophilia, may add to the risk, and should be appropriately managed.

References

Effects of Different Trends on the Development and Outcome of Early Kidney Allograft Dysfunction

Viktor K. Denisov, Vadim V. Zakharov, Eugeny V. Onishchenko, Tatyana S. Golubova, Yana G. Mitsuk, Olga V. Zakharova

Abstract

Objectives: To show the effects of different factors on development and outcome of early kidney allograft dysfunction.

Materials and Methods: Two hundred thirty-one kidney transplant recipients were divided into 2 groups: group 1 (125 patients transplanted from 1999-2004) and group 2 (106 patients transplanted from 2008-2013). Age range was 12 to 62 years (group 1) and 7 to 71 years (group 2). Deceased-donor transplant was more frequent in group 1 (76.8%), and living-donor transplant in group 2 (68.8%). In group 1, transplant was performed for glomerulonephritis or pyelonephritis; in group 2, additional risk factors (18 patients) included diabetes (11 patients), systemic lupus erythematosus (5 patients), amyloidosis (1 patient), and aortic and mitral valve replacement because of bacterial endocarditis (1 patient). In groups 1 and 2, immunosuppression after transplant included cyclosporine, mycophenolate mofetil, and steroids; patients in group 2 also had induction with anti-CD25 monoclonal antibodies.

Results: Primary graft function occurred in 89 patients in group 1 (71.2%) and 83 patients in group 2 (78.3%). Immediately after transplant, delayed graft function included anuria, oliguria, adequate amount of urine, and secondary delayed function (several days of polyuria followed by decreased urine output). Ischemia was a leading cause of delayed renal graft function. Anuria after living-donor transplant was a sign of vascular thrombosis. Rejection was the main cause of secondary delayed graft function, which occurred in only group 1. Survival at 1 year in patients with delayed graft function was 80% in group 1 and 100% in group 2 because of the absence of septic complications.

Conclusions: Despite extension of indications, primary functioning of kidney transplants and patient survival increased. Improved care enables long-term rehabilitation of recipients and expanding criteria for kidney transplant.

Key words: Delayed graft function, End-stage renal disease, Kidney transplant

Introduction

Kidney transplant is a major challenge in children, older adults, and patients who have risk factors such as diabetes mellitus, systemic lupus erythematosus, and long-term maintenance dialysis.1-12 In recent years, more patients who previously were not considered transplant candidates have been referred for kidney transplant. We reviewed the effects of different factors on development and outcome of early kidney allograft dysfunction.

Materials and Methods

Two hundred thirty-one kidney transplant recipients were divided into 2 groups: group 1 (125 patients transplanted from 1999-2004) and group 2 (106 patients transplanted from 2008-2013). Age range was 12 to 62 years (group 1) and 7 to 71 years (group 2). Deceased-donor transplant was more frequent in group 1 (76.8%), and living-donor transplant was more frequent in group 2 (68.8%). Maximum duration of dialysis treatment in patients with anuria...
was 3 years in group 1 and 13 years in group 2. In group 1, transplant was performed for glomerulonephritis or pyelonephritis; in group 2, additional risk factors (18 patients) included diabetes (11 patients), systemic lupus erythematosus (5 patients), amyloidosis (1 patient), and aortic and mitral valve replacement because of bacterial endocarditis (1 patient). Twelve kidney transplants were performed simultaneously with ipsilateral nephrectomy in group 2. In groups 1 and 2, immunosuppression after transplant included cyclosporine, mycophenolate mofetil, and steroids; patients in group 2 also had induction with anti-CD25 monoclonal antibodies.

Results

Primary graft function occurred in 89 patients (71.2%) in group 1 and 83 patients (78.3%) in group 2 (Table 1). With initial renal function, diuresis for the first day after transplant ranged from 2 to 51 liters, and all patients had urine with low specific gravity; patients with delayed renal graft function were more difficult to interpret and treat. Delayed graft function was defined as the need for dialysis during the first week after kidney transplant. Immediately after kidney transplant, 4 different types of clinical course of delayed renal graft function were defined: anuria, oliguria, adequate amount of urine, and secondary delayed function (several days of polyuria followed by decreased urine output including anuria). Ischemia was a leading cause of early delayed renal graft function (Tables 2 and 3). Anuria after living-donor transplant was a sign of vascular thrombosis. Rejection was the main cause of secondary delayed graft function, and this occurred only in group 1. Survival at 1 year in patients with delayed graft function was 80% in group 1 and 100% in group 2, primarily because of the absence of septic complications (Tables 2 and 3).

Discussion

The comparison of the importance of graft ischemia, rejection, thrombosis, infection, and drug-induced complications enables the consideration of ischemia as the main reason for renal graft dysfunction. So that severe renal dysfunction in group 1 with most deceased non-heart beating donor transplants occurred more frequently (28.8%) then it did in group 2 (17.1%) with most living-donor transplants. This is the reason that the absence of kidney function in grafts taken from living donors is a potential sign of vascular thrombosis of the graft or transplant rejection. Insufficient renal graft function in some cases was caused by multiple causes, such as acute rejection in a kidney with underlying tubular damage or infection. In analyzing all cases of delayed graft function, we could not identify cyclosporine nephrotoxicity (with monitoring of cyclosporine concentration in the blood) as a separate cause of renal dysfunction.

In delayed graft function with anuria, it was necessary to consider the differential diagnosis with primary nonfunctioning graft. With primary nonfunctioning graft, the continuation of immunosuppression created the additional risk for the development of sepsis and other severe complications. Doppler ultrasonography was the most available and informative study in patients with equivocal diagnosis. If the cause of oliguria remained

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### Table 1. Kidney Graft Function in the Different Clinical Groups

<table>
<thead>
<tr>
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<tbody>
<tr>
<td>Primary graft function</td>
<td>89 (71.2%)</td>
<td>83 (78.3%)</td>
</tr>
<tr>
<td>Delayed graft function</td>
<td>28 (22.4%)</td>
<td>17 (16%)</td>
</tr>
<tr>
<td>Primary nonfunction graft</td>
<td>8 (6.4%)</td>
<td>6 (5.7%)</td>
</tr>
<tr>
<td>Total</td>
<td>125 (100%)</td>
<td>106 (100%)</td>
</tr>
</tbody>
</table>

### Table 2. Causes and Outcomes of Delayed Graft Function in Group 1

<table>
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</thead>
<tbody>
<tr>
<td>Delayed graft function: anuria</td>
<td>10 (100%)</td>
<td>Ischemia</td>
<td>Rejection</td>
<td>Sepsis</td>
<td>7 (70%)</td>
</tr>
<tr>
<td>Delayed graft function: oliguria</td>
<td>9 (100%)</td>
<td></td>
<td>5 (55.6%)</td>
<td>3 (33.3%)</td>
<td>11 (1%)</td>
</tr>
<tr>
<td>Delayed graft function: adequate amount of urine</td>
<td>6 (100%)</td>
<td></td>
<td></td>
<td></td>
<td>1 (100%)</td>
</tr>
<tr>
<td>Secondary delayed graft function</td>
<td>3 (100%)</td>
<td>1 (33.3%)</td>
<td>2 (66.7%)</td>
<td></td>
<td>1 (33.3%)</td>
</tr>
<tr>
<td>Primary nonfunction graft</td>
<td>8 (100%)</td>
<td>5 (62.5%)</td>
<td>1 (12.5%)</td>
<td>2 (25%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Total</td>
<td>36 (100%)</td>
<td>25 (69%)</td>
<td>8 (22.2%)</td>
<td>1 (0.3%)</td>
<td>2 (5.6%)</td>
</tr>
</tbody>
</table>
primary functioning kidney transplant and patient indications, our data shows that the frequency of efficiency of renal transplant. Programs more accurately and improve the clinical situations, has enabled us to plan treatment including retrospective interpretation of similar dysfunction and predicting the clinical course, was the reason to not perform transplant erythropoietin prescription) was observed, and this concentration of blood hemoglobin (without transplant. In 1 patient, despite anuria, normal level normalizing on day 45 of anuria after kidney resumed and the patient recovered, with creatinine was not observed. Only in 1 patient, diuresis anuria continued > 1 month, graft recovery usually was early, brief hemodialysis sessions without ultrafiltration and heparinization, when possible. If was early, brief hemodialysis sessions without dialysis depended on diuresis dynamics, changes of urea and creatinine concentration in blood and urine tests, and the general state of the patient. Patients with delayed graft dysfunction had an average 5.8 ± 0.7 hemodialysis sessions. With anuria after renal transplant, the most effective treatment was early, brief hemodialysis sessions without ultrafiltration and heparinization, when possible. If anuria continued > 1 month, graft recovery usually was not observed. Only in 1 patient, diuresis resumed and the patient recovered, with creatinine level normalizing on day 45 of anuria after kidney transplant. In 1 patient, despite anuria, normal concentration of blood hemoglobin (without erythropoietin prescription) was observed, and this was the reason to not perform transplant nephrectomy in the subsequent year.

Determination of the cause of early kidney graft dysfunction and predicting the clinical course, including retrospective interpretation of similar clinical situations, has enabled us to plan treatment programs more accurately and improve the efficiency of renal transplant.

In conclusion, despite the extension of indications, our data shows that the frequency of primary functioning kidney transplant and patient survival increased. Improved care enables long-term rehabilitation of recipients and expanding criteria for kidney transplant.

References

Use of Biological Prosthesis in a Patient With Kidney and Pancreas Transplant and a Giant Incisional Hernia: Case Report

Umit Ozcelik,1 Halime Cevik,2 Huseyin Yuce Bircan,1 Alp Demirag1

Abstract

Objectives: The use of synthetic mesh in transplant patients is controversial. Recent studies have shown that biological prostheses have a greater ability to integrate into tissues, resist bacterial colonization, and reduce cytotoxic or allergic reactions, and provide similar functional results, compared with synthetic prostheses. Biological prostheses do not require any reduction or discontinuation of immunosuppressive therapy. We present the case of a kidney and pancreas transplant recipient who had a giant incisional hernia that was treated successfully with a biological prosthesis.

Case Report: A 40-year-old male kidney and pancreas transplant recipient was admitted to our hospital with a giant incisional hernia, 2 years after transplant. The defect on the abdominal wall was 40 × 30 cm. We used 2 biological prostheses (40 × 20 cm and 30 × 20 cm) to close the abdominal wall. The patient was discharged on postoperative day 5 without complications. An abdominal magnetic resonance imaging scan showed complete integrity of the biological prostheses at 1 year after surgery.

Conclusions: Transplant recipients have higher risks with use of synthetic prostheses because of being immunosuppressed, compared with other patients. Recent studies show that biological prostheses provided similar functional results without complications compared with synthetic prostheses. These prostheses are versatile and do not require any changes in immunosuppressive therapy. Therefore, they seem to be a better option than synthetic prostheses. In our opinion, biological prostheses are more safe, effective, and reliable than synthetic prostheses, especially for large incisional hernias in transplant recipients. We believe that further larger studies can support our opinion.

Key words: Abdomen, Reconstruction, Repair, Wound

Introduction

Incisional hernias may occur after any abdominal operation, and the overall incidence of an incisional hernia after abdominal operations is 2% to 13%. Incisional hernias develop in 10% patients who subsequently develop a wound infection. The risks of fascial dehiscence, wound infection, and incisional hernias are higher in organ recipients than they are in other patients. According to a previous report in renal transplant recipients, 4.8% patients developed wound infections and 3.6% patients had a fascial dehiscence or incisional hernia. More than 50% incisional hernias occur within 6 months after surgery. Several risk factors have been defined including obesity, wound infection, hematoma, urinoma, lymphocele, repeat interventions, immunosuppression, diabetes mellitus, advanced age, malnutrition, and smoking history.

The main principle of incisional hernia repair is to restore the anatomic and physiologic integrity of the abdominal wall. Fascial defects with diameter < 6 cm can be repaired primarily, but 30% to 50% defects > 6 cm recur after primary closure. Several types of prosthetic mesh currently are available for

From the Departments of 1General Surgery and 2Radiology, Baskent University School of Medicine, Istanbul, Turkey
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Corresponding author: Alp Demirag, Baskent University School of Medicine, Department of General Surgery, Altunizade mahallesi, Oymaci sokak no. 7, Uskudar, Postal code 34662, Istanbul, Turkey
Phone: +90 216 554 1500 Fax: +90 216 651 9858 E-mail: alpdemirag@yahoo.com


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hernia repair. Although permanent mesh prostheses are considered the best treatment for minimizing hernia recurrence, they have been associated with a high risk of complications due to their nonabsorbable characteristics such as erosion into the abdominal viscera, protrusion, extrusion, adhesion, infection, and bowel fistulae that can lead to more complex and costly surgery.

Biological mesh was introduced as an alternative to synthetic mesh in the 1990s. There are 15 different bioprosthetic materials taken from different types of tissue. Biological mesh prostheses allow neo-vascularization and regeneration due to infiltration of native fibroblasts and they are incorporated into the surrounding tissue. During incorporation, they gradually are degraded and generate a new, metabolically active neofascia to withstand the mechanical forces of the abdominal wall.

The use of synthetic mesh in transplant patients is controversial. Simple, small, and noninfected incisional hernias may be repaired either primarily or with polypropylene mesh with good results. Large cases of fascial dehiscence requiring abdominal reconstruction rarely are discussed in the literature, and no clear treatment method exists for giant incisional hernias in transplant patients. Recent studies have shown that biological prostheses have a greater ability to integrate into tissues, resist bacterial colonization, and reduce cytotoxic or allergic reactions, and provide similar functional results, compared with synthetic prostheses. Biological prostheses do not require any reduction or discontinuation of immunosuppressive therapy.

The purpose of this case report was to present a kidney and pancreas transplant recipient who had a giant incisional hernia that was treated successfully with a biological prosthesis.

**Case Report**

A 40-year-old male kidney and pancreas transplant recipient was admitted to the hospital with a giant incisional hernia. He received a left living-donor kidney transplant 7 years earlier and a pancreas transplant 2 years earlier. The giant abdominal incisional hernia occurred within 6 months after the pancreas transplant. The major risk factors were obesity, diabetes mellitus, and immunosuppressive therapy. The patient’s immunosuppressive therapy included cyclosporine, mycophenolate mofetil, and steroids. Physical examination showed that the defect was 40 cm (vertical) × 30 cm (horizontal). We decided to use a porcine dermal collagen implant to facilitate abdominal wall closure.

The operative procedure started with excision of the scar. The hernia sac was opened carefully, adhesions were gently removed, and dissection was continued inside, to 5 cm in all directions. The sac was excised along the edge of the defect. The 2 porcine dermal collagen mesh prostheses (40 × 20 cm and 30 × 20 cm) (Permacol, Covidien, Mansfield, MA, USA) were sutured to each other, positioned without tension to the edges of the fascia defect, and sutured with 1-0 interrupted polypropylene sutures (Figure 1). We placed 2 Jackson-Pratt drains (Cardinal Health, Dublin, OH, USA) between the mesh construct and abdominal skin. Subcutaneous tissue and skin were closed with interrupted sutures.

Antibiotics were given until postoperative day 5. The patient continued immunosuppressive therapy without any changes. Drains were removed and the patient was discharged on postoperative day 5 without complications. No seroma, hematoma, or wound infection occurred after the operation. An abdominal magnetic resonance imaging scan at 1 year after surgery showed complete integrity of the biological prostheses, and the patient had an excellent functional result (Figure 2). No hernia recurrence was observed at 2-year follow-up after surgery.

**Discussion**

Incisional hernia is a predominant complication in transplant recipients. The incidence of incisional hernias is much higher in patients receiving a combined kidney and pancreas transplant than kidney transplant alone.
It has been claimed that permanent synthetic mesh is safe in renal recipients but there is an increased risk of infection. In inorganic materials are passive, rigid, and adynamic, and they restore the structure but not the function of the abdominal wall. Moreover, inorganic materials are associated with foreign body complications. Half of the patients who develop a recurrence have an infection, and transplant recipients have higher risks of the use of synthetic prostheses because of being immuno-suppressed. Recent studies showed that biological prostheses provided similar functional results without complications compared with synthetic prostheses.

The prosthesis used in the present case (Permacol, Covidien) is a porcine-derived acellular dermal mesh, predominantly composed of type I collagen. After cell removal, it is sterilized by gamma irradiation and packaged in a hydrated state that makes it usable immediately without any preparation. This prosthesis is treated with hexamethylene diisocyanate to increase collagen cross-linking and decrease biodegradability, which might be accelerated in contaminated wounds. Porcine dermis is the closest to human dermis and it is not cytotoxic, hemolytic, pyrogenic, or allergenic, and it does not elicit a foreign body response. It is colonized by host tissue cells and blood vessels that minimize the risk of infection. It is soft and flexible, and it has bilateral smooth surfaces with high tensile strength. It is sold in sheets, allowing it to be cut to shape, and provides the largest grafts available (maximum size, 28 × 40 cm).

In animal studies, a porcine dermal collagen implant produced a substantially weaker inflammatory response and less extensive, less dense adhesions. Although it had significantly lower tensile strength than polypropylene at 30 days after being implanted, at 90 days there was no statistically significant difference. Within 12 weeks after being implanted, biological materials were filled with a regenerated tissue that resembled normal fascia.

Biological mesh is widely used in nontransplant patients. Recurrence rates vary from 0% to 15% in recent reviews, but recurrence rate was lower (2.9%) in clean and clean-contaminated cases and increased with the extent of contamination. Infection was the most common complication with an overall rate of 15.9%, but infection rate with the mesh used in the current study (Permacol, Covidien) was 6.1%. Grafts were removed in only 4.9% infected cases. A meta-analysis of open incisional hernia repair with synthetic mesh showed that a quarter of infections required mesh removal. Biological grafts were associated with a high salvage rate in cases of infection compared with synthetic grafts. The major advantage of biological over synthetic implants is that biological grafts can be used in direct contact with bowel without causing fistulas, and they cause minimal adhesions with successful results even in contaminated or infected wounds.

The operative strategy of incisional hernias in transplant patients is controversial. Recurrence rates vary from 2% to 20% in studies of hernia repair with prosthetic mesh in renal transplant recipients. There are limited data about the use of biological prostheses in transplant patients who have incisional hernias. Coccolini and coworkers reported a 14.3% recurrence rate and 28.6% complication rate. The mean overall complication rate of incisional hernia repair with biological prostheses in the current literature was 9.4% according to their review. Santangelo and associates reported a series of 10 transplant patients with incisional hernia repair using a biological prosthesis without complications or hernia recurrence. Pentlow and coworkers reported satisfactory functional results in 5 pediatric renal transplant recipients with donor size discrepancy who had abdominal wall closure assisted with porcine dermal collagen implants.
In our preliminary experience, a biological prosthesis appears useful, safe, and effective for incisional hernia repair in transplant patients and provides excellent functional results. Major advantages of biological compared with synthetic prostheses include a greater potential to integrate into host tissues, resist bacterial colonization, reduce cytotoxic or allergic reactions, and not to require any reduction or discontinuation of immunosuppressive therapy. In our opinion, biological prostheses are safer, more effective, and more reliable than synthetic prostheses, especially for large incisional hernias in transplant recipients. We believe that further larger studies can support our opinion.

References

Assessment of Myocardial Mechanics in Patients with End-Stage Renal Disease and Renal Transplant Recipients Using Speckle Tracking Echocardiography

Bahar Pirat, Huseyin Bozbas, Vahide Simsek, L. Elif Sade, Burak Sayin, Haldun Muderrisoglu, Mehmet Haberal

Abstract

Objectives: Velocity vector imaging allows quantitation of myocardial strain and strain rate from 2-dimensional images based on speckle tracking echocardiography. The aim of this study was to analyze the changes in myocardial strain and strain rate patterns in patients with end-stage renal disease and renal transplant recipients.

Materials and Methods: We studied 33 patients with end-stage renal disease on hemodialysis (19 men; mean age, 36 ± 8 y), 24 renal transplant recipients with functional grafts (21 men; mean age, 36 ± 7 y) and 26 age- and sex-matched control subjects. Longitudinal peak systolic strain and strain rate for basal, mid, and apical segments of the left ventricular wall were determined by velocity vector imaging from apical 4- and 2-chamber views. The average longitudinal strain and strain rate for the left ventricle were noted. From short-axis views at the level of papillary muscles, average circumferential, and radial strain, and strain rate were assessed.

Results: Mean heart rate and systolic and diastolic blood pressure during imaging were similar between the groups. Longitudinal peak systolic strain and strain rate at basal and mid-segments of the lateral wall were significantly higher in renal transplant recipients and control groups than end-stage renal disease patients. Average longitudinal systolic strain from the 4-chamber view was highest in control subjects (-14.5% ± 2.9%) and was higher in renal transplant recipients (-12.5% ± 3.0%) than end-stage renal disease patients (-10.2% ± 1.6%; P ≤ .001). Radial and circumferential strain and strain rate at the level of the papillary muscle were lower in patients with end-stage renal disease than other groups.

Conclusions: Differences in myocardial function in patients with end-stage renal disease, renal transplant recipients, and normal controls can be quantified by strain imaging. Myocardial function is improved in renal transplant recipients compared with end-stage renal disease patients.

Key words: Chronic kidney disease, Heart, Myocardium, Strain, Strain rate

Introduction

Cardiovascular diseases are the leading causes of death in patients with end-stage renal disease (ESRD). Changes in left ventricular function are common in these patients and are predictors of outcome. Renal transplant improves prognosis in renal transplant recipients (RTR). Several studies have demonstrated improvement in left ventricular systolic function after successful transplant. Even in advanced systolic heart failure, renal transplant leads to an increase in left ventricular ejection fraction (EF) and improves functional status of patients.

Subclinical myocardial disease can be assessed with advanced echocardiographic methods including measurement of tissue velocities, strain, and strain...
rate (SR). Strain and SR provide accurate measurements of contractility, unaffected by myocardial tethering or translation. A feature tracking algorithm incorporating speckle and endocardial border tracking, velocity vector imaging (VVI), provides myocardial velocity, strain, and SR based on 2-dimensional images, independent of Doppler angle.

In this study, we sought to define changes in regional and global left ventricular function in terms of myocardial velocity, systolic strain, systolic SR, and dyssynchrony indices in ESRD patients and RTR with preserved EF using speckle tracking echocardiography. We compared ESRD patients, RTR, and normal controls regarding parameters indicating subclinical myocardial function.

Materials and Methods

Study population
In this study, we enrolled 33 patients with ESRD (age > 18 y) on maintenance hemodialysis therapy, 24 RTR with a functioning graft, and 26 age- and sex-matched healthy controls. Patients with clinical coronary artery disease were not included in the study. Coronary artery disease was defined as the presence of one of the following: typical angina, ST segment, or T wave changes specific for myocardial ischemia, or Q waves on electrocardiogram, wall motion abnormality on echocardiography, a noninvasive stress test revealing ischemia or any perfusion abnormality, or history of a myocardial infarction and/or revascularization. Patients with moderate or severe mitral or aortic regurgitation or stenosis, atrial fibrillation, hypertrophic cardiomyopathy, or poor echocardiographic image quality were excluded from the study.

Demographic data including risk factors for coronary artery disease and cardiovascular medications were evaluated from patient charts. Mean duration of maintenance hemodialysis for ESRD patients, total time on dialysis before transplant, and mean interval between renal transplant and echocardiographic assessment for RTR were noted. Fasting venous blood samples were obtained for biochemical analyses. The institutional review board approved the study protocol, and all patients gave informed consent before enrollment. All of the protocols conformed with the ethical guidelines of the 1975 Helsinki Declaration. Written informed consent was obtained from all subjects.

Echocardiographic image acquisition
All subjects underwent resting echocardiography with an ultrasonography system (ACUSON Sequoia 256, Siemens, Mountain View, CA, USA) and a 3.5-MHz transducer. For patients with ESRD, echocardiography was performed on days between dialysis. Images were acquired from apical 4-chamber, 2-chamber, parasternal long-axis, and parasternal short-axis views at the level of papillary muscles. The 2-dimensional, M-mode, spectral, and color Doppler examinations were performed. Left ventricular end-diastolic and end-systolic diameters, interventricular septal and posterior wall thicknesses, early (E) and late (A) mitral inflow velocities, and deceleration time for mitral E wave were measured. Left ventricular mass was determined using the method of Devereux, and EF was calculated by Simpson biplane method of disks. For each image, 2 to 3 cardiac cycles were acquired at a frame rate of 45 to 50 Hz. Images were stored and transferred to a computer for off-line VVI analysis.

Velocity vector imaging
The off-line software (syngo Velocity Vector Imaging technology, Siemens) provided velocity, strain, and SR from 2-dimensional images. The VVI incorporates speckle tracking, mitral annulus motion, tissue-blood border detection, and periodicity of the cardiac cycle using R-R intervals. Strain assessment by this method was validated in an animal study against sonomicrometry.

Longitudinal systolic velocity, strain, and SR were recorded for lateral, septal, anterior, and inferior walls for each patient. Each wall was divided into basal, mid, and apical segments automatically. In addition to segmental strain and SR, average strain and SR values from 4- and 2-chamber views were noted. Average strain curves obtained from 4-chamber views for patients with ESRD and RTR are shown in Figure 1. Time from onset of QRS to peak systolic velocity, peak systolic strain, and SR for basal, septal, and basal lateral walls were measured, and opposing wall delays were calculated for peak systolic velocity, strain and SR as dyssynchrony indices. From short-axis views at the level of papillary muscles, average circumferential and radial strain and SR were assessed (Figure 2). The average time needed for the analysis by VVI was 4 to 5 minutes for each acquisition.
Statistical analyses

Variables were presented as mean ± SD. Echocardiographic variables from 3 groups were compared using 1-way analysis of variance with Bonferroni adjustment and post hoc analysis. Clinical characteristics of the groups were compared with chi-square test. Values for \( P \leq .05 \) were considered statistically significant. Multivariate linear regression analysis was performed to investigate parameters associated with average strain. The analyses were performed using statistical software (Statistical Package for the Social Sciences, Version 11.0, SSPS Inc., Chicago, IL, USA).

Results

Mean age of patients with ESRD was 36 ± 6 years and RTR was 36 ± 7 years. Prevalence of hypertension was significantly lower in the control group than patients with ESRD and RTR (Table 1). Mean duration of maintenance hemodialysis for ESRD patients was 107 ± 79 months. Total time on dialysis prior to transplant was 62 ± 35 months, and mean interval between renal transplant and echocardiographic assessment was 51 ± 41 months for patients with a functioning graft kidney.

Standard echocardiography showed that mean EF was similar between the study groups (Table 2). Peak systolic velocities of basal segments of septal, lateral, and anterior walls were significantly lower in ESRD patients than controls, but similar between RTR and controls (Table 3). Peak systolic strain values from

Table 1. Baseline Clinical Characteristics of the Groups

<table>
<thead>
<tr>
<th>Variable</th>
<th>Controls ( (n = 26) )</th>
<th>ESRD ( (n = 33) )</th>
<th>RTR ( (n = 24) )</th>
<th>( P \leq )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>36 ± 8</td>
<td>36 ± 6</td>
<td>36 ± 7</td>
<td>.9</td>
</tr>
<tr>
<td>Sex, male</td>
<td>19</td>
<td>24</td>
<td>20</td>
<td>.6</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>26 ± 5</td>
<td>22 ± 3</td>
<td>24 ± 4</td>
<td>.006</td>
</tr>
<tr>
<td>Hypertension (%)</td>
<td>10</td>
<td>68</td>
<td>59</td>
<td>.001</td>
</tr>
<tr>
<td>Hyperlipidemia (%)</td>
<td>20</td>
<td>32</td>
<td>22</td>
<td>.6</td>
</tr>
<tr>
<td>Smoker (%)</td>
<td>45</td>
<td>50</td>
<td>45</td>
<td>.9</td>
</tr>
<tr>
<td>Family history for prematurity CAD (%)</td>
<td>10</td>
<td>9</td>
<td>-</td>
<td>.3</td>
</tr>
<tr>
<td>Systolic BP (mm Hg)</td>
<td>119 ± 10</td>
<td>117 ± 12</td>
<td>122 ± 12</td>
<td>.3</td>
</tr>
<tr>
<td>Diastolic BP (mm Hg)</td>
<td>76 ± 6</td>
<td>75 ± 8</td>
<td>77 ± 8</td>
<td>.6</td>
</tr>
<tr>
<td>Medications</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ß-blocker (No.)</td>
<td>15</td>
<td>28</td>
<td>14</td>
<td>.3</td>
</tr>
<tr>
<td>ACEI (No.)</td>
<td>-</td>
<td>14</td>
<td>9</td>
<td>.2</td>
</tr>
<tr>
<td>Calcium antagonist (No.)</td>
<td>-</td>
<td>33</td>
<td>41</td>
<td>.006</td>
</tr>
<tr>
<td>Statin (No.)</td>
<td>-</td>
<td>19</td>
<td>45</td>
<td>.002</td>
</tr>
<tr>
<td>Aspirin (No.)</td>
<td>5</td>
<td>24</td>
<td>59</td>
<td>.01</td>
</tr>
<tr>
<td>Duration of HD (mo)</td>
<td>NA</td>
<td>107 ± 79</td>
<td>62 ± 35</td>
<td>.02</td>
</tr>
<tr>
<td>Hemoglobin (g/L)</td>
<td>147 ± 15</td>
<td>116 ± 20</td>
<td>141 ± 35</td>
<td>.001</td>
</tr>
<tr>
<td>Creatinine (µmol/L)</td>
<td>70.7 ± 17.6</td>
<td>830.9 ± 274.0</td>
<td>141.4 ± 88.4</td>
<td>.001</td>
</tr>
</tbody>
</table>

Abbreviations: ACEI, angiotensin converting enzyme inhibitor; BMI, body mass index; BP, blood pressure; CAD, coronary artery disease; ESRD, end-stage renal disease; HD, hemodialysis; No., number; RTR, renal transplant recipients
basal, mid, and apical segments of septal, lateral, anterior, and inferior walls were lower in patients with ESRD than controls, and similar patterns of changes were observed for systolic SR (Table 4).

Average systolic strain from 4-chamber and 2-chamber views were highest in control subjects and were higher in RTR than in ESRD patients (4-chamber: control, -14.5% ± 2.9%; RTR, -12.5% ± 3.0%; ESRD, -10.2% ± 1.6%; P < .001) (2-chamber: control, -15.9% ± 2.7%; RTR, -13.4% ± 2.4%; ESRD, -11.4% ± 2.2%; P < .001) (Figure 3). Average systolic SR was similar between controls and RTR and lower in ESRD patients (4-chamber: control, -0.76 ± 0.17 s⁻¹; RTR, -0.77 ± 0.21 s⁻¹; ESRD, -0.62 ± 0.13 s⁻¹; P < .001) (2-chamber: control, 0.85 ± 0.15 s⁻¹; RTR, -0.78 ± 0.19 s⁻¹; ESRD, -0.65 ± 0.12 s⁻¹; P < .001) (Figure 3). Radial and circumferential strain and SR at the level of papillary muscle were lower in patients with ESRD than the other groups (Table 5).

In a multivariate regression model including age, serum creatinine level, left ventricular EF, and hypertension as confounding parameters, average strain was independently associated with creatinine level (β = 0.31; P < .009) and EF (β = -0.41; P < .002).

Table 2. Standard Echocardiographic Findings of the Groups

<table>
<thead>
<tr>
<th>Variable</th>
<th>Controls (n = 26)</th>
<th>ESRD (n = 33)</th>
<th>RTR (n = 24)</th>
<th>P ≤</th>
</tr>
</thead>
<tbody>
<tr>
<td>EDV (mL)</td>
<td>92 ± 13</td>
<td>99 ± 14</td>
<td>94 ± 17</td>
<td>.2</td>
</tr>
<tr>
<td>ESV (mL)</td>
<td>36 ± 7</td>
<td>41 ± 6</td>
<td>38 ± 9</td>
<td>.1</td>
</tr>
<tr>
<td>EF (%)</td>
<td>61 ± 3</td>
<td>58 ± 2</td>
<td>59 ± 4</td>
<td>.06</td>
</tr>
<tr>
<td>Septum thickness (mm)</td>
<td>1.0 ± 0.1</td>
<td>1.2 ± 0.1</td>
<td>1.1 ± 0.07</td>
<td>.001</td>
</tr>
<tr>
<td>Posterior wall thickness (mm)</td>
<td>1.0 ± 0.09</td>
<td>1.2 ± 0.1</td>
<td>1.1 ± 0.08</td>
<td>.001</td>
</tr>
<tr>
<td>LV mass index (g/m²)</td>
<td>89 ± 10</td>
<td>131 ± 27</td>
<td>116 ± 17</td>
<td>.001</td>
</tr>
<tr>
<td>Mitral E (cm/s)</td>
<td>86 ± 15</td>
<td>78 ± 15</td>
<td>84 ± 14</td>
<td>.2</td>
</tr>
<tr>
<td>Mitral A (cm/s)</td>
<td>69 ± 14</td>
<td>81 ± 20</td>
<td>78 ± 17</td>
<td>.08</td>
</tr>
<tr>
<td>E/A</td>
<td>1.2 ± 0.2</td>
<td>1.0 ± 0.4</td>
<td>1.1 ± 0.2</td>
<td>.08</td>
</tr>
</tbody>
</table>

Abbreviations: A, late mitral inflow velocity; E, early mitral inflow velocity; EDV, end-diastolic volume; EF, ejection fraction; ESRD, end-stage renal disease; ESV, end-systolic volume; LV, left ventricle; RTR, renal transplant recipients

Table 3. Peak Systolic Velocities of Basal Segments of 4 Walls of the Study Groups

<table>
<thead>
<tr>
<th>Wall</th>
<th>Controls (n = 26)</th>
<th>ESRD (n = 33)</th>
<th>RTR (n = 24)</th>
<th>P ≤</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lateral</td>
<td>4.2 ± 1.2</td>
<td>3.3 ± 1.0</td>
<td>4.1 ± 1.5</td>
<td>.009</td>
</tr>
<tr>
<td>Septal</td>
<td>4.6 ± 1.0</td>
<td>3.6 ± 0.9</td>
<td>4.2 ± 1.4</td>
<td>.005</td>
</tr>
<tr>
<td>Inferior</td>
<td>4.8 ± 1.4</td>
<td>4.4 ± 1.3</td>
<td>4.8 ± 1.1</td>
<td>.4</td>
</tr>
<tr>
<td>Anterior</td>
<td>4.7 ± 0.9</td>
<td>3.9 ± 1.1</td>
<td>4.1 ± 1.2</td>
<td>.04</td>
</tr>
</tbody>
</table>

Abbreviations: ESRD, end-stage renal disease; RTR, renal transplant recipients

Table 4. Segmental Strain Values of the Groups

<table>
<thead>
<tr>
<th>Region</th>
<th>ESRD (n = 33)</th>
<th>RTR (n = 24)</th>
<th>Controls (n = 26)</th>
<th>P ≤</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal (%)</td>
<td>-9.8 ± 2.7</td>
<td>-14.4 ± 5.5*</td>
<td>-12.9 ± 4.2</td>
<td>.001</td>
</tr>
<tr>
<td>Mid (%)</td>
<td>-10.4 ± 3.0</td>
<td>-12.3 ± 4.3</td>
<td>-15.2 ± 3.1</td>
<td>.001</td>
</tr>
<tr>
<td>Apical (%)</td>
<td>-10.3 ± 3.4</td>
<td>-11.2 ± 4.1</td>
<td>-13.2 ± 5.0</td>
<td>.03</td>
</tr>
<tr>
<td>Septum</td>
<td>-8.5 ± 2.5</td>
<td>-9.8 ± 4.0</td>
<td>-12.9 ± 4.2</td>
<td>.001</td>
</tr>
<tr>
<td>Mid (%)</td>
<td>-11.2 ± 2.8</td>
<td>-12.2 ± 3.3</td>
<td>-15.2 ± 3.4</td>
<td>.001</td>
</tr>
<tr>
<td>Apical (%)</td>
<td>-11.7 ± 3.8</td>
<td>-15.1 ± 6.8*</td>
<td>-15.3 ± 4.3</td>
<td>.009</td>
</tr>
</tbody>
</table>

Abbreviations: ESRD, end-stage renal disease; RTR, renal transplant recipients

Table 5. Radial and Circumferential Strain and Strain Rate at the Level of Papillary Muscle for Each Group

<table>
<thead>
<tr>
<th>Region</th>
<th>ESRD (n = 33)</th>
<th>RTR (n = 24)</th>
<th>Controls (n = 26)</th>
<th>P ≤</th>
</tr>
</thead>
<tbody>
<tr>
<td>Circ. strain (%)</td>
<td>-14.5 ± 3.9</td>
<td>-18.6 ± 5.0*</td>
<td>-17.8 ± 2.8</td>
<td>.004</td>
</tr>
<tr>
<td>Circ. SR (s⁻¹)</td>
<td>-0.8 ± 0.2</td>
<td>-1.1 ± 0.3*</td>
<td>-1.0 ± 0.2</td>
<td>.001</td>
</tr>
<tr>
<td>Radial strain (%)</td>
<td>18.6 ± 8.1</td>
<td>25.9 ± 12.0</td>
<td>25.9 ± 9.5</td>
<td>.037</td>
</tr>
<tr>
<td>Radial SR (s⁻¹)</td>
<td>1.0 ± 0.3</td>
<td>1.4 ± 0.3*</td>
<td>1.3 ± 0.3</td>
<td>.003</td>
</tr>
</tbody>
</table>

Abbreviations: Circ., circumferential; ESRD, end-stage renal disease; RTR, renal transplant recipients; SR, strain rate

*P ≤ .05 when compared with ESRD.
Opposing wall delay in peak systolic velocity, strain, and SR for septal-lateral walls were 30 ± 24 ms, 19 ± 18 ms, and 33 ± 35 ms for controls. Other study groups demonstrated prolongation of this delay, which was more pronounced in patients with ESRD (43 ± 51 ms, 59 ± 49 ms, and 56 ± 54 ms for RTR, and 62 ± 54 ms, 55 ± 44 ms, and 72 ± 68 ms for ESRD patients, P ≤ .03, P ≤ .001 and P ≤ .03 for 3 group comparisons). There was no statistically significant difference between ESRD and RTR groups on post hoc analysis.

Discussion

This study demonstrated that indices of subclinical myocardial disease, including longitudinal, circumferential, and radial strain and SR determined by a new feature tracking algorithm, are impaired in ESRD patients with preserved EF and without overt coronary artery disease. These parameters were improved in subjects who underwent renal transplant.

Cardiovascular diseases are the main cause of death in patients with ESRD, and development of congestive heart failure is the most common complication. Approximately one-third of the patients have cardiac failure at the beginning of dialysis. Myocardial function has been studied extensively using conventional echocardiography parameters. However, because fluid status may show considerable variation in patients with ESRD, it may not be accurate to use load-dependent parameters such as EF. It has been shown that, using tissue Doppler imaging, both systolic and diastolic dysfunction could be demonstrated in hemodialysis patients. In addition, tissue Doppler imaging in comparison with conventional echocardiography was a more sensitive method for the detection of left ventricular diastolic dysfunction and provided additional information in predialysis chronic kidney disease patients. In a substudy of the Multi-Ethnic Study of Atherosclerosis, patients with mild to moderate renal insufficiency and without evidence of clinical heart disease had impaired regional systolic and diastolic function, which was assessed by tagged magnetic resonance imaging.

Edwards and colleagues demonstrated that abnormal longitudinal systolic deformation was present in asymptomatic individuals with early chronic kidney disease without clinical evidence of heart disease. More recently, in a study using 2-dimensional speckle tracking echocardiography with strain analysis, it was demonstrated that global peak longitudinal and circumferential strain were decreased in chronic kidney disease patients. Worsening renal function was associated with a reduction of strain; moreover, hemodialysis patients had better left ventricular systolic function than moderate-advanced chronic kidney disease patients. Wang and associates also confirmed that in hemodialysis patients, myocardial function was impaired in the longitudinal and circumferential directions, despite preserved EF. Similarly, using 2-dimensional speckle tracking echocardiography, we have demonstrated in our study that longitudinal and circumferential strain and SR are impaired in ESRD patients with preserved global systolic function. Furthermore, our study extends these findings, demonstrating that strain in the radial direction and synchronous contraction of the left ventricle were impaired in this patient population. Additionally, we have shown that the above-mentioned indices of regional myocardial function are improved in patients who underwent renal transplant.

The pathophysiologic mechanism underlying the association between renal failure and myocardial dysfunction appears to be multifactorial. The uremic state, characterized by metabolic aberrations, is harmful for cardiac function and structure. Among metabolic alterations, secondary hyperparathyroidism and anemia were proposed as important correlates of cardiac function. Parathyroid hormone stimulates proliferation of cardiac fibroblasts, resulting in myocardial fibrosis. Other potential mechanisms include oxidative stress and inflammation. Microvascular dysfunction, as assessed by impaired coronary flow reserve, also has been implicated in patients with ESRD, which could cause subclinical regional myocardial dysfunction.

A study by Rakhit and coworkers demonstrated that ESRD patients without congestive heart failure have subclinical myocardial disease, and this is associated with adverse outcome. In patients who were dialysis dependent on follow-up, SR significantly decreased and strain remained similar to baseline. For patients who had undergone transplant, mean strain increased and SR did not change significantly compared with baseline. Patients who underwent renal transplant showed...
reduction of wall thickness, reduction of left ventricular volumes, and increases in diastolic tissue velocity and strain. In our study, in addition to longitudinal strain and SR, circumferential and radial strain were investigated and they were all lower in ESRD patients than controls; patients who had renal transplant had values between these 2 groups. In multivariate regression analysis, serum creatinine level was independently associated with longitudinal strain, suggesting that normalization of renal function has favorable effects on left ventricular systolic function.

Recently, in a retrospective analysis of 447 patients, it was shown that global longitudinal strain was an important predictor of all-cause mortality in chronic kidney disease patients. Glomerular filtration rate was independently associated with global longitudinal strain in follow-up at 5 years.

In addition to altered deformation in patients with ESRD, we also found that opposing wall contraction delay (septal-lateral walls) was prolonged in patients with ESRD than normal controls. Gedikli and associates investigated systolic asynchrony in patients with chronic kidney disease using tissue synchronization imaging, and they reported that prevalence of systolic asynchrony was significantly higher in this patient group than in normal controls. Geometric alteration in patients with ESRD related to hypertension, volume overload, and myocardial fibrosis may contribute to the development of systolic dyssynchrony in these patients.

The mechanism by which renal transplant normalizes myocardial function remains a matter of speculation. Successful renal transplant improves uremia, anemia, hyperparathyroidism, and volume overload. Because newer echocardiographic parameters such as strain and SR, as assessed in our study, are relatively load-independent, we speculate that improvement in these parameters in RTR may reflect normalization of subclinical regional myocardial function.

Study limitations include the cross-sectional study design; therefore, we do not present follow-up data of patients with ESRD after they had renal transplant. Similarly, deformation measurements of RTR cannot be compared with these parameters in the same patients when they were on regular hemodialysis before transplant. Because not all patients had coronary angiography, we excluded coronary artery disease clinically in the study patients; however, we do not believe that this limitation would interfere with the comparison of ESRD patients with RTR because clinical characteristics were similar between these groups.

In summary, patients with ESRD who were on regular hemodialysis had subclinical left ventricular systolic dysfunction determined by speckle tracking echocardiography, even when they had preserved EF. Patients who underwent renal transplant had improvement in longitudinal, circumferential, and radial deformation indices. Furthermore, systolic dyssynchrony observed in ESRD patients may improve after renal transplant, but this issue should be tested in further studies.

References


Acute Cardiac Tamponade: An Unusual Cause of Acute Renal Failure in a Renal Transplant Recipient

Naryanan Nampoory, Osama Gheith, Torki Al-Otaibi, Medhat Halim, Prasad Nair, Tarek Said, Ahmed Mosaad, Zakareya Al-Sayed, Ayman Alsayed, Jude Yagan

Abstract

We report a case of slow graft function in a renal transplant recipient caused by uremic acute pericardial effusion with tamponade. Urgent pericardiocentesis was done with an improvement in blood pressure, immediate diuresis, and quick recovery of renal function back to baseline. Pericardial tamponade should be included in consideration of causes of type 1 cardiorenal syndrome in renal transplant recipients.

Key words: End-stage renal failure, Complications, Slow graft function, Uremic pericarditis

Introduction

Pericardial effusion may appear as a transudate, exudate, pyopericardium, or hemopericardium. Large effusions are common with neoplasms, tuberculosis, cholesterol problems, uremic pericarditis, myxedema, and parasitic disease.1 Effusions that develop slowly can be asymptomatic, but rapidly accumulating smaller effusions can cause tamponade. Cardiac tamponade is the decompensated cardiac compression caused by accumulation of fluid and increased intrapericardial pressure.

Renal failure is a common cause of pericardial disease, producing large pericardial effusions in 20% patients.2 There have been 2 types reported1: Uremic pericarditis may occur in 6% to 10% of patients who have advanced renal failure (acute or chronic), and may develop before or shortly after dialysis; it results from inflammation of the visceral and parietal pericardium and correlates with the degree of azotemia3; and dialysis-associated pericarditis may occur in 13% patients who are on maintenance hemodialysis and, occasionally, with chronic peritoneal dialysis because of inadequate dialysis and/or fluid overload.4,5

In surgical tamponade, intrapericardial pressure rises rapidly, within minutes to hours because of hemorrhage. However, a low-intensity inflammatory process may develop for days to weeks before cardiac compression occurs (medical tamponade). Tamponade may present with fever and pleuritic chest pain, but many patients are asymptomatic. Pericardial rubs may persist even with large effusions or may be transient. Due to autonomic impairment in uremic patients, heart rate may remain slow during tamponade despite fever and hypotension. The electrocardiogram does not show the typical diffuse ST-T wave elevations observed with other causes of acute pericarditis due to the absence of myocardial inflammation.6 If the electrocardiogram is typical of acute pericarditis, intercurrent infection must be suspected.

Most patients who have uremic pericarditis respond rapidly to dialysis, if intensified within 1 to 2 weeks, but dialysis must be heparin-free to avoid hemopericardium, hypokalemia, and hypophosphatemia.7,8 Cardiac tamponade and large chronic effusions that are resistant to dialysis must be treated with pericardiocentesis (level of evidence B, class IIa indication) with or without local steroid installation for large, nonresolving symptomatic effusions. Pericardiectomy is indicated only in refractory,
severely symptomatic patients because of potential morbidity and mortality. Within 2 months after renal transplant, pericarditis has been reported in 2.4% patients.9 The present case report highlights possible acute uremic pericardial tamponade in a renal transplant recipient with compromised graft function early after transplant.

Case Report

A 41-year-old Indian woman who had a history of recurrent urinary tract infections and frequent stone passage since 1998 underwent left nephrectomy because of infected nonfunctioning kidney. Her renal function gradually deteriorated until she had end-stage renal disease and she was prepared for preemptive renal transplant with unremarkable echocardiography.

She was admitted for living-related renal transplant from her sister (3 human leukocyte antigen mismatches, negative panel reactive antibodies, and negative crossmatch by complement-dependent cytotoxicity and flow cytometry). Planned immunosuppressive regimen included induction with basiliximab and maintenance with steroids, mycophenolate mofetil, and cyclosporine. She received 1 hemodialysis session the day before transplant with a right internal jugular vein double lumen catheter because of hyperkalemia and metabolic acidosis. She was stable clinically with unremarkable clinical findings, normal thyroid function, and unremarkable findings on the most recent echocardiogram.

In mid-February 2014, a right native nephrectomy was performed in the same session with right iliac renal allotransplant. The surgery was uneventful, but her hemoglobin dropped by 4 g/L over 12 hours. Urgent computed tomography (CT) of the abdomen showed no local bleeding. Despite thrombocytopenia, hemolytic uremic syndrome was excluded because she had a negative blood smear, normal lactate dehydrogenase level, and negative tests for hemolysis. She was treated with a filtered red blood cell transfusion and her hemoglobin became stable. Her urine grew *Escherichia coli*, and she was treated with intravenous piperacillin/tazobactam for 7 days.

On day 2 after transplant, the patient developed unexplained hypotension, low-grade fever, tachycardia, chest pain, and oliguria. The central venous pressure was 16 cm water. The chest radiograph showed mild enlargement of the cardiac shadow (Figure 1). The hemoglobin was stable, and electrocardiography showed sinus tachycardia and a flat wave in lead III in addition to borderline repeated troponin I levels. The kidney graft function was compromised despite a challenge with intravenous fluids and vasopressors. The possibility of septicemic shock was considered high, and we changed the antibiotic to meropenem. She had a past history of deep venous thrombosis of the left lower limb after previous pelvic surgery (hysterectomy), and she had moderate elevation of the D-dimer level; therefore, an urgent ventilation/perfusion scan (Figure 2) was performed but showed low probability for pulmonary embolism. However, it was observed that the cardiac shadow was enlarged compared with the baseline appearance. Bedside echocardiography was performed and showed moderate pericardial effusion with echocardiographic signs of tamponade (Figure 3). Urgent pericardiocentesis with an indwelling drain was performed with hemorrhagic drainage of 255 mL, and further laboratory investigation showed that the fluid was a transudate (Table 1).

At 30 minutes after pericardiocentesis, the blood pressure increased to normal level with
discontinuation of vasopressors. The urine output began to increase with improvement of renal graft function during the subsequent days (Figures 4 and 5). The total drained fluid was 325 mL over 3 days, and when the drainage stopped, as confirmed by an effusion-free echocardiogram, the drainage tube was removed. The patient was discharged with normal graft function and creatinine level of 88 μmol/L.

**Discussion**

This case highlights the importance of heart-kidney interactions in the acute care setting. The renal allograft was slowly recovering because of after renal transplant which was attributed to acute decompensated heart failure as a result of an acute pericardial effusion. The rapid improvement of kidney graft function after pericardiocentesis denoting the complex type I cardiorenal syndrome.10

Pericardial effusion is a complication of chronic kidney disease with untreated advanced uremia.3,11 Other common causes of pericardial effusion include infection, malignancy, autoimmune diseases, medications, trauma, congestive heart failure, and hypothyroidism.12-14 Few cases of acute renal failure secondary to acute pericardial tamponade have been reported, but literature review showed no cases in renal transplant recipients.15-17

Returning venous blood volume creates mean right atrial filling pressure of 6 to 8 mm Hg.14 In cardiac tamponade, rapidly accumulating fluid in the nonelastic pericardial cavity inhibits the filling of the right atrium. After the intrapericardial pressure exceeds the right atrial pressure (and possibly the right ventricular pressure), cardiac output decreases because of reduced filling of the right atrium and ventricle, causing hypotension. Autoregulation of blood flow is necessary to maintain constant organ perfusion despite variations in the arterial pressure. This function is present in all tissues but is particularly pronounced in some organs such as the brain and kidney. Autoregulation of renal blood flow is mainly mediated by myogenic responses and tubuloglomerular feedback. Tubuloglomerular feedback is a regulating mechanism in the kidney that leads to vasoconstriction of the afferent arteriole in response to an increase in the luminal concentration of sodium chloride at the macula densa in the early distal tubule. In addition to myogenic responses and tubuloglomerular feedback, there are several other mechanisms that regulate perfusion including vasopressin, natriuretic peptides, and activation of the renin-angiotensin-aldosterone system. During hypotension, renal vascular resistance decreases to maintain renal blood flow and glomerular filtration by myogenic responses and tubuloglomerular feedback.

Tamponade without ≥ 2 inflammatory signs (typical pain, pericardial friction rub, fever, diffuse

<table>
<thead>
<tr>
<th>Table 1. Laboratory Findings of the Aspirated Pericardial Fluid</th>
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<tbody>
<tr>
<td><strong>Test</strong></td>
</tr>
<tr>
<td>Glucose (mmol/L)</td>
</tr>
<tr>
<td>Lactate dehydrogenase (U/L)*</td>
</tr>
<tr>
<td>Albumin (g/L)</td>
</tr>
<tr>
<td>Cholesterol (mmol/L)</td>
</tr>
<tr>
<td>Gram stain</td>
</tr>
<tr>
<td>Fungal stain</td>
</tr>
<tr>
<td>Nocardia</td>
</tr>
<tr>
<td>Acid-fast bacilli</td>
</tr>
<tr>
<td>Culture</td>
</tr>
<tr>
<td>Cytology</td>
</tr>
</tbody>
</table>

*Lactate dehydrogenase, 193 U/L = 3.22 μkat/L.
ST segment elevation) usually is associated with a malignant effusion (likelihood ratio, 2.9). Electrocardiography may demonstrate diminished QRS and T wave voltages, PR segment depression, ST-T segment changes, bundle branch block, and electrical alternans which is rarely seen in the absence of tamponade. Despite the acute tamponade, the present patient had only T wave changes in lead III.

In chest radiography large effusions are depicted as globular cardiomegaly with sharp margins (“water bottle” silhouette). On well-penetrated lateral radiographs or cine films, pericardial fluid is suggested by lucent lines within the cardio-pericardial shadow (epicardial halo). This sign is useful for the fluoroscopic guidance of pericardiocentesis. The CT, spin-echo, and cine magnetic resonance imaging (MRI) scans also can be used to assess the size and extent of simple and complex pericardial effusions. Effusions measured by CT or MRI typically are larger than observed with echocardiography. In one-third of patients with asymptomatic large pericardial chronic effusion, unexpected cardiac tamponade may develop. Triggers for tamponade include hypovolemia, paroxysmal tachyarrhythmia, and intermittent acute pericarditis. During the present patient, we suspected cardiomegaly during a radioisotope scan of the kidney graft.

The separation of pericardial layers can be detected in echocardiography when pericardial fluid volume >15 to 35 mL. The size of effusions can be graded as small (echo-free space in diastole < 10 mm), moderate (10-20 mm), large (> 20 mm), or very large (> 20 mm and compression of the heart). In the present case, we detected moderate pericardial effusion by bedside echocardiography.

The renal effects of cardiac tamponade may occur before hemodynamic collapse. This occurred in our patient who developed oliguria before tamponade and hemodynamic disturbance.

Increasing the pericardial pressure by 5 mm Hg decreases urinary sodium excretion and increases renin production, but the mean arterial blood flow and glomerular filtration rate remain unchanged. However, a further increase in pericardial pressure reduces both the mean arterial pressure and glomerular filtration rate. In the present patient with progression of tamponade and compromised hemodynamic status, kidney function was further affected. This explains what happened when she developed shock. Kidney autoregulation occurs over a wide range of arterial pressures (80-180 mm Hg), below which the kidney does not autoregulate, and urine flow falls in proportion to reductions in arterial pressure. The subsequent reductions in renal perfusion trigger mechanisms in the kidney to maintain constant renal blood flow and glomerular filtration.

The pericardial effusion might be caused by a known medical condition in 60% cases. We excluded all possible causes of pericardial effusion and we attributed the cause to uremia because of the laboratory findings (Table 1).

Pericardiocentesis is not necessary when the diagnosis can be made without pericardiocentesis, or when the effusions are small or resolving with anti-inflammatory treatment. However, hemodynamic compromise with cardiac tamponade is an absolute indication for drainage. Whenever possible, treatment should be aimed at the underlying cause. Even with idiopathic effusions, extended pericardial catheter drainage (mean 3 ± 2 d; range, 113 d) was associated with lower recurrence rates (6% vs 23%) than in patients without catheter drainage during mean follow-up of 3.8 ± 4.3 years.

We drained our patient for 3 days without any evidence of recurrence for 3 subsequent months, and the uremia (the underlying cause) was resolving rapidly with recovering kidney graft function and basal creatinine 120 μmol/L at discharge. We did not need any further medical therapy or other interventions such as intrapericardial treatment, percutaneous balloon pericardiotomy, or surgery.

In summary, acute uremic pericardial tamponade should be considered as a possible cause of oliguric renal graft dysfunction early after transplant. Strong clinical suspicion for the diagnosis and a low threshold for emergency pericardial drainage are necessary to prevent further graft dysfunction.

References

Effects of Hyperuricemia on Renal Function in Pediatric Renal Transplant Recipients

Cihan Fidan,1 Aslı Kantar,2 Esra Baskın,2 Kaan Gülleroğlu,2 Aydincan Akdur,3 Gökhan Moray,3 Mehmet Haberal3

Abstract

Objectives: Hyperuricemia is common in pediatric renal transplant recipients, and it is associated with poor allograft outcomes. Hyperuricemia occurs early after transplant and is associated with use of diuretics, cyclosporine therapy, a history of hyperuricemia, and decreased glomerular filtration rate. We aimed to investigate causes and effects of hyperuricemia on allograft outcomes in our patients.

Materials and Methods: There were 81 pediatric transplant patients (41 female and 40 male) included in the study. Demographic characteristics and laboratory parameters were recorded. Risk factors for hyperuricemia and the effects of plasma uric acid levels at 3, 6, 12, and 36 months on allograft outcomes were evaluated.

Results: Mean age was 16.9 ± 5.6 years. Mean follow-up after transplant was 3.5 ± 0.47 years. Hyperuricemia was detected in 17.6% patients. A significant negative correlation was observed between 6-month uric acid level and 36-month glomerular filtration rate ($r = -0.33$, $P = .04$; $r = -0.33$, $P = .017$). A significant positive correlation between 3- and 6-month uric acid levels and 36-month plasma creatinine level was observed ($r = -0.44$, $P = .01$; $r = -0.51$, $P = .001$). There was no significant correlation between plasma uric acid level and body mass index, plasma lipid levels, use of diuretics, or immunosuppressive treatment (tacrolimus or cyclosporine).

Conclusions: Uric acid levels may have predictive value in the long-term assessment of renal function. Posttransplant hyperuricemia can be used as a long-term prognostic marker of poor renal outcome. Patients with hyperuricemia should be monitored closely for renal function.

Key words: Children, Graft survival, Kidney transplant, Uric acid

Introduction

Hyperuricemia is common in pediatric renal transplant recipients, and it is associated with poor allograft outcomes, as in adult patients.1,2 In most studies, serum uric acid level was a marker of renal dysfunction.2-6 However, hyperuricemia occurs early after transplant and is associated with use of diuretics, cyclosporine therapy, a history of hyperuricemia, and decreased glomerular filtration rate (GFR).1,3,5,7,8 The effect of hyperuricemia after kidney transplant on graft outcome has not been fully established, but a small number of studies have suggested that an increased serum uric acid level is a prognostic factor for the development of renal allograft impairment.

We aimed to investigate causes and effects of hyperuricemia on allograft outcomes in our pediatric kidney transplant recipients by evaluating patients during 3 years after transplant.

Materials and Methods

Patients

We determined the prevalence of hyperuricemia in pediatric renal transplant recipients for transplants that were performed from December 2000 to
December 2012 in our Transplant Center. There were 81 patients included in the study. Both living- and deceased-donor renal transplant recipients were included. We analyzed patient data retrospectively, and 3-year follow-up data of the patients were evaluated. Uric acid concentration was measured for each patient. The demographic characteristics and laboratory parameters were recorded. All laboratory tests were done in the same laboratory. Hemoglobin values, serum creatinine concentration, fasting blood glucose concentration, cyclosporine concentration, and lipid profile (levels of cholesterol, triglycerides, low-density lipoprotein, and high-density lipoprotein) were evaluated. In these 3 years, we examined serum uric acid levels and GFR of patients at 3, 6, 12, and 36 months after transplant and evaluated associations between the data. Hyperuricemia was defined by level of serum uric acid ≥ 6 mg/dL. Risk factors for hyperuricemia and the effects of serum uric acid levels on allograft outcomes at 3, 6, 12, and 36 months were evaluated.

All patients were given calcineurin inhibitors (cyclosporine or tacrolimus), mycophenolate mofetil, and steroids as the immunosuppressive regimen. If the patient was treated with cyclosporine, the target trough level was 200-300 ng/mL for the first 3 months, 100 to 250 ng/mL for 4 to 12 months, and 100 to 150 ng/mL after 12 months; we used target levels (at 2 hours after the dose) of 800 to 1000 ng/mL in the first 3 months after transplant and 400 to 600 ng/mL after 3 months. For tacrolimus, trough level was 10 to 12 ng/mL for the first 3 months, 6 to 10 ng/mL for 4 to 12 months, and 3 to 6 ng/mL after 12 months.

**Statistical analyses**

Data analyses were performed with statistical software (IBM SPSS for Windows, Version 21.0, IBM Corp., Armonk, NY, USA). The chi-square test was used to compare patient characteristics, and t-test was used to detect differences between groups. The data were expressed as mean ± standard deviation (SD), and differences with $P \leq 0.05$ were considered statistically significant. Numeric variables were reported as number (%).

**Results**

There were 81 pediatric transplant patients (41 female and 40 male) included in the study. The mean age of the patients was 16.9 ± 5.6 years, and the mean age at transplant was 12.4 ± 4.7 years. Patients who underwent transplant received 57 (70.4%) grafts from living donors and 24 (29.6%) grafts from deceased donors (Table 1). The primary renal disease was vesicoureteral reflux (32%), and many patients had no known cause of kidney failure (20%). The other etiologies of kidney failure were focal segmental glomerulosclerosis, polycystic kidney disease, neurogenic bladder, nephrolithiasis, nephropnephrosis, and pyelonephritis (Figure 1).

Hyperuricemia was detected in 14 patients (17.3%) after transplant. The serum uric acid level range was 1.6-10.2 mg/dL. Mean follow-up after transplant was 3.5 ± 0.47 years. The uric acid and serum creatinine levels were measured at 3, 6, 12, and 36 months after kidney transplant, and we estimated GFR simultaneously (Table 2). Hyperuricemia was detected equally in male and female patients.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>3</th>
<th>6</th>
<th>12</th>
<th>36</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uric acid (mg/dL)</td>
<td>4.8 ± 1.6</td>
<td>4.7 ± 1.4</td>
<td>4.6 ± 1.5</td>
<td>4.9 ± 1.8</td>
</tr>
<tr>
<td>Plasma creatinine (mg/dL)</td>
<td>0.86 ± 0.67</td>
<td>0.89 ± 0.63</td>
<td>0.93 ± 0.65</td>
<td>1.2 ± 1.3</td>
</tr>
<tr>
<td>GFR (mL/min/1.73 m²)</td>
<td>81.8 ± 30.2</td>
<td>91.9 ± 31.6</td>
<td>88.9 ± 31.5</td>
<td>79.3 ± 38.3</td>
</tr>
</tbody>
</table>

Abbreviation: GFR, glomerular filtration rate
A significant negative correlation was observed between 6-month uric acid level and 36-month GFR value ($r = -0.33$, $P = .04$). A significant positive correlation between 3- and 6-month uric acid levels and 36-month plasma creatinine level was demonstrated ($r = 0.44$, $P = .01$; $r = 0.51$, $P = .001$). There was no significant correlation between plasma uric acid level and body mass index, plasma lipid levels, use of diuretics, or immunosuppressive treatment (tacrolimus or cyclosporine). Comparison between normouricemic and hyperuricemic groups showed significant differences between the groups in plasma creatinine and GFR at 36 months (Table 3).

**Discussion**

Hyperuricemia is a frequent metabolic disorder after renal, liver, and heart transplants. High uric acid levels can damage endothelial cells, leading to accumulation of inflammatory cells, which can accelerate atherosclerosis. High uric acid levels also may cause tubulointerstitial inflammation and transition of epithelial cells to mesenchymal cells which may cause production of extracellular matrix. These events lead to renal fibrosis, and fibrosis is associated with a decrease in GFR. Elimination of uric acid becomes less efficient with increasing kidney dysfunction. Our study findings demonstrate a significant negative correlation between hyperuricemia and GFR.

The reported prevalence of hyperuricemia in renal transplant recipients has ranged between 15.5% and 84%. In our series, the prevalence of hyperuricemia was 22.2%, and this is similar to results of recent studies. We measured serum uric acid levels at 3, 6, 12, and 36 months after transplant. Although there was a significant correlation between hyperuricemia and GFR at 6 and 36 months, there was no relation at 3 and 12 months. We did not encounter graft loss. In a manner similar to our study, Haririan and coworkers assessed the predictive value of serum uric acid levels to determine graft survival and function during the first year after transplant. Akalin and associates investigated the association between hyperuricemia at 6 months after transplant and clinical outcomes, and Choi and Kwon investigated the association between serum uric acid levels at 3 months after transplant and renal allograft outcomes. These studies all indicated that hyperuricemia may lead to graft dysfunction and loss.

The findings of many studies demonstrated that hyperuricemia is associated with body mass index, serum lipid levels, serum potassium levels, use of diuretics, reduced glomerular filtration rate, use of calcineurin inhibitors, increased recipient age at transplant, and pre-existing history of hyperuricemia and gout. In our study, there was no significant correlation between hyperuricemia and these clinical factors. Similar to some studies, there were no significant differences in the pharmacokinetics of cyclosporine and tacrolimus between patients with and without hyperuricemia.

In addition, some clinical trials have emphasized that hyperuricemia may increase the risk of graft loss, cardiovascular events, and death. In our series, there were no cases of graft loss, cardiovascular events, or death. This may be a result of the small number of patients and short follow-up. These risks should be evaluated with new and larger clinical studies.

In conclusion, posttransplant hyperuricemia can be used as a long-term prognostic marker of poor renal outcome, and patients with hyperuricemia should be monitored closely for renal function.

### Table 3. Comparison Between Hyperuricemic and Normouricemic Groups

<table>
<thead>
<tr>
<th>Variables</th>
<th>Hyperuricemic Patients</th>
<th>Normouricemic Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n = 14)</td>
<td>(n = 67)</td>
</tr>
<tr>
<td>Age (y)</td>
<td>17.25 ± 4.35</td>
<td>16.86 ± 5.65</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>7</td>
<td>33</td>
</tr>
<tr>
<td>Female</td>
<td>7</td>
<td>34</td>
</tr>
<tr>
<td>Immunosuppressive drug</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cyclosporine</td>
<td>5</td>
<td>24</td>
</tr>
<tr>
<td>Tacrolimus</td>
<td>9</td>
<td>43</td>
</tr>
<tr>
<td>Donor type</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Living donor</td>
<td>10</td>
<td>47</td>
</tr>
<tr>
<td>Deceased donor</td>
<td>4</td>
<td>20</td>
</tr>
<tr>
<td>Graft loss</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Follow-up (y)</td>
<td>4.1 ± 2.5</td>
<td>4.2 ± 2.6</td>
</tr>
<tr>
<td>Plasma creatinine (mg/dL)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 mo</td>
<td>0.86 ± 0.26</td>
<td>0.88 ± 0.74</td>
</tr>
<tr>
<td>6 mo</td>
<td>0.96 ± 0.38</td>
<td>0.87 ± 0.68</td>
</tr>
<tr>
<td>12 mo</td>
<td>0.97 ± 0.34</td>
<td>0.92 ± 0.70</td>
</tr>
<tr>
<td>36 mo</td>
<td>1.09 ± 0.29*</td>
<td>1.23 ± 0.51*</td>
</tr>
<tr>
<td>GFR (mL/min/1.73 m²)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 mo</td>
<td>74.82 ± 33.45</td>
<td>81.25 ± 29.44</td>
</tr>
<tr>
<td>6 mo</td>
<td>90.86 ± 37.32</td>
<td>92.83 ± 31.01</td>
</tr>
<tr>
<td>12 mo</td>
<td>73.85 ± 23.63</td>
<td>92.20 ± 30.97</td>
</tr>
<tr>
<td>36 mo</td>
<td>68.03 ± 21.84*</td>
<td>81.70 ± 42.23*</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>21.68 ± 2.87</td>
<td>21.35 ± 4.68</td>
</tr>
<tr>
<td>LDL cholesterol (mg/dL)</td>
<td>102.00 ± 31.72</td>
<td>105.92 ± 58.51</td>
</tr>
<tr>
<td>TG (mg/dL)</td>
<td>126.27 ± 46.01</td>
<td>136.38 ± 92.74</td>
</tr>
<tr>
<td>Total cholesterol (mg/dL)</td>
<td>185.91 ± 33.02</td>
<td>182.71 ± 72.92</td>
</tr>
</tbody>
</table>

**Abbreviation**: BMI, body mass index; GFR, glomerular filtration rate; LDL, low-density lipoprotein; TG, triglycerides

$*P \leq .05.$
References

A Single-Center Experience of Overseas Kidney Transplant for Immunologically High-Risk Patients

Cheol Woong Jung,1* Kwan Tae Park,2* Heungman Jun,1 Su Yeon Kim,3 Su Jin Kim,3 Myung-Gyu Kim,4 Sang-kyung Jo,4 Wonyong Cho,4 Hyoung Kyu Kim4

Abstract

Objectives: We report our experience in treating Mongolian patients transferred to our center in Korea to undergo kidney transplants, including immunologically high-risk patients.

Materials and Methods: Between September 2009 and February 2013, thirty-three Mongolian patients underwent kidney transplants at our center with the approval of the Korean Network for Organ Sharing. Their clinical data were retrospectively collected and analyzed.

Results: The mean age of the transplant recipients was 38.8 ± 10.5 years, and the causes of end-stage renal disease were glomerulonephritis (5), diabetes mellitus (2), hypertension (3), and unknown (25). These cases included ABO incompatibility, high levels of sensitization, and retransplant, at frequencies of 9, 12, and 9. Basiliximab (30) or antithymocyte globulin (2) was administered as the induction therapy, and combination regimens of plasmapheresis with or without intravenous immunoglobulins and/or rituximab were used in some high-risk patients. The mean follow-up after kidney transplant was 12.87 ± 11.7 months. During the follow-up, antibody-mediated rejection and graft failure occurred in 2 patients. In addition, cytomegalovirus infection, calcineurin inhibitor toxicity, and BK viremia developed in 1 patient each. The mean estimated glomerular filtration rates at 1, 6, and 12 months after kidney transplant were 88.2 ± 26.9 mL/min/1.73 m², 67.5 ± 14.9 mL/min/1.73 m², and 63.9 ± 21.1 mL/min/1.73 m². In addition, subgroup analysis revealed that ABO-incompatible and immunologically high-risk recipients had comparable renal function status during the follow-up.

Conclusions: We found that an overseas kidney transplant program in Korea could be conducted safely, even in high-risk patients. Therefore, a proper cooperation and transfer system for these high-risk patients between neighboring countries might help in providing improved medical care in this setting.

Key words: ABO-incompatible, High-risk kidney transplant, Overseas kidney transplant, Sensitization

Introduction

Although kidney transplant (KT) is the best treatment option to enhance the quality of life of patients with end-stage renal disease (ESRD), severe global inequalities in access to transplant surgery still exist because, in the transplant health care system, developing countries have not adopted essential legislation and established governmental support. To improve transplant systems in such countries, it would be of great help to network with experienced centers that can share their systems and protocols. Especially in countries with insufficient experience with performing KT in high-risk patients, a transfer system between neighboring countries could be a reasonable alternative for patients with ESRD and incompatibility with a live donor.
In Mongolia, chronic kidney disease is common. Recently, researchers reported that 13.9% of the Mongolian population have proteinuria or an estimated glomerular filtration rate (eGFR) < 60 mL/min/1.73 m² as calculated with the Modified Diet in Renal Disease Study equation. In addition, approximately 200 people in Mongolia are newly diagnosed with ESRD every year, despite the country’s having a small population of about 3 million people. However, owing to a limited number of functioning dialysis units, many patients in Mongolia with ESRD cannot be maintained on dialysis therapy, which leads to difficulty in management and more frequent requirement for KT. Although KT has been conducted successfully in Mongolia since 2006, little attention has been paid to KT for high-risk patients there. Therefore, in Mongolia, more experience with ABO-incompatible and highly sensitized cases is required to achieve an enhanced KT program. Since 2009, our center in Korea has operated an overseas KT program for Mongolian patients, including those who are at risk immunologically. In this article, we report our experience with this program.

Materials and Methods

Between September 2009 and February 2013, thirty-three KT were performed on Mongolian patients at the Korea University Anam Hospital. We retrospectively analyzed the medical records of these patients. All procedures were carried out with living donors and conducted with the approval of the national allocation system (Korea Network for Organ Sharing [KONOS]). KONOS evaluates the relation strictly between the donor and recipient to prevent any kind of commercial donation. The study was approved by the Ethical Review Committee of the Institute. All of the protocols conformed to the ethical guidelines of the 1975 Helsinki Declaration. Written informed consent was obtained from all subjects.

Before KT, each patient was evaluated by performing a series of laboratory and radiologic tests, including human leukocyte antigen (HLA) typing, HLA cross-matching, anti-HLA antibody testing, ABO blood typing, abdominal computed tomography, and echocardiography. Anti-HLA antibodies were assessed by performing Luminex assays (Tepnel Lifecodes, Stamford, CT, USA) using class I and class II panel reactive antibody (PRA) identification kits, and highly sensitized recipients were defined as having a PRA level ≥ 50%.

In ABO-incompatible cases, we used rituximab and multiple sessions of plasmapheresis (double-filtration plasmapheresis or total plasma exchange) according to the level of ABO antibody titer before KT and obtained an isoagglutinin titer ≤ 1:8 on the day of the KT.

For the initial immunosuppressant, basiliximab (90.9%) or antithymocyte globulin (6.1%) (1.5 mg/kg/d for 4 or 5 days) was used to induce immunosuppression with triple-maintenance drugs (prednisolone, calcineurin inhibitor, and an antimetabolite). In the combination regimens, plasmapheresis, with or without intravenous immunoglobulins and/or rituximab, was also used in some cases. All patients were periodically followed by both transplant centers in Mongolia and at our center every 3 to 6 months. They were also in constant contact with our medical staff via e-mail.

Kidney biopsies were performed in our center when renal function decreased after KT, and they were analyzed using the Banff 07 classification.

Results

Baseline patient characteristics

The baseline patient characteristics are summarized in Table 1. Among the 33 KT cases, 9 were retransplants (27.3%), 12 involved highly sensitized recipients (36.4%), 10 had 4 or more HLA mismatches (30.3%), and 9 involved an ABO-incompatible donor (27.3%). Although the causes of ESRD were unknown in 75.8% of the cases, the most common primary renal disease was glomerulonephritis. Four patients (12.1%) were hepatitis B surface antigen (HBsAg)-positive, and 12 were hepatitis C antibody (36.4%) (HCVAb)-positive. All transplant recipients who were hepatitis virus-positive had normal liver function and showed no evidence of liver cirrhosis before undergoing KT. In addition, all HBsAg-positive recipients were administered antiviral prophylaxis after KT. Donors who were hepatitis C virus (HCV)-positive donors were matched with recipients who were HCV-positive, and donors who were hepatitis B virus (HBV)-positive were involved when their DNA test was negative for HBV and the recipient displayed a high HBV antibody titer (> 10 mIU/mL).
Clinical patient outcomes

All recipients were followed for a minimum of 3 months after KT (mean follow-up 12.87 ± 11.7 mo). In the early posttransplant period, delayed graft function occurred in 1 patient. The mean eGFRs at 1, 6, and 12 months after KT were 88.2 ± 26.9 mL/min/1.73 m², 67.5 ± 14.9 mL/min/1.73 m², and 63.9 ± 21.1 mL/min/1.73 m². The renal function of the Mongolian patients was not significantly different from that of the Korean patients in the same period. In addition, in ABO-incompatible cases, the mean eGFRs at 1, 6, and 12 months were 79.2 ± 38.1 mL/min/1.73 m², 67.8 ± 20.9 mL/min/1.73 m², and 62.1 ± 27.4 mL/min/1.73 m², showing similar short-term outcomes compared to those of ABO-compatible cases (Figure 1A). Patients were defined as “high risk” if they had 1 or more of the following characteristics: second transplant, PRA > 50%, 4 or more HLA mismatches, and ABO incompatibility. A total of 22 patients were included in the high-risk group, and their mean eGFRs at 1, 6, and 12 months were 85.4 ± 30.3 mL/min/1.73 m², 68.7 ± 16.8 mL/min/1.73 m², and 63.2 ± 22.5 mL/min/1.73 m² (Figure 1B).

During the follow-up, a posttransplant biopsy was conducted a total of 11 times across a total of 5 recipients who experienced acute or sustained renal dysfunction. In those cases, acute tubular necrosis and acute rejection were found in 1 and 4 patients. Borderline acute T-cell–mediated rejection was present in 2 patients, and another 2 patients had acute antibody-mediated rejection. One case with acute antibody-mediated rejection occurred 17 months after KT in a highly sensitized recipient (PRA I/II > 50%), and the other occurred 3 weeks after KT in an ABO-incompatible recipient. Their renal function improved slightly after treatment; however, their clinical response was not sustained for long, and their renal function gradually deteriorated. A follow-up biopsy showed chronic active antibody-mediated rejection, and graft failure occurred in these 2 patients. In addition, cytomegalovirus infection, calcineurin inhibitor toxicity, and BK viremia developed in 1 patient each; however, their renal function stabilized

Table 1. Baseline Patient Characteristics

<table>
<thead>
<tr>
<th>Variable</th>
<th>Data</th>
</tr>
</thead>
<tbody>
<tr>
<td>Donor age (y)</td>
<td>46.6 ± 11.1</td>
</tr>
<tr>
<td>Recipient age (y)</td>
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<tr>
<td>Donor sex (% male)</td>
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<tr>
<td>Recipient sex (% male)</td>
<td>54.5</td>
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<tr>
<td>Donor BMI (kg/m²)</td>
<td>27.6 ± 4.1</td>
</tr>
<tr>
<td>Recipient BMI (kg/m²)</td>
<td>22.9 ± 5.1</td>
</tr>
<tr>
<td>Donor eGFR (mL/min/1.73 m²)</td>
<td>96.4 ± 17.7</td>
</tr>
<tr>
<td>Donor HBsAg (+), n (%)</td>
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</tr>
<tr>
<td>Recipient HBsAg (+), n (%)</td>
<td>4 (12.1)</td>
</tr>
<tr>
<td>Donor HCVAb (+), n (%)</td>
<td>2 (6.1)</td>
</tr>
<tr>
<td>Recipient HCVAb (+), n (%)</td>
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</tr>
<tr>
<td>Retransplant (%)</td>
<td>27.3</td>
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<td>Cause of ESRD (%)</td>
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<td>GN</td>
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<td>DM</td>
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<tr>
<td>HTN</td>
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<td>PRA, n (%)</td>
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<td>0%</td>
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<td>1%-49%</td>
<td>2 (6.1)</td>
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<tr>
<td>≥ 50%</td>
<td>12 (36.4)</td>
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<td>Anti-ABO titer of recipient, n</td>
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<td>&lt; 1:32</td>
<td>3</td>
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<tr>
<td>≥ 1:32</td>
<td>6</td>
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<tr>
<td>HLA mismatch, n (%)</td>
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<tr>
<td>≤ 3</td>
<td>23 (69.7)</td>
</tr>
<tr>
<td>&gt; 3</td>
<td>10 (30.3)</td>
</tr>
</tbody>
</table>

Abbreviations: BMI, body mass index; DM, diabetes mellitus; eGFR, estimated glomerular filtration rate; ESRD, end-stage renal disease; GN, glomerulonephritis; HBsAg, hepatitis B surface antigen; HCVAb, hepatitis C virus antibody; HLA, human leukocyte antigen; HTN, hypertension; PRA, panel reactive antibody

Data are given as average ± standard deviation, percentage (%), or number (n) as appropriate.

Clinical patient outcomes

All recipients were followed for a minimum of 3 months after KT (mean follow-up 12.87 ± 11.7 mo). In the early posttransplant period, delayed graft function occurred in 1 patient. The mean eGFRs at 1, 6, and 12 months after KT were 88.2 ± 26.9 mL/min/1.73 m², 67.5 ± 14.9 mL/min/1.73 m², and 63.9 ± 21.1 mL/min/1.73 m². The renal function of the Mongolian patients was not significantly different from that of the Korean patients in the same period. In addition, in ABO-incompatible cases, the mean eGFRs at 1, 6, and 12 months were 79.2 ± 38.1 mL/min/1.73 m², 67.8 ± 20.9 mL/min/1.73 m², and 62.1 ± 27.4 mL/min/1.73 m², showing similar short-term outcomes compared to those of ABO-compatible cases (Figure 1A). Patients were defined as “high risk” if they had 1 or more of the following characteristics: second transplant, PRA > 50%, 4 or more HLA mismatches, and ABO incompatibility. A total of 22 patients were included in the high-risk group, and their mean eGFRs at 1, 6, and 12 months were 85.4 ± 30.3 mL/min/1.73 m², 68.7 ± 16.8 mL/min/1.73 m², and 63.2 ± 22.5 mL/min/1.73 m² (Figure 1B).

During the follow-up, a posttransplant biopsy was conducted a total of 11 times across a total of 5 recipients who experienced acute or sustained renal dysfunction. In those cases, acute tubular necrosis and acute rejection were found in 1 and 4 patients. Borderline acute T-cell–mediated rejection was present in 2 patients, and another 2 patients had acute antibody-mediated rejection. One case with acute antibody-mediated rejection occurred 17 months after KT in a highly sensitized recipient (PRA I/II > 50%), and the other occurred 3 weeks after KT in an ABO-incompatible recipient. Their renal function improved slightly after treatment; however, their clinical response was not sustained for long, and their renal function gradually deteriorated. A follow-up biopsy showed chronic active antibody-mediated rejection, and graft failure occurred in these 2 patients. In addition, cytomegalovirus infection, calcineurin inhibitor toxicity, and BK viremia developed in 1 patient each; however, their renal function stabilized

Figure 1. The Mean Estimated Glomerular Filtration Rates (eGFRs) at 1, 6, and 12 Months After Transplant

Subgroup analysis revealed that ABO-incompatible (A) or immunologically high-risk (B) recipients had comparable renal function status during the follow-up.
Discussion

As reported previously, there has been an increasing burden of ESRD in Mongolia. However, the management of patients with ESRD there has been limited by a lack of proper dialysis units. Korea and Mongolia are very close countries, both geographically and culturally; therefore, in 2009, our hospital opened a KT program for Mongolian patients with ESRD. We provided transplant staff who speak Mongolian and tried to minimize the burden of high medical costs for these patients. We also visited Mongolia regularly for education and consultation with Mongolian medical staff and were in constant contact with the patients through e-mail. As a result of these efforts, a total of 33 patients underwent KT in our center during the study period. In our analysis, we found the following patient characteristics. They were relatively young (mean age 38.8 ± 10.5 y), and, although it was difficult to know the exact cause of ESRD in many cases, glomerulonephritis was the most common renal disease (immunoglobulin A nephropathy in 4 patients and suspicion of glomerulonephritis in 2 patients). These patients had especially high rates of HBV and HCV infection (42.4%).

Mongolia is known to have the highest rate of hepatocellular carcinoma in the world, and recent studies have shown that 10% to 15% of the general population have HBV or HCV infection. Therefore, hepatitis-associated glomerulonephritis might be a common cause of ESRD in Mongolia. In the past, hepatitis infection was a hurdle to successful KT, but, with the availability of more effective antiviral agents, successful viral suppression and better outcomes can be achieved for KT recipients with HBV. We also used antiviral agents in HBV-positive recipients after KT to keep their liver function stable during the follow-up. In patients with HCV, because the safety and effectiveness of antiviral treatment after KT have not yet been verified, more careful evaluation is important, and antiviral treatment should be considered before performing the transplant. However, there are complex factors to be considered in the selection of hepatitis-positive donors, owing to issues with viral transmission and donor safety. Recent organ shortages have led to active use of kidneys from HBV-positive donors, especially in geographic areas in which HBV infection is endemic. We also performed KT only when donor DNA tests for HBV were negative and the recipient was immune to HBV. The recipients remained HBsAg-negative 11 months after KT.

Controversy remains regarding the use of kidneys from HCVAb-positive donors; however, their use could be considered for HCVAb-positive recipients. In a recent prospective study, researchers demonstrated the long-term safety of this strategy. In our study, the patients who were HCV-positive and received kidneys from donors who were HCV-positive also had stable renal function during the follow-up. Therefore, in Mongolia, where HBV and HCV are endemic, it is necessary to consider the use of organs from hepatitis-positive donors as one option to more actively decrease the current organ shortage.

In this study, approximately 66.7% of the recipients were immunologically at high risk. We used induction immunosuppressants in all high-risk cases and desensitization protocols in some, including ABO-incompatible KT. Although severe antibody-mediated rejection developed in 2 recipients and their responses to treatment were poor, most of the recipients had excellent short-term outcomes. In addition, subgroup analysis revealed that ABO-incompatible and immunologically high-risk recipients had comparable renal function during the follow-up.

In Mongolia, data on KT for high-risk patients is insufficient. Additionally, some of the important immunologic tests and treatment strategies, such as plasmapheresis, are not yet available. Therefore, an overseas transfer system between neighboring countries for high-risk patients, as well as the availability of KT for HLA- and ABO-incompatible patients in experienced centers, could be a reasonable alternative for patients with ESRD who have incompatibility with live donors. Moreover, the advent of the Internet and the smartphone has made it possible to easily contact patients remotely and respond to emergencies. In this study, we completed follow-up with most recipients via regular e-mails, and some of them visited us whenever they were asked to or wanted to. Our results suggest that an overseas transfer program for KT should be considered, especially for high-risk patients in countries where the experience in performing KT is limited.
not enough, although a long-term follow-up study is required to verify the safety of such programs. We also remain interested in and are continuing our efforts toward improving the transplant system by transferring our KT experience to a transplant team in Mongolia.

Conclusion

We found that an overseas transplant program in Korea for patients who need KT could be conducted safely, even in high-risk patients. Therefore, proper cooperation and a transfer system for those high-risk patients between neighboring countries might help in providing improved medical care in this setting.

References

Living-Donor Kidney Transplant From Hepatitis B Surface Antigen-Positive Donors to Hepatitis B Antibody-Positive Recipients Without Hepatitis B Immunoglobulin Prophylaxis in an Endemic Country

Heungman Jun,1 Myung Gyu Kim,2 Kwan Tae Park,3 Cheol Woong Jung1

Abstract

Objectives: Living-donor kidney transplant from donors who are chronically infected with hepatitis B virus can be considered as a possibility to compensate for insufficiency of organ transplants, particularly in a hepatitis B virus endemic country. In this study, the safety and efficacy were reviewed retrospectively in living-donor kidney transplant from donors who were chronically infected with hepatitis B virus.

Materials and Methods: In the years between 2012 and 2013, we transplanted 4 renal grafts from hepatitis B surface antigen-positive living donors to antihepatitis B antibody-positive recipients. Lamivudine was prescribed for recipients after transplant without hepatitis B immunoglobulin.

Results: In 1-year follow-up, there were no abnormal findings in the levels of renal and liver enzymes, and there was no unwanted seroconversion to positive hepatitis B surface antigen.

Conclusions: When combined with careful hepatitis B virus-monitoring, renal grafts from hepatitis B surface antigen-positive living donors can be transplanted to hepatitis B antibody-positive recipients, without the need for hepatitis B immunoglobulin prophylaxis, in a hepatitis B virus endemic country.

Key words: End-stage renal disease, Infectious diseases, Lamivudine

Introduction

There is a disparity between the number of patients on waiting lists and transplantations globally. Due to the shortage of renal grafts and the good outcome of hepatitis B virus (HBV) infection with medication, kidney transplant with HBV-infected deceased donors has been accepted generally.1-4 However, kidney transplant from HBV-infected living donors has been avoided due to the possibility of graft instability and HBV transmission, except in some studies.5

In this study, living-donor kidney transplant from chronically HBV-infected donors was considered as a possibility to compensate for insufficiency of organ transplants, particularly in an HBV-endemic country where many potential donors are already infected.

Materials and Methods

In the years between 2012 and 2013, we transplanted 4 renal grafts from living donors who were positive for hepatitis B surface antigen (HBsAg) to antihepatitis B antibody-positive recipients. We retrospectively analyzed clinical outcomes and hepatitis viral status after kidney transplant. In preoperative evaluation of the recipients, the titers of antihepatitis B surface antibody (anti-HBs Ab) ranged 54 to > 1000 IU/L, and the immunity of recipient was attributed to natural immunity. Viral profiles of donors showed negative hepatitis B extracellular antigen (HBeAg), positive antihepatitis B extracellular antibody (anti-HBe Ab), and positive
antihepatitis B core antibody (anti-HBc Ab) immunoglobulin G (IgG) (Table 1).

Lamivudine was prescribed for recipients after transplant. But hepatitis B immunoglobulin was not prescribed. In all cases, basiliximab for induction agent and tacrolimus, mycophenolate mofetil, and steroid for maintenance agent were used. In 1 of 4 cases, the living donor was ABO incompatible. All patients were monitored for liver and renal function and hepatitis B viral status including HBsAg and anti-HBs Ab titer every 3 months during 1 year after transplant. In this center, the HBV-infected recipients from HBs Ag positive donors took lamivudine for 3 months.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Case 1</th>
<th>Case 2</th>
<th>Case 3</th>
<th>Case 4</th>
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<td>54/28</td>
<td>43/46</td>
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<tr>
<td>Donor HBsAg</td>
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<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Donor HBeAg / anti-HBe Ab</td>
<td>/+</td>
<td>/+</td>
<td>/+</td>
<td>/+</td>
</tr>
<tr>
<td>Donor anti-HBc Ab (IgM / IgG)</td>
<td>/+</td>
<td>/+</td>
<td>/+</td>
<td>/+</td>
</tr>
<tr>
<td>Recipient HBsAg</td>
<td></td>
<td></td>
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<tr>
<td>Recipient anti-HBs Ab titer (IU/L)</td>
<td>&gt; 1000</td>
<td>477</td>
<td>137</td>
<td>54</td>
</tr>
</tbody>
</table>

Abbreviations: anti-HBc Ab, antihepatitis B core antibody; anti-HBe Ab, antihepatitis B extracellular antibody; anti-HBs Ab, antihepatitis B surface antibody; HBsAg, hepatitis B surface antigen; IgG, immunoglobulin G; IgM, immunoglobulin M

### Results

Mean age of donor and recipient were 37.5 and 45.5 years. The recipients were 2 men and 2 women. The follow-up was 18.0 ± 8.79 months. In serial follow-up, there were no abnormalities of renal and liver enzymes. At 1 year after kidney transplant, liver enzymes including aspartate aminotransferase (mean, 32.52 ± 14.57 U/L) and alanine aminotransferase (mean, 35.07 ± 17.42 U/L), and renal function tests including blood urea nitrogen (mean, 2.34 ± 0.82 mmol/L) and creatinine (mean, 94.48 ± 37.08 μmol/L), were normal. No patients had unwanted seroconversion to positive HBsAg without hepatitis B immunoglobulin. There were no events of graft rejection, HBV activation, or mortality.

### Discussion

Accepting kidney transplant from HBsAg-positive donors has been used as a means to achieve a wider donor pool in countries in which HBV is endemic. Most reported experience involves deceased donors.4 Renal transplant from HBsAg positive donors to HBsAg negative recipients successfully using natural immunity began to emerge in endemic areas from 1988.6 Living-donor kidney transplant from HBsAg-positive donors to hepatitis B antibody-positive recipients were reported in only 45 cases in Hong Kong, Saudi Arabia, Turkey, and the United States.1-3,5,6 These data came from endemic areas, where the prevalence of natural immunity was high.

To minimize the risk of infectious complications, HBsAg-positive recipients often are administered prophylactic antiviral drugs such as lamivudine and hepatitis B immunoglobulin.7,8 In a meta-analysis in 2005, the seroconversion of seropositive HBsAg from negative in kidney recipients showed increased graft failure and mortality.9 With respect to lamivudine and hepatitis B immunoglobulin, there was no standard strategy.2 However, Chung and coworkers recommended lamivudine treatment for 12 months with known HBV DNA positive status of donor.7 The risk for HBV reactivation in recipients with immunologic markers of past infection (HBsAg-negative, anti-HBc Ab-positive, anti-HBs Ab-positive) is < 5%.10 After transplant, the risk of infectious complications with HBV depends on the infectious status of the donor and recipient. Therefore, it is important to evaluate the risk of infectious complications measured that can be applied different detailed examination than a uniform guideline after transplant.11 Strict risk assessment is needed.

An absolute protective threshold of anti-HBs Ab titer is not yet defined in kidney recipients for hepatitis B immunoglobulins. Careful monitoring strategy can be applied to avoid unnecessary drug administration and various adverse events and to identify patients at risk for HBV reactivation.11

Sumethkul and associates suggested that the positivity of HBsAg in donors was not associated with evidence of active liver disease after kidney transplant in HBV-endemic areas with 10-year follow-up.12 Many authors suggested that allocation of renal grafts from donors with HBsAg may be permitted in HBV endemic areas.12,13 In a recent study, Tuncer and coworkers suggested that HBsAg positivity is not a contraindication for living-donor kidney donation.5

In summary, when combined with careful HBV-monitoring, renal grafts from HBsAg-positive living
donors can be transplanted to hepatitis B antibody-positive recipients without the need for hepatitis B immunoglobulin prophylaxis in an HBV-endemic country.

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Evaluation of Late Antibody-Mediated Rejection (C4d-Mediated Rejection): A Single-Center Experience

Erhan Tatar,1 Adam Uslu,2 Cenk Simsek,2 Enver Vardar3

Abstract

Objectives: There has been no improvement in long-term graft survival rates in renal transplant-recipients during the past decade. We evaluated patients who underwent renal transplant and experienced late (≥ 3 years) antibody-mediated rejection, after an immunologically uneventful course early after transplant.

Materials and Methods: Between 2003 and 2010, twenty-one of 312 patients who had kidney transplants at our center were diagnosed with antibody-mediated rejection according to the Banff 97 criteria. The patients' information from their files was retrospectively evaluated.

Results: Of the 7 male and 3 female patients (mean age, 33 ± 11, range, 18-52 y), 5 received deceased-donor kidneys, and 5 had living-related donor kidneys. The average basal and third-year serum creatinine levels were 1.24 ± 0.31 mg/dL and 1.36 ± 0.43 mg/dL (P < .001). The mean follow-up until rejection was 64 ± 23 months (range, 37-101 mo). Medical history revealed recurrent bacterial infections in 4, cytomegalovirus infection and post-transplant diabetes each in one patient and drug withdrawal in 2 patients. For this reason, maintenance immunosuppressive therapy was reduced and/or replaced. In kidney biopsies, 6 patients had acute findings of antibody-mediated rejection, and chronic features were predominant in 4 cases. Renal function improved in 8 patients after treatment, but rejection remained progressive in 2 patients. Three patients lost their grafts during follow-up. Noncompliance was the cause of graft loss in 2 cases. In the remaining 7 patients, the mean follow-up after rejection treatment was 18 ± 14 months (range, 6-48 mo), and the average serum creatinine level was 3.0 ± 0.93 mg/dL (range, 2.3-4.7).

Conclusions: Late antibody-mediated rejection can emerge soon after the modification of immunosuppressive drug dosages and may be responsible for graft dysfunction or loss.

Key words: C4d-mediated rejection, Chronic rejection, Graft survival, Late antibody-mediated rejection, Renal transplant

Introduction

Deceased-donor grafts survive as well as living-related kidney transplants during the first year. The survival rate is almost 90%. However, this rate is only about 50% at 10 years after transplant.1,2 Developments in transplant immunology and immunosuppressive therapy have led to a significant decrease in the incidence of acute rejection and rejection-induced early graft loss. However, there has been no significant improvement in long-term graft survival during the past decade.1,2 Recent publications have emphasized the importance of antibody-mediated rejection (AMR) as a major cause in late graft loss.3,4

The purpose of this study is to evaluate the kidney transplants performed in our center between January 2003 and December 2010, of whom had an uneventful course during the first 3 years after surgery, but presented with late graft dysfunction caused by biopsy-proven C4d-mediated rejection.
Materials and Methods

Among 312 patients who underwent renal transplantation at our center between January 2003 and December 2010, twenty-one patients with a diagnosis of C4d-mediated rejection were evaluated. Of those; 10 patients with good and stable graft function in the first 3 years, but had had deteriorating graft function due to late C4d-mediated rejection were analyzed.

Results

Of the 10 patients, 5 received deceased-donor and 5 living-donor kidneys. 7 patients were men and 3 were women with a mean age of 40±10 years (range, 23-56 y). The mean age of the donors was 48 ± 18 years (range, 10-66 y). One patient received an expanded-criteria donor kidney. The primary kidney diseases of the patients were unknown in 4, chronic glomerulonephritis in 4, chronic tubulointerstitial nephritis in 1, and amyloidosis in 1. All recipients were serologically negative with regard to hepatitis B and C. Those with living-related donor kidneys received 1 haplotype-matched graft. The average mismatch was 3.8 ± 0.4 in patients who received deceased-donor kidneys. Pretransplant panel reactive antibody levels were present in 7 cases, and both class I and class II antibody titers were negative. Implantation biopsies were present in 7 cases, and 3 had donor-derived mild histologic injury. Delayed graft function (mean 11.2 ± 6.7, range 3-20 d) was observed in 5 patients. Induction immunosuppressives were antithymocyte globulin in 9 cases and basiliximab in 1 case. Maintenance immuno-suppression and SCr levels throughout the follow-up are shown in the Table. The clinical histories of the patients revealed recurrent bacterial infections with high fever in 4 patients and cytomegalovirus infection in 1 patient. Additionally, 2 patients were noncompliant and stopped taking immunosuppressive drugs, and 1 patient had posttransplant diabetes. Immediately before rejection, it was found that maintenance doses of immunosuppressive therapy had been significantly reduced and only 1 patient had been treated with prednisolone + mycophenolate mofetil + tacrolimus (Table). The follow-up duration until rejection was 64 ± 23 months (range, 37-101 mo). The average SCr values about 6 months earlier and at the time of rejection were 1.4 ± 0.3 mg/dL (range, 0.7-1.9 mg/dL) and 2.7 ± 1.0 mg/dL (range 2-5.6) (P < .001). The mean SCr value after treatment was 2.5 ± 0.9 mg/dL (range 1.6-4.4).

In 8 patients, a decrease of SCr was detected early after treatment, and 2 patients had a progressive course. One patient had early graft loss. Rejection therapy was composed of 2 g/kg intravenous immunoglobulin (IVIG) for every patient. Six patients received 3- or 5-session double-filtration plasmapheresis. In addition to IVIG, 8 patients had pulse steroid treatment, 5 patients received antithymocyte globulin, and 3 patients had rituximab therapy. Apart from the 1 patient with early graft loss, the mean follow-up after rejection treatment was 21 ± 15 months (range, 5.2-48 mo). Among the graft losses, 1 was early, 1 other occurred at 45 months after treatment, and one was due to the patient’s noncompliant cessation of immunosuppressive medication 15 months after rejection treatment. Panel reactive antibody titers were positive in all patients with graft loss, but positive in only 50% of the remaining 7 patients with functioning grafts (Table). The mean follow-up of 7 patients after treatment was 18 ± 14 months (range, 6-48 mo). The average SCr level was 3.0 ± 0.93 mL/dL (range, 2.3-4.7), and the mean quantitative proteinuria level was 1.7 ± 1.4 g/d.

Discussion

Late graft dysfunction is a major problem in the daily practice of organ transplant. Death with a functioning graft and chronic allograft nephropathy
(also termed interstitial fibrosis and tubular atrophy) are decreasing in frequency as the most common causes of graft loss.5,6 Today, immune factors have come to the forefront in the pathogenesis of chronic allograft nephropathy.3,6,7 In patients with biopsy-proven chronic allograft nephropathy, acute rejection within the first year, infection, and/or the use of low-dose immunosuppression have been reported to be significant causative factors leading to long-term graft loss.3,8 Immune-mediated injury in the pathogenesis of chronic allograft nephropathy may be due to low-dose immunosuppression.3,8 Similarly, 1 of every 3 patients develop de novo donor-specific antibodies after renal transplant, and these lead to immunologically mediated late graft loss.7 The early uneventful course observed in our cases are attributable to negative pretransplant panel reactive antibody levels in 7 patients, each of whom was at low risk immunologically; first transplant and at least 1 haplotype histocompatibility in all patients. The development of late humoral rejection, even in these low-risk patients, may have been associated with drug withdrawal (2 patients), mTOR-based triple immunosuppression (3 patients), double immunosuppression (1 patient), or azathioprine containing regimen (1 patient), and, more consistently, may have been caused by immunosuppressive drug dosage modifications for treating and preventing recurrent infections.

There are various modalities for treating humoral rejection, such as pulse steroids, antilymphocyte therapy, plasmapheresis and immunoadsorption, IVIG, rituximab, bortezomib, and eculizumab.8-10 Data on the most effective treatment are insufficent. Treatment efficacy is especially low in patients with late AMR with biopsy-proven chronic histologic changes. There are many studies in the literature on the effectiveness of plasmapheresis, IVIg, and rituximab treatment in different combinations as rituximab-IVIg or plasmapheresis-IVIg.9-10 However, there are no definitive data, particularly on the optimal dose of IVIg therapy, and total doses varying from 400 mg/kg to 2 g/kg have been proposed.

When considering the dual effect, such as replacement of gamma globulin loss and neutralization of antibodies against rebound phenomenon after plasmapheresis, IVIg emerges as an important treatment option, especially in combination therapy with plasmapheresis. In addition IVIg decreases infection risk during AMR treatment. Most of our patients had recurrent infections before AMR, but 2 g/kg IVIG treatment yielded good and safe responses, and no severe infectious complications were observed during follow-up. High-dose IVIg therapy (2 g/kg) combined with plasmapheresis in patients with late occurring AMR seems to be a good treatment option. Novel small-scale studies on the efficacy of bortezomib in patients with late AMR refractory to conventional treatment have been reported in the literature.8-10 The results of the BORTEJECT study, a randomized, controlled trial designed to evaluate the treatment effectiveness of bortezomib in patients with late AMR, are eagerly awaited.11

C4d-mediated rejection may be responsible for late graft dysfunction, even in renal transplant patients with low immunological risk. Low-dose

### Table 1. Characteristics and Outcomes of Patients

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Age / Gender</th>
<th>TX Date / Type</th>
<th>3-Year Serum Creatinine (mg/dL)</th>
<th>3-Year Posttransplant Immunosuppressive Complication</th>
<th>Rejection Date / PRA</th>
<th>Rejection Serum Creatinine (mg/dL)</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>28 / F</td>
<td>2004 / LR</td>
<td>1.25</td>
<td>P + MMF + CSA</td>
<td>2012 Chronic / +/-</td>
<td>2.20</td>
<td>Alive with functioning graft</td>
</tr>
<tr>
<td>2</td>
<td>18 / F</td>
<td>2005 / DD</td>
<td>0.8</td>
<td>MMF + TAC</td>
<td>2013 Acute / +/-</td>
<td>5.60</td>
<td>Alive with functioning graft</td>
</tr>
<tr>
<td>3</td>
<td>42 / F</td>
<td>2008 / LR</td>
<td>0.9</td>
<td>P + MMF + CSA</td>
<td>2012 Chronic / +/-</td>
<td>2.20</td>
<td>Alive with functioning graft</td>
</tr>
<tr>
<td>4</td>
<td>32 / M</td>
<td>2008 / LR</td>
<td>1.4</td>
<td>P + MMF + SIR</td>
<td>2013 Acute / +/-</td>
<td>2.60</td>
<td>Alive with functioning graft</td>
</tr>
<tr>
<td>5</td>
<td>39 / M</td>
<td>2007 / DD</td>
<td>2.0</td>
<td>P + MPS + SIR</td>
<td>2010 Acute / +/-</td>
<td>2.00</td>
<td>Alive on hemodialysis</td>
</tr>
<tr>
<td>6</td>
<td>21 / M</td>
<td>2003 / DD</td>
<td>1.25</td>
<td>P + AZA + CSA</td>
<td>2013 Acute / +/-</td>
<td>2.50</td>
<td>Alive on hemodialysis</td>
</tr>
<tr>
<td>7</td>
<td>30 / M</td>
<td>2008 / DD</td>
<td>1.6</td>
<td>P + MMF + EVO</td>
<td>2012 Chronic / +/-</td>
<td>2.61</td>
<td>Alive on hemodialysis</td>
</tr>
<tr>
<td>8</td>
<td>50 / M</td>
<td>2010 / DD</td>
<td>2.1</td>
<td>P + MMF + TAC</td>
<td>2013 Chronic / +/-</td>
<td>2.80</td>
<td>Alive with functioning graft</td>
</tr>
<tr>
<td>9</td>
<td>52 / F</td>
<td>2005 / DD</td>
<td>1.3</td>
<td>P + MMF + SIR</td>
<td>2009 Chronic / +/-</td>
<td>3.00</td>
<td>Alive on hemodialysis</td>
</tr>
<tr>
<td>10</td>
<td>27 / M</td>
<td>2003 / DD</td>
<td>1.0</td>
<td>P + AZA + CSA</td>
<td>2009 Acute / +/-</td>
<td>2.60</td>
<td>Alive on hemodialysis</td>
</tr>
</tbody>
</table>

**Abbreviations:** AZA, azathioprin; CMV, cytomegalovirus; CSA, cyclosporin A; DD, deceased-donor; EVO, everolimus; F, female; LR, living-related; M, male; MMF, mycophenolate mofetil; NODAT, new onset diabetes after renal transplant; P, prednisolone; PRA, panel reactive antibody; SIR, sirolimus; TAC, tacrolimus.
immunosuppression and drug withdrawal are important predisposing factors of C4d-mediated rejection.

References

Bone Marrow Biopsy in Patients With Renal Transplant: Spectrum of Findings and Diagnostic Use

Pelin Börcek,1 B. Handan Özdemir,1 Eylem Akar Özkân,1 F. Zeynep Taşlıca,1 Mehmet Haberal2

Abstract

Objectives: Renal transplant may be complicated by cytopenia, fever of unknown etiology, or hematolymphoid malignancies. Bone marrow biopsy may be indicated to evaluate these complications. However, to the best of our knowledge, no previous study has systematically documented the characteristics of bone marrow biopsy in these patients. The present study reports the range of bone marrow findings in renal transplant recipients.

Materials and Methods: We selected 85 patients who underwent bone marrow biopsy among 1745 renal transplant recipients who had transplant at Başkent University from January 1990 to December 2013. The files of these patients were reviewed for age, sex, age at renal transplant, underlying renal disease, donor type, immunosuppressive therapy, presence or absence of acute humoral or cellular rejection, duration between transplant and bone marrow biopsy, indication for bone marrow biopsy, and histopathologic diagnoses of bone marrow biopsies.

Results: The most common cause of renal insufficiency leading to transplant in this patient group was unknown etiology, observed in 24 patients (28.2%). The most common indication for bone marrow biopsy was blood cytopenia, detected in 56 patients (65.9%). Neoplastic involvement of the bone marrow was detected in 6 patients (7.1%), all of which were hematolymphoid malignancies. Corticosteroids were the most commonly used immunosuppressive agents, administered to all patients.

Conclusions: Bone marrow biopsy provides important information in renal transplant recipients, especially in cases of neoplastic bone marrow involvement, specific inflammation, and amyloidosis, which are uncommon in this patient group. The overall diagnostic use is related to the individual situation of each patient.

Key words: Anemia, Cytopenia, Kidney transplant

Introduction

Renal transplant may be complicated by persistent fever or cytopenia. Renal transplant recipients also are susceptible to the development of hematolymphoid malignancies, and bone marrow biopsy may be indicated for malignancy staging. Therefore, bone marrow biopsy is not infrequently performed in this patient group. However, to the best of our knowledge, no previous study has systematically documented the characteristics of bone marrow biopsy in these patients. The present study reports the range of bone marrow findings in renal transplant recipients.

Materials and Methods

Patients

Patients who underwent bone marrow biopsy were selected among 1745 kidney transplant recipients from January 1990 to December 2013 in Başkent University. Clinical findings of these patients were reviewed from patient files, including age, sex, age at renal transplant, underlying renal disease, donor type, immunosuppressive therapy, presence or...
absence of acute humoral or cellular rejection, duration between transplant and bone marrow biopsy, indication for bone marrow biopsy, and histopathologic diagnoses of bone marrow biopsies.

Statistical analyses
Descriptive and comparative statistical analyses were conducted with statistical software (SPSS for Windows, Version 16.0, SPSS Inc., Chicago, IL, USA).

Results
There were 85 patients who underwent bone marrow biopsy after kidney transplant. In the 85 patients, 57 patients (67.1%) were male and 28 patients (32.9%) were female. The mean age at renal transplant was 32.0 ± 12.3 years. The graft source was a living-related donor in 54 patients (63.5%) and deceased donor in 31 patients (36.47%). The mean age at bone marrow biopsy was 36.5 ± 13.1 years (range, 4-65 y), and 7 patients (8.2%) were aged < 19 years at bone marrow biopsy. The mean duration between transplant and the requirement for bone marrow biopsy was 59.1 ± 57.8 months (range, 1-250 mo).

The most common cause of renal insufficiency leading to transplant in this patient group was unknown, observed in 24 patients (28.2%). The most common known underlying disease was primary glomerulopathy (19 patients [22.4%]). The other underlying diseases, in order of decreasing frequency, were pyelonephritis (6 patients [7.0%]), diabetes mellitus (6 patients [7.0%]), polycystic kidney disease (5 patients [5.9%]), Alport syndrome (4 patients [4.7%]), systemic hypertension (4 patients [4.7%]), familial Mediterranean fever (4 patients [4.7%]), nephrolithiasis (4 patients [4.7%]), vesicoureteral reflux (3 patients [3.5%]), cystinosis (2 patients [2.4%]), tubulointerstitial nephritis (2 patients [2.4%]), systemic lupus erythematosus (1 patient [1.2%]), and renal agenesis (1 patient [1.2%]) (Table 1).

Indications for bone marrow biopsy were fever of unknown origin in 18 patients (21.2%), cytopenia in 56 patients (65.9%), and staging evaluation for posttransplant lymphoproliferative disorder (PTLD) in 11 patients (12.9%).

In the 85 bone marrow biopsies, 6 biopsies (7.1%) showed neoplastic infiltration, including 3 biopsies performed for staging of PTLD (Table 2). There were 2 bone marrow biopsies that were characterized by diffuse blast infiltration; and bone marrow was infiltrated with diffuse large-B-cell lymphoma in 1 patient. The frequency of bone marrow involvement by PTLD in our series of renal transplant recipients was 3 of 11 patients (27.3%), and no neoplastic involvement of bone marrow other than hematolymphoid malignancies was observed. All 4 patients (4.7%) who underwent renal transplant due to familial Mediterranean fever had amyloidosis in their bone marrow biopsies. Granulomatous inflammation was observed in 3 bone marrow biopsies (3.5%); in these patients, 1 patient had systemic lupus erythematosus and 1 patient had nephrolithiasis as the underlying etiology (both patients were investigated for fever of unknown origin), and 1 patient had cryptogenic origin of renal insufficiency and was biopsied for pancytopenia. In the other bone marrow biopsies, 34 biopsies (40.0%) were normocellular for patient age, 23 biopsies (27.1%) were hypocellular, and 9 biopsies (10.6%) were hypercellular (Table 2). In 1 patient, there was infiltration of bone marrow histiocytes with foreign material secondary to ingestion of a traditional herbal medication in a renal transplant recipient of unknown underlying cause.

The most common immunosuppressive drugs were corticosteroids, which were used in all patients.
Additionally, 41 patients (48.23%) used cyclosporine and 14 patients (16.47%) used tacrolimus. There were 30 patients (35.29%) who received triple immunosuppressive therapy (calcineurin inhibitor, antimetabolite, and corticosteroids).

There were 2 of 85 patients (2.6%) who experienced acute humoral rejection, 20 patients (23.5%) who had acute cellular rejection, and 3 patients (3.5%) who had both acute humoral and cellular rejection.

When cases were grouped as neoplastic (6 patients [7.1%]), nonneoplastic (75 patients [88.2%]), and amyloidosis (4 patients [4.7%]) according to bone marrow biopsy histology, there were no significant differences in mean duration between transplant and bone marrow sampling; presence or absence of acute humoral and cellular rejection; presence of interfering cytomegalovirus, hepatitis C virus, or Epstein-Barr virus infections; use of cyclosporine, tacrolimus, or sirolimus as immunosuppressive medication; or indication of bone marrow biopsy grouped as investigation of cytopenia, fever of unknown origin, or staging for hematolymphoid malignances (all relevant \( P \) values were above .05).

Discussion

Anemia is a common complication of renal transplant and may occur due to a variety of causes including impaired renal function, immunosuppressive medication, donor and recipient age, and impaired iron homeostasis.\(^1,2\) Immunosuppressive drugs, especially azathioprine and mycophenolate mofetil, frequently cause bone marrow suppression resulting in anemia, leukopenia, and thrombocytopenia.\(^3\) Bone marrow biopsy may be required in renal transplant patients to rule out neoplastic infiltration in the bone marrow or primary bone marrow pathology, diagnose specific infections involving the bone marrow, and determine the extent of myelosuppression. However, we have not encountered any systematic studies on findings of bone marrow biopsies from renal transplant recipients. Therefore, the present study is a preliminary report which outlines the features of bone marrow biopsies in renal transplant recipients.

Cryptogenic renal insufficiency and primary glomerulopathies were the leading underlying etiologic factors in renal transplant recipients who required bone marrow sampling. Bone marrow biopsies were most commonly taken from patients who were being investigated for cytopenia, and the most frequent histopathologic finding was normocellular bone marrow. Involvement of bone marrow by neoplastic processes was infrequent, and no neoplasms resulted from a solid-organ primary neoplasm.

Bone marrow biopsy provided useful information for renal transplant recipients, and diagnostic utility was related to the individual situation of each patient. Further studies are needed to specify the histologic characteristics and utility of bone marrow biopsy in this patient group.

References

The Role of a Single Center Experience in Azerbaijan’s Nephrology Field

Hikmet Ismayilov,1 Shahin Qadimov,1 Kamil Muslumov,1 Khanbaba Huseynov,2 Fariz Babayev3

Abstract

Objectives: There is a saying that, to evaluate the level of medicine in a country, one should pay attention to its level of uronephrology service – specifically, the level of renal replacement therapy, hemodialysis, peritoneal dialysis, and transplant. With the increasing number of patients requiring renal replacement therapy, it is the utmost duty of modern medicine to structure and organize it. Since its establishment in 2000, MedServis Private Medical Centre has dedicated its main services to hemodialysis, renal replacement therapy, healthcare in other branches of medicine, follow-ups, and preparing patients for transplant.

Materials and Methods: We compared statistical indicators of the patients who have been enlisted in reports of Azerbaijan, MedServis Private Medical Centre, which is in Azerbaijan, and European Renal Association-European Dialysis and Transplant Association between 2000 and 2013. The statistics of the patients reported in MedServis Private Medical Centre’s account for the past 14 years were categorized with respect to several factors.

Results: During its activity in the past 14 years, we received 5894 new patients at MedServis Private Medical Centre (male, 58.9%; female, 41.1%). Of all the patients received, children were 1.6% patients. In all patients, 9% patients had acute kidney failure and 91% had chronic kidney disease.

Conclusions: MedServis Private Medical Centre has contributed importantly to nephrology in Azerbaijan, with its first-in-the-country services in remote hemodialysis, hemodialysis of children, close-up control of the pregnancy of a woman with 6 years of hemodialysis, and successful delivery of the first permanent catheter during 14 years of activities.

Key words: End-stage renal failure, Hemodialysis, Peritoneal dialysis, Transplant

Introduction

There is a concept in the medical literature that, to evaluate a country’s health care, one should be paying attention to its uronephrology services. To be more precise, one should evaluate the level of renal replacement therapy, hemodialysis, peritoneal dialysis, and transplant in the country. Forming a well-established structure of renal replacement therapy is one of the key elements of modern medicine. When patients with terminal kidney insufficiency are not under a renal replacement therapy regimen, their morbidity and mortality indicators escalate. With application of renal replacement therapy, life expectancy of such patients increases by decades.

According to statistical indicators, the number of patients who have terminal kidney insufficiency and are in need of renal replacement therapy has been increasing markedly. According to the World Health Organization 2011 reports, there are 2786000 people around the world who are under renal replacement therapy, and this number is steadily increasing. Prevalence varies between different countries. In 2009, prevalence in Europe was 730 patients per million population (pmp), Ukraine was 101 pmp, Russia was 170 pmp, Belgium was 1193 pmp, Spain was 1507 pmp, and Turkey was 828 pmp.
In 1970, the first hemodialysis center in Azerbaijan and the second hemodialysis center in the Union of Soviet Socialist Republics was created at the Republican Clinic Urological Hospital named after M. Javadzade, and the first kidney transplant surgery was performed later in that hospital by academic M. Javadzade. The second hemodialysis center was created in 1985 under the IV Medical Office of Cabinet Ministers (then known as medical commission). In 1995, a hemodialysis department was started with activities in Central Hospital of Oil Workers, which provided care for 40 patients.

In the year that MedServis Private Medical Centre was founded, there were only 10 to 15 hemodialysis machines that served 40 to 60 hemodialysis patients nationwide in a country of 8000000 people. Starting its activities under such conditions, our center was recognized and valued by Azerbaijan’s health care society for its successful work within a short time. Since then, more than 6500 patients have had hemodialysis therapy due to chronic and acute kidney insufficiency. It is worthwhile mentioning that most hemodialysis patients who have applied for therapy at Medservis Private Medical Centre were in bad health condition, and as a result of a well programmed therapy regimen, their situation recovered and became better and stable. Most patients are continuing their therapy in various hemodialysis centers that have been opened in different regions of Azerbaijan. During these years, our center organized personnel education abroad, and people were sent to professional development trainings in leading medical institutions in Turkey, Iran, and Commonwealth of Independent States countries. Staff members who have improved their skills as doctors, midlevel medical providers, and technical service personnel currently are working at Medservis Private Medical Centre and other leading medical centers in Azerbaijan.

One of the major problems that our center had when it started its activities was the lack of medical working protocols that were consistent with global standards. These protocols regulate processes such as the beginning of hemodialysis therapy, adequacy of hemodialysis, viral hepatitis, anemia, and renal osteodystrophy. We organized our activities with the therapist-prophylactic office of Azerbaijan Republic Health Ministry, based on protocols that were developed by the National Kidney Foundation-Disease Outcomes Quality Initiative, European Renal Association-European Dialysis and Transplant Association, and Baskent University.

Based on exact statistics, we have received 6500 new patients within 14 years of our activity, including 3729 adult males (58.9%), 2675 adult females (41.1%), and 96 pediatric patients (1.6%). Of all patients, 5919 patients (91%) had chronic kidney disease stage 5 and 581 patients (9%) had acute kidney injury. Causes of acute kidney injury were obstetric, gynecologic, and obstructive issues.

With respect to etiologic factors, 1917 patients (29.5%) had diabetic nephropathy, 1852 patients (28.5%) had chronic glomerulonephritis, 1137 patients (17.5%) had chronic pyelonephritis, 903 patients (13.9%) had hypertonic nephrosclerosis, 338 patients (5.2%) had polycystic kidney disease, 39 patients (0.6%) had amyloidosis, 130 patients (2%) had other causes of kidney disease, and 184 patients (2.8%) had kidney diseases of unknown cause.

When patients presented for the first time, catheters were inserted into central veins as the main venous line, including the internal jugular vein in 5765 patients (88%), subclavian vein in 650 patients (10%), and femoral vein in 65 patients (1%). There were 49 patients (1%) who had a previously placed arteriovenous fistula. In children and patients who could not have an arteriovenous fistula placed, permanent catheters were placed for the first time in Azerbaijan. During these years, 825 patients had permanent catheters placed, and 5 patients had vascular prostheses placed in veins.

Due to viral hepatitis prophylaxis, which is a major problem for dialysis centers, the hepatitis profile was identified on all evaluated patients at Medservis Private Medical Centre. There were 226 patients (4.52%) who had hepatitis B surface antigen, 259 patients (5.18%) who had antihepatitis C virus antibodies, 5 patients (0.1%) who had antihepatitis D virus antibodies, and 23 patients (0.46%) who had mixed infection. We also had a patient who had human immunodeficiency virus. In addition, liver function tests were evaluated monthly and hepatitis markers were evaluated every 3 months. Hepatitis patients and hepatitis carriers had hemodialysis in separate rooms. We have been performing vaccination for our medical personnel and regular patients since the center was founded.

When evaluating for bone mineral disease prophylaxis, parathyroid hormone level was tested on all patients and was > 400 pg/mL in 4430 patients (88.6%).
Detailed information about kidney transplant was given to our patients who had chronic kidney disease stage 5 and who did not have any contraindications for transplant. During this time all our patients who applied to our clinics were prepared for kidney transplant and their health status was followed regularly according to the modern protocols mentioned earlier. Our patients have had successful kidney transplant surgery in Iran, Turkey, Russia, and Azerbaijan. We currently have information about 285 patients. Among all patients who had transplant, 1.72% patients were children, 39.65% were female, 60.35% were male, and 1.8% patients had transplant even before preemptive hemodialysis.

In conclusion, compared with the year 2000 when we started, better centers currently operate in Azerbaijan that follow modern standards. The level of nephrology services has increased, and there currently are hemodialysis centers in almost every region of Azerbaijan. Kidney transplant is successfully performed at Republican Clinic Urological Hospital Named After Academic Dr. Javadzade and at the Central Hospital of Oil Workers. Peritoneal dialysis services are being established. At Medservis Private Medical Centre, we consider that our contribution is to improve the level of nephrology services to the current stage.

References

Association Between Panel Reactive Antibody and Antiendothelial Cell Antibody Positivity in Kidney Transplant Patients

Bilkay Baştürk,1,2 Bircan Kantaroğlu,2 Z. Aytül Noyan,3 Sedat Yıldırım,4 Çağla Sarıtürk5

Abstract

Objectives: Endothelium is the major tissue for hyperacute and acute rejection. Binding of antibody to endothelium activates several immunologic mechanisms. Antiendothelial cell antibodies are a group of nonhuman leukocyte antigen antibodies that may play a role in the induction of an immunologic reaction that triggers inflammation. The aim of this study was to investigate whether there was an association between antiendothelial cell antibody positivity and panel reactive antibody positivity in renal transplant patients.

Materials and Methods: In this study, we investigated the association between antiendothelial cell antibodies and panel reactive antibody Class I class II crossmatch positivity in patients, and compared these results with results from 100 healthy volunteers. All serum samples were analyzed by bead-based technology for calculated panel reactive antibody positivity; in addition, slides were used, each containing human umbilical vein endothelial cells and capillary-rich tissue for antiendothelial cell antibody positivity.

Results: Antiendothelial cell antibodies was positive in 48 of 89 patients (panel reactive antibody Class I class II negative), 22 of 35 patients (class I-positive), 25 of 39 patients (class II-positive), 26 of 40 (class I-class II positive), and 37 of 57 serologic and flow cytometry crossmatch-positive patients (P ≤ .016), and ultimately, in 122 of 205 patients and 25 of 100 volunteers (P ≤ .001). Antiendothelial cell antibody positivity was more frequent in panel reactive antibody-positive than negative patients and the control group.

Conclusions: Binding of antiendothelial cell antibodies to endothelial cells may activate complement by the classical pathway and cause up-regulation of adhesion molecules. This study questioned the antigenic specificity of antiendothelial cell antibodies. Our study results showed that antiendothelial cell antibodies may play an important role for graft destruction, independent of panel reactive antibody and crossmatch positivity.

Key words: End-stage renal disease, Human leukocyte antigen, Non-HLA antibody

Introduction

Antibodies against the endothelium can damage the graft cells via a complement-dependent mechanism and/or stimulation of a proinflammatory and proliferation signaling mechanism. Endothelium expresses both human leukocyte antigen (HLA) and non-HLA antigens. Antiendothelial cell antibodies (AECAs) are a family of non-HLA antibodies that target the endothelial cells via antigenic determinants on the cell membrane. These antibodies are mainly immunoglobulin G (IgG) antibodies binding through the Fab domain. The AECAs effects are well-known in autoimmune diseases and some inflammatory processes. They might contribute to the pathogenesis of systemic vasculitis-associated diseases by activation of endothelial cells, direct cytotoxic effects due to complement-dependent cytotoxicity, indirect
cytotoxic effects secondary to antibody-dependent cytotoxicity, induction of coagulation, induction of apoptosis through the binding of phospholipids or heat shock protein, or induction of endothelial cell activation. The targeted antigens of AECA are a large group including matrix proteins and extracellular molecules. The AECAs induce the expression of adhesion molecules, E-selectin, intracellular adhesion molecule 1, vascular cell adhesion molecule 1, some interleukins including interleukin 1, interleukin 6, and interleukin 8, and monocyte chemoattractant protein. Panel reactive antibody (PRA) is defined as the percentage of HLA antigens of a panel reacting with a patient’s serum. The presence of anti-HLA antibodies are at high risk for acute and chronic antibody mediated rejection. Studies have shown that a substantial effect of antibody ligation of class I molecules on the surface of endothelial cells is the induction of proliferation. Anti-HLA class II antibodies play an important role for chronic rejection by stimulating endothelial cell proliferation. The aim of this study was to retrospectively investigate whether there was an association between AECA positivity and PRA positivity in renal transplant patients.

Materials and Methods

Subjects and assays
In this retrospective study, we investigated the association between AECA and PRA class I and class II positivity in patients. For this purpose, we compared the results from 205 patients and 100 healthy volunteers (previous AECA study results). All samples collected from patients were stored at -20°C until testing. Samples were analyzed by bead-based technology, with screening and identification for class I and II and single antigen for donor-specific antibodies, for calculated PRA positivity. For AECA positivity, each sample was diluted 1:10 in phosphate-buffered saline (PBS) and polysorbate (Tween, Sigma-Aldrich, St. Louis, MO, USA). We used slides containing biochips coated with frozen sections of human umbilical vein endothelial cells (HUVEC) and capillary-rich tissue such as skeletal muscle (Euroimmun, FB 1960-1005-2, Lübeck, Germany). The test specificity for HUVEC was 100% and monkey skeletal muscle was 100%; the sensitivity for HUVEC was 100% and monkey skeletal muscle was 97%. Frozen sections of primate skeletal muscle and cultivated HUVEC covering the reaction areas of the biochip slides were incubated with a dilute serum sample. A reaction was considered positive when specific antibodies (IgG) attached to the antigens. The bound antibodies were stained with fluorescein-labeled antihuman antibodies and visualized by fluorescence microscopy (Figure 1). Results were determined qualitatively and quantitatively.

Statistical analyses
Statistical analysis was performed using a statistical package (SPSS, Version 17.0, SPSS Inc., Armonk, NY, USA). Data were summarized according to the frequency distribution. The categorical variables between groups were analyzed with chi-square test or Fisher exact test. Values of \( P \leq .05 \) were considered statistically significant.

Results
The results showed that the PRA-positive (PRA ≥ 60%) patient group has increased AECA levels. The AECA test was positive in 48 of 89 patients (PRA class I class II negative; \( P = 1.00 \)), 22 of 35 patients (PRA class I-positive; \( P = .047 \)), 25 of 39 patients (PRA class II-positive; \( P = .005 \)), 26 of 40 patients (PRA class I class II positive, \( P = .0001 \)), and 37 of 57 of the
serologic and flow cytometry (FCM) crossmatch-positive patients \( (P = .016) \). The AECA test was positive in 122 of 205 patients and 25 of 100 volunteers \( (P < .001) \) (Table 1). The AECA positivity was more frequent in PRA-positive patients than the PRA-negative patient and control groups and independent from PRA mean fluorescence intensity (MFI) values. The PRA positivity was more frequent in female than male patients (Figure 2). No significant correlation was found between MFI value or donor-specific antibodies and occurrence of AECA.

**Discussion**

In our study, we showed that PRA-positive renal transplant patients had increased levels of AECA. The AECA recognized constitutively expressed or cytokine induced antigens such as \( \beta_2 \)-glycoprotein I, platelet factor 4, DNA, and membrane or cytoskeleton proteins such as tubulin, vimentin, laminin, vinculin, and annexin V.\(^8\) Multiple pathogenic roles of AECA have been elicited in diseases that involve the vascular system such as vasculitis and atherosclerosis.\(^9\) Previous studies showed that the development of AECAs was significantly associated with cytomegalovirus infection and especially associated with the level of cytomegalovirus antigenemia positivity.\(^10,11\) The AECAs may trigger an inflammatory process and induce endothelial cell apoptosis and necrosis.\(^12\) The AECAs are detected in a wide range of clinical pathologies that involve the vascular system, including autoimmune disease, infectious disease, and vasculopathies, but they also can be observed in healthy individuals.\(^13,14\) Alloimmune-mediated injury of the endothelium leads to the aberrant expression of self proteins and subsequent generation of autoantibodies. It is accepted that blood group antigens and HLA class I and II are not targets for AECA-associated autoimmune disease.\(^15\) The AECAs may be joined to destructive potential of PRA on graft endothelial cells.

In conclusion, alloimmune-mediated injury of the endothelium leads to the aberrant expression of self proteins and subsequent generation of autoantibodies. Occurrence of AECAs is not significantly associated with acute vascular rejection alone, but may be related to PRA positivity and/or may aggravate the inflammatory process together.

**References**


Fatal Outcome After Renal Transplant in a Pediatric Patient With Noonan Syndrome

Coskun Araz,1 Ebru Kaval,1 Adnan Torgay,1 Gokhan Moray,2 Mehmet Haberal2

Abstract
Noonan syndrome is a congenital, common, hereditary disorder. Facial dysmorphism, growth retardation, and various heart defects are typical clinical features. In patients with minor cardiac pathology, life expectancy is normal. We report a case of renal transplant in a pediatric patient with Noonan syndrome that ended with death of the patient. Our patient presented with unexpected and refractory postoperative neurological complications that were unresponsive to intensive therapy, and the patient died because of secondary complications.

Key words: Anesthesia, Complication, End-stage renal disease, Genetics

Introduction
Noonan syndrome is a genetically heterogeneous, congenital, and developmental disorder characterized by dysmorphic facial features, short stature, and a wide spectrum of congenital heart and other defects.1-3 The estimated incidence of Noonan syndrome is 1 in 1000 to 2500 live births, and the syndrome equally affects both sexes. A gene for Noonan syndrome has been mapped to chromosome number 12. Noonan syndrome may be accompanied by varied skeletal, neurological, genitourinary, hematologic, or dermatologic findings.1-4

We present a case report of a 4-year-old boy with end-stage renal disease secondary to vesicoureteral reflux and Noonan syndrome who died after renal transplant.

Case Report
A 4-year-old boy with a clinical diagnosis of Noonan syndrome and end-stage renal disease was scheduled for renal transplant from his mother. The patient had Noonan syndrome with typical facial dysmorphism, low growth percentile, and hypertrophic cardiomyopathy. He had no clinically important skeletal deformity, Mallampati score class 2, and mouth opening was 4 cm. The patient had no findings of cardiac decompensation and his neurological condition was normal. He also had α-thalassemia, and hemoglobin level was 7.1 g/dL after transfusion of 150 mL red blood cells. The serum electrolytes, glucose level, organ function tests, coagulation profile, and platelet level were normal. The preoperative echocardiography revealed nonobstructive hypertrophic cardiomyopathy. None of his family members had characteristics of Noonan syndrome.

After 8 hours without food or drink, he was premedicated intravenously with 2 mg midazolam and taken to the operating room. Standard invasive monitoring was performed with 5-lead electrocardiogram, radial artery catheter, pulse oximeter, left subclavian central venous catheter, and esophageal heat probe. Antibiotic prophylaxis (ampicillin [50 mg/kg] and gentamicin [2 mg/kg]) was administered 30 minutes before induction of anesthesia. The first measurement of blood pressure was 175/95 mm Hg. After administration of fentanyl and midazolam, the blood pressure decreased to 135/80 mm Hg. Anesthesia induction was performed with ketamine (1 mg/kg) and atracurium (0.4 mg/kg).
and the trachea was intubated with a 4.5-mm cuffed endotracheal tube. Anesthesia was maintained with sevoflurane (1.0 – 1.5 minimum alveolar concentration) in a mixture of 50% oxygen and 50% air and intravenous infusion of remifentanil (0.05 μg/kg/min). According to the institutional routine protocol, a bolus of methylprednisolone (1 mg/kg) and infusion of basiliximab (1 mg/kg during 1 hour) were administered at the induction of anesthesia, and mannitol (0.5 g/kg) and furosemide (1 mg/kg) were given intravenously during the vascular anastomosis. After an uneventful and successful operation, the patient was extubated in the operating room and he was transferred to the surgical intensive care unit for hemodynamic and neurological monitoring. The surgery time was 270 min, and bleeding during surgery was 100 mL.

After an uneventful early postoperative course, he developed severe hypertension (195/115 mm Hg) and a generalized tonic-clonic seizure at postoperative hour 14. He was given empiric anti-hypertensive (calcium channel blockers) and antiepileptic drugs (phenytoin). The serum sodium level was 119 mg/dL, and he was reintubated because of decreased level of consciousness.

During the first 48 hours after surgery, his convulsions repeated several times, and he was treated with sodium and erythrocyte replacement. Magnetic resonance imaging (MRI) scan revealed intracranial pressure and intracranial edema. On the second postoperative day, decompressive craniectomy and external ventricular drainage were performed. In the follow-up MRI, intracranial hemorrhage and ischemic lesions were seen.

On the following day, the patient’s consciousness and clinical condition did not show any improvement. He had a tracheotomy performed, and he developed various infections. He remained in the intensive care unit until he died at 4 months after surgery secondary to severe septic shock and multi organ failure syndrome. However, his cardiac and renal functions did not deteriorate during the 4 postoperative months.

Discussion

Noonan syndrome, first reported by J.A. Noonan in 1963, is a clinical disorder with autosomal dominant inheritance and sporadic occurrence. The clinical presentation of Noonan syndrome is variable. The affected cases may have different clinical features such as typical facial properties (hypertelorism, proptosis, micrognathia, down-slanting palpebral fissures, and low posterior hair line), congenital heart defects (pulmonary stenosis, hypertrophic cardiomyopathy, and atrial septal defects), short stature, webbed neck, chest or skeletal deformities, undescended testes, mild intellectual disability, and hematologic anomalies (clotting defects and myeloproliferative disorders).1-5 The clinical diagnosis of Noonan syndrome can be made using van der Burgt6 criteria. Our patient satisfied these criteria including facial features, growth retardation, and cardiac dysfunction (hypertrophic cardiomyopathy). Difficult airway treatment and mechanical ventilation due to facial dysmorphism, webbed neck, and vertebral and thoracic anomalies were described in previous reports.2,7 Endotracheal intubation and mechanical ventilation were performed in our patient without any difficulty. Our patient was known to have heart dysfunction and his cardiac condition could have been a risk for anesthesia. To avoid cardiac decompensation, measures were taken to prevent acute changes in systemic vascular resistance, heart rate, and contractility.

Genitourinary anomalies are detected in 11% patients with Noonan syndrome including renal pelvic dilation, renal hypoplasia or ectopia, and cryptorchidism. Fertility may be normal or diminished. Undescended testes in male patients can occur in 77% patients.1,8,9 Our patient had end-stage renal disease due to vesicoureteral reflux since age 1 year. He had received peritoneal dialysis initially, and he was treated with hemodialysis for the 6 months before transplant.

Severe psychological or neurological problems are not common in Noonan syndrome. Minimal mental or motor retardation may occur. Partial hearing loss, speech disorders, and visual problems may be observed. Recurrent convulsions may be observed in 13% patients.1,8,10 The most important cause of poor outcome in our patient was unexpected development of neurological complications. Early after surgery, he had stable cardiac, respiratory, and neurological condition, and renal graft function was excellent. However, he had sudden tonic-clonic seizures at 14 hours after surgery. An increase in intracranial pressure followed by ischemic and hemorrhagic complications were the main reasons for the deterioration of the patient. The early seizures
may have been due to electrolyte imbalance. The patient’s urinary output was high on the first postoperative day (10 mL/kg/h). This marked diuresis probably was the reason for the electrolyte imbalance. He subsequently developed serious infections and died. No cardiac problems or complications related to the transplanted kidney was observed until multiorgan failure developed from sepsis at the terminal stage.

In conclusion, Noonan syndrome can affect multiple organs or systems and the anesthesiologist may confront various challenges. The most frequent difficulties are airway problems, cardiac anomalies, musculoskeletal anomalies, and bleeding diathesis. Although neurological complications are rare, they are important because they can cause severe morbidity and mortality. Potential susceptibility of these patients should be considered during major surgery, especially when rapid fluid shifts, electrolyte imbalance, or other neurological problems may occur. Poor outcomes can be prevented with increased awareness of these complications and careful patient evaluation. Careful preoperative assessment may help anesthesiologists prepare for potential problems during anesthesia.

References

Dermal Tophus: A Complication of Gout in a Kidney Transplant Recipient

Ebru Hatice Ayvazoglu Soy,1 Emre Karakaya,1 Arzu Karatas Togral,2 Aydincan Akdur,1 Gokhan Moray,1 Mehmet Haberal1

Abstract
Gout is a chronic metabolic disease caused by disturbance of purine metabolism that leads to hyperuricemia. Hyperuricemia prevalence after renal transplant is reported as 19% to 84% in different studies. Tophaceous gout in renal transplant recipients is a consequence of increased hyperuricemia. Although tophus formation in skin and soft tissues is an indicator of chronic gout (also referred to as tophaceous gout), tophi may be the first sign of gout. In this study, we report a case of a 62-year-old male renal transplant recipient who had tophi as the first clinical sign of gout. After confirming gout diagnosis, cyclosporine was changed to sirolimus, and allopurinol was added to therapy to decrease uric acid levels. In conclusion, hyperuricemia is a common complication in renal transplant recipients. Presentation might be atypical, and diagnosis can be challenging.

Key words: End-stage renal disease, Hyperuricemia, Tophaceous gout

Introduction
Gout is a chronic metabolic disease caused by disturbance of purine metabolism that results in hyperuricemia. Increased serum uric acid levels cause monosodium urate crystal deposition in joints, kidneys, and soft tissues and an inflammatory response.1 2 If gout is not recognized and treated, it progresses through 4 clinical stages: asymptomatic hyperuricemia, acute gout, interval gout, and chronic tophaceous gout. Chronic gout is associated with heavy alcohol intake, diuretic use, obesity, hypertension, and renal impairment.3

Hyperuricemia prevalence after renal transplant is reported as 19% to 84% in different studies.4 In renal transplant recipients, the prevalence of gout is more than in the general population and reported between 3.5% and 28%.5 Gout in renal transplant patients is described as more aggressive with early onset, fast tophaceous progression, and involvement of unusual joints such as the hip, shoulder, and sacroiliac joints. Tophaceous gout in renal transplant recipients is a consequence of increased hyperuricemia. Previous studies focused mostly on renal transplant with increased hyperuricemia due to cyclosporine use, impaired renal function, and diuretic therapy.6 8 The association between hyperuricemia and azathioprine or mycophenolate mofetil (MMF) use has not been described.

Although tophus formation in skin and soft tissues is an indicator of chronic gout, also referred to as tophaceous gout, tophi may be the first sign of gout. In this study, we report a case of a renal transplant recipient who had tophi as the first clinical sign of gout.

Case Report
A 62-year-old man was admitted to our clinic with gradually increasing multiple, painful subcutaneous nodular lesions. He received renal transplant from his cousin 2 years ago. The etiology of renal failure
was diabetes mellitus. Past medical history included hypertension and coronary artery disease. He received cyclosporine (2.5 mg/kg/d; body weight, 60 kg), MMF (30 mg/kg/d), and prednisolone (10 mg/d) since transplant. Laboratory studies showed that the level of creatinine was 1.4 mg/dL, urea was 32 mg/dL, and uric acid was 10 mg/dL (normal range, 3 to 7.2 mg/dL).

On physical examination, he had white, firm, painful subcutaneous lesions on the palmar aspect of the tips of the fingers, right ear helix, and right elbow (Figure 1). These white subcutaneous lesions were suspected to be gout tophi or dystrophic calcifications. For diagnosis, we performed punch biopsy of the lesions. Pathologic examination confirmed the presence of nodular conglomerates of amorphous material, surrounded by histiocytes. Polarized light microscopy showed needle-shaped monosodium urate crystals, consistent with tophaceous gout (Figure 2). Bacterial smears and cultures were negative. Radiographic examination of the hands revealed osteoporosis and vascular calcifications without any erosive defect or sign of osteomyelitis (Figure 3). After confirming the diagnosis of gout, cyclosporine was changed to sirolimus (3 mg/d), and allopurinol (300 mg/d) was added to therapy to decrease uric acid levels.

Discussion

It has been reported in several studies that the prevalence of gout, one of the oldest forms of arthritis, is increasing worldwide. A previous study showed a prevalence of gout of 1.4% (male:female ratio, 3.6:1).9 Disease prevalence increases with increased age, and is 4% in men aged 75 to 84 years. Although gout is characterized by chronic hyperuricemia, several risk factors (such as purine-rich alimentation, heavy alcohol intake, obesity, hypertension, diuretic therapy, and reduced renal clearance) also play a role in pathogenesis. After renal transplant, the prevalence of gout is more than in the general population, ranging from 3.5% to 28%.10 Immunosuppressive agents, such as

Figure 1. Tophaceous Gout

![Figure 1. Tophaceous Gout](image)

(A and B) Multiple firm, white papules and nodules on the digits of both hands. (C) Subcutaneous nodule on the right ear helix.

Figure 2. Tophaceous Gout

![Figure 2. Tophaceous Gout](image)

(A) Nodular conglomerates consisted of amorphous material surrounded by histiocytes. (B) Polarized light microscopy showed needle-shaped monosodium urate crystals.
cyclosporine and tacrolimus, play a role in inducing hyperuricemia and gout in renal transplant recipients.\textsuperscript{11} Cyclosporine induces renal arterial vasoconstriction and causes a decrease in tubular urate secretion. The mean time between transplant and onset of gout ranges from 12 to 72 months, with an earlier onset with cyclosporine use.\textsuperscript{10} Our patient had a long history of hypertension that had been treated with different diuretics for several years, and he also used cyclosporine for 12 years.

Untreated gout progresses through 4 clinical stages. Asymptomatic hyperuricemia progresses to acute gout attacks as monoarthritis involving mostly the first metatarsophalangeal joint. Pain, swelling, redness of the joints, onset in early morning, and progression during the following 24 to 48 hours are pathognomonic features of these attacks. Fever and leukocytosis can accompany these findings. Dactylitis can develop and may take weeks to resolve. With time, attacks become more frequent and involve more joints. The last stage of the disease is tophus formation. Our patient had these tophi for 5 years without any joint involvement. As in our patient, in rare and exceptional cases, gout tophi may be the first symptom of the disease. Tophi can manifest at any location, preferentially at the distal interphalangeal joints, olecranon bursa, dorsal surface of the proximal interphalangeal joints, metacarpophalangeal joints, and dorsum of the toes. Although finger pads are less commonly involved, tophi were observed mostly on the finger pads in our patient.\textsuperscript{2} Differential diagnosis of gout depends on the stage of the disease. When evaluating arthralgia, arthritis, or myelopathy in a transplanted patient, the possibility of gout should be considered.

Treatment of gout in transplant recipients is challenging due to the potential for drug interactions and adverse events. Colchicine and/or nonsteroidal anti-inflammatory drugs are standard treatment for acute attacks of gout. Systemic corticosteroids or intraarticular corticosteroid injections are more suitable in renal transplant recipients. Xanthine oxidase inhibitors also are effective, especially for prophylaxis of gout. Azathioprine metabolism is xanthine oxidase-dependent, and coadministration of azathioprine and cyclosporine may increase the risk of bone marrow toxicity; a change from azathioprine to MMF can be considered in this case. Treatment in our patient included allopurinol and a change from cyclosporine to sirolimus because he was using MMF.

In conclusion, hyperuricemia is a common complication among renal transplant recipients. Clinical gout usually occurs several years after renal transplant. The presentation might be atypical, and diagnosis can be challenging. Treatment of renal transplant patients for gout should be adjusted individually due to possible drug interactions and the risks of adverse events.

References

Prevalence and Outcome of Herpes Zoster Infection in Renal Transplant Recipients

Mahir Kırnap,1 Aydıncan Akdur,1 Hatice Ebru Ayvazoğlu Soy,1 Hande Arslan,2 Sedat Yıldırım,1 Gökhan Moray,1 Mehmet Haberal1

Abstract

Objectives: Varicella zoster virus (VZV) is an important pathogen after renal transplant. The aim of this study is to assess the outcome of disseminated Varicella zoster virus infection in renal transplant recipients and to determine potential risk factors for mortality.

Materials and Methods: From January 2001 to January 2014, we performed 1614 renal transplants at our institution. Varicella zoster virus infection was diagnosed in 41 patients (2.5%). Median time of diagnosis of Varicella zoster virus was 5 years after transplant (range, 3 mo to 13 y).

Results: Thirty-seven patients (90%) had dermatomal distribution of Varicella zoster virus, 4 patients (10%) had disseminated Varicella zoster virus infection. After diagnosis of Varicella zoster virus immunosuppressive therapy was reduced and patients received acyclovir. Cutaneous lesions were healed with a scar in 7 cases (17%). Two patients (5%) developed postherpetic neuralgia. Seventy percent of cases were diagnosed within 5 years, and 92% were diagnosed within 10 years after transplant. Mortality due to Varicella zoster virus was 2% (n = 1). Visceral involvement found to be a risk factor for mortality. Prophylactic acyclovir or gancyclovir therapy following transplantation reduced Varicella zoster virus infection. However, Varicella zoster virus seropositivity did not influence fatal outcome.

Conclusions: Early initiation of antiviral therapy may prevent development of complication and visceral dissemination of disease. Active immunization should be applied for all seronegative patients before organ transplant.

Key words: Varicella zoster virus, Infection, Transplant

Introduction

Varicella-zoster virus (VZV) is a human, double-stranded DNA herpesvirus. Primary infection presents as acute varicella or chicken pox, a usually benign illness in children acquired from direct contact with a skin lesion or exposure by airborne spread from respiratory droplets. After primary infection, VZV establishes latency in dorsal root ganglia, and can reactivate years or decades later as herpes zoster (HZ) or shingles.1,2 The risk of shingles increases with altered cell-mediated immune responses, which occur naturally as a result of aging or in immunocompromised patients. In solid-organ transplant recipients, the incidence of reactivation is 10- to 100-fold higher than the general population, ranging from 1% to 12%.3,4 The typical clinical presentation of zoster is a painful, localized, unilateral, vesicular rash involving ≤ 2 adjacent dermatomes.2

The VZV is an important pathogen after renal transplant. The risk of an individual for development of infection after renal transplant is determined by the relation between the epidemiologic exposure of the individual and the state of immunosuppression that determines the individual’s susceptibility to infection.5 Infection with VZV causes 2 clinically different forms of disease: (1) primary disease (varicella or chicken pox), characterized by vesicular lesions on the trunk, head, or extremities; and (2) HZ...
(shingles), characterized by a painful unilateral vesicular eruption that rarely may be disseminated. The aim of this study was to assess the outcome of disseminated VZV infection in renal transplant recipients and to determine potential risk factors for mortality.

**Materials and Methods**

**Patients**
From January 2001 to January 2014, we performed 1614 renal transplants at our institution. The VZV infection was diagnosed in 41 patients (2.5%). Median time of diagnosis of VZV was 5 years after transplant (range, 3 mo to 13 y).

**Data collection**
The following information was collected: age, sex, date of transplant, cause of nephropathy, induction therapy, immunosuppressive regimen, treated rejection episodes, pretransplant VZV serologic status, presentation of VZV disease (including fever, cutaneous, gastrointestinal, and neurologic symptoms), complications (including hepatitis, pancreatitis, pneumonitis, neurologic problems, and disseminated intravascular coagulation [DIC]), antiviral and immunoglobulin (Ig) treatment, time from transplant to infection, time from onset of symptoms to treatment, and outcome (death or favorable outcome). The pretransplant VZV serologic status was recorded as seropositive, seronegative, or unknown and was based on laboratory testing for VZV immunoglobulin G (IgG). The diagnosis was made on clinical grounds and/or VZV seroconversion. The study was approved by the Ethics Committee of Medicine of Baskent University.

**Immunosuppressive protocols**
The immunosuppressive protocols included anti-proliferative drugs, steroids, tacrolimus, and cyclosporine. Steroid dosage at transplant was 0.5 mg/kg/d and was tapered to the maintenance dosage of 0.05 mg/kg/d. Induction treatment was used occasionally (18.4% patients). Rejection episodes were treated with steroids (10 mg/kg) that were given in 3 to 5 boluses.

**Statistical analyses**
We used descriptive statistics. Results were presented as median (percentile 25-percentile 75) or number (%). The 2-tailed *t* test or Mann-Whitney test was used for comparison between groups, depending on the distribution. Patient survival rates were calculated according to Kaplan-Meier method. Statistical analyses were performed using statistical software (SPSS for Windows, Version 18.0, SPSS Inc., Armonk, NY, USA). All tests were 2-tailed, and *P* ≤ .05 was considered significant.

**Results**
There were 37 patients (90%) who had dermatomal distribution of VZV and 4 patients (10%) who had disseminated VZV infection (Figure 1). After diagnosis of VZV, immunosuppressive therapy was reduced and patients received acyclovir. Cutaneous lesions were healed with scar in 7 patients (17%). There were 2 patients (5%) who developed postherpetic neuralgia. Most (70%) patients were diagnosed within 5 years, and 92% patients were diagnosed within 10 years after transplant. Mortality due to VZV occurred in 1 patient (2%). Visceral involvement was a risk factor for mortality. Varicella-zoster virus prevalence is reported between 1% and 12% in the literature; however our center VZV prevalence was reduced to 1% with ganciclovir and acyclovir therapy after transplant. However, VZV seropositive status did not affect fatal outcome (Table 1).

Serologic information about previous immunization status was available in 30 patients; 5 patients had VZV IgG prior to admission, and 11 patients were not immune to VZV. Symptoms at presentation and visceral complications occurred equally in both groups. There was no difference in mortality between patients with primary varicella and patients with reactivating VZV (not significant).

**Discussion**
The VZV infection is a rare but potentially serious complication in renal transplant recipients. Lethal outcomes of VZV infection have been reported. Our results demonstrated a low prevalence of VZV infection in renal transplant recipients (3.51%) compared with other studies that reported a prevalence of 3% to 10%. Previous studies showed that female sex was a risk factor for developing HZ in liver transplant recipients, but 63% renal transplant patients who developed VZV infection were male.
The frequency and intensity of VZV infection is associated with the intensity of immunosuppression. Introduction of mycophenolate mofetil to immunosuppressive protocols improved graft survival. However, an increased incidence of different viral infections was reported. According to our results, introduction of mycophenolate mofetil to immunosuppressive protocols resulted in higher incidence and severity of VZV disease.

Early therapy of choice is oral acyclovir and reduction of mycophenolate mofetil dose. We believe that the dosage adjustment and finding an upper limit of the therapeutic range of mycophenolic acid, above which the risk of different viral infections is increased, should be determined for mycophenolate mofetil therapy at least in patients who received increased immunosuppression early after transplant.

There were 70% patients who were exposed to intensive immunosuppressive treatment before VZV infection; they had an induction drug, steroid bolus therapy for treatment of acute rejection, or a high calcineurin inhibitor concentration. This is consistent with previous observations that intensive immunosuppression is a risk factor for development of VZV infection. There were 4 cases (10%) of disseminated HZ recorded, which is a higher proportion than observed in other studies. A mortality rate of 34% was described in patients with disseminated HZ. We have 1 mortality due to disseminated VZV infection, and this low number was probably due to fast recognition of the infection and initiation of antiviral therapy.

Risk factors for mortality included a longer time between transplant and VZV infection. This may reflect a longer duration of time under immunosuppression, and possibly a follow-up less rigid than early after transplant. Primary varicella potentially has a fatal outcome in immunocompromised patients. In cases with VZV reactivation, physicians can be wrongfully reassured by the previous immune status of the patient. Although limited by publication bias, this study emphasizes the possible fatal outcome in patients who have disseminated reactivation, which places these patients at risk of mortality equal to patients who present with acute varicella.

We reported a low rate of postherpetic neuralgia (5%) in our patients but a high rate of cutaneous scarring (17%). Other authors reported postherpetic neuralgia in 42.7% solid-organ recipients. Active immunization for VZV-seronegative patients before transplant should be performed. High-dose acyclovir therapy and reduction of immunosuppression are primary treatment for VZV infection. Timely initiation of therapy may prevent development of complications and the visceral form of disease. Based on our experience with the development of chickenpox, we suggest active immunization for all seronegative patients before organ transplant.
References

Minimal-Access Kidney Transplant

Mohie E. Omar

Abstract

Objectives: Minimal-access kidney transplant is not a new approach, however this approach is a good option for obese patients because access is difficult and often associated with wound complications and prolonged recovery.

Materials and Methods: Minimal-access kidney transplant uses an inguinal incision that is placed 4–6 cm above the pubic bone that extends to 2.5-cm lateral to the mid-inguinal point. Once the skin and subcutaneous tissues are opened, the external oblique is split in the same direction as the wound. Then, the oblique and transverse abdominal muscles are split at the lateral edge of the wound, the abdominal muscles are separated from the lateral border of the rectus muscle, and the inferior epigastric vessels and round ligament are tied and cut. Mobilizing the peritoneum upward exposes the iliac vessels. Then, dissect the space between the urinary bladder and rectus muscle to create a pouch, which accommodates the kidney. The renal vessels should be clearly visible, while the kidney itself is hidden in the subrectus pouch. Suitable retractors are needed to perform these procedures. Then, arterial anastomosis can be performed. The clamps are released after testing the arterial and venous anastomoses. After securing hemostasis, the kidney can be left in the pouch, rotated laterally, or remain in the middle of the wound. Close only the external oblique muscle.

Results: This technique requires minimal assistance and a small incision. An illustrative photo and diagram are included along with the full demographic data of the patients.

Conclusions: Engrafting kidneys into obese patients via the minimal-access approach is feasible, safe, and demonstrates comparable outcomes to other methods; however, more studies are needed.

Key words: Renal transplant, Minimal access

Introduction

Minimal-access kidney transplant is not a new approach; however this approach is suitable for obese patients because access is difficult and often associated with wound complications and prolonged recovery.

Surgical technique

Minimal-access kidney transplant requires an inguinal incision 4 to 6 cm above the pubic bone to 2.5-cm lateral to the mid-inguinal point. Once the skin and subcutaneous tissue are opened, the external oblique is split in the same direction as the wound. The oblique and transverse abdominal muscles are then split at the lateral edge of the wound and separated from the lateral border of the rectus muscle, and the inferior epigastric vessels and round ligament are tied and cut. The peritoneum is then mobilized and the iliac vessels are dissected. Next, dissect the space between urinary bladder and rectus muscle to create a pouch that will accommodate the kidney during anastomosis. The renal vessels should be clearly visible, while the kidney itself is hidden in the subrectus pouch. Suitable retractors are essential to perform these procedures. Start by performing arterial anastomosis, which can be performed using the classic eversion
technique or from within. Venous anastomosis can be performed from within. The clamps are released after testing the arterial and venous anastomoses for any bleeding. Hilar bleeding points can be very easily accessed when the kidney is in subrectus position. After securing hemostasis, the kidney can be left in the pouch, laterally rotated, or maintained in the middle of the wound. The external oblique muscle is the only muscle that is closed.

Discussion

The hockey stick, inguinal Gibson, and midline incisions are standard incisions used during kidney transplant.\textsuperscript{2,3}

Surgeons have tried to use minimal-access surgery to perform transplants, however hurdles include the complexity of the required vascular anastomoses (which requires ample space to achieve adequate precision) and preventing graft thrombosis. Second, the short vessels associated with living donor and right kidneys are well-recognized surgical challenges of kidney transplant. Various approaches that use minimal incisions have been suggested as ways to achieve good accessibility and accuracy. The first approach was published in 2006 by Øyle, who suggested using a small groin incision (7-9 cm) to place the kidney in the lateral extraperitoneal space; anastomoses can then be performed from within.\textsuperscript{4} The second minimal approach was reported by Mun, who suggested using a laparoscopically assisted technique to help visualize the anastomosis within the narrow operating field.\textsuperscript{5} Then, the total laparoscopic renal transplant technique came to match the laparoscopic donor nephrectomy.\textsuperscript{6}

All minimal techniques share certain contraindications, including obesity, atherosclerotic vessels, repeat transplant, and a deep pelvis, and are associated with certain difficulties such as controlling bleeding points that result from the intrinsically limited maneuvers required to access the kidney from different angles. Deceased-donor kidneys are seldom used because they are associated with bleeding from the fat around the hilum.

Conclusions

Placing the kidney in the subrectus space while performing anastomosis allows the surgeon to accurately and safely perform kidney transplant via a small incision.

References

The 2-Stage Liver Transplant: 3 Clinical Scenarios

Ender Gedik,1 Murat Bıçakçıoğlu,2 Emrah Otan,3 Hüseyin İlksen Toprak,4 Burak Işık,3 Cemalettin Aydın,3 Cüneyt Kayaalp,3 Sezai Yılmaz3

Abstract
The main goal of 2-stage liver transplant is to provide time to obtain a new liver source. We describe our experience of 3 patients with 3 different clinical conditions. A 57-year-old man was retransplanted successfully with this technique due to hepatic artery thrombosis. However, a 38-year-old woman with fulminant toxic hepatitis and a 5-year-old boy with abdominal trauma had poor outcome. This technique could serve as a rescue therapy for liver transplant patients who have toxic liver syndrome or abdominal trauma. These patients required intensive support during long anhepatic states. The transplant team should decide early whether to use this technique before irreversible conditions develop.

Key words: Deceased donor, End-stage liver disease, Living donor, Retransplant, Toxic liver syndrome

Introduction
A 2-stage liver transplant involving total hepatectomy and temporary portacaval shunt requires intensive perioperative care, especially during prolonged anhepatic periods. The main goal of this technique is to enable more time to obtain a new liver source. This procedure was first performed in 1988 for a patient with multiorgan failure including graft failure.1 The main indications are acute conditions such as fulminant hepatic failure, primary graft failure, severe hepatic trauma, and spontaneous hepatic rupture secondary to HELLP syndrome (syndrome of hemolysis, elevated liver enzymes, and low platelet count) and preeclampsia.1,2

The most important indication for this technique is toxic liver syndrome, characterized by total hepatic necrosis that mimics systemic inflammatory response syndrome. Severe cardiorespiratory failure, neurologic dysfunction, and acute kidney injury accompany this syndrome.1,3 The necrotic liver is removed at the first stage and, when available, liver transplant is performed at the second stage.

We describe our experience with 3 patients who had 3 different clinical conditions. All patients were treated at Inonu University from 2011 to 2013.

Case Report

Case 1
A 57-year-old man underwent living-related donor liver transplant due to hepatitis B virus and hepatocellular carcinoma. After successful liver transplant, he was admitted to the transplant intensive care unit on mechanical ventilation. The next day, he was evaluated by Doppler ultrasonography and had hepatic artery thrombosis. He had reoperation with laparotomy on day 2, and we decided retransplant. However, there was no additional liver source. His liver was totally excised, and portacaval shunt was performed. After a 48-hour anhepatic phase, he was retransplanted from a deceased donor. There was no need for extracorporeal therapy because standard treatments were sufficient. He was discharged from the hospital on day 15 after the second liver transplant and had a good outcome.
Case 2
A 38-year-old woman was admitted to our center. A living-donor liver transplant due to fulminant toxic hepatitis caused by ingestion of unknown amounts of wild mushrooms was necessary, but a donor graft was not available. Perioperative treatment included high-dose inotrope therapy for hypotension and continuous venovenous hemofiltration (CVVHF) for acute kidney injury. We decided to perform total hepatectomy and portacaval shunt due to her near-fatal status. The purpose of this option was to attempt to prevent damage to the body by the nonfunctional liver and immunologic reactions. The patient was acidotic, hypotensive, anuric, and hypothermic during the operation. After the operation, extracorporeal liver support was initiated (Molecular Adsorbent Recycling System [MARS], Gambro, Baxter Corp, Stockholm, Sweden) and high-dose vasopressor therapy, fluid therapy, and CVVHF were continued. However, she died 12 hours after surgery.

Case 3
A 5-year-old boy underwent urgent deceased-donor liver transplant due to abdominal trauma caused by a fall down stairs. He had been operated due to severe liver laceration at another center 1 year earlier. After liver transplant, he was discharged from the hospital with good outcome. After 11 months, he was admitted to the transplant intensive care unit. He had poor health status and severe jaundice. Erythrocyte suspension, albumin infusion, and plasma exchange were started. He was diagnosed with severe biliary stricture. Percutaneous transluminal cholangiography was performed and a biliary drainage catheter was inserted. Liver biopsy was performed and he was diagnosed with rejection. A living-related retransplant was performed. During the first postoperative week, hepatic artery thrombosis was diagnosed and he was reoperated. At reoperation, we observed that the total liver blood supply was interrupted. However, there was no additional liver source for retransplantation. The only available option was total hepatectomy and temporary portacaval shunt. In the intensive care unit, extracorporal liver support (MARS, Gambro) therapy and CVVHF were performed. Urgent liver notification was performed, and he was successfully retransplanted with a deceased-donor graft 50 hours later. However, during the retransplant operation, he developed refractory lung edema. He was readmitted to the transplant intensive care unit. He was ventilated using the airway pressure release ventilation mode. Maximal doses of vasopressor therapy and CVVHF were continued. However, he died 6 hours after surgery due to severe cardiorespiratory collapse.

Discussion
We described our experience with two-stage liver transplant. All patients had different clinical scenarios. They had total hepatectomy and temporary portacaval shunt. Case 2 did not have a liver transplant because a donor was not available. Case 1 and case 3 had retransplant with deceased-donor grafts after total hepatectomy. Despite liver transplant in case 3, he died 6 hours after surgery. Case 1 was discharged from the hospital with a good prognosis.

The 2-stage liver transplant procedure has been used since 1988. There are conflicting results reported with this procedure. Some studies showed that portacaval shunt may increase perioperative mortality. Other studies reported that survival is increased when liver transplant is available. In the previously described indications for this procedure, 2 indications are important: toxic liver syndrome and hepatic rupture with marked bleeding. Treatment of toxic liver syndrome mainly is supportive and includes mechanical ventilation, vasoactive agents, diuretics, and renal replacement therapy.

Acute hepatic necrosis with multiorgan failure has a mortality rate of approximately 100%. The etiology of multiorgan failure is not explained clearly. The pathophysiologic mechanism is based on metabolites and endogenous vasoactive substances that are released from the toxic liver. Experimental studies have shown the effects of cardiosuppressor agents in ischemic and anoxic conditions. Toxic liver syndrome occurs in acute liver failure and decompensating chronic liver disease. Necrotic liver should be removed and transplant should be performed before irreversible changes occur from multiorgan failure. The main purpose of this strategy is hemodynamic and metabolic stabilization of the patient before transplant. A previous study described a 6-month-old baby with
living-related transplant and recommended this procedure to provide additional time in treating emergency conditions.6

Various clinical studies have shown that total hepatectomy and portacaval shunt may provide hemodynamic and metabolic stabilization of patients by portal venous drainage. The most commonly used shunt is the transjugular intrahepatic portosystemic shunt. Varotti and coworkers reported an alternative temporary porto-middle hepatic vein shunt technique for 2-stage liver transplant.9

As a result of 2-stage liver transplant, there is longer anhepatic time. There is no consensus about the safest duration of the anhepatic period. A goal of two-stage liver transplant technique is to minimize the anhepatic period as much as possible. The reported anhepatic times vary between different case reports (Ringe and associates, 41 h; Bentdal and coworkers, 60 h; Patrono and associates, 6.58-72.5 h).3,6,7 Anhepatic times > 48 hours are associated with poor outcomes. Arora and coworkers reported anatomic anhepatic time 67 hours for a patient with primary nonfunction, and this is the longest anhepatic time in the literature.10 Anhepatic times < 30 hours are well tolerated.5

There are several problems during the anhepatic period, and patients require maximal intensive care. The common problems include hypoglycemia, hypothermia, hypocalcemia, oliguria, acute kidney injury, and coagulation disorders.5 Existing problems may contribute to worsening of the anhepatic state. Treatment options include intravenous glucose replacement for hypoglycemia and heating the patient with blankets and warm intravenous fluids and blood products for hypothermia.5

The risk of rapid development of acute kidney injury and oliguria is common in the first 24 hours. Early acute kidney injury predicts poor outcome and sepsis. Previous workers reported that urine output increased after total hepatectomy but decreased at 30 hours, and they recommended diuretic therapy and CVVHF.2,8 Therapy with CVVHF improves metabolic and fluid imbalance in these patients.1 All 3 patients in the present study received diuretic therapy, and 2 patients received CVVHF.

The citrate load due to administration of blood and blood products may cause hypocalcemia-induced coagulation disorders in long anhepatic states. Metabolic acidosis, anemia, hypomagnesemia, and hypothermia may contribute to coagulation defects.2 Laboratory evaluation of these disorders may reflect poor status, but these defects may be improved with fresh frozen plasma and fibrinogen replacement.2,5 Our team successfully treated coagulation defects in our patients.

Sepsis and multiorgan failure are the main causes of mortality from prolonged anhepatic periods. Sepsis is more fatal later in the course. Strict isolation techniques, routine surveillance cultures, and antibiotic prophylaxis for surgery are useful precautions against sepsis.2,5 The mortality rate of sepsis was 25% in the case series of Ringe and coworkers.3 Our patients had no sepsis, but case 2 died from multiorgan failure.

Extracorporeal support of the liver is another important option in 2-stage liver transplant. Nonbiologic systems (MARS, Gambro) may remove toxic substances, provide time to plan for liver transplant, and reduce intracranial pressure.2 We used this therapy in cases 2 and 3, but case 3 did not have enough time for this therapy due to refractory lung edema.

In summary, we described our limited experience with 2-stage liver transplant. This technique could serve as rescue therapy for liver transplant patients with toxic liver syndrome or abdomino trauma. These patients require intensive support in long anhepatic states. The transplant team should decide about using this technique in a timely manner before irreversible conditions develop.

References


Extracorporeal Membrane Oxygenation After Living-Related Liver Transplant

Ender Gedik,1 Muhammet Reha Çelik,2 Emrah Otan,3 Olcay Murat Dişli,4 Nevzat Erdil,4 Yaşar Bayındır,5 Ramazan Kutlu,6 Sezai Yılmaz3

Abstract

Various types of extracorporeal membrane oxygenation methods have been used in liver transplant operations. The main indications are portopulmonary or hepatopulmonary syndromes and other cardiorespiratory failure syndromes that are refractory to conventional therapy. There is little literature available about extracorporeal membrane oxygenation, especially after liver transplant. We describe our experience with 2 patients who had living-related liver transplant. A 69-year-old woman had refractory aspergillosis pneumonia and underwent pumpless extracorporeal lung assist therapy 4 weeks after liver transplant. An 8-month-old boy with biliary atresia underwent urgent liver transplant; he received venoarterial extracorporeal membrane oxygenation therapy on postoperative day 1. Despite our unsuccessful experience with 2 patients, extracorporeal membrane oxygenation and pumpless extracorporeal lung assist therapy for liver transplant patients may improve prognosis in selected cases.

Key words: End-stage liver disease, Pumpless extracorporeal lung assist, Extracorporeal support

Introduction

Extracorporeal membrane oxygenation (ECMO) has been a rescue therapy since 1965 in neonates, children, and adults. The main indication for ECMO therapy is support of cardiac and respiratory systems when conventional therapies have failed. There are 2 types of ECMO procedures. Venovenous ECMO (VV-ECMO) is used in patients with respiratory failure, and venoarterial ECMO (VA-ECMO) supports both cardiac and respiratory systems.1 Pumpless extracorporeal lung assist (PECLA) method is an arteriovenous method that uses the patient’s own cardiac output.2

Different ECMO methods have been used in liver transplant operations. The main indications for ECMO therapy with liver transplant are portopulmonary or hepatopulmonary syndrome and other types of cardiorespiratory failure refractory to conventional therapy. There is little literature available about ECMO, especially after liver transplant.3

We present our experience with 2 patients who were treated at Inonu University in 2013.

Case Reports

Case 1

A 69-year-old woman underwent successful living-related liver transplant due to autoimmune hepatitis. At 4 weeks after transplant, she was readmitted to the intensive care unit because of acute respiratory failure and sepsis. She was intubated and mechanically ventilated. Acinetobacter baumannii and Aspergillus fumigatus were isolated from a deep tracheal aspirate culture, and she was treated with antibiotics. On hospital day 5, bilateral pleural drainage tubes were inserted due to massive pleural effusion. Continuous venovenous hemofiltration (CVVHF) was initiated for acute kidney injury. Thoracic computed tomography scan showed bilateral aspergilloma. Her arterial blood gas values and clinical status deteriorated on conventional
mechanical ventilation. Rescue therapy with PECLA (iLA Membrane Ventilator, Novalung GmbH, Heilbronn, Germany) was planned. On hospital day 10, she had 15-French right arterial and 17-French left venous cannulas inserted into the femoral vessels with fluoroscopic guidance. She was anticoagulated with unfractionated heparin (activated clotting time, 180-200 s). The arterial blood gas values and lung mechanics markedly improved within 2 days. The CVVHF was continued simultaneously. Both therapies were continued for 7 days with lung improvement. There were no cannula-related or bleeding complications during ECMO therapy. However, she died on hospital day 27 due to refractory septic shock.

Case 2
An 8-month-old boy underwent urgent living-related liver transplant from his mother and hepatico-jejunostomy due to congenital extrahepatic biliary atresia. He had an acute fulminant onset of chronic liver failure, bilateral pneumonia, and hepatopulmonary syndrome. The surgical transplant team used the mother’s left lobe. After a successful, uncomplicated operation, he was admitted to the transplant intensive care unit. He was intubated and mechanically ventilated (biphasic positive airway ventilation mode). The Murray score was 3.

The arterial blood gas values and clinical condition deteriorated suddenly on postoperative day 1. He had marked arterial hypoxemia. A blood culture revealed carbapenem-resistant Klebsiella pneumoniae. Antibiotic therapy was started. On postoperative day 3, despite invasive mechanical ventilation and maximum inotropic therapy with dopamine, noradrenaline, and dobutamine, his cardiopulmonary status deteriorated and he was at risk for cardiac arrest. Therefore, we started urgent VA-ECMO therapy. Right 8-French common carotid arterial and 16-French internal jugular venous cannulas were inserted surgically in the operating room. The ECMO pump (Novalung) (flow rate, 0.8-1.0 L/min) was connected to the cannulas. He was heparinized (target activated clotting time, 180-200 seconds). He was transported to the transplant intensive care unit.

With high dose inotropic support and ECMO therapy, his cardiopulmonary status was improved on ECMO day 1. However, his clinical status deteriorated on ECMO day 2. We used airway pressure release ventilation mode. The vascular anastomoses of the graft were patent. Graft failure developed on ECMO day 3. There was no new liver transplant graft available. Despite maximum inotropic therapy and ECMO, he died on ECMO day 7 due to multiorgan failure and severe collapse. There were no cannula-related or bleeding complications during ECMO therapy.

Discussion
We described our unsuccessful experience with 2 cases after living-related liver transplant. Although ECMO is a well-known rescue method in critical care patients, there are limited data about perioperative ECMO therapy in liver transplant patients. Most reports include case series or retrospective studies. Park and associates reported the largest available series about ECMO in adult liver transplant patients. They performed 1076 liver transplants in 3 years, mostly (87.9%) living-related transplants, and 18 transplant recipients hadVV-ECMO therapy. There were 6 patients who had undergone deceased-donor liver transplant. In 7 patients, ECMO therapy was used preoperatively, and the other 11 patients received therapy within 1-61 months after transplant. The main indication was refractory respiratory failure due to pneumonia (12 patients) and acute respiratory distress syndrome (6 patients). The survivor group remained alive at 13-21 months after therapy. The authors concluded that ECMO was a rescue therapy for adult liver transplant patients with refractory pulmonary dysfunction.

Our patient (case 1) had refractory pulmonary dysfunction due to aspergillosis. We preferred PECLA in this case. The patient had no circulatory shock, she tolerated PECLA well, and she quickly responded to therapy. To our knowledge, she is the first reported PECLA case after liver transplant. There are several case reports about the use of postoperative VV-ECMO in adult liver transplant patients. Auzinger and coworkers advocated the use of ECMO for severe refractory hypoxemia after liver transplant for hepatopulmonary syndrome. They concluded that future well-designed multicenter studies are needed. They used circuits up to 3 weeks without systemic anticoagulation. This experience should be verified with future trials. We have no experience with VV-ECMO for liver transplant patients. Our patient who had ECMO...
The driving force of the system is the pressure gradient, and an external oxygen source. The main flow resistance, arterial and venous cannulas, a flow system includes a membrane oxygenator with low induced thrombocytopenia. At 7 days after transplantation due to biliary atresia and heparin-bridge therapy to transplant. The patient was discharged on day 48, and VV-ECMO was a bridge therapy to transplant.

There are limited data about pediatric ECMO cases. Landsman described a 3-year-old girl with liver failure due to biliary atresia and heparin-induced thrombocytopenia. At 7 days after transplant, the patient had laparotomy and hepatic artery thrombectomy, and heparin infusion was started; 3 days later, hepatectomy and temporary portacaval shunt were performed. At 19 hours after hepatectomy, the patient had retransplant and splenectomy with VV-ECMO support. This was the first reported case with VV-ECMO for urgent liver transplant; although 2-stage liver transplant and ECMO were used for this patient, she died 4 days later.

Son and associates described a 5-year-old girl with fulminant Wilson disease and severe pulmonary hemorrhage who underwent successful liver transplant following VA-ECMO; this was the first reported case with VA-ECMO for urgent liver transplant. Fleming and coworkers reported successful ECMO therapy for a 12-year-old male with hepatopulmonary syndrome after transplant. Fujita and associates described the youngest child in the literature reviewed by our team, a 10-week-old dependent child. Anesth Analg. 2010;111(5):1275-1278.

The first clinical use of PECLA was in 1996. This system includes a membrane oxygenator with low flow resistance, arterial and venous cannulas, a flow monitor, and an external oxygen source. The main driving force of the system is the pressure gradient between the arterial and venous circulation. The advantages of PECLA compared with conventional ECMO therapy include the lower risk of blood trauma and coagulation disorders with PECLA. Despite these advantages, blood flow is dependent on cardiac output. The PECLA allows lung protective ventilation and improves alveolar gas exchange. Diseased lung sections may have better recovery because mechanical ventilation can be downgraded, and iatrogenic lung injury from barotrauma and volutrauma can be reduced. The main indications for this technique are hypercapnic respiratory failure, acute respiratory distress syndrome, trauma, and life threatening influenza-like H1N1 infection. Flörchinger and coworkers reported 159 patients with acute respiratory distress syndrome and severe pneumonia; the mean support time was 8.5 days, 30-day mortality was 13.6%, and they concluded that the best indication for use of PECLA was severe hypercapnia and moderate hypoxia. Our patient who had PECLA (case 1) satisfied these indications.

In summary, despite our unsuccessful experience with these 2 cases, ECMO and PECLA therapy for liver transplant patients may improve prognosis in selected cases.

References


Blood Glucose Regulation During Living-Donor Liver Transplant Surgery

Ender Gedik,1 Hüseyin İlksen Toprak,2 Erdinç Koca,3 Taylan Şahin,4 Ülkü Özgül,2 Mehmet Özcan Ersoy2

Abstract

Objectives: The goal of this study was to compare the effects of 2 different regimens on blood glucose levels of living-donor liver transplant.

Materials and Methods: The study participants were randomly allocated to the dextrose in water plus insulin infusion group (group 1, n = 60) or the dextrose in water infusion group (group 2, n = 60) using a sealed envelope technique. Blood glucose levels were measured 3 times during each phase. When the blood glucose level of a patient exceeded the target level, extra insulin was administered via a different intravenous route. The following patient and procedural characteristics were recorded: age, sex, height, weight, body mass index, end-stage liver disease, Model for End-Stage Liver Disease score, total anesthesia time, total surgical time, and number of patients who received an extra bolus of insulin. The following laboratory data were measured pre- and postoperatively: hemoglobin, hematocrit, platelet count, prothrombin time, international normalized ratio, potassium, creatinine, total bilirubin, and albumin.

Results: No hypoglycemia was noted. The recipients exhibited statistically significant differences in blood glucose levels during the dissection and neohepatic phases. Blood glucose levels at every time point were significantly different compared with the first dissection time point in group 1. Excluding the first and second anhepatic time points, blood glucose levels were significantly different as compared with the first dissection time point in group 2 (P < .05).

Conclusions: We concluded that dextrose with water infusion alone may be more effective and result in safer blood glucose levels as compared with dextrose with water plus insulin infusion for living-donor liver transplant recipients. Exogenous continuous insulin administration may induce hyperglycemic attacks, especially during the neohepatic phase of living-donor liver transplant surgery. Further prospective studies that include homogeneous patient subgroups and diabetic recipients are needed to support the use of dextrose plus water infusion without insulin.

Key words: Dextrose, Insulin, Recipient, Operation

Introduction

Living-donor liver transplant (LDLT) is an important treatment option for end-stage liver disease (ESLD). With the introduction of this procedure, the number of patients awaiting liver transplant has decreased in recent years.1 Similar to other major and long-lasting surgical operations, patients who undergo LDLT have a significant risk of hyperglycemia. The hyperglycemic effect of these operations may be stress-induced or persistent,2,3 and patients with preoperative diabetes mellitus experience greater difficulties.2,4

The detrimental effects of hyperglycemia have been well demonstrated in medical and surgical intensive care unit patients and during the perioperative period. Hyperglycemia worsens patient prognosis, particularly after cerebrovascular events and cardiovascular surgery.2,5 The detrimental effects

From the 1Baskent University Faculty of Medicine, Department of Anesthesiology and Reanimation, Ankara, the 2Inonu University Faculty of Medicine, Department of Anesthesiology and Reanimation, Malatya, the 3Malatya State Hospital, Department of Anesthesiology and Reanimation, Malatya, and the 4Ersin Arslan State Hospital, Department of Anesthesiology and Reanimation, Gaziantep, Turkey

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Corresponding author: Ender Gedik, MD, Baskent University Faculty of Medicine, Department of Anesthesiology and Reanimation, 06490 Ankara, Turkey

Phone: +90 312 212 6868, ext. 4841 Fax: +90 312 223 7333

E-mail: gedikender@gmail.com


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of hyperglycemia in liver transplant patients include poor graft survival and increased numbers of infections.6-8 There is a paucity of data regarding blood glucose regulation in liver transplant patients, especially those who undergo LDLT. The vast majority of studies are abstracts of congress and retrospective studies performed in large centers.9,10

The management of hyperglycemia during the perioperative period is always a challenge to anesthesiologists, surgeons, and intensivists. Over the years and throughout the literature, controversy has surrounded the blood glucose target levels and methods for controlling it. The majority of clinicians have accepted the 2001 Leuven Intensive Insulin Therapy Trial as a landmark in this area.11 In this study, Berghe et al demonstrated that intensive insulin therapy (target blood glucose concentration of 80-110 mg/dL) improves the survival of cardiac surgical intensive care unit patients. This work led to many other controlled and uncontrolled studies in this area, with the majority reporting no difference with respect to mortality.2,12,13 Later, it was demonstrated that tight glucose control is associated with substantial dilemmas, such as hypoglycemia. Perioperative clinicians are well aware that hypoglycemia is more detrimental than hyperglycemia, especially in terms of the risk of neurologic sequelae.2,5 A large multicenter trial (Normoglycemia in Intensive Care Evaluation-Survival Using Glucose Algorithm Regulation, NICE-SUGAR) was recently published and demonstrated absolute increases in mortality and hypoglycemic attacks with intensive insulin therapy. The new target blood glucose level has again been changed to “nostalgic” levels (140-180 mg/dL).14

The liver plays a central role in regulating whole-body metabolism. Thus, ESLD leads to major alterations in glucose metabolism. In addition, complex interactions arise during liver transplant surgery with respect to these metabolic processes. Specifically, removal of the old liver and adaptation of the new liver disturbs glucose control during the intraoperative period.4,15

In this study, we aimed to compare the effects of 2 different regimens (5% dextrose in water and 5% dextrose in water plus insulin) on blood glucose levels during the dissection, anhepatic, and neohepatic phases of LDLT.

Materials and Methods

The study was approved by the Institutional Ethics Committee of the Medical Faculty of Inonu University. All of the protocols conformed with the ethical guidelines of the 1975 Helsinki Declaration. Written informed consent was obtained from all subjects. A total of 120 patients (14-64 years old, American Society of Anesthesiologists score II/III) scheduled for LDLT recipient surgery were enrolled and randomly allocated to the 5% dextrose in water (D5W) plus insulin (lispro, Humulin R, 100 IU/mL; Eli Lilly and Company, Indianapolis, IN, USA) infusion group (group 1, n = 60) or the D5W infusion group (group 2, n = 60) using a sealed envelope technique. The infusion for group 1 comprised 500 mL D5W plus 5 IU insulin and was continuously infused at 100 mL/h. The infusion of group 2 comprised plain 500 mL D5W and was continuously infused at 10 mL/h. The patient flow chart is shown in Figure 1. There was no potassium supplementation in either group. Intravenous methylprednisolone was administered intraoperatively at 250 and 100 mg during the dissection and anhepatic phases according to the Inonu University immunosuppressive protocol. All operations for this study were performed between October 2010 and August 2011. We excluded recipients who had previously undergone solid-organ transplant or had preoperative diabetes mellitus or acute liver failure.

All recipients were anesthetized using standard anesthetic techniques and monitored with devices including continuous 5-lead electrocardiography, pulse oximetry, capnography, a radial arterial line for blood pressure monitoring, nasopharyngeal temperature, and bispectral index monitoring (model A-2000TM, Aspect Medical System, Newton, MA, USA). A pulmonary artery catheter was placed when deemed necessary according to preoperative echocardiography pressure values. The internal jugular vein was used for central venous pressure monitoring. Large-bore antecubital vein catheters or central venous 8/8.5-F introducer sheaths were used for rapid fluid or blood and blood product replacement. Anesthesia was maintained using isoflurane or desflurane and remifentanil as an analgesic with cisatracurium as a muscle relaxant. All patients were mechanically ventilated (Dräger, Cato, Medizintechnik GmbH D-23542, Lübeck, Germany) with air/oxygen (50%/50%) to maintain an end-tidal carbon dioxide partial pressure of 30 to 40 mm Hg. Fluids, blood products,
and catecholamines were administered at the discretion of the attending anesthesiologist. The same surgical team performed all recipient operations. After the operation, all recipients were transferred to the intensive care unit. The same anesthesiologist collected all of the recipients' operative data.

The following patient and procedure characteristics were recorded: age (y), sex (male/female), height (m), weight (kg), body mass index (kg/m²), type of ESLD, Model for ESLD score, total anesthesia time (min), total surgical time (min), and number of patients who received an extra bolus of insulin. The following laboratory data were measured pre- and postoperatively: hemoglobin, hematocrit, platelet count, prothrombin time, international normalized ratio, potassium, creatinine, total bilirubin, and albumin.

Three phases of the recipient surgery were analyzed: the dissection phase, which was from the induction of anesthesia until portal vein cross-clamping; the anhepatic phase, from cross-clamping until unclamping of the portal vein; and the neohepatic phase, which was from portal vein unclamping until the end of skin closure. Blood glucose levels were measured and recorded 3 times during each phase with a calibrated blood glucose meter and glucose test strip (Lever Chek, TD-422; Muenster, Germany) using arterial blood samples. The target blood glucose level was 150 mg/dL. When the blood glucose level of a patient exceeded the target level, an extra bolus of insulin was administered by a different intravenous route (1 IU insulin for each 25 mg/dL increase in blood glucose concentration).

**Statistical analyses**

Data were presented as mean values ± SD. For continuous variables, the Mann–Whitney U test was used, and the chi-square or Fisher exact test was
adopted for categorical variables. For within-group analysis, the Wilcoxon signed-rank test was used. Differences were regarded as statistically significant when \( P < .05 \). All calculations were performed using SPSS 16.0 software (Armonk, NY, USA).

**Results**

The demographic, surgical, and anesthetic characteristics of the recipients are shown in Table 1. The patients in group 2 were younger than those in group 1 (\( P < .05 \)). Overall, 75% of patients in group 1 and 10% of patients in group 2 received an extra bolus of insulin (\( P < .05 \)). The other characteristics were similar between the 2 groups.

The intraoperative hematologic data are shown in Table 2. The mean platelet counts in group 2 were higher than those in group 1 at the end of surgery (platelet 2, \( P < .05 \)).

The intraoperative biochemical data are shown in Table 3. There were no statistically significant differences between the 2 groups.

The intraoperative blood glucose levels are presented in Table 4 and Figure 2. We observed significantly higher blood glucose levels in group 1 compared to group 2 during the anhepatic and neohepatic phases (\( P < .05 \)).

### Table 1. Recipient Characteristics

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Group 1 (( n = 60 ))</th>
<th>Group 2 (( n = 60 ))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>46.0 ± 12.7</td>
<td>38.8 ± 15.3*</td>
</tr>
<tr>
<td>Sex (male/female)</td>
<td>38/22</td>
<td>39/21</td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.7 ± 9.3</td>
<td>1.7 ± 11.2</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>69.0 ± 13.6</td>
<td>66.5 ± 16.7</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>24.4 ± 4.1</td>
<td>23.8 ± 5.4</td>
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<tr>
<td>Type of ESLD</td>
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<td></td>
</tr>
<tr>
<td>Cancer</td>
<td>9</td>
<td>6</td>
</tr>
<tr>
<td>Idiopathic</td>
<td>10</td>
<td>12</td>
</tr>
<tr>
<td>Metabolic</td>
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<td>2</td>
</tr>
<tr>
<td>Viral</td>
<td>30</td>
<td>35</td>
</tr>
<tr>
<td>Other</td>
<td>7</td>
<td>5</td>
</tr>
<tr>
<td>MELD score</td>
<td>14.5 ± 8.4</td>
<td>16.0 ± 8.1</td>
</tr>
<tr>
<td>Total anesthesia time (min)</td>
<td>524.3 ± 64.0</td>
<td>548.8 ± 71.6</td>
</tr>
<tr>
<td>Total surgical time (min)</td>
<td>545.3 ± 63.1</td>
<td>575.7 ± 69.5</td>
</tr>
<tr>
<td>Patients who received an extra bolus of insulin</td>
<td>45</td>
<td>6</td>
</tr>
</tbody>
</table>

**Abbreviations:** BMI, body mass index; ESLD, end-stage liver disease; MELD, model for end-stage liver disease

*Group 1 versus Group 2: \( P < .05 \).

### Table 2. Recipients’ Intraoperative Hematologic Data

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Group 1 (( n = 60 ))</th>
<th>Group 2 (( n = 60 ))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemoglobin 1 (g/dL)</td>
<td>11.0 ± 2.2</td>
<td>11.2 ± 2.0</td>
</tr>
<tr>
<td>Hemoglobin 2 (g/dL)</td>
<td>9.0 ± 1.9</td>
<td>9.5 ± 1.6</td>
</tr>
<tr>
<td>Hematocrit 1 (%)</td>
<td>32.9 ± 7.0</td>
<td>34.0 ± 6.2</td>
</tr>
<tr>
<td>Hematocrit 2 (%)</td>
<td>26.9 ± 5.7</td>
<td>28.7 ± 5.1</td>
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<tr>
<td>Platelet 1 (x 10^12/mL)</td>
<td>107.1 ± 84.0</td>
<td>116.0 ± 85.2</td>
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<tr>
<td>Platelet 2 (x 10^12/mL)</td>
<td>66.9 ± 43.4</td>
<td>89.0 ± 61.6*</td>
</tr>
<tr>
<td>PT 1 (s)</td>
<td>21.7 ± 15.2</td>
<td>18.9 ± 6.8</td>
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<tr>
<td>PT 2 (s)</td>
<td>39.1 ± 22.0</td>
<td>35.2 ± 16.5</td>
</tr>
<tr>
<td>INR 1</td>
<td>2.0 ± 1.4</td>
<td>1.7 ± 0.6</td>
</tr>
<tr>
<td>INR 2</td>
<td>3.7 ± 2.0</td>
<td>3.2 ± 1.4</td>
</tr>
</tbody>
</table>

**Abbreviations:** INR, international normalized ratio; PT, prothrombin time

*Group 1 versus Group 2: \( P < .05 \).

### Table 3. Recipients’ Intraoperative Biochemical Data

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Group 1 (( n = 60 ))</th>
<th>Group 2 (( n = 60 ))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Potassium 1 (mEq/L)</td>
<td>3.8 ± 0.6</td>
<td>4.0 ± 0.8</td>
</tr>
<tr>
<td>Potassium 2 (mEq/L)</td>
<td>3.9 ± 0.6</td>
<td>4.0 ± 0.8</td>
</tr>
<tr>
<td>Creatinine 1 (mg/dL)</td>
<td>0.8 ± 0.7</td>
<td>0.7 ± 0.4</td>
</tr>
<tr>
<td>Creatinine 2 (mg/dL)</td>
<td>0.8 ± 0.6</td>
<td>0.7 ± 0.4</td>
</tr>
<tr>
<td>Total bilirubin 1 (mg/dL)</td>
<td>4.8 ± 3.8</td>
<td>6.6 ± 4.9</td>
</tr>
<tr>
<td>Total bilirubin 2 (mg/dL)</td>
<td>4.2 ± 3.7</td>
<td>5.9 ± 4.8</td>
</tr>
<tr>
<td>Albumin 1 (g/dL)</td>
<td>2.7 ± 0.6</td>
<td>2.9 ± 0.7</td>
</tr>
<tr>
<td>Albumin 2 (g/dL)</td>
<td>1.8 ± 0.5</td>
<td>1.7 ± 0.5</td>
</tr>
</tbody>
</table>

**Abbreviations:** INR, international normalized ratio; PT, prothrombin time

*Group 1 vs. group 2: \( P < .05 \).

† N2 vs. N3: \( P < .05 \).

‡ any measurements versus D1: \( P < .05 \).

### Table 4. Recipients’ Intraoperative Blood Glucose Concentrations (mmol/L)

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Group 1 (( n = 60 ))</th>
<th>Group 2 (( n = 60 ))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dissection 1</td>
<td>6.46 ± 1.93</td>
<td>6.29 ± 3.69</td>
</tr>
<tr>
<td>Dissection 2</td>
<td>7.53 ± 2.11*</td>
<td>7.68 ± 3.22*</td>
</tr>
<tr>
<td>Dissection 3</td>
<td>7.89 ± 2.07*</td>
<td>7.33 ± 2.09*</td>
</tr>
<tr>
<td>Anhepatic 1</td>
<td>7.98 ± 1.94*</td>
<td>6.53 ± 1.92*</td>
</tr>
<tr>
<td>Anhepatic 2</td>
<td>7.77 ± 2.19*</td>
<td>6.41 ± 2.04*</td>
</tr>
<tr>
<td>Anhepatic 3</td>
<td>8.21 ± 2.29*</td>
<td>6.93 ± 2.33*</td>
</tr>
<tr>
<td>Neohepatic 1</td>
<td>9.59 ± 2.67*</td>
<td>8.07 ± 2.52*</td>
</tr>
<tr>
<td>Neohepatic 2</td>
<td>9.72 ± 2.64*</td>
<td>7.91 ± 2.33*</td>
</tr>
<tr>
<td>Neohepatic 3</td>
<td>9.42 ± 2.54*†</td>
<td>7.79 ± 2.14*†</td>
</tr>
</tbody>
</table>

*Group 1 vs. group 2: \( P < .05 \).

† N2 vs. N3: \( P < .05 \).

‡ any measurements versus D1: \( P < .05 \).

**Figure 2. Intraoperative Patient Blood Glucose Levels**

Blood glucose levels were significantly different \((P < .05)\) from those measured at the dissection 1 time point in group 1. With the exception of the anhepatic 1 and 2 measurements, blood glucose levels were significantly different \((P < .05)\) as compared with the dissection 1 time point in group 2. We performed intraphase (dissection, anhepatic, and neohepatic) analyses of blood glucose levels in both groups. During each phase, we compared the initial level with the other measurements (eg, dissection 1 with
dissection 2 and dissection 1 with dissection 3). The statistical analyses revealed that the only difference was between the neohepatic 2 and 3 measurements in group 1 ($P < .05$).

No patients developed hypoglycemia (blood glucose < 60 mg/dL). The mean blood glucose levels were 116 to 175 mg/dL in group 1 and 113 to 145 mg/dL in group 2.

**Discussion**

A total of 120 LDLT recipients were prospectively enrolled in this study. There was no intraoperative mortality. We found that isolated DW5 infusion was superior to DW5 plus insulin infusion in terms of hyperglycemia management.

Intraoperative hyperglycemia management has been studied for other major operations. Gandhi et al examined 400 cardiac surgery patients during the intraoperative period. They compared intensive insulin therapy with conventional treatment and found that intensive insulin therapy did not reduce perioperative death or morbidity. The mean glucose levels were approximately 111 mg/dL in both groups.

Cammu et al evaluated 20 patients who underwent tumor hepatectomy. They used the modified Atlanta protocol, and the lower and upper intraoperative blood glucose concentrations were set at 85 and 110 mg/dL. Blood glucose levels ranged from 65 to 176 mg/dL, indicating that the modified Atlanta protocol is a safe and efficient therapy.

Sato et al examined 52 patients who underwent major liver resection. They compared glucose plus insulin therapy with conventional treatment. In both groups, 27% of the patients were diabetic. The majority of glucose measurements from nondiabetic patients were in the range of 63 to 110 mg/dL in both groups. They concluded that glucose plus insulin therapy more effectively maintained normoglycemia than standard therapy.

Maeda et al examined 30 patients who underwent hepatic resection. They continuously measured blood glucose levels using an artificial pancreas. Both diabetic and nondiabetic patients were included in the study. They did not use insulin during surgery, and the Pringle maneuver was used for hepatic resection. Glucose levels immediately and profoundly increased after unclamping the hepato-duodenal ligament. The authors hypothesized that glucose variation after unclamping might involve glycogen breakdown within hepatocytes due to hypoxia.

The negative effects of hyperglycemia in liver transplant have been retrospectively evaluated in numerous studies. Ammori et al evaluated 184 diabetic and nondiabetic liver transplant recipients. They reported that intraoperative hyperglycemia was associated with an increased risk of postoperative infection and mortality. The authors selected a blood glucose level of 150 mg/dL as the threshold between strict and poor glucose control, which was also the target level in our study.

Park et al evaluated 680 diabetic and nondiabetic liver transplants. They began insulin infusion if the patient’s blood glucose concentration was > 200 mg/dL. Severe intraoperative hyperglycemia (blood glucose ≥ 200 mg/dL) was found to be an independent risk factor for postoperative surgical site infection.

Xia et al used a divided insulin dose regimen as an effective treatment for hyperkalemic patients during the dissection phase. The total patient population was 717, and 50 of the patients received the divided dose regimen. The authors found that this therapy was useful for preventing increased serum potassium levels. In our study, the patient potassium levels were within the normal range and comparable between the 2 groups.

Wallia et al retrospectively studied 113 liver and 31 kidney-liver recipients. The cutoff glucose concentration was 200 mg/dL. They reported that immediate postoperative hyperglycemia was associated with an increased risk of liver allograft rejection. Keegan et al examined 158 recipients and found that early postoperative insulin protocol use resulted in a mean glucose concentration of 149 mg/dL, which was comparable with our target level.

There is no universal cut-off for glucose deviations in surgical patients. There is some vigilance for diabetic surgical patients, but the preoperative prevalence rate of undiagnosed glycemic dysfunction is unknown. We examined nondiabetic recipients, and the main limitation of this study is the absence of a screening test for poor glycemic control, such as measuring hemoglobin A1c or lactate level.

Another limitation of our study is the difference between the mean ages of the groups. The patients in
group 2 were younger than those in group 1. Although we used a randomization process for patient selection, the use of larger patient populations should prevent this limitation in future studies. A final limitation is recipient heterogeneity according to ESLD type. Further studies can overcome this problem by including patients with similar types of ESLD (eg, only viral or metabolic).

There is some paucity of clinical data regarding intraoperative blood glucose regulation in liver transplant recipients. Previous studies were retrospective or designed to evaluate the perioperative period. In addition, the authors studied deceased donor recipients. Despite these limitations, our findings should inform future well-designed studies of intraoperative glycemic control during LDLT.

Patients with ESLD exhibit normal hepatic glucose production. However, these patients demonstrate exaggerated hyperglycemic and hyperinsulinemic responses following glucose challenge, which can induce peripheral insulin resistance. Hypoglycemia only occurs in the setting of acute liver failure.4 We excluded patients with acute liver failure from the present study, and there were no recipients with baseline hypoglycemia.

Progressive hyperglycemia occurs during the dissection and anhepatic phases despite concurrent insulin concentration increases.4 In our study, the recipients in group 1 exhibited a similar pattern, but the anhepatic 1 and 2 measurements were similar to the baseline levels in group 2 patients. We believe that this was due to concurrent insulin injection in group 1 patients; it is possible that the subjects developed resistance due to exogenous insulin administration.

Anesthesiologists who work with liver transplants are familiar with the hyperglycemic effects that occur when portal vein unclamping is followed by an immediate stepwise increase in glucose concentration. The effect of the reperfusion (neohepatic) phase on glucose levels has been demonstrated in previous studies. The sudden hyperglycemia is due to glucose release by the graft liver.4 Our recipients exhibited a similar pattern, but group 2 patients showed more acceptable blood glucose levels. A possible beneficial effect of plain DW5 infusion was again observed in group 2 patients.

Transplant recipients are at major risk for hyperglycemia. Additional investigations of hyperglycemia prevention and management are needed.10 We hope that our study will provide some knowledge in this area.

In conclusion, plain D5W infusion (10 mL/h) may effectively promote safer blood glucose levels as compared with D5W plus insulin infusion (100 mL/h) for LDLT recipients. Continuous exogenous insulin administration may induce hyperglycemic attacks, especially during the neohepatic phase of LDLT surgery. Further prospective studies that include homogeneous patient subgroups and diabetic recipients are needed to clarify this issue.

References


Abstract

Objectives: Hemodynamic monitoring is vital during liver transplant surgeries because distinct hemodynamic changes are expected. The continuous noninvasive arterial pressure (CNAP) monitor is a noninvasive device for continuous arterial pressure measurement by a tonometric method. This study compared continuous noninvasive arterial pressure monitoring with invasive direct arterial pressure monitoring in living-liver donors during transplant.

Materials and Methods: There were 40 patients analyzed while undergoing hepatic lobectomy for liver transplant. Invasive pressure monitoring was established at the radial artery and continuous noninvasive arterial pressure monitoring using a finger sensor was recorded simultaneously from the contralateral arm. Systolic, diastolic, and mean arterial pressures from the 2 methods were compared. Correlation between the 2 methods was calculated.

Results: A total of 5433 simultaneous measurements were obtained. For systolic arterial blood pressure, 55% continuous noninvasive arterial pressure measurements were within 10% direct arterial measurement; the correlation was 0.479, continuous noninvasive arterial pressure bias was -0.3 mm Hg, and limits of agreement were 32.0 mm Hg. For diastolic arterial blood pressure, 50% continuous noninvasive arterial pressure measurements were within 10% direct arterial measurement; the correlation was 0.630, continuous noninvasive arterial pressure bias was -0.4 mm Hg, and limits of agreement were 21.1 mm Hg. For mean arterial blood pressure, 60% continuous noninvasive arterial pressure measurements were within 10% direct arterial measurement; the correlation was 0.692, continuous noninvasive arterial pressure bias was +0.4 mm Hg, and limits of agreement were 20.8 mm Hg.

Conclusions: The 2 monitoring techniques did not show acceptable agreement. Our results suggest that continuous noninvasive arterial pressure monitoring is not equivalent to invasive arterial pressure monitoring in donors during living-donor liver transplant.

Key words: CNAP, Liver transplant, Living donors, Blood pressure

Introduction

During every surgical procedure irrespective of the type of anesthesia, it is mandatory to monitor the arterial blood pressure, heart rate, and oxygen saturation. Oxygen saturation and heart rate are measured continuously, but blood pressure is assessed every 3 to 10 minutes by a noninvasive intermittent oscillometric technique. However, in major surgery or high-risk patients, continuous arterial blood pressure measurement using an indwelling arterial line is preferred for close monitoring. In this respect, intra-arterial blood pressure (IABP) measurement is considered the best method.¹ However, placement of an arterial catheter...
is susceptible to several complications such as vascular or nerve trauma, hematoma, infection, and occlusion.1-3

For several decades, monitors for continuous noninvasive arterial pressure (CNAP) measurement have been developed, and various studies have investigated the accuracy of these devices and compared them with direct arterial pressure measurement. However, these noninvasive monitors have not yet been used in clinical anesthesia practice. Therefore, a beat-to-beat, noninvasive, and reliable technique for tracking arterial blood pressure is desirable.4

The first investigations for CNAP measurements were developed by Peñáz and coworkers in the 1970s.5 Recently, a device (Infinity CNAP, Draeger Medical Systems, Drager Medical GmbH, Lübeck, Germany) has been developed to provide CNAP measurements. Previous studies suggested that the CNAP monitor may be superior to the intermittent oscillometric measurements because it can detect rapid changes better in different procedures. The validation of the CNAP monitor has been performed in different procedures including cardiac angiography, abdominal surgery, cardiac surgery, and neurosurgery.6,7

During anesthesia for liver donation in living-donor liver transplant surgery, arterial catheters routinely are inserted for hemodynamic monitoring in our clinic. The aim of this study was to evaluate the agreement between simultaneous CNAP monitoring and invasive direct arterial pressure monitoring in donors during living-donor liver transplant.

Materials and Methods

Subjects
This study was approved by Baskent University Institutional Review Board and Ethics Committee (project number KA12/170). Written informed consent was obtained from all patients. For the present study, 40 adult patients undergoing hepatic lobectomy for living-donor liver transplant by a single team were recruited from February 2012 to March 2014 at the Baskent University Ankara Hospital. Exclusion criteria were the presence of vascular occlusion, surgery, or disease (such as Raynaud syndrome) of the upper extremities or an anatomic deformity of the distal forearm.

Procedure
After an 8-hour starving period, all patients were premedicated orally with midazolam (0.1 mg/kg) 1 hour before the induction of anesthesia. In the operating room, standard monitoring was applied to all patients including 5-lead electrocardiogram, noninvasive blood pressure monitor, and pulse oximetry. The anesthetic treatment was performed according to the standard procedures of our department with propofol, fentanyl, and non-depolarizing neuromuscular blockers (rocuronium). Cefazolin, methylprednisolone, and ranitidine were given at induction. After endotracheal intubation, mechanical ventilation was started using volume-controlled ventilation. Ventilation variables were adjusted (respiratory rate, 8-16/min; tidal volume, 4-7 mL/kg) to maintain pulse-oximetry saturation > 95% and end-tidal carbon dioxide pressure 32 to 38 mm Hg with sevoflurane (1-1.5 minimum alveolar concentration in 40% oxygen and 60% air mixture).

A central venous catheter was peripherally inserted into the brachial veins and a 20-gauge arterial catheter was inserted into the radial artery under general anesthesia for hemodynamic monitoring. All pressure lines were connected to a pressure transducer (Biometrix B.V., Breda, The Netherlands) and the transducer was placed and set to zero at the mid thoracic level. To eliminate errors from damping and frequency change, the natural frequency and damping coefficient for each system was determined by the flush method.8,9

After these monitoring settings, appropriately sized cuff CNAP sensors (Infinity CNAP, Draeger) were placed on the patient’s index and middle fingers of the same extremity as the arterial catheter. A brachial noninvasive blood pressure (NIBP) cuff for calibration was placed on the contralateral arm and measurements were performed at 30-minute intervals. A single finger cuff was inflated at a time, and inflation of the cuffs was rotated between the 2 fingers every 30 minutes. After all monitoring procedures were completed, all CNAP and IABP data were manually recorded simultaneously at 2-minute intervals for systolic, diastolic, and mean pressures.

Statistical analyses
All simultaneously recorded IABP and CNAP measurements were compared. The agreement criterion for the CNAP value was set to within ± 10%
IABP value. The accuracy of CNAP was assessed using analysis of bias and limits of agreement. Bias was the mean difference between CNAP and IABP. Differences were calculated by subtracting CNAP values from IABP values. The limits of agreement were described using the mean of the differences (mean ± SD) × 1.96. Pearson product moment correlation was used to assess the relation between CNAP and invasive IABP measurements, and Bland-Altman plots were constructed to assess the agreement between CNAP and invasive IABP measurements. Data analysis was performed using a statistical program (SPSS, Version 17.0, SPSS Inc., Chicago, IL, USA). Continuous variables were reported as mean ± SD.

Results

There were 40 patients who had donor hepatectomy for liver transplant and were enrolled in this study (Table 1). A total of 5433 concomitant paired measurement sets, with 1 set being a group of systolic, diastolic, and mean pressures by CNAP and IABP, were recorded and compared separately for systolic, diastolic, and mean arterial pressures. The average number of measurement sets per patient was 136 ± 41 in this study. There were no complications due to CNAP, and none of the patients reported any complaints about CNAP after surgery. The compatibility of direct arterial blood pressures and CNAP for systolic, diastolic, and mean pressures in terms of percentage difference, correlation, analyses of bias, and limits of agreement for all patients in combination were tabulated (Table 2). The corresponding Bland-Altman plots showed the comparison of measurements of systolic, diastolic, and mean arterial pressures with IABP and CNAP (Figure 1-3).

Table 1. Patient Characteristics*

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>34.9 ± 7.9 (24-53)</td>
</tr>
<tr>
<td>Sex (male/female)</td>
<td>19/21</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>72.9 ± 11.9 (49-104)</td>
</tr>
<tr>
<td>ASA physical status 1/2</td>
<td>7/33</td>
</tr>
<tr>
<td>Side of lobectomy (right/left)</td>
<td>14/26</td>
</tr>
</tbody>
</table>

*Abbreviations: ASA, American Society of Anesthesiologists
*N = 40 patients. Data reported as mean ± SD (range, minimum-maximum) or number.

Table 2. Comparison of Continuous Noninvasive Arterial Pressure and Direct Arterial Blood Pressures

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Blood Pressure</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Systolic</td>
</tr>
<tr>
<td>CNAP within 10% direct arterial blood pressure</td>
<td>55%</td>
</tr>
<tr>
<td>Correlation (r)</td>
<td>0.479</td>
</tr>
<tr>
<td>CNAP mean difference (mm Hg)</td>
<td>-0.3</td>
</tr>
<tr>
<td>Limits of agreement (± 2 SD)</td>
<td>32.0</td>
</tr>
</tbody>
</table>

*Abbreviations: CNAP, continuous noninvasive arterial pressure

Discussion

The use of living-donor liver transplant became increasingly popular because the number of deceased donors for solid-organ transplants was limited. Despite the benefits to the recipients, there is a risk of major complications, including mortality.
risk, for solid-organ donors.\textsuperscript{11} Therefore, donor safety and comfort is the major ethical issue. Reduction of invasive procedures contributes to increased patient safety. However, reducing these interventions can be accomplished only by replacing them with reliable and validated methods.\textsuperscript{3} In this report, we investigated the compatibility between 2 hemodynamic monitoring techniques including direct IABP and CNAP. The main result of our prospective, observational clinical trial was that CNAP monitoring was not equivalent to direct IABP monitoring in donors for living-donor liver transplant.

Several previous reports emphasized that > 20\% hypotensive episodes during surgery were missed and another 20\% were detected with a delay by NIBP.\textsuperscript{12} These findings indicate that closer monitoring should be done, especially in critically ill or high risk patients.\textsuperscript{3,12} Existence of important complications of arterial catheters has led to a search for devices that can make noninvasive and continuous measurement of blood pressure possible.\textsuperscript{1,3} The basic principle of these devices is the vascular unloading technique introduced first by Penáz and coworkers in 1973.\textsuperscript{13,14} In recent years, several devices have been developed for measuring blood pressure noninvasively and continuously. The CNAP device tested in this study uses volume unloading technology. This device monitors blood flow into the finger. By encircling finger cuffs, it senses the blood flow oscillations and converts them to waveforms and numeric values.

There are several studies that have compared CNAP and IABP, the best method, in different patient populations and have shown reliability of CNAP. Schramm and colleagues reported that CNAP could be safely used in older and high risk patients.\textsuperscript{7} Similar results were reported in other studies for CNAP use in the prone position, children, and intensive care patients who received inotropic support.\textsuperscript{3,15} However, Gayat and associates showed that CNAP rapidly reflected changes in blood pressure but failed to be in concordance with arterial pressure measurements.\textsuperscript{16} Ilies and associates showed in another study that measurements obtained by CNAP at different stages of anesthesia were unreliable.\textsuperscript{12}

In our study, CNAP was used in living donors during liver transplant for liver transplant. After anesthesia induction and patient stabilization, CNAP was properly placed and measurements were obtained. Invasive IABP and CNAP measurements obtained from the same monitor every 2 minutes were recorded manually. All patients were either young or middle-aged patients in ASA class 1 or 2. A total 5433 data sets for 40 patients were recorded. The comparison of CNAP and direct IABP measurements showed that the ratios of systolic, diastolic, and mean blood pressure measurements in 10\% safety limits were 55\%, 50\%, and 60\%. An analysis of data showed that CNAP measured blood pressure with limits of agreement ± 32.0, ± 21.1, and ± 20.8 mm Hg for systolic, diastolic, and mean arterial pressures. These limits were too wide to substitute CNAP for direct IABP values. The reason for this finding might be the hemodynamic changes during surgery, with an intraoperative change in mean arterial pressure of > 20\% to 30\% compared with preoperative values. Similar to our results, Findlay and coworkers reported that a noninvasive arterial blood pressure monitor (Vasotrac, Biopac Systems, Goleta, CA, USA) was not an accurate substitute for direct IABP monitoring during liver transplant surgery.\textsuperscript{17} The authors explained that the differences observed in their results, in contrast with other previously published studies, were because of the cardiovascular pathophysiology of their patients undergoing liver transplant; their patients had high cardiac output, low peripheral resistance, and alterations in arterial wall compliance that may affect the accuracy of the noninvasive (Vasotrac) measurements.\textsuperscript{17}

During the use of CNAP, finger sensor cuffs are placed on the fingers in turn to decrease pressure in
the fingers. We set this interval of rotation at 30 minutes. Ilies and associates revealed no findings regarding the adverse events of CNAP, but long-term use may be associated with vascular occlusion, ischemia, and pain. We did not observe such complications in our patients. After CNAP was placed on the patient’s arm, when movement of the sensors occurred, we observed that intrasensor pressure changes were directly reflected on the pressure traces on the monitor. The values obtained during these changes were omitted from the records. However, the frequency and causes of interruptions of the waves were not recorded.

Limitations of the present study include the unavailability of automatic recording of the data, the small number of measurements per patient, and not taking hypotensive attacks and arrhythmias into consideration.

In conclusion, we observed that CNAP and invasive IABP monitoring did not show acceptable agreement. The CNAP monitoring was not equivalent to invasive IABP monitoring in living liver donors during transplant.

References

Epstein-Barr Virus DNAemia in Iranian Liver Transplant Recipients and Assessment of Its Variation in Posttransplant Lymphoproliferative Disorder Patients by Quantitative Polymerase Chain Reaction Assay

Marzieh Jamalidoust,1,2 Bita Geramizadeh,3 Gholamreza Pouladfar,1 Mandana Namayandeh,1 Sadaf Asaie,1 Nasrin Aliabadi,1 Saman Nikeghbalian,4 Mazyar Ziyaeyan1

Abstract

Objectives: Epstein-Barr virus primary infection and/or reactivation may play a major role in the incidence of posttransplant lymphoproliferative disorder in organ recipients. We assessed Epstein-Barr virus viral load in liver transplant patients suspected of having Epstein-Barr virus/ post-transplant lymphoproliferative disorder at specified times after transplant and evaluated the clinical findings and posttransplant complications.

Materials and Methods: In the 696 patients who underwent liver transplant in this retrospective study, Epstein-Barr virus viral load was examined intermittently in 127 liver transplant recipients who were suspected to have Epstein-Barr virus infection/disease. Sampling was performed during 4 years from July 2009 to May 2013 using real-time polymerase chain reaction assay. Clinical and pathologic data were gathered by reviewing medical records.

Results: There were 78 of the 127 suspected patients (61%) who exhibited Epstein-Barr virus DNAemia and 19 patients had posttransplant lymphoproliferative disorder. The median EBV viral load of posttransplant lymphoproliferative disorder patients was significantly higher than unaffected patients. Posttransplant lymphoproliferative disorder was diagnosed clinically in 34 subjects (4.9%). Estimated mortality rate of posttransplant lymphoproliferative disorder patients was 35% during 1.5-year follow-up after transplant.

Conclusions: Monitoring Epstein-Barr virus load may enable detection of Epstein-Barr virus infection/disease in liver transplant patients suspected of having the virus, even several weeks before the onset of any clinical manifestations, especially in pediatric patients who have high incidence and mortality from posttransplant lymphoproliferative disorder.

Key words: DNA, Hepatic failure, Immunosuppression, Virology

Introduction

Epstein-Barr virus (EBV) is a member of Herpesviridae family, Gammaherpesvirinae subfamily, and Lymphocryptovirus genus and is known as Human herpesvirus 4.1,2 It can infect smooth muscle cells, natural killer cells, B lymphocytes, and T lymphocytes. This ubiquitous virus 90% adults worldwide.3-5 It is associated with different malignancies and disorders such as Hodgkin lymphoma, non-Hodgkin lymphoma, Burkitt lymphoma, nasopharyngeal carcinoma,
gastric carcinoma, and posttransplant lymphoproliferative disorders (PTLDs). 6,7

In immunocompetent individuals, EBV causes infectious mononucleosis, an acute but self-limited disease that affects children and young adults. Although the severity of infectious mononucleosis is not correlated with EBV viral load, immuno-compromised patients such as patients undergoing organ transplant may have EBV DNAemia that may cause a variety of clinical conditions including a nonspecific viral syndrome, mononucleosis, or PTLD. 8,9

The PTLD is a life-threatening condition that requires urgent treatment and occurs up to 10 years after transplant. 10 The highest morbidity of this complication is within the first year after transplant, with a high incidence in the first 6 months. 11,12 The disease is associated with a wide range of clinical symptoms and may range from a self-restricted proliferation to a severe fulminant disorder. It is classified histologically into 4 groups: (1) early lesions, (2) polymorphic PTLD, (3) monomorphic PTLD, and (4) classic Hodgkin-lymphoma-type PTLD. 3

Based on clinical and histologic features, the diagnosis of PTLD and its differentiation from organ rejection often are difficult, and use of a sensitive test such as real-time polymerase chain reaction (PCR) is recommended. 13-15 The high EBV viral load in the plasma and/or peripheral blood mononuclear cells can serve as a reliable marker for development of PTLD. Different studies show a positive correlation between EBV viral load and severity of PTLD. 16-19

In this study, we quantified the EBV viral load by real-time PCR in patients after liver transplant who were suspected to have EBV/PTLD at the only center for liver transplant in Iran. We report demographic data and outcome of patients associated with PTLD.

Materials and Methods

Study setting, transplant patients, and samples
Medical records of 696 patients who underwent liver transplant at Nemazee Hospital, Shiraz, Iran, from July 2009 to May 2013, were examined retrospectively in this study. Liver transplant accounted for 75% transplants performed during this period at Nemazee Hospital. Routine immuno-suppressive regimen after transplant was triple therapy with tacrolimus, mycophenolate mofetil, and prednisolone.

There were 510 specimens in 127 patients that were assessed for EBV DNAemia at Professor Alborzi Clinical Microbiology Research Center, the major referral center for this examination. Sampling time varied in different patients based on clinical manifestations from 1 day to 1.5 years after transplant. Blood samples (average, 4 samples; range, 1-10 samples) were tested for suspected patients. Medical record review showed that 34 patients were affected by PTLD based on clinical data and World Health Organization criteria. 20

Nucleic acid extraction and measurement of Epstein-Barr virus viral load by real-time polymerase chain reaction
Viral DNA was extracted from 200 μL serum by a simple and effective column-based DNA extraction kit (Invisorb Spin Virus DNA Mini Kit, Stratagene, Birkenfeld, Germany), according to instructions from the manufacturer. Real-time PCR was used as a sensitive and specific method to determine EBV serum sample loads against a serial dilution of a standard that had known EBV DNA content. To quantify EBV DNA, we used a commercially available system (TaqMan Universal Master Mix, Life Technologies, Carlsbad, CA, USA). Each 25λ reaction volume contained: 1 × reagent (TaqMan Universal Master Mix), forward and reverse primers (15 pmol each), probe (TaqMan); 5λ DNA template, and water added up to 25λ. Thermocycling conditions were 50°C for 2 minutes; 95°C for 10 minutes; 95°C for 15 seconds, and 60°C for 1 minute for 40 cycles. The sensitivity of the test was 10 copies/λ specimen.

Statistical analyses
Data analyses were performed with statistical software (SPSS for Windows, Version 16.0, SPSS Inc., Chicago, IL, USA). Statistical significance was defined by P ≤ .05.

Results

The mean age of 696 patients undergoing liver transplant was 28.53 ± 0.66 years (range, 1 mo to 75 y). The male: female ratio was 1.74 (442 males;
254 females). Underlying diseases leading to liver transplant were varied, most commonly end-stage hepatitis B virus infection (Figure 1). In 39% patients, the reasons for liver transplant were unknown.

**Figure 1.** Underlying Disease Leading to Liver Transplantation

The EBV genome was detected in 78 of 127 patients suspected of having EBV/PTLD, including 19 PTLD and 59 non-PTLD patients. A significant difference was observed between the median of maximum EBV loads in PTLD patients (4035 copies/mL) and non-PTLD patients (500 copies/mL; SE ± [standard error]; \( P \leq 0.05 \)). Evaluation of the changes in EBV load in 8 PTLD patients (at least 5 measurements per patient) showed that the peak viral load varied between 13 and 360 days after transplant (Figure 2). In the PTLD cases, 13 patients were living and 6 patients died; in the non-PTLD patients, 57 patients were living and 2 patients died. A 2-year-old boy with maximum EBV load 234,576 copies/mL was among the dead non-PTLD patients.

The incidence of PTLD was 34 patients in 696 transplant patients (4.9%); in 15 patients, there were no medical records with data about EBV load. In the 34 patients, 30 were aged < 12 years (88.2%). Death occurred in 11 of 34 patients (35%) and only 1 patient who died was an adult (Table 1).

**Figure 2.** Epstein-Barr Virus Load in 8 Patients Who Had Posttransplant Lymphoproliferative Disorder During the Immunosuppression Interval

According to histopathology reports, most lesions in the PTLD patients were not classified (77%) which 1 was associated with non-Hodgkin lymphoma (4.3%). Among the classified lesions, 6 were monomorphic PTLD including 5 diffuse large B-cell lymphoma (DLBCL) (22%) and 1 mucosa-associated lymphoid tissue (MALT) B-cell PTLD (4.3%). There was 1 Hodgkin lymphoma PTLD (4.3%), and no patients had polymorphic PTLD or early lesion (Table 1).

**Discussion**

There are 40,000 organ transplants performed annually worldwide, most frequently kidney, liver, lung, and heart transplant in decreasing order of frequency. In our center, the only referral center for liver transplant in Iran, liver transplant is performed more commonly than other organ transplants.

Widespread consumption of immunomodulating agents may occur to prevent organ rejection, but this may cause unwanted opportunistic infections including different parasitic, fungal, bacterial, and viral infections, particularly *Herpesviridae* members such as...
cytomegalovirus, and EBV. At present, it is proven that quantitative PCR is important in the early diagnosis of EBV-associated disorders, even several months before the onset of clinical manifestations. In the present study, according to the protocol in our center, EBV load was assessed in 127 suspected patients of 696 patients who received liver organ transplant between 2009 and 2013. The EBV loads in these patients were monitored 1 to 10 times after transplant, based on the clinical presentations. The data showed that the median of maximum viral loads were significantly higher in the PTLD patients (4035 copies/mL) than nonaffected patients (500 copies/mL), consistent with several previous studies.3-5

As we detected in this study, a wide range of EBV loads were observed in liver transplant patients. This was because of involvement of multiple host factors such as age of transplant, active primary infection, different underlying disease, immunosuppressive drug regimen, and intensity. Therefore, it is more acceptable to monitor EBV load than consider a cutoff value to treat patients, as recommended in other studies.22-26

In 8 PTLD patients who had serial viral loads available, large variation in maximum load (4370 to 286 844 copies/mL) and time of maximum load (13-360 d after transplant) were observed, which is consistent with other studies.27,28

Infection with EBV causes a wide range of clinical manifestations including a nonspecific viral syndrome, infectious mononucleosis, and PTLD. The PTLD, which is the most important EBV-associated disorder, can be prevented by modulation of the immune system, autologous expanded T-cell infusion, and/or use of anti-EBV drugs such as rituximab.12,30 The incidence of PTLD in liver recipients in this study was 4.9% (34 of 696 patients); this is in agreement with other studies (1.6%-15%). In another study conducted in our center from 2003 to 2010, the incidence of PTLD was 0.9% (5 patients with PTLD in 550 liver transplant

### Table 1. Characteristics of Patients with Posttransplant Lymphoproliferative Disorder Lesions

<table>
<thead>
<tr>
<th>Complication After Liver Transplant</th>
<th>Patient Number</th>
<th>Mean Viral Load (No. of Samples)</th>
<th>Sex/Age (y)</th>
<th>Underlying Disease</th>
<th>Range of EBV Load</th>
</tr>
</thead>
<tbody>
<tr>
<td>PTLD (n = 26) (76.4%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PTLD (n = 5) (22%)</td>
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<tr>
<td>MALT n = 1 (4.3%)</td>
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</tr>
<tr>
<td>Non-Hodgkin lymphoma (n = 1) (4.3%)</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Hodgkin-lymphoma-type PTLD (n = 1) (4.3%)</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

**Abbreviations:** DLBCL, diffuse large B-cell lymphoma; EBV, Epstein-Barr virus; F, female; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; M, male; MALT, mucosa-associated lymphoid tissue; PFIC, progressive familial intrahepatic cholestasis; PTLD, posttransplant lymphoproliferative disorder

*These patients died.
patients).\textsuperscript{31} This difference may be explained by the use of more potent immunosuppressive regimens in the later years and higher index of suspicion of our clinicians to diagnose PTLD.

The mortality of our patients with PTLD was high (11 of 34 patients [35%]), which is within the reported range of 15% to 55%.\textsuperscript{32,33} High mortality indicates that current treatment modalities such as immunomodulation and antiviral therapy did not reverse the immunopathologic background that caused PTLD.\textsuperscript{34} The mortality rate of PTLD among liver transplant recipients was somehow similar to those in previous report [11 of 696 patients (1.58%) vs (3 of 550 patients (0.54%)].\textsuperscript{31,35}

As shown in several studies, the incidence of PTLD is higher in pediatric than adult liver transplant patients.\textsuperscript{1,3} The Scientific Registry of Transplant Recipients (SRTR) reported that 82% PTLDs occur in children aged < 17 years.\textsuperscript{36} In the current study, more than 88% (30 of 34 patients) of PTLD cases occurred in children aged < 12 years. This may have been caused by EBV seronegativity, primary infection occurring from a latent EBV-infected donor passenger leukocyte, or by close contact with healthy individuals in the community after transplant.\textsuperscript{19,34}

Although it is difficult to subcategorize PTLD lesions,\textsuperscript{30,37} the most common PTLD lesion in our patients was monomorphic (5 of 34 patients), which is similar to data from other studies including a previous study in our center (3 of 5 patients).\textsuperscript{30,31,37} A previous study reported that the monomorphic type occurred in 31%, polymorphic type in 19%, and hyperplastic form of early lesions in 1% patients.\textsuperscript{38}

Because the highest PTLD incidence and mortality were seen in pediatric liver transplant recipients, timely diagnosis of this complication before appearance of clinical symptoms is important.\textsuperscript{29} Preventing PTLD by monitoring EBV loads after transplant should be considered, especially in infants, young children, and other patients who are at high risk of developing EBV infection.\textsuperscript{39}

References


Evaluation of Safety and Efficacy of Liver Biopsy Following Liver Transplant

Mahir Kırnap, Aydincan Akdur, Nihan Haberal Reyhan, Cüneyt Aytekin, Ali Harman, Sedat Yıldırım, Gokhan Moray, Mehmet Haberal

Abstract

Objectives: Liver biopsy is a diagnostic tool for liver pathology after liver transplant. However, biopsy can cause life-threatening complications. There is limited knowledge about efficacy and complications of liver biopsy after liver transplant. Our aim was to evaluate the risk and benefit of liver biopsy after liver transplant and quality of biopsy specimens.

Materials and Methods: We retrospectively analyzed all liver biopsies performed after liver transplant between January 2000 and October 2014. All patients were monitored for minimum 24 hours after biopsy.

Results: We performed 245 liver biopsies in 159 liver transplant patients. Fifteen biopsies (6%) were nondiagnostic. In the samples, there were 102 cases (41%) of acute rejection, 79 cases (35%) of cholangitis, and 49 cases (20%) of cholestasis observed. Complications after biopsy were seen in 23 patients (9%) and biopsies. There were 7 patients who had severe abdominal pain followed by fever. We diagnosed 4 patients who had intercostal/subcapsular bleeding and 12 patients who had vasovagal reaction. All patients were treated with analgesic agents and monitored for 24 hours. No blood transfusion or surgery was required.

Conclusions: Liver biopsy after liver transplant is an invasive diagnostic tool for liver pathology. However, it can be used safely in experienced centers.

Key words: Complication, Hepatic failure, Treatment, Vasovagal reaction

Introduction

Liver biopsy is a diagnostic tool for liver pathology after liver transplant. Since the 1980s, the number of image-guided percutaneous procedures, including tissue biopsies and fluid aspirations, has markedly increased despite the advances in imaging techniques and serologic investigations. In many liver diseases, liver biopsy still is the best option to make the diagnosis, determine prognosis, and assist in determining the treatment plan. The increased popularity of liver biopsy is because it is less invasive and has lower risk than surgery, has high diagnostic accuracy, and is cost effective.1,2

Although liver biopsy is an easy procedure for hospitalized patients and outpatients, some complications or technical failures may occur in 5% patients, including pain that causes hospital admission, bleeding that necessitates transfusion or surgery, pneumothorax, failure to obtain tissue, and obtaining other tissues.3,5 However, attempts should be made to increase safety and decrease complications of liver biopsy.2,5 There is a statistically significant decrease in the complication rate of liver biopsy when performed with ultrasonography guidance.6-8 However, biopsy can cause life-threatening complications. There is limited knowledge about the efficacy and complications of liver biopsy following liver transplant.

Ultrasonography-guided percutaneous random core needle biopsy of the liver is considered a safe and effective procedure.9 The procedure is important in the characterization and treatment of patients.
with liver disease, and it is used routinely to assess rejection in liver transplant recipients. Therefore, data regarding efficacy and safety are becoming more important in achieving national patient safety goals.

Our aim was to evaluate the complications and quality of biopsy specimens obtained by percutaneous liver biopsy using a commercially available biopsy needle in patients after liver transplant who were scheduled for treatment in our center.

**Materials and Methods**

**Patients**

We retrospectively analyzed all liver biopsies performed after liver transplant between January 2000 and October 2014. All patients were monitored for minimum 24 hours after biopsy. There were 245 liver biopsy procedures in 159 patients that were performed under real-time ultrasonography guidance. In these 245 procedures, 125 biopsies (51%) were performed in men and 120 biopsies in women (49%).

**Biopsy protocol**

All ultrasound-guided percutaneous random core needle biopsies of the liver in our institution were performed using a standardized protocol and guidelines (Figure 1). These guidelines adhered to the practice guideline for the performance of image-guided percutaneous needle biopsy. Patients undergoing percutaneous biopsy were required to have an international normalized ratio < 1.5 and platelet count > 50 ×10⁹/L. When the platelet count was < 50 ×10⁹/L, patients received transfusion before and during the procedure.

Informed consent for the procedure was obtained from all patients. An access route to the liver was identified using ultrasonography, choosing a subcostal route whenever possible; an epigastric route into segment II, III, or IV, or a right subcostal route, was preferred to an intercostal route. Intravenous conscious sedation was given to all patients who were hemodynamically stable; conscious sedation was not given to hemodynamically unstable patients or patients who declined conscious sedation. All biopsies were performed after administration of local anesthetic (1% lidocaine mixed with epinephrine, 8 parts, and sodium bicarbonate, 2 parts) at the intended puncture site with a 25-gauge needle and along the needle tract with a 22-gauge spinal Boston Scientific needle. Any hemorrhage after the procedure was documented; diagnosis of hemorrhage after the procedure was made by ultrasonography or computed tomography (CT) in all cases. The CT scan or ultrasonogram was performed at the discretion of the attending radiology staff, only after a patient complained of pain or had unstable vital signs, and we do not know the number of patients who had asymptomatic hemorrhage. Infection was suspected and recorded as a complication when the patient had febrile symptoms or sepsis after the procedure.

**Results**

We performed 245 liver biopsies in 159 liver transplant patients. There were 15 biopsies (6%) that were reported as insufficient. All biopsy procedures were performed on liver transplant recipients who had elevated liver function tests at biopsy.

There were 102 biopsies (41%) that showed acute rejection, 79 biopsies (35%) with cholangitis, and 49 biopsies (20%) with cholestasis. Complications after biopsy were observed after 23 biopsies (9%). There were 7 patients who had severe abdominal pain that was followed by fever. We diagnosed 4 patients with intercostal/subcapsular bleeding and 12 patients with vasovagal reaction. All patients were treated with analgesic agents and monitored for 24 hours. No blood transfusion or surgery was required (Table 1).

**Table 1. Complications of Liver Biopsy After Liver Transplant**

<table>
<thead>
<tr>
<th>Complications</th>
<th>No. of Patients¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abdominal pain</td>
<td>7</td>
</tr>
<tr>
<td>Fever</td>
<td>7</td>
</tr>
<tr>
<td>Intercostal/subcapsular bleeding</td>
<td>4</td>
</tr>
<tr>
<td>Vasovagal reaction</td>
<td>12</td>
</tr>
</tbody>
</table>

¹N = 23 patients. Data reported as number.
Discussion

Although noninvasive imaging of the liver, such as CT and magnetic resonance imaging, can identify liver transplant rejection or other diffuse liver disorders, random percutaneous core needle biopsy of the liver is important to detect and characterize liver disease. Patients seen immediately before or after liver transplant often are anxious, and a procedure is important that is quick, safe, and requires minimal monitoring after the procedure. However, guided percutaneous liver biopsy with a specialized cutting (Tru-Cut) biopsy needle is an effective, safe, and cost-effective procedure that is performed primarily by radiologists. The percutaneous ultrasonography-guided liver biopsy after liver transplant should be performed in all indicated cases by an experienced operator. Liver biopsy after liver transplant is an invasive diagnostic procedure for liver pathology, but it can be used safely in experienced centers.

The results of our study confirm the safety of real-time, ultrasonogram-guided percutaneous biopsy of the liver. Although complications occurred in 9% procedures, most complications were minor and only 1 outpatient (< 0.2%) was subsequently hospitalized. Furthermore, many complications did not require further intervention. No deaths were attributed to the procedure.

References

Efficacy of Cell Saver Use in Living-Donor Liver Transplant

Mahir Kırnap,¹ Tugan Tezcaner,¹ Hatice Ebru Ayvazoğlu Soy,¹ Aydincan Akdur,¹ Sedat Yıldırım,¹ Adnan Torgay,² Gökhan Moray,¹ Mehmet Haberal¹

Abstract

Objectives: Liver transplant currently is the best treatment option for end-stage liver disease. During liver transplant, there is major blood loss due to surgery and primary disease. By using a cell saver, the need for blood transfusion is markedly reduced. In this study, we aimed to evaluate the efficacy of cell saver use on morbidity and mortality in living-donor liver transplant.

Materials and Methods: We retrospectively evaluated 178 living-donor liver transplants, performed from 2005 to 2013 in our center. Child-Turcotte-Pugh A patients, deceased-donor liver transplants, and liver transplants performed for fulminant hepatic failure were not included in this study. Intraoperative blood transfusion was done in all patients to keep hemoglobin level between 10 and 12 g/dL. Cell saver was used in all liver transplants except in patients with malignancy, hepatitis B, and hepatitis C.

Results: We included 126 patients in the study. Cell saver was used in 84 liver transplants (66%). In 42 patients (34%), liver transplant was performed without a cell saver. In living-donor liver transplant with cell saver use, 10 mL/kg blood (range, 2-50 mL/kg blood) was transfused from the cell saver; in addition, 5 to 10 mL/kg allogeneic blood was transfused. In living-donor liver transplant without cell saver, 20 to 25 mL/kg allogeneic blood was transfused.

Conclusions: During liver transplant, major blood transfusion is needed because of surgery and primary disease. Cell saver use markedly decreases the need for allogeneic blood transfusion and avoids adverse events of massive transfusion.

Key words: Blood, End-stage liver disease, Transfusion

Introduction

Liver transplant currently is the best treatment option for end-stage liver disease. Patients undergoing liver transplant often present with coagulopathy, have massive intraoperative blood loss, and require large amounts of blood transfusion. Massive transfusion has been correlated with morbidity and reduced survival. Various surgical and anesthesic procedures have been used for the past 20 years to prevent excessive bleeding and reduce blood use. Presently, 40% operations are performed without blood transfusion.¹² During liver transplant, there is a major amount of blood loss because of surgery and primary disease. By using a cell saver, the need for blood transfusion is markedly reduced. Although massive transfusion is related to postoperative morbidity, there has not been a defined relation between the number of packed red cells transfused and survival.³⁴

Liver transplant is associated with major use of allogeneic blood products, which places major demands on finite resources and increases recipient exposure to viral, bacterial, and protozoal diseases associated with transfusion—undesirable events in immunosuppressed patients.¹ During this procedure, abnormal bleeding typically occurs as a consequence of surgery, hemostatic dysfunction, and portal hypertension. The cause of abnormalities of
hemostasis is multifactorial, including deficits in platelets and coagulation factors related to the existing liver disease and increased fibrinolysis. Lack of tissue plasminogen activator clearance during the anhepatic phase, and the burst release of tissue plasminogen activator associated with reperfusion of the ischemic graft, result in large amounts of circulating tissue plasminogen activator, with consequent pathologic activation of the fibrinolytic system.

Intraoperative red blood cell salvage (IRBCS) with autologous transfusion is not used routinely because cost effectiveness is a major concern. In this study, we aimed to evaluate the efficacy of cell saver use on morbidity and mortality in living-donor liver transplant.

Materials and Methods

We retrospectively evaluated 178 living-donor liver transplants that were performed from 2005 to 2013 in our center. Child-Turcotte-Pugh A patients, deceased-donor liver transplants, and liver transplants performed for fulminant hepatic failure were not included in this study. Intraoperative blood transfusion was done in all patients to keep hemoglobin levels from 10 to 12 g/dL. A cell saver (Figure 1) was used in all liver transplants except in patients with malignancy, hepatitis B, and hepatitis C.

Blood transfusions were administered on the basis of clinical and hemodynamic criteria. The replacement of other blood components was not analyzed; volume replacement was performed with crystalloid and colloid solutions. Anesthetic induction and maintenance was performed with a combination of intravenous drugs such as propofol, fentanyl, and pancuronium. Hemodynamic monitoring included an arterial line and pulmonary artery catheter. Body temperature was maintained with warming blankets and intravenous fluid warmers.

The analyzed variables for recipients included age, sex, disease, Child-Turcotte-Pugh classification, body weight, height, warm ischemic time, and Model for End-Stage Liver Disease (MELD) score. Statistical analyses were performed using t test, Cox hazard regression, Kaplan-Meier method, and log rank test.

Results

We included 126 patients in the study. A cell saver was used in 84 liver transplants (66%). In 42 patients (34%), liver transplant was performed without a cell saver (Table 1). In living-donor liver transplant with cell saver use, 10 mL/kg blood (range, 2-50 mL/kg blood) was transfused from the cell saver; in addition, 5 to 10 mL/kg allogeneic blood was transfused. In living-donor liver transplant without a cell saver, 20 to 25 mL/kg allogeneic blood was transfused (Figure 2).

![Figure 1. Cell Saver Device](image)

**Figure 1. Cell Saver Device**

**Table 1. Demographic Characteristics of Patients**

<table>
<thead>
<tr>
<th>Variable</th>
<th>With Cell Saver</th>
<th>Without Cell Saver</th>
<th>P ≤</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>21</td>
<td>23</td>
<td>.12</td>
</tr>
<tr>
<td>Sex (male/female)</td>
<td>45/39</td>
<td>18/24</td>
<td>.18</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>27</td>
<td>24</td>
<td>.1</td>
</tr>
<tr>
<td>Warm ischemia time (min)</td>
<td>200</td>
<td>210</td>
<td>.18</td>
</tr>
<tr>
<td>Surgical time (h)</td>
<td>8</td>
<td>8.5</td>
<td>.1</td>
</tr>
<tr>
<td>MELD score</td>
<td>20</td>
<td>22</td>
<td>.1</td>
</tr>
<tr>
<td>Child-Turcotte-Pugh score</td>
<td>8</td>
<td>9</td>
<td>.1</td>
</tr>
<tr>
<td>Transfusion from cell saver (mL/kg)</td>
<td>10</td>
<td>-</td>
<td>.001</td>
</tr>
<tr>
<td>Allogeneic blood transfusion (mL/kg)</td>
<td>5-10</td>
<td>20-25</td>
<td>.001</td>
</tr>
</tbody>
</table>

**Abbreviation:** MELD, model for end-stage liver disease

![Figure 2. Allogeneic Blood Transfusion in Living-Donor Liver Transplant](image)

**Figure 2. Allogeneic Blood Transfusion in Living-Donor Liver Transplant**

Discussion

Although the number of patients is small for significant statistical analysis, this study gives us an important opportunity to evaluate the transfusion practice and strategies employed in liver transplant.
Most patients in both groups were Child-Turcotte-Pugh B, and median MELD score was 20 ± 5 in patients with IRBCS and 22 ± 3 in patients without IRBCS.

The patients had cirrhosis, predominantly of moderate severity. The median duration of surgery was an average 30 minutes less in patients with than without IRBCS. This coincided with the experience gained by the surgical team. The blood loss during surgery also reflected this technical refinement, with less intraoperative blood loss in the group with IRBCS (with cell saver, 5-10 mL/kg allogeneic blood was transfused; without cell saver, 20-25 mL/kg allogeneic blood was transfused). When IRBCS was used, > half of the blood lost was recovered (600 ± 300 mL) and this was mostly available for reinfusion after processing (400 ± 250 mL). We also observed a substantial reduction in fresh frozen plasma transfusion and a lesser reduction in platelet requirement in the IRBCS group. Technical refinements also were partly responsible for the reduction in heterologous transfusion requirements. Although significant statistical studies could not be performed, there seemed to be a definite tendency toward reduced transfusion requirement with the use of IRBCS.

The presence of hepatocellular carcinoma was considered a contraindication for the use of IRBCS because of the theoretical risk of reintroducing neoplastic cells into the circulation. Although a recent study did not detect any difference in the incidence of neoplastic recurrence with the use of IRBCS, this remains a concern and should be confirmed.3 Perhaps in the future, the use of IRBCS can be extended to patients with hepatocellular carcinoma.

The use of IRBCS or any other strategy that reduces the demand for heterologous transfusion is welcome because it reduces exposure to transmissible infectious diseases—viral, bacterial, and protozoal.5 Routine use of thromboelastography in the operating room is another way to reduce transfusion of blood components, especially fresh frozen plasma and platelets. This method may reduce transfusion in liver transplant and reduce surgical costs.

In this study, as in other reports, the use of IRBCS showed a tendency to reduce transfusion of blood components. In a prospective study published recently, the use of IRBCS reduced transfusions and was cost effective.6 Despite the fact that major blood use has been related to reduced survival after liver transplant, massive transfusion has not been considered an independent predictor of postoperative prognosis.7

During liver transplant, major amounts of blood transfusion are needed because of surgery and primary disease. Cell saver use markedly decreases the need for allogeneic blood transfusion and avoids adverse effects of massive transfusion.

In summary, IRBCS has the potential to reduce the need for heterologous transfusion, reduces the risks of disease transmission, and may reduce cost. If all methods available for reducing transfusion were used, liver transplant could possibly be a safer and less costly procedure.

References

Synthetic Graft for Reconstruction of Middle Hepatic Vein Tributaries in Living-Donor Liver Transplant

Refaat Kamel, Yasser Hatata, Karim Hosny, Khaled Amer, Mohamed Taha

Abstract

Objectives: In middle hepatic vein dominant livers, the anterior segment of the right lobe of the liver (segments V and VIII) drains mainly into the middle hepatic vein. In these donors, when right lobe grafts are procured without the middle hepatic vein, the graft may harbor large segment V and/or VIII veins that need reconstruction to avoid graft congestion and subsequent graft dysfunction. Draining these middle hepatic vein tributaries using autologous or cryopreserved vessels is a solution, despite the possible difficulties of their preparation. However, these vessels are not always available. Our objective was to evaluate the effectiveness and safety of using a synthetic vascular graft.

Materials and Methods: Between January 2012 and October 2013, eighteen adult recipients underwent living-donor liver transplant using right lobe grafts without the middle hepatic vein at Dar Al Fouad Hospital, 6th of October City, Egypt. All grafts had a large tributary of the middle hepatic vein. Eight-mm ringed expanded polytetrafluoroethylene vascular grafts were used to drain 15 segment V vein tributaries and 3 segment VIII vein tributaries directly to the inferior vena cava. Follow-up was done using duplex ultrasound to evaluate the patency of the vascular graft and the liver congestion and the liver function tests including liver enzymes.

Results: Intraoperative Duplex ultrasound confirmed patency and absent segmental congestion in all 18 recipients. The vascular graft patency was 17/18 at 1 week (94.4%) and 15/18 at 1 month (83.3%). No recipients developed graft infection at 1 month.

Conclusions: Synthetic vascular expanded polytetrafluoroethylene grafts could be used effectively and safely in middle hepatic vein tributary reconstruction to overcome the unavailability of autologous or cryopreserved vessel grafts or just to avoid the additional burden of recovering autologous grafts thus simplifying the procedure.

Key words: Segment V, Segment VIII, Expanded polytetrafluoroethylene, Venous congestion, Venous outflow

Introduction

Living-donor liver transplant (LDLT) was first reported in 1989. Living-donor liver transplant has become common at many transplant centers worldwide. Right lobe (RL) grafts provide more liver volume; thus, providing an adequate graft-to-recipient weight ratio (GRWR) to match the recipient’s metabolic needs. However, to preserve a satisfactory liver remnant to the donor, the middle hepatic vein (MHV) may often not be included in RL grafts. In donors with MHV dominant livers, a RL graft without a MHV will harbor the ostia of large venous tributaries of the anterior segment (segments V and VIII). Anterior segment venous congestion will occur if the venous outflow from these veins is not restored, which may lead to early graft dysfunction, sepsis, and mortality. To avoid this anterior segment congestion, various types of drainage options have been reported. One option is
a RL graft including MHV if the donor remnant allows. The use of interposition vessels for reconstruction of MHV tributaries avoids jeopardizing donor safety. However, cryopreserved vessels are not always available and autologous vessels preparation can sometimes be complicated and time consuming. Thus, synthetic vessel grafts could be used to overcome the unavailability of vessel grafts and to simplify the procedure. We report our experience for drainage of anterior segment tributaries using synthetic expanded polytetrafluoroethylene (ePTFE) grafts in right lobe liver grafts.

Materials and Methods

Between January 2012 and October 2013, eighteen adult recipients underwent LDLT and received a RL graft with a large MHV tributary at Dar Al Fouad Hospital in 6th of October City, Egypt. Fifteen patients required reconstruction of a segment V vein (V5) and 3 patients required reconstruction of a segment VIII vein (V8). All veins were reconstructed using ringed synthetic ePTFE grafts of 8 mm diameter. The use of these grafts was approved by the ethics committee before this work began, and the protocols conformed to the ethical guidelines of the 1975 Helsinki Declaration. Written informed consent was obtained from patients or their guardians including approval of the protocol of treatment and the anonymous use of the data for research purposes.

Pretransplant evaluation

Pretransplant data of the recipients and donors is shown in Table 1. Preoperative assessment of MHV tributaries involved multislice computed tomographic (CT) angiography with 3-dimensional reconstructions in all patients as well as MeVis imaging (MeVis Medical Solutions AG, Bremen, Germany) which additionally provided detailed information about the percentage of liver volume drained by each tributary, thus knowing the significance of each MHV tributary (Figure 1).

Donor surgery

During parenchymal transection in the donor hepatectomy any major MHV branches greater than 5 mm in diameter were identified and tied with silk ligature. This allowed quantification of the purple discoloration that occurred because of congestion of the draining segment.

Table 1. Demographic Data of the 18 Recipients Who Received LDLT With Synthetic Grafts and Their Donors

<table>
<thead>
<tr>
<th>Recipient Information</th>
<th>18:0 (male: female)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Recipient age (y)</td>
<td>51.8 ± 11.2†</td>
</tr>
<tr>
<td>Recipient body weight (kg)</td>
<td>71.2 ± 9.8†</td>
</tr>
<tr>
<td>Original liver disease</td>
<td>Viral hepatitis (18/18 (100%))</td>
</tr>
<tr>
<td>Others</td>
<td>0/18 (0%)</td>
</tr>
<tr>
<td>MELD score</td>
<td>20.1 ± 8.7†</td>
</tr>
<tr>
<td>Donor Information</td>
<td>16.2 (male: female)</td>
</tr>
<tr>
<td>Donor age (y)</td>
<td>26.8 ± 9.8†</td>
</tr>
</tbody>
</table>

Abbreviations: MELD, model for end stage liver disease† mean ± standard deviation.

Figure 1. Three Dimensional MeVis Reconstruction Showing a Potential Right Lobe Graft Without the Middle Hepatic Vein With a Large Segment V Vein (Arrow) Draining Into the Middle Hepatic Vein

Back table

At the back table, the graft was perfused with a histidine-tryptophan-ketoglutarate solution via the portal vein. Congested segments retained their blood content despite clear fluid draining from the right hepatic vein orifice, and thus appeared as patches of dark discoloration. If the congested area was more than 20% of the anterior segment then the temporary ligature on the V5 or V8 was removed, which was followed by a gush of blood. Perfusion via the portal vein was continued until the segmental vein fluid was clear (Figure 2). The size of the vein was measured along with weighing the liver to calculate the GRWR, which along with the recipient Model for End stage Liver Disease (MELD) score aided in deciding whether venous drainage was needed. High MELD score and low GRWR favored venous reconstruction. Then, an end-to-end anastomosis was performed between the V5 or V8 hepatic vein and 1 end of a synthetic 8 mm ePTFE vascular graft using 6-0 polypropylene continuous sutures (Figure 3).
Recipient surgery
In the recipient the usual steps were performed. The diseased liver was removed, and MHV and left hepatic vein orifices were closed. Then, the inferior vena cava (IVC) was nearly totally clamped with a Satinski double angled partial side-occluding clamp. The recipient right hepatic vein (RHV) orifice was divided caudally to create a larger opening that fits the longitudinal dimension of the graft RHV. Anastomosis of the RHV to the IVC was done using 5-0 polypropylene continuous sutures. The portal vein anastomoses was done using 6-0 polypropylene continuous sutures. Then, reperfusion was done before the ePTFE to IVC anastomosis.

Synthetic graft
At this point, there was a clamp occluding the ePTFE graft. We could visualize the size of the congested segment (Figure 4). A partial side occluding vascular clamp was applied to the IVC caudal to the RHV anastomosis. Then a longitudinal venotomy on the anterior wall of the IVC at least 1.3 cm long matching the 26 mm circumference of the 8 mm ePTFE graft was made, to which the ePTFE synthetic graft was anastomosed. The anastomosis was completed with 5-0 polypropylene continuous sutures (Figure 5). Intra-operative resolution of congestion of the segment drained was immediately noticed after anastomosis declamping (Figure 6). Intraoperative duplex ultrasonography was performed to immediately assess the patency of the MHV branches after the hepatic artery anastomosis was completed (Figure 7). No additional anticoagulant therapy was administered during or after the operation.

Results
Intraoperative recipient and graft data are shown in Table 2. Intraoperative Duplex ultrasonography confirmed patency of all ePTFE grafts and showed absent congestion in their corresponding drained segments. The ePTFE graft patency was assessed daily and monitored for 1 month. After 1 week 17/18 recipients (94.4%) had patent grafts. After 1 month graft patency was documented in 15/18 recipients (83.3%). One patient with no flow on the third postoperative day showed marked elevation of the liver enzymes with persistent high output ascites in the drains. The patient did not require any intervention and was managed conservatively and

Table 2. Intraoperative and Graft Information

<table>
<thead>
<tr>
<th>Operative Information</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Graft weight (g)</td>
<td>730 ± 195†</td>
</tr>
<tr>
<td>GRWR (%)</td>
<td>0.93 ± 0.42†</td>
</tr>
<tr>
<td>Operative time (min)</td>
<td>520.8 ± 129.5†</td>
</tr>
</tbody>
</table>

*Abbreviations: GRWR, graft-to-recipient weight ratio
†mean ± standard deviation.
had uneventful recovery. The 2 patients with no flow after 1 month had uneventful postoperative courses. No mortality or major morbidity including infections was documented in this group of patients.

Discussion

This study aimed at testing the patency of synthetic grafts in reconstruction of MHV tributaries in adult RL LDLT. In addition, the risk and incidence of infection was observed. Venous congestion due to MHV tributaries outflow obstruction in RL grafts can be prevented by a vascular graft between the MHV tributaries and the recipient's IVC.\textsuperscript{11,12} This is more important when GRWR is below 1% as the liver graft regeneration is significantly lower when MHV tributary flow is not restored.\textsuperscript{13,14}

In addition to the autologous vessels, which have complications during or after recovery,\textsuperscript{15} other grafts such as cryopreserved veins and arteries have been used for this purpose.\textsuperscript{5-10} Cryopreserved vessel grafts are associated with a good short-term outcome (2-3 mo) after transplant.\textsuperscript{9,10} However, cryopreserved grafts are not available in many countries as Egypt.

Damaged sinusoids in congested areas of the right lobe will recover and function 1 week after transplant because intrahepatic venous collaterals develop.\textsuperscript{5} Thus, the long-term patency of ePTFE grafts for MHV tributaries drainage may not be a crucial issue, as the liver graft recovers adequate graft function within 2 weeks. Also delayed occlusions of vascular grafts after 2 weeks did not cause any graft dysfunction or clinical problems during long-term follow-up. This observation was found in our study, as late occlusion of the ePTFE graft neither caused congestion in the liver graft nor any clinical abnormalities. In another study,
although the 4-month patency rate was less than 50% none had complications related to graft congestion.\textsuperscript{16}  

Patency of cryopreserved vessels is thought to be higher than ePTFE grafts because a large percentage of luminal endothelial cells that remain viable at implantation are invaded with fibroblasts, making them less thrombogenic.\textsuperscript{17} In a recent study, a composite graft using a synthetic graft and an artery patch to improve patency time was tried.\textsuperscript{18}

Infection of synthetic grafts may be a concern; however, the recipient peritoneal cavity is not an infectious cavity, and contamination is uncommon because in all our cases we do duct-to-duct biliary anastomosis. While some studies show less infection with cryopreserved vessels versus ePTFE,\textsuperscript{17} other studies had no incidence of infection in their group of ePTFE patients.\textsuperscript{16,19}

Our technique in RL grafts with a main RHV and a right inferior hepatic vein is performing both these anastomoses before liver reperfusion. However, we perform the end-to-side anastomosis between the synthetic graft and the IVC after reperfusion of the liver. This allows us to see the congested segment size and to take a stitch in posterior anastomotic wall if needed and allows the liver to expand to estimate the IVC venotomy site and the graft length more accurately to avoid kinks and twists. It also decreases the warm ischaemia time. The use of ringed (reinforced) grafts versus nonringed grafts helps to avoid kinks and twists.

In conclusion, the patency rate in the early postoperative period of the ePTFE graft was satisfactory. Late obstruction of the ePTFE graft had no impact on congestion in the anterior segment or patient outcome. Therefore, the ePTFE grafts which are readily available and are easily handled may be useful for anterior segment vein drainage in living-donor liver transplant without serious complications.

References

Seizure as a Neurologic Complication After Liver Transplant

Eda Derle,1 Seda Kibaroğlu,1 Ruhsen Öcal,1 Mahir Kırnap,2 Münire Kılınç,1 Sibel Benli,1 Mehmet Haberal2

Abstract

Objectives: Seizure is a common complication after liver transplant and has been reported to occur in up to 42% of patients in different case series. Multiple factors can trigger seizures, including immunosuppressive toxicity, sepsis, metabolic imbalance, and structural brain lesions. The aim of this retrospective study was to evaluate seizure types and associated factors in adult liver transplant patients.

Materials and Methods: We retrospectively evaluated the medical records of 142 adult patients who received a liver transplant between 2005 and 2013. We recorded demographic data, immunosuppressive treatment, seizure type, cause, recurrence, and treatment.

Results: Of the 146 patients, 23 (15.7%) had a seizure after the liver transplant. This group included 10 females and 13 males, with ages ranging between 18 and 63 (39.9 ± 14.8 y). Generalized tonic-clonic seizures were the most common, occurring in 20 patients (87%). We observed complex partial seizure and status epilepticus in 1 and 2 patients.

Immunosuppressive drug-related seizure occurred in 8 patients (34.8%) with normal drug blood levels, and all but 1 of these patients experienced seizure within the first week after transplant. Multiple factors (26.1%), metabolic imbalance (17.4%), structural lesion (13%), and sepsis (8.7%) were the other factors identified as underlying conditions.

Conclusions: In conclusion, seizure occurred in a significant proportion of patients who underwent liver transplant. Immunosuppressive drugs were the most common factor associated with seizure occurrence and drug cessation prevented seizure recurrence.

Key words: Seizure, Liver transplant, Seizure cause, Immunosuppressive drug toxicity

Introduction

Postoperative neurologic complications can occur in liver transplant (LT) patients, with seizure being the second most common.1-4 Although the seizure rate has decreased in recent studies, previous case series reported seizure prevalence rates as high as 42%.5,6 There are various causative factors underlying seizures, including immunosuppressive drug toxicity, metabolic disturbance, infection, and structural brain lesions.6 Generalized seizures are the most common type in this patient population.6,7 It is difficult to establish accurate reasons for seizures in LT patients because multiple factors may influence their onset; therefore, the aim of this retrospective study was to evaluate seizure type and associated factors in adult LT patients.

Materials and Methods

We retrospectively evaluated the medical records of 142 adult patients who underwent LT between 2005 and 2013 at Baskent University hospital. Of these, 23 experienced seizures after LT. We recorded their demographic data, immunosuppressive treatment,
seizure type, immunosuppressive drug level at the time of seizure, cause, recurrence, and seizure treatment.

The possible seizure causative factors considered were immunosuppressive drug toxicity, metabolic disturbance, infection, and structural lesion. Causative factors were determined by performing extensive evaluations in all 23 patients, including neurologic examinations and blood tests for metabolic disturbance, infection, and drug levels. Brain imaging for structural lesions and electroencephalography was performed if available. Patients were considered to have immunosuppressive drug toxicity if there was no other possible explanation for seizure onset rather than drugs, even the drug level within normal limits. The possible cause was classified as multifactorial in patients who had 2 or more precipitating factors at the time of seizure onset. Seizure type was determined based on clinical observations.

Statistical analyses
Statistical analyses were performed with SPSS software (version 11.0 for Windows, SPSS, Inc, Chicago, IL, USA). Data are expressed as percentages for categorical variables and as means ± SD for continuous variables.

Results
Of the 142 patients who underwent LT, 23 (15.7%) experienced postoperative seizure. Among the 23 patients, there were 10 females and 13 males with ages ranging between 18 and 63 years (39.9 ± 14.8). The liver tissue was from a living donor in 19 cases (82.6%) and a deceased donor in 4 cases (17.4%). The indications for LT and the patients’ clinical findings are listed in Table 1.

Of the 23 patients, 20 (87%) had generalized tonic-clonic seizures, 2 (8.7%) had status epilepticus, and 1 (4.3%) had a complex partial seizure. We determined that immunosuppressive drug-related seizure (34.8%) was the most common etiology, followed by multifactorial cause (26.1%). Immunosuppressive drug levels in all 23 patients were within therapeutic ranges at the time of seizure onset. Patient 23 was determined to have multifactorial cause because at the time of brain magnetic resonance imaging, which showed lesions compatible with posterior reversible leukoencephalopathy syndrome, she also had acute renal failure and malignant hypertension. In 4 patients (17.4%), metabolic disturbance was established as an underlying cause; 1 had hypoglycemia, 1 had hypocalcemia, and the other 2 had elevated liver enzyme levels. Structural lesions

### Table 1. Clinical Findings of LT Patients with Seizure

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Sex</th>
<th>Age*</th>
<th>Primary Liver Disease</th>
<th>Immunosuppressive Treatment</th>
<th>Seizure Onset Time†</th>
<th>Seizure Type</th>
<th>Seizure Cause</th>
<th>Seizure Treatment</th>
<th>Recurrence Imaging</th>
<th>Brain</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>M</td>
<td>57</td>
<td>HBV + HCC</td>
<td>Sirolimus</td>
<td>32 mo</td>
<td>GTC</td>
<td>Sepsis</td>
<td>DPH</td>
<td>No</td>
<td>ND</td>
</tr>
<tr>
<td>2</td>
<td>M</td>
<td>34</td>
<td>PSC</td>
<td>TAC</td>
<td>8 d</td>
<td>GTC</td>
<td>Sepsis</td>
<td>LEV</td>
<td>No</td>
<td>ND</td>
</tr>
<tr>
<td>3</td>
<td>F</td>
<td>48</td>
<td>Wilson</td>
<td>TAC + MMF</td>
<td>7 d</td>
<td>SE</td>
<td>Metabolic</td>
<td>Propofol</td>
<td>Yes</td>
<td>Diffuse swelling</td>
</tr>
<tr>
<td>4</td>
<td>F</td>
<td>53</td>
<td>HBV</td>
<td>TAC + MMF</td>
<td>16 d</td>
<td>GTC</td>
<td>Metabolic</td>
<td>-</td>
<td>No</td>
<td>Nonspecific</td>
</tr>
<tr>
<td>5</td>
<td>M</td>
<td>48</td>
<td>HBV</td>
<td>TAC</td>
<td>1 d</td>
<td>GTC</td>
<td>Metabolic</td>
<td>-</td>
<td>No</td>
<td>ND</td>
</tr>
<tr>
<td>6</td>
<td>M</td>
<td>55</td>
<td>HBV</td>
<td>Sirolimus</td>
<td>12 mo</td>
<td>CP</td>
<td>Metabolic</td>
<td>LEV</td>
<td>Yes</td>
<td>Nonspecific</td>
</tr>
<tr>
<td>7</td>
<td>F</td>
<td>18</td>
<td>Cryptogenic</td>
<td>TAC + MMF</td>
<td>16 d</td>
<td>GTC</td>
<td>Structural</td>
<td>LEV</td>
<td>Yes</td>
<td>Air embolism + infarct</td>
</tr>
<tr>
<td>8</td>
<td>F</td>
<td>27</td>
<td>Cryptogenic</td>
<td>TAC + MMF</td>
<td>45 d</td>
<td>GTC</td>
<td>Structural</td>
<td>DPH</td>
<td>Yes</td>
<td>Air embolism</td>
</tr>
<tr>
<td>9</td>
<td>M</td>
<td>63</td>
<td>Alcoholic</td>
<td>CsA</td>
<td>3 mo</td>
<td>GTC</td>
<td>Structural</td>
<td>LEV</td>
<td>Yes</td>
<td>Cryptococcal abscess</td>
</tr>
<tr>
<td>10</td>
<td>F</td>
<td>26</td>
<td>FIC</td>
<td>CsA</td>
<td>19 d</td>
<td>GTC</td>
<td>Drug toxicity</td>
<td>LEV</td>
<td>No</td>
<td>Nonspecific</td>
</tr>
<tr>
<td>11</td>
<td>M</td>
<td>29</td>
<td>Cryptogenic</td>
<td>TAC + MMF</td>
<td>4 d</td>
<td>GTC</td>
<td>Drug toxicity</td>
<td>-</td>
<td>No</td>
<td>Normal</td>
</tr>
<tr>
<td>12</td>
<td>M</td>
<td>38</td>
<td>HBV</td>
<td>TAC + MMF</td>
<td>6 d</td>
<td>GTC</td>
<td>Drug toxicity</td>
<td>LEV</td>
<td>No</td>
<td>Normal</td>
</tr>
<tr>
<td>13</td>
<td>M</td>
<td>19</td>
<td>Budd-Chiari</td>
<td>TAC + MMF</td>
<td>5 d</td>
<td>GTC</td>
<td>Drug toxicity</td>
<td>LEV</td>
<td>No</td>
<td>Nonspecific</td>
</tr>
<tr>
<td>14</td>
<td>F</td>
<td>37</td>
<td>HBV</td>
<td>TAC + MMF</td>
<td>5 d</td>
<td>GTC</td>
<td>Drug toxicity</td>
<td>-</td>
<td>No</td>
<td>Nonspecific</td>
</tr>
<tr>
<td>15</td>
<td>F</td>
<td>32</td>
<td>Autoimmune</td>
<td>TAC</td>
<td>3 d</td>
<td>GTC</td>
<td>Drug toxicity</td>
<td>-</td>
<td>No</td>
<td>PRES</td>
</tr>
<tr>
<td>16</td>
<td>F</td>
<td>18</td>
<td>ALF</td>
<td>TAC</td>
<td>2 d</td>
<td>GTC</td>
<td>Drug toxicity</td>
<td>LEV</td>
<td>Yes</td>
<td>Normal</td>
</tr>
<tr>
<td>17</td>
<td>M</td>
<td>40</td>
<td>HBV</td>
<td>TAC + MMF</td>
<td>1 d</td>
<td>GTC</td>
<td>Drug toxicity</td>
<td>DPH</td>
<td>Yes</td>
<td>PRES</td>
</tr>
<tr>
<td>18</td>
<td>M</td>
<td>26</td>
<td>Budd-Chiari</td>
<td>TAC + MMF</td>
<td>8 mo</td>
<td>SE</td>
<td>Multifactorial</td>
<td>Propofol</td>
<td>Yes</td>
<td>Nonspecific</td>
</tr>
<tr>
<td>19</td>
<td>M</td>
<td>49</td>
<td>HBV + HCC</td>
<td>TAC + MMF</td>
<td>20 mo</td>
<td>GTC</td>
<td>Multifactorial</td>
<td>LEV</td>
<td>Yes</td>
<td>Normal</td>
</tr>
<tr>
<td>20</td>
<td>M</td>
<td>55</td>
<td>HCC</td>
<td>TAC</td>
<td>13 d</td>
<td>GTC</td>
<td>Multifactorial</td>
<td>LEV</td>
<td>Yes</td>
<td>Subacute infarct</td>
</tr>
<tr>
<td>21</td>
<td>F</td>
<td>58</td>
<td>PBC</td>
<td>TAC + MMF</td>
<td>11 mo</td>
<td>GTC</td>
<td>Multifactorial</td>
<td>LEV</td>
<td>Yes</td>
<td>Diffuse swelling</td>
</tr>
<tr>
<td>22</td>
<td>M</td>
<td>27</td>
<td>Wilson</td>
<td>TAC + MMF</td>
<td>12 d</td>
<td>GTC</td>
<td>Multifactorial</td>
<td>DPH</td>
<td>Yes</td>
<td>Nonspecific</td>
</tr>
<tr>
<td>23</td>
<td>F</td>
<td>52</td>
<td>Cryptogenic</td>
<td>TAC</td>
<td>2 mo</td>
<td>GTC</td>
<td>Multifactorial</td>
<td>LEV</td>
<td>No</td>
<td>Normal/PRES (follow-up)</td>
</tr>
</tbody>
</table>

Abbreviations: ALF, acute liver failure; CP, complex partial; CsA, cyclosporin A; DPH, diphenylhydantoin; FIC, familial intrahepatic cholestasis; GTC, generalized tonic clonic; HBV, hepatitis B; HCC, hepatocellular carcinoma; LEV, levetiracetam; LT, liver transplant; MMF, mycophenolate mofetil; ND, not done; PBC, primary biliary cirrhosis; PRES, posterior reversible leukoencephalopathy syndrome; PSC, primary sclerosing cholangitis; SE: status epilepticus; TAC, tacrolimus

*Age at transplant.
†Time since LT.
were detected in 3 patients (13%). Two had air embolisms due to catheter removal, and 1 of these patients also had a subacute ischemic infarct in a posterior brain region. The third patient had a residual lesion following a cryptococcal brain abscess. Seizures in 2 patients (8.7%) were related to sepsis.

Twelve patients (52.2%) had recurrent seizure. We found that seizures due to structural lesions and multifactorial causes tended to be recurrent (100% and 83.3%), whereas most drug-related seizures were single episodes (75%).

We preferred to prescribe levetiracetam as an antiepileptic drug for most patients (12/23, 52.2%). Diphenylhydantoin was administered to 4 patients (17.4%) and propofol to 2 patients (8.7%) with refractory status epilepticus. Five patients were not treated with antiepileptic drugs.

**Discussion**

Seizure is a common complication after LT. The reported incidence of seizure ranges from 2.8% to 42% in different case series, although recent publications have stated that the incidence of seizure in transplant patients tends to decrease over time.

Generalized seizure was the most common type in both our study and the majority of published case series. Only 1 study reported a higher frequency of partial seizure. The determination of seizure type is based on clinical observations and electroencephalography findings when available. It is difficult to determine the true incidence of focal and secondary generalized seizures because they can easily be overlooked. For that reason, the rates of focal seizures may be higher than reported. Convulsive and nonconvulsive status epilepticus are also rare in transplant patients. Nonconvulsive status epilepticus should be considered in patients with impaired consciousness, and electroencephalography is a useful diagnostic tool in these cases.

Seizure in LT patients typically occurs in the early postoperative period. One study described a bimodal distribution of seizure occurrence: within the first week and between 5 and 16 weeks after transplant. We did not observe a bimodal distribution in the present series, but the majority of our patients experienced seizures within the first 4 weeks after LT.

It is important to establish seizure cause to plan the best treatment. Various factors such as immunosuppressive drug toxicity, metabolic factors, infection, and structural lesions may precede seizure in LT patients. The diagnostic evaluations should consist of extensive examinations including blood tests for metabolic parameters and immunosuppressive drug levels, electroencephalography, and neuroimaging to accurately identify the underlying factor(s). Brain magnetic resonance imaging is the recommended modality to assess for structural abnormalities that cause seizure as in the general population. Cerebrospinal fluid examination is also suggested in patients with suspected central nervous system infection.

Immunosuppressive drug toxicity is the main causative factor in the literature describing seizure in LT patients. Immunosuppressive drug-related seizures may occur independently or in association with posterior reversible leukoencephalopathy syndrome, even in patients on therapeutic drug levels. In these patients, reducing the dosage or changing drugs are the recommended treatment approaches. In our study group, the most common causative factor was immunosuppressive drugs, and the blood levels of these drugs were within normal ranges at the time of seizure in all 8. Two of these patients also had hyperintense lesions in parieto-occipital regions on T2-weighted and fluid-attenuated inversion recovery magnetic resonance images, which was compatible with posterior reversible leukoencephalopathy syndrome.

Seizure recurrence was associated with the underlying cause. Seizures that develop as a result of immunosuppressive drug toxicity or metabolic factors such as electrolyte imbalance or hypoglycemia do not usually recur if treatment is promptly administered. Conversely, seizure due to other factors such as organ rejection, sepsis, or cerebrovascular disease has a poor prognosis.

Medical treatment of seizure is an important issue in LT patients, but no randomized controlled trials have tested antiepileptic drugs in this population. Pharmacokinetic properties are important to consider when selecting an appropriate drug to treat seizures. Some antiepileptic drugs such as carbamazepine, oxcarbazepine, phenobarbital, and diphenylhydantoin may reduce the blood levels of cyclosporine, tacrolimus, and steroids. The newer antiepileptic drugs, especially levetiracetam,
are recommended as a first-line therapy in LT patients.\textsuperscript{14,16}

In conclusion, seizure is a common complication after LT. Preventive approaches such as close control of metabolic parameters and immunosuppressive drug levels may reduce seizure frequency in these patients. In seizures do occur, prompt treatment should be administered according to the underlying factor(s). If necessary, levetiracetam is the recommended first-line antiepileptic drug to prevent seizure recurrence.

References

Neurologic Complications After Liver Transplant: Experience at a Single Center

Eda Derle,¹ Seda Kibaroğlu,¹ Ruhsen Öcal,¹ Mahir Kıran,² Ufuk Can,¹ Sibel Benli,¹ Mehmet Haberal²

Abstract

Objectives: Neurologic complications occur frequently after liver transplants. Up to 43% of patients experience severe postsurgical neurologic complications. These complications are significantly associated with longer hospital stay, morbidity, and mortality. The aim of this retrospective study was to evaluate the type and incidence of neurologic complications after liver transplants in adult patients.

Materials and Methods: We retrospectively evaluated the medical records of 176 adult patients who had undergone liver transplants between 1995 and 2013. We recorded the demographic data, type of neurologic complications, type, and level of immunosuppressive treatment, and cause of liver failure.

Results: Our study sample consisted of 48 deceased-donor liver transplants and 128 living-donor transplants (n = 176). Fifty-three of the patients (30.1%) were female. The age range of the total sample was 18 to 66 years (mean age, 43.1 ± 13.7 y). As immunosuppressive treatment, most patients received tacrolimus alone (52%) or tacrolimus combined with mycophenolate mofetil (33%). Neurologic complications occurred in 74 of the patients (42%). The most common neurologic complications were diffuse encephalopathy (22.2%) and seizure (14.2%). Other neurologic complications were posterior reversible encephalopathy (1.7%), peripheral neuropathy (1.7%), cerebrovascular disease (1.1%), and central nervous system infection (1.1%). Age, cause of liver failure, and type of transplant were not associated with occurrence of neurologic complications.

Conclusions: There was a high incidence of neurologic complications after liver transplants. Diffuse encephalopathy and seizure were common complications. Physicians should be aware of the high risk of neurologic complications after liver transplants. Factors such as immunosuppressive toxicity and metabolic imbalance that predispose patients to neurologic complications after liver transplants should be evaluated immediately, and treatment of postoperative neurologic complications should be initiated as early as possible.

Key words: Liver transplant, Neurologic complication, Encephalopathy, Seizure

Introduction

Liver transplant (LT) is the primary treatment option for patients with end-stage liver disease, and it has been performed since 1963.¹ Neurologic complications (NCs) are a common postoperative problem in patients who undergo LT, with up to 43% experiencing severe NCs.¹ Neurologic complications can occur at any time after LT, especially in the early period after surgery.²,³ Neurologic complications can be caused in several ways, such as by surgery, immunosuppressive treatment, and metabolic disturbance.³ Diffuse encephalopathy, seizure, cerebrovascular disorder, neuromuscular disease, central nervous system infection, and central pontine myelinolysis (CPM) are the major
complications. Diffuse encephalopathy is the most common NC in patients who undergo LT, and CPM is a characteristic complication. These complications may lead to longer hospital stay, mortality, and morbidity.

The aim of this retrospective study was to evaluate the types and incidence of NCs after LT in adult patients at a single tertiary center.

Materials and Methods

We retrospectively evaluated the medical data of 176 adult patients who had undergone LT between 1995 and 2013 in Baskent University Hospital, Ankara, Turkey. We reviewed the following data from the medical records: age at the time of LT, sex, cause of hepatic failure, type of NC, possible precipitating factor of the NC, immunosuppressive drug type, and the drug level in blood at the time of the NC.

Statistical analyses were performed with SPSS software (SPSS: An IBM Company, version 11.0, IBM Corporation, Armonk, NY, USA). Data are expressed as percentages for the categorical variables and as means and standard deviations for the continuous variables. The chi-square test was used to compare categorical variables, and the t test was used for continuous variables. P values < .05 were considered significant. The study was approved by the Ethical Review Committee of the Institute. All of the protocols conformed with the ethical guidelines of the 1975 Helsinki Declaration.

Results

Of the 176 included patients, 53 were female (30.1%) and 123 were male (69.9%). Age at the time of LT ranged from 18 to 66 years (mean age, 43.1 ± 13.7 y).

The primary liver disease diagnoses are summarized in Table 1. The miscellaneous group includes Budd-Chiari syndrome (n = 6), hydatid cyst (n = 3), acute liver failure (n = 3), Caroli disease (n = 1), congenital hepatic fibrosis (n = 1), familial intrahepatic cholestasis (n = 1), von Gierke disease (n = 1), portal hypertension (n = 1), Alagille syndrome (n = 1), hemangiosarcoma (n = 1), and familial hypercholesterolemia (n = 1). Living-donor transplants were performed in 128 patients (72.7%), and the remaining transplants were from deceased donors.

Immunosuppressive treatment was based on calcineurin inhibitors in 174 patients. Of these 174 patients, 92 received tacrolimus alone (52%), 58 received tacrolimus combined with mycophenolate mofetil (33%), 21 received cyclosporine alone (11.3%), 3 received cyclosporine combined with mycophenolate mofetil (1.7%), 1 received sirolimus (0.6%), and 1 received no immunosuppressive treatment because of postoperative complications.

Of the 176 patients, 74 experienced NCs after surgery (42%). These patients constituted the NC(+) group; the remaining 102 patients constituted the NC(−) group. The mean age of the patients did not differ significantly between the 2 groups (NC(−) vs NC(+) 43.4 ± 13.2 y vs 42.9 ± 14.5 y; P = .811). There was no significant difference between these 2 groups regarding primary liver disease (P = .99) (Table 2) or immunosuppressive treatment (P = .245) (Table 3).

Neurologic complications occurred in 54 patients (42.2%) who underwent living-donor transplants and in 20 patients (41.7%) who had deceased-donor transplants. There was no significant difference according to graft type (P = .95).

Neurologic complications occurred within the first month after LT in 55 patients (33.2%), between 1 and 6 months in 11 patients (6.3%), and after 6 months in 8 patients (4.5%). The most common NC was diffuse encephalopathy, which occurred in 39 patients (22.2%). Immunosuppressive treatment-related mental status changes were observed in 16 of these 39 patients (41%). Full recovery was achieved

<table>
<thead>
<tr>
<th>Cause of Liver Transplant</th>
<th>Patients, n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hepatitis B with or without hepatocellular carcinoma</td>
<td>78 (44.3)</td>
</tr>
<tr>
<td>Hepatitis C with or without hepatocellular carcinoma</td>
<td>21 (11.9)</td>
</tr>
<tr>
<td>Cryptogenic</td>
<td>17 (9.7)</td>
</tr>
<tr>
<td>Wilson disease</td>
<td>12 (6.8)</td>
</tr>
<tr>
<td>BPC/PSC</td>
<td>10 (5.7)</td>
</tr>
<tr>
<td>Alcoholic</td>
<td>10 (5.7)</td>
</tr>
<tr>
<td>Autoimmune</td>
<td>8 (4.5)</td>
</tr>
<tr>
<td>Miscellaneous</td>
<td>20 (11.4)</td>
</tr>
<tr>
<td>Total</td>
<td>176 (100)</td>
</tr>
</tbody>
</table>

Abbreviation: PBC, primary biliary cirrhosis; PSC, primary sclerosing cholangitis

<table>
<thead>
<tr>
<th>Cause of Liver Transplant</th>
<th>Patients with NCs, n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hepatitis B with or without hepatocellular carcinoma</td>
<td>30 (38.5)</td>
</tr>
<tr>
<td>Hepatitis C with or without hepatocellular carcinoma</td>
<td>9 (42.9)</td>
</tr>
<tr>
<td>Cryptogenic</td>
<td>7 (41.2)</td>
</tr>
<tr>
<td>Wilson disease</td>
<td>12 (6.8)</td>
</tr>
<tr>
<td>BPC/PSC</td>
<td>5 (50)</td>
</tr>
<tr>
<td>Alcoholic liver disease</td>
<td>5 (50)</td>
</tr>
<tr>
<td>Autoimmune disease</td>
<td>4 (50)</td>
</tr>
<tr>
<td>Miscellaneous</td>
<td>9 (45)</td>
</tr>
</tbody>
</table>

Abbreviation: NC, neurologic complication; PBC, primary biliary cirrhosis; PSC, primary sclerosing cholangitis
in these 16 patients by switching the drug from one to another. In the other 23 patients (59%), different precipitating factors were detected, such as metabolic imbalance and infection.

Seizures occurred in 25 patients (14.2%), and within 1 month after LT in 16 (64%) of them. Posterior reversible leukoencephalopathy syndrome (PRES) occurred in 3 patients (1.7%). Posterior reversible leukoencephalopathy syndrome was associated with immunosuppressive drug therapy in 2 patients, 1 of whom had been receiving tacrolimus and the other cyclosporine. Peripheral neuropathy developed in 3 patients (1.7%). It was focal in 1 patient and generalized in 2. One of the patients with generalized neuropathy was diagnosed with Guillain-Barré syndrome due to immunosuppressive drug treatment. Two patients (1.1%) experienced cerebrovascular disease; 1 had ischemia, and the other had intracerebral hemorrhage. Central nervous system infection occurred in 2 patients (1.1%); 1 had viral encephalitis, and the other had cryptococcal meningitis. The mortality rates were 47.3% (n = 35) in the NC(+) group and 15.8% (n = 16) in the NC(−) group. The mortality rate was significantly higher in the NC(+) group (P = .000).

Discussion

Neurologic complications are a common problem in patients who undergo LT, and they cause significant morbidity and mortality. The incidence of NCs after LT reported in the literature varies. Published reports indicate that up to 43% of patients experience severe NCs after the procedure. In our present study, NC frequency was within the limits of previously reported rates. We considered all NCs that occurred at any time during follow-up. The highest rate was observed in the first month after LT, which is compatible with previous reports in the literature.

We did not find a significant correlation between primary liver disease and NCs. Some of the results reported in the literature resemble ours; however, Lewis and associates found a higher incidence in patients with alcoholic liver disease and those with primary biliary cirrhosis. In our study, the incidence of NCs did not differ according to immunosuppressive treatments, as also had been reported elsewhere in the literature. The incidence of NCs was previously reported to be lower in patients who underwent living-donor LTs than in patients who had deceased-donor LTs; however, we were unable to detect such a relation.

Diffuse encephalopathy was the most common neurologic manifestation after LT in our study, a finding which is reflected in other studies in the literature. The rate of diffuse encephalopathy reported in the literature varies between 10% and 62%; in our study, 22.2% of our patients (n = 39) experienced different levels of mental status changes.

There are several factors associated with encephalopathy, such as metabolic disturbance, drug neurotoxicity, infection, seizure, and cerebrovascular disease. Also, a higher risk of encephalopathy after LT was reported in patients with metabolic liver disease, alcohol abuse-related hepatic failure, and history of severe hepatic encephalopathy. The mechanisms underlying encephalopathy are not clear, although the most common finding in neuropathologic studies is diffuse anoxic-ischemic changes.

Seizure is the second most common NC after LT. Recently, researchers have reported that the incidence of seizure tends to decrease over time in patients who have undergone LT. In most of the patients, seizure occurs in the early period after surgery. Twenty-five patients (14.2%) in our study experienced seizures, and 64% of the seizures occurred within the first month after LT, which is compatible with previous reports in the literature. Various factors may precipitate seizures, such as immunosuppressive drug toxicity, metabolic imbalance, infection, and structural brain lesions. Close monitoring of metabolic parameters and immunosuppressive drug levels is recommended for prevention of seizure occurrence in this setting.

Posterior reversible leukoencephalopathy syndrome has been reported in about 5% of patients who undergo LT. Posterior reversible leukoencephalopathy syndromes consist of headache, seizure, visual disturbance, focal neurologic deficits, and mental status changes. Diagnosis of PRES is based on neuroimaging features that show bilateral
hyperintense lesions caused by vasogenic edema, especially in posterior regions of the brain. Several factors can be associated with the development of PRES, but the most prominent factor in patients who have undergone LT is the neurotoxic effect of calcineurin inhibitors. We identified postoperative PRES in 1.7% of the patients in our study, but it is hard to establish the true incidence of the syndrome in a retrospective study.

Neuromuscular complications such as peripheral neuropathy and plexus injuries are less common after LT. Peripheral neuropathy and plexopathy frequently occur because of pressure or traction. Peripheral neuropathy was detected in 1.7% of our patients (n = 3).

The incidence of central nervous system infection after LT has been estimated to be 5%; however, it has been reported in fewer than 2% of the patients in recent series. Opportunistic infections due to viruses and fungi are common, and the risk is highest between 1 and 6 months after LT. Two of our patients experienced central nervous system infections; 1 had viral encephalitis and the other had cryptococcal meningitis.

Cerebrovascular complications occurred in about 4% of the patients in previously reported clinical series. In our study, cerebrovascular complications developed in 2 patients (1.1%); an ischemic lesion was detected in 1 patient, and intracranial hemorrhage occurred in the other. In the literature, intracranial hemorrhages have been reported more commonly than ischemic strokes as NCs after LT.

Central pontine myelinolysis is a characteristic NC after LT. It was reported to have occurred in about 2% of the cases in previously published clinical series. The incidence of CPM after LT in 1 series was attributed to large amounts of fluid replacement during surgery and rapid correction of hyponatremia. The clinical presentation of CPM can vary from asymptomatic to quadripareisis and coma. We did not find that CPM developed in any of our patients. However, neuroimaging studies were not obtained for all patients; therefore, we may not have detected CPM in some patients.

In conclusion, NCs are a common problem after LT. Some of the complications can be prevented by careful assessment of precipitating factors such as metabolic imbalance and immunosuppressive drug toxicity. Physicians who treat these patients should be aware of the high risk for NCs. Early recognition and management of NCs may improve patient morbidity and survival.

References
Role of Bronchoalveolar Lavage in Diagnosis of Fungal Infections in Liver Transplant Recipients

Merih Tepeoğlu,1 Alev Ok Atılgan,1 B. Handan Özdemir,1 Mehmet Haberal2

Abstract

Objectives: Pulmonary fungal infections remain the most important cause of morbidity and mortality in liver transplant recipients. Fast and accurate causative diagnoses are essential for a good outcome. Bronchoscopy with bronchoalveolar lavage frequently is performed to diagnose pulmonary infections in immunocompromised patients. The aim of this study was to evaluate the diagnostic use of bronchoalveolar lavage in liver transplant recipients with pulmonary infections.

Materials and Methods: We retrospectively analyzed the data of 408 patients who underwent liver transplant from January 1990 to December 2012. Patients who underwent bronchoalveolar lavage after transplant were included in this study.

Results: There were 18 of 408 liver transplant recipients (4.41%) who underwent bronchoalveolar lavage after transplant. The mean age was 49.5 ± 18 years. In 5 patients (27.8%), fungal microorganisms were observed in the cytology of bronchoalveolar lavage specimens, including Aspergillus fumigatus in 3 patients and Candida albicans in 2 patients. Death occurred in 4 of 5 patients (80%) with fungal infections. No association was observed between the presence of fungal infection and clinical and radiographic findings of the patients.

Conclusions: Bronchoscopy with bronchoalveolar lavage is a useful, noninvasive diagnostic tool for the rapid diagnosis of infections in solid-organ transplant recipients.

Key words: Aspergillus fumigatus, Candida albicans, Cytology, Pulmonary

Introduction

Solid-organ transplant recipients are at a high risk for infectious complications, and the most common infections include pulmonary fungal infections.1,2 These infections are associated with major morbidity and mortality. Therefore, rapid diagnosis of fungal infections in immunocompromised patients is important.3 Bronchoscopy with bronchoalveolar lavage (BAL) is an important tool for the diagnosis of pulmonary infections. This study sought to analyze the effectiveness of BAL in establishing the diagnosis of pulmonary infections in liver transplant recipients.

Materials and Methods

Patients

This study was approved by the Institutional Review Board at Başkent University. Patients with BAL were selected retrospectively from 408 liver allograft recipients who underwent liver transplant from January 1990 to December 2012 at Başkent University. Clinical findings of these patients were obtained from patient charts including age, sex, immunosuppressive regimen, clinical symptoms, thoracic computed tomography results, bronchoscopic findings, culture results of BAL fluid, complete blood count, age at transplant, and time between transplant and BAL. The baseline (preoperative to postoperative) immunosuppression protocol included tacrolimus or cyclosporine with low-dose steroids. For tacrolimus, the posttransplant...
target whole blood trough level was 10 to 12 ng/mL during the first 2 weeks, and the dose subsequently was tapered. Sirolimus was administered only in selected patients. Biopsy-proven rejection was treated with steroid bolus therapy.

**Statistical analyses**

The data analysis was performed with statistical software (SPSS for Windows, Version 16.0, SPSS Inc., Chicago, IL, USA). Average values were reported as mean ± SD and analyzed with Kruskal-Wallis test and Mann-Whitney U test. Values of \( P \leq .05 \) were considered statistically significant.

**Results**

During follow-up, only 18 of 408 liver transplant recipients (4.41%) underwent BAL after transplant. Of these 18 patients, 16 patients were (88.9%) male and 2 patients were (11.1%) female. The mean patient age was 49.5 ± 18 years (age range, 10-72 y) at the time of bronchoscopy. The underlying liver diseases were hepatocellular carcinoma secondary to viral hepatitis (8 patients), cryptogenic cirrhosis (3 patients), Wilson disease (2 patients), alcoholic cirrhosis (2 patients), autoimmune hepatitis (2 patients), and Alagille syndrome (1 patient). The graft source was a living-related donor in 10 patients (55.6%) and a deceased donor in 8 patients (44.4%). For immunosuppressive therapy, 6 patients (33.3%) received cyclosporine (mean dose, 162 ng/mL), 9 patients (50%) received tacrolimus (mean dose, 10.78 ng/mL), and 3 patients (16.7%) received sirolimus (mean dose, 10.33 ng/mL). In the 18 patients, acute rejection was observed only in 4 patients (22.2%). There were 8 patients (44.4%) who had diabetes mellitus before liver transplant.

The clinical symptoms were fever in 14 patients (77.8%) and cough in 4 patients (22.2%). In radiographic examination, lung infiltrates were observed in 16 patients (88.9%) and lung nodules were detected in the other 2 patients (11.1%). Bronchoscopic findings included increased secretions (5 patients), necrotic plaque (4 patients), and edematous mucosa (1 patient), and bronchoscopy otherwise was unremarkable (8 patients). In 5 patients (27.8%), fungal microorganisms were observed in BAL samples; 3 fungi were *Aspergillus fumigatus* and 2 were *Candida albicans*. In the cytology, *Aspergillus* was demonstrated with the presence of septate, 45°-branching hyphae, and *Candida albicans* was observed with pseudohyphae and multiple budding yeasts (Figure 1). The average interval between transplant and infection was 1.33 ± 0.5 months for *Aspergillus* infections and 1 month for *Candida* infections. All patients with fungal infection were men aged > 50 years. In the 5 patients, 3 patients were receiving cyclosporine and 2 patients were receiving tacrolimus-based immunosuppressive therapy. None of these patients had an acute rejection episode. The mean white blood cell count was 9.3 \( \times 10^9 \)/L in patients who had fungal infection and 6.6 \( \times 10^9 \)/L in patients who did not have fungal infection. Although the white blood cell count was higher in patients with fungal infections, the difference was not statistically significant (\( P > .05 \)). The clinical and radiographic findings of the patients who had fungal infections in BAL cytology are summarized in the table (Table 1).

In the 18 patients who had BAL, the other 13 patients had nonspecific findings on cytologic examination. Synchronous transbronchial biopsy was taken only in 5 patients; the histologic diagnosis in 4 patients was nonspecific and in 1 patient, histologically invasive aspergillosis was reported which also was observed in BAL cytology (Figure 1). Microbiologic culture was performed in all 18 patients, and only 2 patients (11.1%) had positive

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**Figure 1. Fungal Microorganisms in Bronchoalveolar Lavage Cytology**

(A, B) *Aspergillus fumigatus*. Septated hyphae with 45° branching (hematoxylin-eosin, original magnification ×100).

(B) *Aspergillus fumigatus*. Septated hyphae with 45° branching (Giemsa, original magnification ×40).

(C) Septated hyphae of invasive aspergillosis in lung biopsy (hematoxylin-eosin, original magnification ×200).

(D) *Candida albicans*. Pseudohyphae and budding yeasts (arrows) (hematoxylin-eosin, original magnification ×600).
cultural, including 1 culture that showed *Aspergillus fumigatus* and the other culture that showed *Candida albicans*; both had already been detected in cytology. There were 3 patients who had positive cytology for fungi that were not detected in the cultures. In 3 patients who had positive BAL cytology, galactomannan level was investigated and was normal.

All fungal infections were treated with antifungal agents; 3 were treated with amphotericin B and the other 2 were treated with fluconazole and caspofungin. The antifungal treatment was started during the first 5 days in all patients, but shortly after BAL, 4 of the 5 patients who had invasive fungal infection had died. The mortality rate with aspergillosis was 100%, and these patients died at mean 10.66 days after diagnosis. For *Candida*, the mortality rate was 50%; 1 patient died after 86 days and the other patient still is alive after 72 months.

In our study, no significant differences were detected in donor type (living-related or deceased-donor transplant), cause of primary liver disease, clinical or radiographic findings, immunosuppressive treatment, or presence of acute rejection between patients who had or did not have fungal infection.

**Discussion**

Fungal infections are the most important cause of morbidity and mortality in liver transplant patients. The most important reason for the high mortality is the difficulty in early diagnosis because of the low index of suspicion and nonspecific clinical and radiographic findings. Therefore, rapid and accurate diagnosis is essential for a good outcome. As a useful tool, flexible bronchoscopy with BAL is simple, safe, fast, and reliable. This procedure has been used extensively as a diagnostic procedure in transplant recipients with suspected pulmonary infections, especially opportunistic infections.

*Candida* and *Aspergillus* species are the most important causes of invasive fungal infections in solid-organ transplant recipients, and are associated with almost 100% mortality. In most studies, the infection started during the first 6 months after transplant. In our study, *Aspergillus* was the most common cause of fungal infection in liver recipients, consistent with other studies, and the average interval between transplant and infection was 1.33 ± 0.5 months with *Aspergillus* and 1 month with *Candida*. Therefore, the time between transplant and fungal infection is shorter in our study than previous reports in the literature. The mortality rate of invasive aspergillosis is 50% to 100%, and the mortality from *Candida* infections is less than the mortality from *Aspergillus* infections. In our study, the mortality rate in aspergillosis was 100%, and the patients died at mean 10.66 days after diagnosis. The mortality rate of our patients who had *Candida* was 50%; 1 patient died at 86 days after diagnosis and the other patient still is alive after 72 months.

The most important risk factors for fungal infections in liver transplant recipients include high-dose immunosuppressive regimens, prolonged duration of transplant surgery, broad spectrum antibiotics, and acute rejection episodes. In our study, there was no significant association between the presence of fungal infection and the clinical or radiographic findings or immunosuppressive regimen that the patients received.

The BAL is the preferred method to investigate pulmonary infections in immunosuppressed patients. The procedure has low risk and can be

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**Table 1. Clinical and Radiographic Findings in Patients Who Had Fungal Infections Diagnosed in Bronchoalveolar Lavage Specimens**

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age/sex</th>
<th>Time after transplant (mo)</th>
<th>Symptoms</th>
<th>Computed Tomography Findings</th>
<th>Bronchoscopy Biopsy</th>
<th>BAL Culture</th>
<th>Fungus</th>
<th>Diabetes Mellitus</th>
<th>Immunosuppressive Regimen</th>
<th>Donor Type</th>
<th>Leukocyte Count (&lt;10⁹/L)</th>
<th>Neutrophil (%)</th>
<th>C-Reactive Protein</th>
<th>Treatment</th>
<th>Outcome, Follow-up, Cause of Death</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>64/male</td>
<td>1</td>
<td>Fever</td>
<td>Infiltrate, right-upper lobe</td>
<td>Necrotic plaque</td>
<td>No</td>
<td>Positive Aspergillus fumigatus</td>
<td>Ablative</td>
<td>Cyclosporine</td>
<td>Living</td>
<td>9.7</td>
<td>92</td>
<td>69</td>
<td>Amphotericin B</td>
<td>Died, 5 days, sepsis</td>
</tr>
<tr>
<td>2</td>
<td>56/male</td>
<td>1.5</td>
<td>Fever</td>
<td>Infiltrate, right-lower lobe</td>
<td>Necrotic plaque</td>
<td>No</td>
<td>Negative Aspergillus fumigatus</td>
<td>Ablative</td>
<td>Cyclosporine</td>
<td>Deceased</td>
<td>21</td>
<td>61</td>
<td>110</td>
<td>Amphotericin B</td>
<td>Died, 24 days, sepsis</td>
</tr>
<tr>
<td>3</td>
<td>63/male</td>
<td>1</td>
<td>Fever</td>
<td>Cough, right-upper lobe</td>
<td>Nonspecific</td>
<td>No</td>
<td>Negative Candida albicans</td>
<td>Present</td>
<td>Cyclosporine</td>
<td>Living</td>
<td>82</td>
<td>78</td>
<td>61</td>
<td>Fluconazole</td>
<td>Died, 86 days, sepsis</td>
</tr>
<tr>
<td>4</td>
<td>53/male</td>
<td>2</td>
<td>Fever</td>
<td>Infiltrate, left lobe</td>
<td>Plaque</td>
<td>Negative Aspergillus fumigatus</td>
<td>Ablative</td>
<td>Tacrolimus</td>
<td>Living</td>
<td>65</td>
<td>88</td>
<td>230</td>
<td>Caspofungin</td>
<td>Died, 3 days, sepsis</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>29/female</td>
<td>1</td>
<td>Fever</td>
<td>Infiltrate, left lobe</td>
<td>Percutaneous secretion</td>
<td>No</td>
<td>Positive Candida albicans</td>
<td>Ablative</td>
<td>Tacrolimus</td>
<td>Deceased</td>
<td>10.1</td>
<td>85</td>
<td>111</td>
<td>Amphotericin B</td>
<td>Alive</td>
</tr>
</tbody>
</table>
safely performed in most patients, including those with hypoxia. However, the value of the cytologic examination of BAL specimens is controversial because of low sensitivity. In the study of Rañó and coworkers, BAL was performed in 135 cases and a specific diagnosis was obtained in 68 cases, with diagnostic yield 51%. The diagnosis of infection was made by culture or cytologic evaluation of BAL specimens. In these cases, 12% were fungal infections and the most common organism was *Aspergillus fumigatus*. As a result, the authors emphasized the importance of the use of noninvasive bronchoscopic procedures, and noted that BAL provided the highest diagnostic yield in diagnosing pulmonary infiltrates in immunocompromised patients.

Another large study about pulmonary infections diagnosed by BAL was published by Joos and associates: 1066 immunocompromised patients underwent BAL, including 173 solid-organ transplant recipients, and the diagnostic yield of BAL in diagnosing aspergillosis was 4%. The most common infections in this population (n = 1066) were bacterial and viral. Similar results were reported in the study of Reichenberger and coworkers, who evaluated the diagnostic yield of BAL in renal transplant recipients; microorganisms were isolated from 69% BAL cases, but most were bacterial and viral, and *Aspergillus* was detected only in 1 case.

Hohenadel and associates investigated the role of BAL in 95 immunocompromised patients who had hematologic disorders with pneumonia. Pathologically relevant isolates were observed in 65% cases; BAL provided the definitive diagnosis in only 29 cases (31%), and 11% of these infections were fungal. In a study by Al-Za’abi and coworkers in immunosuppressed patients (96% lung transplant recipients), the most common identified organism was *Aspergillus* species, and 17% *Aspergillus* cases were detected by BAL cytologic examination. However, in a study by Pugliese and associates who investigated fungal infections in all solid-organ recipients (37.7% liver recipients), *Candida albicans* infections were the most common in their population, and the most frequently contaminated biological sample was from BAL (25.3%).

Although the number of the patients who underwent BAL in our study was limited (18 of 408 patients), the diagnostic yield of BAL in detecting fungal infections was high (27.8%). The clinical and radiographic findings of the patients in the current study were nonspecific; therefore, BAL was useful in detecting fungal infections in our liver transplant recipients.

In conclusion, the mortality of fungal infections in liver transplant recipients is high. Early diagnosis and aggressive antifungal therapy is essential for successful treatment. Therefore, BAL is valuable as a noninvasive method for detecting fungal microorganisms in liver transplant recipients.

**References**

Postoperative Effects of Intraoperative Hyperglycemia in Liver Transplant Patients

Özgür Kömürçü,1 Aynur Camkıran Fırat,1 Şerife Kaplan,1 Adnan Torgay,1 Arash Pirat,1 Mehmet Haberal,2 Gülnaz Arslan1

Abstract

Objectives: The aim of this study was to determine the effects of intraoperative hyperglycemia on postoperative outcomes in orthotopic liver transplant recipients.

Materials and Methods: After ethics committee approval was obtained, we retrospectively analyzed the records of patients who underwent orthotopic liver transplant from January 2000 to December 2013. A total of 389 orthotopic liver transplants were performed in our center, but patients aged < 15 years (179 patients) were not included in the analyses. Patients were divided into 2 groups based on their maximum intraoperative blood glucose level: group 1 (patients with intraoperative blood glucose level < 200 mg/dL) and group 2 (patients with intraoperative blood glucose level > 200 mg/dL). Postoperative complications between the 2 groups were compared.

Results: There were 58 patients (37.6%; group 1, blood glucose < 200 mg/dL) who had controlled blood glucose and 96 patients (62.3%; group 2, blood glucose > 200 mg/dL) who had uncontrolled blood glucose. The mean age and weight for groups 1 and 2 were similar. There were no differences between the 2 groups regarding the duration of anhepatic phase (P = .20), operation time (P = .41), frequency of immediate intraoperative extubation (P = .14), and postoperative duration of mechanical ventilation (P = .06). There were no significant differences in frequency of patients who had postoperative infectious complications, acute kidney injury, or need for hemodialysis. Mortality rates after liver transplant were similar between the 2 groups (P = .81).

Conclusions: Intraoperative hyperglycemia during orthotopic liver transplant was not associated with an increased risk of postoperative infection, acute renal failure, or mortality.

Key words: Complications, End-stage liver disease, Glucose, Infection

Introduction

Perioperative high blood glucose level is a risk factor for serious intraoperative and postoperative complications. Uncontrolled blood glucose levels in diabetic patients may lead to serious organ damage, depending on the duration of diabetes. Surgery-related stress response also has been associated with various postoperative complications because administered pharmacologic agents may cause high perioperative blood glucose.

Liver transplant is a complex process in terms of surgery, anesthetic approach, and metabolic changes. These patients may have failure of different organ systems, depending on duration of liver failure. Various abnormalities might be observed in the endocrine system in patients with liver failure associated with low or high blood levels of hormones that are synthesized and catabolized in the liver. Hypoglycemia or hyperglycemia may affect all systems negatively and may be observed in these patients.

Intraoperative blood glucose regulation is important. High blood glucose levels that may be
observed for various reasons during liver transplant
may negatively affect postoperative morbidity and
mortality. Many treatments such as immuno-
suppressive drugs used after liver transplant, blood
and blood product replacements, mechanical
ventilation, invasive procedures for diagnosis and
treatment, and catheterizations are risk factors for
various infections.

In this study, we aimed to determine the effects of
intraoperative hyperglycemia on postoperative
outcomes in orthotopic liver transplant recipients.

Materials and Methods

Our study included patients having elective
orthotopic liver transplant due to liver failure
between January 2001 and December 2013 at Başkent
University Faculty of Medicine. A total of 389
patients had liver transplant in our center. Patients
aged < 15 years were excluded (179 patients).
Patients were divided into 2 groups according to
their maximum intraoperative blood glucose level:
group 1 included patients with maximum
intraoperative blood glucose < 200 mg/dL and group
2 included patients with maximum intraoperative
blood glucose > 200 mg/dL. Postoperative
complications were compared between the 2
groups.

The same anesthetic and surgical techniques
were used in all patients. They were admitted to the
intensive care unit at the end of surgery. The same
surgical and anesthesia team performed all liver
transplants. After operation, the patients were
routinely maintained on mechanical ventilation
until spontaneous breathing effort was evident and
hemodynamic stability had been achieved. Patients
were assessed in detail 1 day before the operation
and informed about the anesthesia. Patients were
premedicated with midazolam (0.1 mg/kg oral) and
hydroxyzine hydrochloride (1 mg/kg oral) 1 hour
before the operation. All patients were taken to the
operating room and routinely monitored with a 5-
channel electrocardiogram, pulse oximeter, heat
probe, and invasive blood pressure measurement.

Thiopental (3-6 mg/kg intravenous) and
fentanyl citrate (1-2 µg/kg intravenous) were
administered for induction of general anesthesia,
and vecuronium bromide (0.1 mg/kg intravenous)
was administered to attain sufficient muscle
relaxation before intubation. Isoflurane (0.5%-1%)
was administered in a mixture of 50% oxygen and
50% air for maintenance of anesthesia. Calcium,
dopamine, and remifentanil hydrochloride infusion
were initiated for controlled hypotension. Vascular
access was established with a double-lumen
hemodialysis catheter with suitable diameter and
length, and the widest intravenous cannula possible
was used to provide rapid fluid resuscitation.
Radial artery cannulation was performed for
invasive blood pressure follow-up and femoral
artery cannulation for monitoring of pulse-induced
contour cardiac output (PiCCO, Philips,
Amsterdam, Netherlands).

Measurements were performed with pulse-
induced contour cardiac output at the beginning of
operation, 2 hours later, before, during, and after
and the anhepatic phase, and before intubation.
Fluid and blood replacement decisions were made
according to pulse-induced contour cardiac output.
Thromboelastogram was used in patients for whom
intraoperative hemostasis could not be ensured or
cogulopathy was considered.

Hemodynamic parameters (systolic and diastolic
arterial blood pressure, heart rate, and oxygen
saturation) were recorded in the anesthesia record at
5-minute intervals. Postoperative pain control was
ensured with intravenous morphine (0.3 mg/h basal
infusion; 15 minutes locking time) using a patient-
controlled analgesia machine.

Hemodynamic stability (absence of hypotension)
and normothermia were the primary criteria
required for extubation following liver transplant.
Other criteria were an awake patient obeying verbal
commands, sufficient ventilation (respiratory rate
< 30 breaths/min with a good respiration pattern;
oxxygen saturation > 95% at room air; end-tidal
carbon dioxide pressure, 30-40 mm Hg), complete
resolution of neuromuscular blockade, normal
arterial blood gas results, and sufficient hemostasis.
All patients were taken to intensive care following
extubation in the operating room, and monitoring
included electrocardiogram, oxygen saturation,
respiratory rate, urination, radial artery pressure,
and central venous pressure. Respiratory
physiotherapy, prophylactic antibiotics, nutritional
support, fluid infusion, and supportive oxygen to
keep oxygen saturation > 94% were administered in
the intensive care unit.

Preoperative data collected included age (mo),
sex, height (cm), weight (kg), liver failure (including
duration and etiology), abdominal acid, hepato-pulmonary syndrome, hepatorenal syndrome, concomitant systemic diseases, donor degree of kinship, and Child-Pugh classification. Preoperative laboratory parameters included complete blood count, aspartate aminotransferase, alanine aminotransferase, total and indirect bilirubin, blood urea nitrogen, creatinine, electrolytes, activated partial thromboplastin time, prothrombin time, international normalized ratio, blood glucose levels, hepatitis markers, and preoperative biopsy. Intraoperative data included fluids, blood, and blood products administered; maximum and minimum systolic, diastolic, and mean blood pressure, heart rate, and central venous pressure; oxygen saturation; the worst blood gas pH, partial pressure of oxygen, carbon dioxide, and lactate levels recorded; the frequency of hypotension and bradycardia; vasopressor or antihypertensive therapy (20% of baseline values that require 6 or vasopressor support of blood pressure values as hypotension; 50 beats/min or below the required atropine for bradycardia was defined as heart rate values) (Hypotension was defined as a mean blood pressure less than 60 mm Hg or vasopressor requirement or mean blood pressure less than 20% of the baseline value; bradycardia was defined as a heart rate less than 50 beats/min or use of atropine); graft weight; intraoperative urine output; duration of anesthesia; and intraoperative blood glucose measurements. Postoperative data included intensive care unit stay, postoperative mechanical ventilation required, duration of mechanical ventilation, postoperative renal injury, lung injury, graft loss, revision surgery and indications, postoperative blood and blood products administered, postoperative pulse steroid therapy, number of rejection episodes, postoperative white blood cell count and C-reactive protein level, culture results, and mortality.

Statistical analyses
All data were analyzed with statistical software (Statistical Package for the Social Sciences, Version 17.0, SSPS Inc., Chicago, IL, USA). The 2 groups were compared using chi-square and Mann-Whitney tests. Logistic regression analysis was performed with variables of clinical and statistical significance for postoperative infection. The data were expressed as mean ± standard deviation (SD). Statistical significance was defined by $P \leq .05$.

Results
There were 58 patients (37.6%) (group 1, blood glucose < 200 mg/dL) who had controlled blood glucose and 96 patients (62.3%) (group 2, blood glucose >200 mg/dL) who had uncontrolled blood glucose (Table 1). The mean age and weight were similar for groups 1 and 2 (Table 1). There were more diabetic patients in group 1 (27 patients) than group 2 (3 patients; $P \leq .001$). There were no differences between the 2 groups in the duration of anhepatic phase, operation time, frequency of early (immediate) intraoperative extubation, or postoperative duration of mechanical ventilation (Table 1).

There were 60 (50) of the 154 patients (38.9%) who had postoperative infectious complications. The most frequent infectious complications for the 2 groups were pneumonia, followed by urinary tract infection, surgical site infection, and cholangitis (Table 2). Mortality rates after liver transplant were similar between the 2 groups (Table 2). There were no significant differences in frequency of acute kidney injury between group 1 (31 patients [53.4%]) and group 2 (45 patients [46.9%]; $P = .43$) or need for postoperative hemodialysis between group 1 (13 patients [22.4%]) and group 2 (25 patients [26%]; $P = .61$).

**Discussion**
We analyzed the effects of intraoperative hyperglycemia on postoperative outcomes in orthotopic
Glycemia. Vasodilation might be due to the vasodilation may be impaired due to hyper-oxide synthase gene synthesis associated with collagenase activity and decreased wound collagen. Reperfusion bovine aortic endothelial cells. Inducible nitric oxide synthase gene activation of inducible nitric oxide synthase gene and neutrophils. Additionally, hyperglycemia bactericidal functions of macrophages, granulocytes, and neutrophils. Infection is 1 of the important factors associated with morbidity and mortality in liver transplant recipients. In addition, wound site healing and wound site infections were more frequent in diabetic patients in various studies. In another study, heart surgery patients with high blood glucose levels had more complications following surgery despite that absence of any other risk factors. In our study, infection and mortality rates were similar between the 2 groups.

Hyperglycemia may have negative effects on the immune system. Hyperglycemia negatively affects bactericidal functions of macrophages, granulocytes, and neutrophils. Additionally, hyperglycemia impairs the functions of immunoglobulins as a result of negative effects on proteins. In addition, wound site healing may be delayed because of increased collagenase activity and decreased wound collagen. These factors may help explain the association between infections and hyperglycemia. Hyperglycemia increases reactive oxygen production in bovine aortic endothelial cells. Inducible nitric oxide synthase gene synthesis associated with vasodilation may be impaired due to hyperglycemia. (Vasodilation might be due to the activation of inducible nitric oxide synthase gene synthesis due to hyperglycemia.) Reperfusion damage following liver transplant in hyperglycemic patients might be aggravated with impaired nitric oxide synthase activity.

Adult liver transplant patients in the postoperative period of intraoperative blood glucose high mortality and morbidity in our study we examined the effect on blood glucose during surgery height did not show adverse effects on the development of the infection. (Intraoperative blood glucose in adult liver transplant patients is associated with high mortality and morbidity in the post-operative period. In our study, elevation of blood glucose during surgery not shown adverse effects on the development of infection in the postoperative period.) There are no guidelines for treatment of glucose levels during the perioperative period.

There were some limitations to our study. There are many factors that may facilitate transplant in patients with postoperative infection. Considering all these factors, we did not standardize the 2 groups completely. Other limitations of the study included the possibly limited clinical data, retrospective design, analysis of registry data, frequent missing data, and misclassification of important exposures.

In conclusion, according to this study, intraoperative hyperglycemia during liver transplant was not associated with an increased risk of postoperative infection, acute renal failure, or mortality.

References


Abstract

Objectives: Living-donor liver transplant has become a viable option and an important source of hepatic grafts. The goal of this study is to establish postoperative pulmonary complications of liver donation surgery in our center.

Materials and Methods: Data from 188 subjects (median age, 33.7 ± 8.4 y; male/female, 51.1%/48.9%) who had liver donation surgery from 1988 to 2013 were analyzed retrospectively. Patient demographic and clinical features were recorded. Postoperative complications and the correlation of risk factors for postoperative pulmonary complications were investigated.

Results: The incidence of early postoperative complications was 17% (n = 32), and 16 of these patients had postoperative pulmonary complications (8.5%); 2 of the postoperative pulmonary complications were detected on the day of surgery and the other 14 complications were observed between the second and seventh day after surgery. Most postoperative pulmonary complications were minor complications including atelectasis, pleural effusion, and pneumonia. There was 1 major postoperative pulmonary complication: pulmonary embolism that occurred on the fourth day after surgery in 1 patient. Late pulmonary complications also were reviewed and no late postoperative pulmonary complications were observed. There was no significant difference in early and late postoperative pulmonary complications between ex-smokers and smokers. Postoperative atelectasis was significantly higher in patients with body mass index ≤ 20 kg/m² than patients with body mass index > 21 kg/m² (P = .027). In our study population, no postoperative mortality was recorded.

Conclusions: We believe that preoperative weight reduction strategies and early mobilization with postoperative respiratory physiotherapy could be important factors to reduce postoperative pulmonary complications in liver donors.

Key words: Atelectasis, End-stage liver disease, Pleural effusion, Pneumonia, Transplant

Introduction

Living-donor liver transplant (LDLT) has been an innovative surgical treatment option in patients with liver failure since 1989.1,2 Although this procedure is common today in many transplant centers, the risk of morbidity and mortality for the donor continues to be a leading concern for surgeons and donors.3

Although adult-to-adult LDLT outcomes for postoperative complications are similar to donor transplant recipients, there are many concerns about donor morbidity reported.1-7 Until now, many studies have been published, reporting median 16% adult donor morbidity after LDLT.1 The reported severe postoperative complications are mostly abdominal, and pulmonary complications have a lower incidence. Potential postoperative pulmonary complications (POPC) of donors include pneumonia, atelectasis, pleural effusion, and pulmonary embolism.8-11

Nowadays, liver transplant is the main treatment option with a better result of survival than other treatment methods for end-stage liver patients.
Unfortunately, there are many more patients waiting for liver transplants than there are available deceased-donor organs. Therefore, LDLT is an important source of liver grafts for these patients but there are some risks for the donors. Therefore, liver donor candidates have some understandable concerns while making a decision about donating their organ, even though they would save and/or improve the quality of life of a transplant candidate recipient.

This study was performed to evaluate respiratory complications of liver donation surgery in our center. We also aimed to investigate the factors affecting postoperative respiratory complications on living liver donors.

Materials and Methods

Patients and evaluation
The medical records of adult liver donors who had donation surgery at Baskent University Hospital between 1988 and 2013 were analyzed retrospectively. The study was approved by the Ethical Review Committee of the Institute. All of the protocols conformed with the ethical guidelines of the 1975 Helsinki Declaration. The demographic features, smoking status, comorbid diseases, medications, and pulmonary function tests (PFTs) were recorded. The actual value and percentage of forced vital capacity (FVC%), forced expiratory volume in the first second (FEV₁%), FEV₁/FVC ratio, and forced midexpiratory flow (FEF₂₅%-₇₅%) were obtained. Preoperative sequential lesions on chest radiography and oxygen saturation as measured by pulse oximetry were noted. The type of inhalational anesthetic agent used during the operation, anatomic side of liver resection, duration of surgery, length of stay in the intensive care unit, and hospital stay were noted in the database.

Early (within 1 month) and late (≥ 1 month after surgery) pulmonary complications were recorded. Pleural effusion, pneumonia, respiratory insufficiency, atelectasis, pulmonary embolism, diaphragmatic eventration, and bronchospasm were all considered pulmonary complications. These data were obtained from physical examination, chest radiographs, and thoracic computed tomography scans. The findings on thoracic computed tomography were grouped as pleural effusion, consolidation, atelectasis, coexisting consolidation, and combinations of these radiographic findings. Bronchoscopic lavage culture results were noted in patients who underwent bronchoscopic examination.

Statistical analyses
Data analysis was performed with statistical software (IBM SPSS Statistics for Windows, Version 20.0, IBM Corp., Armonk, NY, USA). Continuous variables were expressed as mean or median ± standard deviation. The chi-square test was used to compare qualitative variables. Spearman rank correlation was used to analyze correlation between quantitative variables. All \( P \) values were 2-sided, and \( P \leq .05 \) was considered statistically significant.

Results

A total of 188 living liver donors, including 92 females (48.9%) and 96 males (51.1%), were analyzed in the study (Table 1). The mean age of patients was 33.7 ± 8.4 years. There were various comorbidities in 13 patients (6.9%) including chronic obstructive pulmonary disease (COPD), asthma, hypertension, rheumatoid arthritis, and diabetes mellitus (Table 1). Preexisting pulmonary disease was observed in 3 donors, but none of them had any respiratory complaints before the donation surgery.

There were 84 donors (44.7%) who never smoked, 67 donors (35.6%) who were current smokers (10.65 ± 10.36 pack-years) before surgery, and 37 donors (19.7%) who were ex-smokers (10.64 ± 10.4 pack-years) before surgery.

| Table 1. Demographic and Clinical Characteristics of the Patients |
|-------------------------|----------------|
| Demographics            | Patients (n = 188) (%) |
| Age (y)                 | 33.7 ± 8.4 |
| Sex (F/M)               | 48.9% / 51.1% |
| Smoking status (NS/CS/ES)| 44.7% / 35.6% / 19.7% |
| Smoking history (pack-years)| 10.64 ± 10.4 |
| Body mass index (kg/m²) | 24.5 ± 4.0 |
| Chronic drug usage      | |
| Total                   | 10 (5.0) |
| PPI                     | 1 (0.5) |
| Inhaler drug            | 2 (1.1) |
| Antihypertensive        | 2 (1.1) |
| Antihyperlipidemic      | 1 (0.5) |
| Thyroid hormone replacement | 2 (1.1) |
| NSAID                   | 2 (1.1) |
| Systemic disease        | 13 (7) |
| Hypertension            | 2 (1.1) |
| Hyperlipidemia          | 1 (0.5) |
| Asthma                  | 3 (1.6) |
| COPD                    | 1 (0.5) |
| Hypothyroidism          | 3 (1.6) |
| DM                      | 1 (0.5) |
| Acute rheumatic fever   | 2 (1.1) |

Abbreviations: COPD, chronic obstructive pulmonary disease; CS, current smoker; DM, diabetes mellitus; ES, ex-smoker; F, female; M, male; NS, never smoked; NSAID, non-steroidal anti-inflammatory drug; PPI, proton pump inhibitor.

*Data reported as median ± SD or number of patients (%).
donors (19.7%) who were ex-smokers. The POPCs were detected in 7 current smokers (10.4%) and 7 patients who never smoked (8.3%). There was no statistical correlation observed between smoking status and POPC occurrence ($P > .05$).

There were some pathologic findings in 47 patients (25%) in preoperative chest radiographs. The PFTs showed normal results except in 1 patient who had COPD and PFTs that showed mild airway obstruction. All donor preoperative oxygen saturation values by pulse oximetry were normal ($96.8 \pm 1.1$ mm Hg).

The type of hepatectomy procedures were listed in Table 2. Mean duration of donor surgery was $7.6 \pm 1.7$ hours. There were 3 types of inhalational anesthetics used during surgery (Table 3). There was no correlation between POPC and type of inhalational anesthetics, duration of surgery, or type of hepatectomy ($P > .05$).

Total hospitalization length of stay of the donors was $7.02 \pm 4.5$ days. Most patients (176) were discharged from the intensive care unit to the regular service within 24 hours. Early mobilization and respiratory physiotherapy were performed from the first day after surgery in 186 patients (98.9%). The incidence of early postoperative complications was 17% (n = 32), and 16 of these patients (8.5%) had POPC; of these, 2 POPC were detected within the first day of surgery and the others were detected between the second and seventh day after surgery. Most POPC were minor complications including atelectasis, pleural effusion, and pneumonia. There was 1 major POPC, which was a pulmonary embolism that occurred on the fourth day after surgery (Table 4). Atelectasis accompanied by pleural effusion (n = 5) was the most common POPC, and isolated atelectasis (n = 3) and pleural effusion (n = 3) were the second most common POPCs. The other early POPC were pneumonia (n = 2), pneumonia accompanied by pleural effusion (n = 1), pneumonia accompanied by atelectasis (n = 1), and pulmonary embolism accompanied by both pneumonia and pleural effusion (n = 1). The total length of hospital stay was statistically correlated with POPC ($P = .03$) (Table 5). Postoperative atelectasis was significantly higher in patients with body mass index > $21$ kg/m$^2$ compared with patients with body mass index $\leq 20$ kg/m$^2$ ($P = .027$) (Table 6).

<table>
<thead>
<tr>
<th>Table 2. Type of Hepatectomy Performed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hepatectomy</td>
</tr>
<tr>
<td>Left hepatectomy</td>
</tr>
<tr>
<td>Left lobectomy</td>
</tr>
<tr>
<td>Left lobectomy + cholecystectomy</td>
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<tr>
<td>Left segmentectomy</td>
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<tr>
<td>Left segmentectomy + cholecystectomy</td>
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<tr>
<td>Right hepatectomy</td>
</tr>
<tr>
<td>Right lobectomy</td>
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<tr>
<td>Right lobectomy + cholecystectomy</td>
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<tr>
<td>Right segmentectomy</td>
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<td>Right segmentectomy + cholecystectomy</td>
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<table>
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<tr>
<th>Table 3. Inhalational Anesthetics Used During Liver Donation Operation</th>
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<tbody>
<tr>
<td>Anesthetic Agent</td>
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<tr>
<td>---------------------------</td>
</tr>
<tr>
<td>Isoflurane + N$_2$O</td>
</tr>
<tr>
<td>Isoflurane + air</td>
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<tr>
<td>Sevoflurane + N$_2$O</td>
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<tr>
<td>Sevoflurane + air</td>
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<tr>
<td>Desflurane + air</td>
</tr>
<tr>
<td>Desflurane + N$_2$O</td>
</tr>
</tbody>
</table>

**Abbreviations:** N$_2$O, nitrous oxide

Total hospitalization length of stay of the donors was $7.02 \pm 4.5$ days. Most patients (176) were discharged from the intensive care unit to the regular service within 24 hours. Early mobilization and respiratory physiotherapy were performed from the first day after surgery in 186 patients (98.9%). The incidence of early postoperative complications was 17% (n = 32), and 16 of these patients (8.5%) had POPC; of these, 2 POPC were detected within the first day of surgery and the others were detected between the second and seventh day after surgery. Most POPC were minor complications including atelectasis, pleural effusion, and pneumonia. There was 1 major POPC, which was a pulmonary embolism that occurred on the fourth day after surgery (Table 4). Atelectasis accompanied by pleural effusion (n = 5) was the most common POPC, and isolated atelectasis (n = 3) and pleural effusion (n = 3) were the second most common POPCs. The other early POPC were pneumonia (n = 2), pneumonia accompanied by pleural effusion (n = 1), pneumonia accompanied by atelectasis (n = 1), and pulmonary embolism accompanied by both pneumonia and pleural effusion (n = 1). The total length of hospital stay was statistically correlated with POPC ($P = .03$) (Table 5). Postoperative atelectasis was significantly higher in patients with body mass index $> 21$ kg/m$^2$ compared with patients with body mass index $\leq 20$ kg/m$^2$ ($P = .027$) (Table 6).

<table>
<thead>
<tr>
<th>Table 4. Postoperative Pulmonary Complications in Liver Donors</th>
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<tbody>
<tr>
<td>Postoperative Pulmonary Complications</td>
</tr>
<tr>
<td>No. (%)</td>
</tr>
<tr>
<td>----------------------------------------------------------------</td>
</tr>
<tr>
<td>Total</td>
</tr>
<tr>
<td>Atelectasis</td>
</tr>
<tr>
<td>Pneumonia</td>
</tr>
<tr>
<td>Pleural effusion</td>
</tr>
<tr>
<td>Atelectasis + pleural effusion</td>
</tr>
<tr>
<td>Pneumonia + pleural effusion</td>
</tr>
<tr>
<td>Pneumonia + atelectasis</td>
</tr>
<tr>
<td>Pulmonary embolism + pleural effusion + pneumonia</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Table 5. Variables Evaluated for Postoperative Pulmonary Complications Occurrence*</th>
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</thead>
<tbody>
<tr>
<td>Variable</td>
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<tr>
<td>------------------------------------</td>
</tr>
<tr>
<td>Age, median (y)</td>
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<tr>
<td>Sex</td>
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<tr>
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<td>Female</td>
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<tr>
<td>Body mass index (kg/m$^2$)</td>
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<tr>
<td>≤ 20</td>
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<td>&gt; 21</td>
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<tr>
<td>Type of hepatectomy</td>
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<tr>
<td>Left</td>
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<tr>
<td>Right</td>
</tr>
<tr>
<td>Smoking status</td>
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<tr>
<td>Smoker</td>
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<tr>
<td>Nonsmoker</td>
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<tr>
<td>Ex-smoker</td>
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<tr>
<td>Anesthetic agent</td>
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<tr>
<td>Isoflurane + N$_2$O</td>
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<tr>
<td>Isoflurane + air</td>
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<tr>
<td>Sevoflurane + N$_2$O</td>
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<td>Sevoflurane + air</td>
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<tr>
<td>Desflurane + air</td>
</tr>
<tr>
<td>Desflurane + N$_2$O</td>
</tr>
<tr>
<td>Hospitalization length of stay (d)</td>
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</tbody>
</table>

**Abbreviations:** N$_2$O, nitrous oxide

*Data reported as mean or median ± SD or number of patients (%). All percentages are given relative to the total patient number (n = 188).
Discussion

Despite the development of technical advances and the increasing experience about liver resection surgery in specialized centers, the surgery remains a concern because of the risk of postoperative morbidity and mortality. The safety profile of hepatectomy probably can be improved if surgeons and medical staff have comprehensive knowledge of the expected complications and expertise in treatment. This study was performed to demonstrate the possible pulmonary complications in living-liver donors who underwent liver donation surgery in our transplant center.

Smoking has a major effect on outcomes after organ transplant for donors and recipients. The poor effects of smoking on outcomes of surgical procedures, particularly in solid-organ transplants, are well known. Smoking increases all-cause mortality, including problems with the cardiovascular system and infections that may account for most deaths in liver recipients. Some studies have shown beneficial effects of smoking cessation on liver and kidney recipients. However, there are insufficient data about smoking-related morbidity and mortality in liver donors.

In our study population, 104 patients (55.3%) were smokers and 84 patients (46.7%) were non-smokers. We observed no correlation between smoking status and early or late POPC. In our center, we recommend the cessation of smoking 8 weeks before the surgery for both donors and recipients to decrease postoperative complications, as recommended in the literature. Therefore, we suggested that this result could be attributed to our strict smoking cessation program for donors before the surgery.

Postoperative atelectasis or pulmonary infections most commonly present 3 to 5 days after the surgery because of insufficient inspiration limited by abdominal pain and poor effects of anesthesia on respiratory muscles. Atelectasis is the most common pulmonary complication in living-liver donors (range, 13%-26%). In 2 recent studies, POPC also was evaluated, but there were no data about atelectasis occurrence rate. In the present study, atelectasis was detected only in 3 patients (1.6%). As in many centers, respiratory physiotherapy and early mobilization strategies are being performed from the first day of surgery in our center. We believe that our strict postoperative treatment including early mobilization, respiratory physiotherapy (incentive spirometry, chest physical therapy, and hyperinflation therapy), and postoperative analgesic techniques could be responsible for this lower incidence of early postoperative atelectasis.

Obesity is a well-known risk factor for POPC after noncardiac surgery. Obese liver recipients have an increased risk of perioperative complications and reduced long-term survival. In practice guidelines from the American Association for the Study of Liver Diseases and American Society of Transplantation, preoperative dietary counseling is recommended for obese liver transplant candidates (≥ World Health Organization class 1). A recent study has shown that obesity was not an independent risk factor for POPCs after hepatic resection. In the present study, preoperative body mass index of donors was related to postoperative pulmonary atelectasis as a patient-related risk factor. Therefore, we suggested that living-liver donor candidates should be evaluated for a weight-reducing dietary program before donating, to reduce POPCs.

Liver surgery has not been described as a high risk factor for thromboembolic disease. A transient hypercoagulable state has been described after hepatectomy in living liver donors, despite standard prophylaxis with low-molecular weight heparin.
The probable mechanism of hypercoagulability after hepatectomy is the release of massive amounts of factor VIII and/von Willebrand factor and activation of the coagulation cascade because of the cut in the liver parenchyma. Pulmonary embolism could be a life-threatening postoperative problem in donors. Postoperative pulmonary embolism has been reported in a few studies with a different incidence in living-liver donors.\(^2,3,11,26\)

In our study, pulmonary embolism incidence was 1.8%. This lower rate could be attributed to our prophylactic treatment, including daily subcutaneous injection of low molecular weight heparin sodium started on the day after early and persistent mobilization, and compression sleeves, for all donors in our center. We concluded that the donors should be considered at high risk for developing thromboembolic disease and should be given appropriate anticoagulant prophylaxis as recommended in the literature.\(^21\) Moreover, all donor candidates should be evaluated for other known thrombosis risk factors such as obesity, smoking, drug use, and familial or personal history of thromboembolic disease.

In our study, there were no statistical relations between PFT results and POPC. This result could be explained by the selection of donor candidates with normal lung function before donation. There was 1 patient in our study group who had mild airway obstruction on PFTs, and this patient developed no POPC. In addition, no correlation was found between comorbid diseases (n = 13) and POPC in our study group. This could be explained by the precisely controlled comorbid diseases before surgery.

In this study, total length of hospitalization was 7.02 ± 4.5 days and significantly correlated with POPC occurrence in donors. This result could be explained by an extended hospital stay to diagnose and treat POPCs in these patients.

In conclusion, LDLT is a promising treatment method for chronic liver failure patients. As with any extensive surgery involving general anesthesia, there are possible pulmonary complications of the anesthesia and surgery on liver donors, but these complications are very rare. Our study indicates that the incidence of donor complications could be decreased by some important methods including respiratory physiotherapy, weight control, and prophylaxis for venous thromboembolism.

References


A Single-Center Retrospective Clinicopathologic Study of Endomyocardial Biopsies After Heart Transplant at Baskent University Hospital in Ankara, 1993-2014

Ayşen Terzi,1 Atilla Sezgin,2 Zeynep Tunca,1 Ebru Deniz,1 Ebru Şebnem Ayva,1 Nihan Haberal Reyhan,1 Halduş Müderrisoğlu,3 Binnaz Handan Özdemir1

Abstract

Objectives: The purpose of this study was to investigate the frequency and prognostic importance of acute cellular rejection after heart transplant.

Materials and Methods: All 84 heart transplant patients at our center from January 1993 to January 2014, including all 576 endomyocardial biopsies, were evaluated with retrospective review of clinical records and endomyocardial biopsies. Routine and clinically indicated endomyocardial biopsies after heart transplant were graded for acute cellular rejection (2005 International Society for Heart and Lung Transplantation Working Formulation). Survival analysis was performed using Kaplan-Meier method.

Results: There were 61 male (73%) and 23 female recipients. Median age at heart transplant was 29 years (range, 1-62 y). Posttransplant early mortality rate was 17.9% (15 patients). In the other 69 patients, 23 patients died and 46 patients (66.7%) were alive at mean 69.3 ± 7.2 months after heart transplant. Mean follow-up was 35.4 ± 29.8 months (range, 0.07-117.5 mo). Mean 8.4 ± 4.2 endomyocardial biopsies (range, 1-19 biopsies) were performed per patient. Median first biopsy time was 7 days (range, 1-78 d). The frequency of posttransplant acute cellular rejection was 63.8% (44 of 69 patients) by histopathology; 86% patients experienced the first episode of acute cellular rejection within 6 months after transplant. There were 18 patients with acute cellular rejection ≥ grade 2R on ≥ 1 endomyocardial biopsy in 44 patients with acute cellular rejection. No significant difference was observed between survival rates of patients with grade 1R or ≥ grade 2R acute cellular rejection, or between survival rates of patients with or without diagnosis of any grade of acute cellular rejection. Acute cellular rejection was not related to any prognostic risk factor.

Conclusions: Acute cellular rejection had no negative effect on heart recipient long-term survival, but it was a frequent complication after heart transplant, especially within the first 6 months.

Key words: Acute cellular rejection, Cardiac, Outcome, Survival

Introduction

Acute cellular rejection (ACR) is an inflammatory response, comprised mainly of lymphocytes, directed against the transplanted organ. Recognition of rejection in the transplanted heart is based on direct histologic examination of allograft tissue samples obtained by endomyocardial biopsy. An international histopathologic grading system for cardiac allograft biopsies was adopted by the International Society for Heart Transplantation in 1990. The revision of the 1990 Working Formulation for the standardization of nomenclature in the
diagnosis of heart rejection was reported by the International Society for Heart and Lung Transplantation (ISHLT) Working Group in 2005. This report summarized the revised consensus classification for cardiac allograft rejection with a multidisciplinary review of the cardiac biopsy grading system. The revised (R) categories of cellular rejection were: Grade 0R, no rejection (no change from 1990); Grade 1R, mild rejection (1990 Grades 1A, 1B, and 2); Grade 2R, moderate rejection (1990 Grade 3A); and Grade 3R, severe rejection (1990 Grades 3B and 4). The ISHLT Working Group recommended that grade 2R should be the threshold for treatment.

The effect of ACR episodes on the survival of heart transplant recipients is controversial. Previous studies supported the notion that recurrent ACR episodes increase the probability of allograft coronary artery disease and possibly decrease long-term survival when identified after the first year, particularly in pediatric heart transplant recipients. However, another study group found that ≥ 3 episodes of acute antibody-mediated rejection resulted in a statistically significant increase in cardiovascular mortality. In contrast, ACR episodes did not increase the risk of cardiovascular mortality. The frequency of acute rejection has declined with new maintenance immunosuppression options. This study was performed to investigate the frequency of ACR after heart transplant in our center and its prognostic importance in consideration of the current histopathologic grading system for cardiac allograft biopsies.

Materials and Methods

Patients
A retrospective clinicopathologic evaluation was performed of all endomyocardial biopsies of patients who underwent first-time allograft heart transplant at Başkent University Hospital in Ankara from January 1993 to January 2014. In the 21 years, 84 patients had heart transplant at the Department of Cardiovascular Surgery, and 15 patients died of early posttransplant complications.

The other 69 patients were followed by routine or additional clinically indicated endomyocardial biopsies. Routine endomyocardial biopsies were performed weekly for the first 2 weeks, monthly between the second and tenth week, and every 3 months between 10 weeks and 1 year. This study included 576 endomyocardial biopsies in 69 recipients. All endomyocardial biopsies that were diagnosed with any grade of ACR were reviewed and graded according to the 2005 ISHLT Working Formulation. The assessments of endomyocardial biopsies were performed only by light microscopy. All endomyocardial biopsy specimens included 2 or 3 tissue pieces. Light microscopy was performed with 5-μm sections from paraffin-embedded tissue using hematoxylin-eosin and histochemical staining methods including Masson trichrome. All biopsy specimens were evaluated with ≤ 6 microscopic sections per tissue piece.

Clinical data about presentation and follow-up were retrieved from patient and hospital records. Patients were followed by echocardiography and dobutamine stress echocardiography.

Statistical analyses
Continuous variables were presented as mean ± standard deviation, and categorical variables were presented as percentage and compared using chi-square test. Nonparametric tests (Wilcoxon rank sum test) were used for the relation between prognostic parameter and existing ACR. Multiple logistic regression was used to compute the risk of experiencing ACR and the relative odds ratios for 8 main prognostic groups. These groups were identified as demographic or clinical features that possibly had prognostic importance for heart transplant recipients (Table 1). The survival analysis was performed using Kaplan-Meier method and log-rank test. Grade 1R ACR and ACR ≥ grade 2R were labeled as degree 1 and degree 2, respectively, in Kaplan-Meier curves (Figure 1 and 2). Statistical significance was set at P ≤ .05. The analyses were performed using statistical software (SPSS, Version 15.0, SPSS Inc., Chicago, IL, USA).

Results

Demographic and clinical characteristics
There were 61 male (73%) and 23 female (27%) recipients. Median age at time of heart transplant was 29 years (range, 1 to 62 y). There were 2 patients who had combined cardiac and renal transplant. The 2 most frequent reasons for heart transplant were idiopathic dilated cardiomyopathy (39 patients) and ischemic heart disease (16 patients) (Table 2). Biatral
anastomosis was performed for orthotopic heart transplant in all patients. Hemofiltration was used routinely during the operations. In all cases, standard triple immunosuppressive therapy without induction therapy was used; the patients received corticosteroids, cyclosporine, and mycophenolate.
mofetil. Sirolimus was employed in some cases due to impaired renal function. For patients who had ACR ≥ grade 2R rejection, treatment consisted of pulse intravenous corticosteroids. Cyclosporine was replaced with tacrolimus for the treatment of grade 1R rejection.

Complications after heart transplant in 69 recipients were shown in Table 3. Mean follow-up was 35.4 ± 29.8 months (range, 0.07-117.5 mo). There were 15 of 84 recipients (17.9%) who died of early posttransplant complications. The other 69 patients were followed; 23 patients died and the other 46 patients (66.7%) were alive at mean 69.3 ± 7.2 months after heart transplant. In the posttransplant midterm or late phases, the known causes of death were sudden death (3 patients), sepsis (3 patients), and heart failure (2 patients). Other clinical data of the 69 recipients with follow-up were summarized (Table 4).

Endomyocardial biopsy
During follow-up, mean 8.4 ± 4.2 endomyocardial biopsies (range, 1-19 biopsies) were performed per patient. First biopsy was performed at median 7 days (range, 1 to 78 d). The frequency of posttransplant ACR diagnosed with histopathology was 63.8% (44 of 69 patients). Eighty-six percent of patients experienced their first episode of ACR in the early phase after transplant (first 6 months). There were 18 patients who had ACR ≥ grade 2 on ≥ 1 endomyocardial biopsy. Thus, the frequency of patients who experienced moderate or high grade ACR was 26% of 69 recipients.

Survival
The survival rates in the fifth and tenth years were 58.3% and 54.8% for all 84 recipients. The 5- and 10-year survival rates were 71% and 66.7% for 69 surviving recipients after exclusion of patients who had early posttransplant mortality. No significant difference was observed between survival rates of patients with grade 1R ACR and ACR ≥ grade 2R (Figure 1) or between survival rates of patients who had diagnosis of any grade of ACR and patients who did not have ACR (Figure 2) on their endomyocardial biopsies. Experience of having ACR was not significantly related to any prognostic risk factor (P > .05). No statistical difference was observed by logistic regression among the prognostic groups for risk of existence of ACR (Table 1).

Discussion
In this study, we observed higher frequency of ACR (63.8%) and ACR ≥ grade 2R (26%) than other similar studies in the current literature, especially those reported since 2003. Some authors suggested that the frequency of histologic rejection has decreased with current immunosuppression. Several studies showed that acute rejection is less frequent with a tacrolimus, than cyclosporine-based regimen. Our study included the 10 years before 2003, when new immunosuppressive regimens were not used. Standard posttransplant triple immunosuppressive

### Table 2. Etiology of Cardiac Disease

<table>
<thead>
<tr>
<th>Recipient Diagnosis</th>
<th>No. of Patients (%)</th>
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<tbody>
<tr>
<td>Dilated cardiomyopathy-idiopathic</td>
<td>39 (46.4)</td>
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<tr>
<td>Ischemic heart disease</td>
<td>16 (19.1)</td>
</tr>
<tr>
<td>Restrictive cardiomyopathy</td>
<td>10 (11.9)</td>
</tr>
<tr>
<td>Congenital heart disease</td>
<td>9 (10.7)</td>
</tr>
<tr>
<td>Dilated cardiomyopathy caused by adriamycin</td>
<td>3 (3.6)</td>
</tr>
<tr>
<td>Myocarditis</td>
<td>3 (3.6)</td>
</tr>
<tr>
<td>Valvular ischemic heart disease</td>
<td>2 (2.3)</td>
</tr>
<tr>
<td>Hypertrophic cardiomyopathy</td>
<td>1 (1.2)</td>
</tr>
<tr>
<td>Malignant arrhythmia</td>
<td>1 (1.2)</td>
</tr>
<tr>
<td>Total recipient number</td>
<td>84 (100)</td>
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</tbody>
</table>

### Table 3. Complications After Heart Transplant in 69 Recipients With Follow-Up

<table>
<thead>
<tr>
<th>Complications</th>
<th>No. of Patients (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acute postoperative renal dysfunction</td>
<td>13 (18.80)</td>
</tr>
<tr>
<td>Cardiovascular complications</td>
<td>18 (26.10)</td>
</tr>
<tr>
<td>Minor cerebrovascular accident</td>
<td>4</td>
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<tr>
<td>Pulmonary or peripheral thromboembolism</td>
<td>4</td>
</tr>
<tr>
<td>Pericardial effusion</td>
<td>3</td>
</tr>
<tr>
<td>Pericardial tamponade</td>
<td>2</td>
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<tr>
<td>Reoperation because of bleeding</td>
<td>2</td>
</tr>
<tr>
<td>Right ventricular aneurysm after endomyocardial biopsy</td>
<td>1</td>
</tr>
<tr>
<td>Temporary atrioventricular block</td>
<td>1</td>
</tr>
<tr>
<td>Arrhythmia</td>
<td>1</td>
</tr>
<tr>
<td>Infections</td>
<td>9 (13.00)</td>
</tr>
<tr>
<td>Urinary tract infections</td>
<td>3</td>
</tr>
<tr>
<td>Pulmonary aspergillosis</td>
<td>2</td>
</tr>
<tr>
<td>Cytomegalovirus infection</td>
<td>2</td>
</tr>
<tr>
<td>Herpes zoster</td>
<td>1</td>
</tr>
<tr>
<td>Mediastinitis</td>
<td>1</td>
</tr>
<tr>
<td>Late complications</td>
<td>6 (8.70)</td>
</tr>
<tr>
<td>Malignancy</td>
<td>4 (5.80)</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>2 (2.90)</td>
</tr>
<tr>
<td>Total number of recipient with complication</td>
<td>46 (66.7)</td>
</tr>
</tbody>
</table>

### Table 4. Clinical Data of 69 Recipients With Follow-Up

<table>
<thead>
<tr>
<th>Clinical Feature</th>
<th>Mean ± SD</th>
<th>Missing Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pulmonary vascular resistance (U)</td>
<td>2.8 ± 1.8</td>
<td>22</td>
</tr>
<tr>
<td>Left ventricle ejection fraction (%)</td>
<td>26.3 ±14.3</td>
<td>44</td>
</tr>
<tr>
<td>Total ischemic period (min)</td>
<td>218.6 ± 60.2</td>
<td>6</td>
</tr>
<tr>
<td>Aortic cross clamp time (min)</td>
<td>91.1 ± 17.5</td>
<td>5</td>
</tr>
<tr>
<td>Total bypass time (h)</td>
<td>5.3 ± 1.3</td>
<td>12</td>
</tr>
<tr>
<td>Blood loss in the first 24 h (mL)</td>
<td>811.4 ± 528.2</td>
<td>55</td>
</tr>
<tr>
<td>Mechanical ventilation time (h)</td>
<td>98.3 ± 150.5</td>
<td>54</td>
</tr>
<tr>
<td>Hospital stay (d)</td>
<td>28.5 ±14.7</td>
<td>55</td>
</tr>
<tr>
<td>Follow-up (mo)</td>
<td>35.4 ±29.8</td>
<td>0</td>
</tr>
</tbody>
</table>
therapy not including tacrolimus have been used in our center since 2003. Thus, no recipient included in this study initially had a tacrolimus-based regimen; cyclosporine was replaced with tacrolimus in cases of grade 1R rejection diagnosed with histopathology. Moreover, we estimated that noncompliance with intake of immunosuppressive drugs contributed slightly to the high ACR rate in the Turkish population. Another report from our center included 13 heart transplant patients from 2003-2007, and the acute rejection rate was lower (31%) but the rate of ACR ≥ grade 2R was high; all 4 patients with ACR experienced grade 3A (2R) ACR.10 However, the mean follow-up of this study was only 18.6 months (range, 1 to 38 mo), and the number of cases was very small.

A current similar study from Skane University Hospital in Sweden showed proportionally more first-year ACR ≥ grade 2 in endomyocardial biopsies in heart transplant patients from 1988-1999 (9.6%) than 2000-2010 (5.5%).11 This study showed low frequency and severity of first-year ACR with 6.5% routine endomyocardial biopsies and 14.1% additional clinically indicated endomyocardial biopsies showing ACR ≥ grade 2. They studied only posttransplant first year biopsies and they searched the percentage of ACR ≥ grade 2-positive biopsy specimens in all first year biopsies.

In contrast, we researched the ACR rate on the basis of recipients who had the diagnosis of any grade ACR on ≥ 1 endomyocardial biopsy. We observed that ACR was a frequent complication in heart transplant recipients in our center, especially within 6 months after heart transplant. There were 86% recipients who had ACR, with their first episode of ACR within 6 months after surgery. This can be explained by frequent routine endomyocardial biopsies that may allow early detection of ACR in this period and the use of a standard triple immunosuppressive therapy, cyclosporine-based, without induction therapy in all recipients and with improved immunosuppressive adjustments.

The 5- and 10-year survival rates were 71% and 66.7% for 69 surviving recipients, when posttransplant early mortality was excluded in our study. These results, as an experience of a single center, were compatible with others in the current literature.8,12

There are many different factors that possibly have an effect on survival of allograft organ recipients. Some demographic and clinical factors were recorded and evaluated for a relation to ACR experience, but no relevant factors were noted statistically. Söderlund and associates observed that proportionally more (P < 0.05) first-year cases of ACR ≥ grade 2 in endomyocardial biopsies were observed in heart transplants with pediatric (11.3%) than adult (7.1%) donors, and in sex-mismatched (10.4%) than sex-matched (6.3%) heart transplants.11 They did not observe a statistically significant relation with first-year ACR ≥ grade 2 and other risk factors such as age of recipient, difference in age between recipient and donor, sex of recipient, sex of donor, ABO-matching between recipient and donor, and maintenance immunosuppression in patients alive at discharge. None of these risk factors had a statistically significant relation to ACR experience in our study (Table 1). They also found that 5- and 10-year survival was lower in heart transplant patients with ≥ 1 compared with 0 first-year ACR ≥ grade 3A/3B. We did not observe a significant difference between survival rates of patients with ACR ≥ grade 2R and patients who had no diagnosis of any grade of ACR (Figure 1 and 2). Chin and associates identified a progressive decrease in survival with more frequent rejection episodes and late rejection (even when asymptomatic) after transplant in pediatric heart transplant recipients.3 They suggested that recurrent rejection is a risk factor for mortality after pediatric heart transplant, particularly when complicated by hemodynamic compromise. Our study included 27 pediatric recipients (aged < 18 y) that comprised 39.1% of all 69 surviving recipients. We studied pediatric and adult recipients together, and we observed that ACR experience had no negative effect on heart recipient survival. However, pediatric recipients may be the topic of a further research study with clinico-pathologic parameters.

References

Invasive Pulmonary Aspergillosis in Heart Transplant Recipients

Elif Küpeli, Gaye Ulibay, Sevil Bayram Akkurt, Füsün Öner Eyüboğlu, Atilla Sezgin

Abstract

Objectives: Invasive pulmonary aspergillosis is the most common invasive mycosis in heart transplant recipients. Early clinical recognition of this complication is difficult and laboratory data is not specific. Our aim was to study the characteristics of invasive pulmonary aspergillosis infections in heart transplant recipients.

Materials and Methods: Between 2007 and 2013, there were 82 patients who underwent heart transplant at our institution, including 6 patients who were diagnosed with invasive pulmonary aspergillosis. Medical records of these patients were reviewed for demographic, clinical, and radiographic features, microbiology data, serum galactomannan levels, antifungal treatment, and overall outcomes.

Results: The most common species causing the infection was Aspergillus fumigatus. The infection was encountered irrespective of the duration since the transplant. Bronchoalveolar lavage with positive culture for Aspergillus species and/or abnormal serum galactomannan level was suggestive of invasive pulmonary aspergillosis.

Conclusions: In our opinion, empiric antifungal therapy should be commenced as soon as invasive pulmonary aspergillosis is suspected in heart transplant recipients to reduce mortality. Although the duration of antifungal therapy for invasive pulmonary aspergillosis is debatable, heart transplant recipients may require long-term therapy to avoid recurrence.

Key words: Aspergillus fumigatus, Fungus, Galactomannan, Lung infection

Introduction

Invasive fungal infections are an important cause of morbidity and mortality in the recipients of solid-organ transplant. In this population, infection with Aspergillus species can produce several clinical presentations including sinusitis, tracheobronchitis, pneumonia, necrotizing cellulitis, brain abscess, or disseminated disease. In heart transplant recipients, the fungus Aspergillus most frequently causes pneumonia and is the opportunistic pathogen with the highest attributable mortality. Although invasive aspergillosis is a serious disease in this population, little is known about its natural history.

We performed a retrospective chart review of patients who underwent heart transplant at our institution and developed invasive pulmonary aspergillosis (IPA). The objective of our study was to further establish the characteristics of IPA infections in this population.

Materials and Methods

Study population

Patients who underwent heart transplantation at Başkent University School of Medicine between 2007 and 2013 and developed IPA were identified from our transplant database and clinical laboratory information system. Demographic and clinical variables were abstracted onto a data form. These variables included patient age, sex, primary diagnosis, date of transplant, serum galactomannan level, known risk factors for IPA, incidence of
rejection, cytomegalovirus infection, immunosuppressive regimen, radiographic features, prophylactic maneuvers, follow-up, outcomes, bronchoscopy data, and microbiology data. Follow-up information was obtained on all patients for a minimum 2 years after transplant.

**Diagnostic criteria**

Cases of IPA were identified according to the Clinical Practice Guidelines of the Infectious Diseases Society of America for IPA. The diagnosis was considered definite when the patient had positive histology and culture of a sample obtained from the same site or negative histology (or none done) and positive culture results of a sample obtained by protocol-specified invasive techniques such as bronchoalveolar lavage (BAL), bronchial washings, brushings, or needle aspiration. The diagnosis was considered probable when the heart transplant recipients with unexplained respiratory symptoms had abnormal chest radiographic findings and an invasive procedure was contraindicated; had 2 positive cultures of either sputum or throat samples or 1 positive culture or smear result of any bronchoscopy specimen; or met criteria for definite invasive aspergillosis in any other organ system. Results are expressed as “galactomannan index,” by comparison to the cutoff control. Galactomannan indexes of 1 or higher are regarded as positive. Positive serum galactomannan level suggested the diagnosis of IPA in which false positive results were excluded. Some antibiotics may cause false-positive results. The greatest problem has been in patients receiving piperacillin-tazobactam, amoxicillin or amoxicillin-clavulanate.

**Statistical analyses**

Data from case forms were extracted, entered into a central database, and analyzed.

**Results**

**Subjects**

We identified 6 patients with IPA in 82 patients who underwent heart transplant between 2007 and 2013 at our institution. The mean age of the entire heart transplant recipient group was 33.8 ± 18 years (range, 2-61 y) and there were 58 males and 24 females. Selection criteria for recipients and donors were as described previously and were constant during the study. Operative techniques did not markedly change during the study.

**Immunosuppression, rejection, and prophylaxis**

Each patient received prednisone, either azathioprine or mycophenolate mofetil, and either tacrolimus or cyclosporine as part of the antirejection regimen. All patients were on trimethoprim and sulfamethoxazole prophylaxis for Pneumocystis jiroveci pneumonia.

**Invasive pulmonary aspergillosis cases**

All IPA cases were male patients with a mean age of 52.5 ± 9.7 years (range, 38-61 y). The most common indication for heart transplant was dilated cardiomyopathy (83.3%) (Table 1). The most common symptoms of IPA were fever (n = 4) and cough (n = 3). Serum galactomannan level was abnormally high in 4 of 6 patients (Table 1). Aspergillus fumigatus grew in 50% cases on BAL culture. The onset of IPA varied between 30 days to 3 years after heart transplant. Each patient had abnormalities on chest radiography and thoracic computed tomography (Table 2). Multiple nodular consolidations were observed in all patients. A cavity was detected only in 1 patient. Voriconazole was prescribed in all patients for treatment (range, 1-12 mo) (Table 2). Only 1 patient (case 4) required the addition of intravenous amphotericin B due to resistant fever. Only 1 patient died because of the IPA infection.

**Discussion**

Since the introduction of heart transplantation as a therapeutic modality for end-stage congestive heart failure in 1968, Aspergillus has been recognized as a major opportunistic pathogen after transplant. During the past 30 years, major improvements have been introduced in immunosuppressive protocols, treatment of rejection, and prophylaxis against opportunistic infections. Since the introduction of cyclosporine in 1980, several immunosuppressive protocols have been suggested for heart transplant recipients. Initially, most patients who underwent heart transplant received prednisone, azathioprine, and cyclosporine as part of their immunosuppressive regimen. Subsequently, other immunosuppressive agents were added including muromonab-CD3 in June 1987 and mycophenolate mofetil and tacrolimus in February 1994. Ganciclovir was added as prophylaxis against cytomegalovirus in January 1987,
without cough, usually within 3 months after inhaled amphotericin B. However, inhaled amphotericin B prophylaxis has not been implemented at our institution.

Before the introduction of cyclosporine, the overall incidence of IPA was 27% and mortality from IPA was 60%. More recently, these numbers have decreased to 8% and 36%. It also has been suggested that the incidence of IPA has decreased markedly in heart transplant recipients. Cavitation or the halo sign may be present either at the time of detection of the nodule or may develop during the evolution of the nodule or may develop during the early diagnosis of IPA, we did not encounter this finding in our patients. This could be due to the small number of patients in our study.

Risk factors for invasive aspergillosis include prolonged neutropenia, neutrophil function deficits, corticosteroid therapy, graft-versus-host disease, and cytomegalovirus infection. In contrast with patients who have hematologic malignancies, neutropenia is not a risk factor for IPA in heart transplant recipients. None of our patients with IPA were neutropenic (absolute neutrophil count < 500 cells/mm³) at the time of diagnosis. The radiographic findings of IPA in our patients were similar to those described in the literature. Multiple pulmonary nodules are the most common radiographic manifestation and were present in all our patients. A cavitating nodule was present in only 1 of our patients but has been described to occur more frequently in this group of patients. Cavitating lungs may improve the outcomes of patients with IPA. Most of our patients also presented with these symptoms. These symptoms are vague and nonspecific but might be of value in interpreting a positive culture result for Aspergillus species, particularly within 3 months after transplant. In the present study, 50% cases were diagnosed during the first 90 days after heart transplant.

inhalation. It is very likely that these pharmacologic modifications decreased the incidence of invasive aspergillosis in heart transplant recipients.

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In conclusion, the incidence, morbidity, and mortality of IPA have steadily declined during the past 20 years. This is due most likely to the introduction of newer antirejection regimens and antifungal prophylaxis including inhaled amphotericin B. It also appears that these modifications have led to delayed appearance of IPA in this group. Patients with IPA usually present with respiratory symptoms and fever within the first 90 days after transplant. The most common radiographic findings include single or multiple pulmonary nodules. Cavitation or the halo sign may not always be present. Presence of neutropenia is not essential for the development of IPA. The advent of 2 new antifungal drugs with marked activity against Aspergillus species, voriconazole and caspofungin, may improve the outcomes of patients with IPA.

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Although the duration of antifungal therapy for IPA is controversial, some patients may require therapy for a long duration to prevent recurrent IPA infection.\textsuperscript{7,20} The development of IPA should be suspected in heart transplant recipients who present with fever and respiratory symptoms during the first 3 months after transplant, have a positive culture result for \textit{Aspergillus} species, and have abnormal radiographic findings, especially nodules, even in the absence of neutropenia.

\section*{References}

Long-Term Pulmonary Infections in Heart Transplant Recipients

Elif Küpeli, Gaye Ulubay, Esma Sevil Akkurt, Füsun Öner Eyüboğlu, Atilla Sezgin

Abstract

Objectives: Pulmonary infections are life-threatening complications in heart transplant recipients. Our aim was to evaluate long-term pulmonary infections and the effect of prophylactic antimicrobial strategies on time of occurrence of pulmonary infections in heart transplant recipients.

Materials and Methods: Patients who underwent heart transplantation between 2003 and 2013 at Baskent University were reviewed. Demographic information and data about immunosuppression and infectious episodes were collected.

Results: In 82 heart transplant recipients (mean age, 33.85 y; 58 male and 24 female), 13 recipients (15.8%) developed pulmonary infections (mean age, 44.3 y; 9 male and 4 female). There were 12 patients who had dilated cardiomyopathy and 1 patient who had myocarditis before heart transplantation; 12 patients received immunosuppressive therapy in single or combination form. Pulmonary infections developed in the first month (1 patient), from first to third month (6 patients), from third to sixth month (1 patient), and > 6 months after transplantation (5 patients). Chest computed tomography showed consolidation (unilateral, 9 patients; bilateral, 4 patients). Multiple nodular consolidations were observed in 2 patients and a cavitary lesion was detected in 1 patient. Bronchoscopy was performed in 6 patients; 3 patients had Aspergillus fumigatus growth in bronchoalveolar lavage fluid, and 2 patients had Acinetobacter baumannii growth in sputum. Treatment was empiric antibiotics (6 patients), antifungal drugs (5 patients), and both antibiotics and antifungal drugs (2 patients); treatment period was 1-12 months in patients with invasive pulmonary aspergillosis.

Conclusions: Pulmonary infections are the most common cause of mortality in heart transplant recipients. A. fumigatus is the most common opportunistic pathogen. Heart transplant recipients with fever and cough should be evaluated for pulmonary infections, and invasive pulmonary aspergillosis should be suspected if these symptoms occur within the first 3 months. Immediately starting an empiric antibiotic is important in treating pulmonary infections in heart transplant recipients.

Key words: Acinetobacter baumannii, Aspergillus fumigatus, Heart transplantation, Immunosuppression

Introduction

Pulmonary infections are commonly observed after heart transplantation. Pneumonia may occur in 30% patients. In an analysis from Mayo Clinic, the incidence decreased over three 5-year periods from 40% to 18%. Risk factors for pneumonia include longstanding heart failure, subclinical multiple organ failure, potential postoperative extracorporeal assistance, and heavy immunosuppression. Postoperative lung dysfunction may result from a complicated surgical procedure, phrenic nerve and diaphragmatic dysfunction, hemorrhage, re-expansion at the surgical site, pulmonary embolism, and infections. Other factors include prolonged intubation, the effects of sternotomy on respiration, and intensive immunosuppression.
After early graft failure, infections are the second leading cause of death in heart transplant recipients, and the lungs rank first as the infection site. Pneumonia occurs within the first 4 to 6 months after transplantation and may occur from nosocomial infection, community-acquired bacteria, opportunistic microorganisms, and cytomegalovirus. The incidence ranges from 20% to 30% and mortality rate is 30%.3-6 The highest fatality rate is recorded for Aspergillus infections (62%), despite a substantial decrease in incidence and mortality in the most recent series.7

We sought to evaluate long-term pulmonary infections in heart transplant recipients and to evaluate the effect of prophylactic antimicrobial strategies on time of occurrence of pulmonary infections after heart transplantation.

Materials and Methods

Study population
Patients who underwent orthotopic heart transplantation from 2003 to 2013 at Baskent University were reviewed. Demographic information, type of immunosuppression, perioperative infectious prophylaxis, follow-up clinical information, incidence of rejection, and the type of infectious episodes were collected from our transplantation database and clinical laboratory information system. Infections were listed according to the type of organism, organ involved, time of onset after transplant, and clinical outcome. Available bronchoscopy and microbiology data also were collected. The study was approved by the local ethics committee.

Diagnostic criteria
Lower respiratory tract infection was defined as the presence of cough with purulent sputum production, temperature > 38°C, and leukocytosis with response to appropriate antimicrobial therapy. Pneumonia was defined to include these symptoms and signs and new lung infiltrates on radiography. Although microbiologic studies were ordered, a positive result was not mandatory to confirm the diagnosis.

Cases of invasive pulmonary aspergillosis (IPA) were identified according to the Clinical Practice Guidelines of the Infectious Diseases Society of America for IPA.8 The diagnosis was considered definite if the patient had positive histology and culture results of a sample obtained by protocol-specified invasive techniques (bronchoalveolar lavage [BAL], bronchial washings, brushings, or needle aspiration). The diagnosis was considered probable if;

1. The heart transplant recipient with unexplained respiratory symptoms had abnormal chest radiographic findings and
2. if an invasive procedure was contraindicated, the patient should have 2 positive cultures of sputum or throat samples or 1 positive culture result of a smear of a bronchoscopy specimen, or
3. the patient should met criteria for definite invasive aspergillosis in any other organ system.8 Positive serum galactomannan levels also suggested the diagnosis of IPA in which false positive results were excluded. Some antibiotics may cause false-positive results. The greatest problem has been in patients receiving piperacillin-tazobactam, amoxicillin, or amoxicillin-clavulinate.

Statistical analyses
Data from case forms were extracted, entered into a central database, and analyzed.

Results

Patients
In a total of 82 heart transplant recipients (mean age, 33.85 y; range, 2-31 y; 58 male and 24 female), 13 patients (15.8%) developed pulmonary infections (mean age, 44.3 y; range, 20-61 y; 9 male and 4 female) (Table 1).

Indications for heart transplant
The indications for heart transplant in patients with pulmonary infections were dilated cardiomyopathy in 12 patients and myocarditis in 1 patient (Table 1).

Prophylaxis and immunosuppressive treatment
All patients received trimethoprim, sulfamethoxazole, and valganciclovir prophylaxis, and 13 patients received immunosuppressive therapy in single or combination form (Table 1).

Clinical manifestations
Pulmonary infections developed in the first month (1 patient), from first to third month (6 patients), from
third to sixth month (1 patient), and > 6 months after transplant (5 patients). Presenting symptoms were cough and/or fever (Table 1).

**Radiographic manifestations**
Chest computed tomography showed unilateral consolidation in 9 patients and bilateral consolidation in 4 patients. Multiple nodular consolidations were observed in 2 patients, and a cavitory lesion was detected in 1 patient (Table 2).

**Microbiologic manifestations**
Bronchoscopy was performed in 6 patients. There were 3 patients who had *Aspergillus fumigatus* growth in BAL fluid, and 2 patients had *Acinetobacter baumannii* growth in sputum (Table 2). Six patients developed pulmonary infections between the first to third month, 5 patients after 6 months, 1 patient in the first month, and 1 patient between the third and sixth months after heart transplantation. The other 8 patients had no growth in BAL (3 patients), sputum (3 patients), deep tracheal aspirate (1 patient), or pleural effusion (1 patient).

**Treatment**
Treatment was empiric antibiotics (6 patients), antifungal drugs (5 patients), and both antibiotics and antifungal drugs (2 patients); treatment period was 1 to 12 months in patients with IPA.

**Prognosis**
There were 4 patients who completely improved, 3 patients who remained on antifungal treatment, 3 patients who died because of rejection, and 3 patients who died because of pulmonary infection.

**Discussion**
Pulmonary infection is a common complication after heart transplantation. Despite improvements in the prevention of pneumonia, occurrence of pneumonia was independently predictive of mortality in this population. In this study, the incidence of pulmonary infection was 15.8% in heart transplant recipients.

The risk for pulmonary infection increases due to elevated immunosuppression. The immuno-

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**Table 1. Demographics of the Patients, Indications for Heart Transplantation, Prophylaxis, and Immunosuppressive Drug Regimen**

<table>
<thead>
<tr>
<th>Case</th>
<th>Age (y)</th>
<th>Sex</th>
<th>Indication</th>
<th>Perioperative Prophylaxis</th>
<th>Immunosuppressive Drugs</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>50</td>
<td>Male</td>
<td>Dilated cardiomyopathy</td>
<td>Trimethoprim/sulfamethoxazole valganciclovir</td>
<td>Prednisolone mycophenolate mofetil cyclosporine</td>
</tr>
<tr>
<td>2</td>
<td>50</td>
<td>Female</td>
<td>Dilated cardiomyopathy</td>
<td>Trimethoprim/sulfamethoxazole valganciclovir</td>
<td>Prednisolone mycophenolate mofetil</td>
</tr>
<tr>
<td>3</td>
<td>22</td>
<td>Female</td>
<td>Dilated cardiomyopathy</td>
<td>Trimethoprim/sulfamethoxazole valganciclovir</td>
<td>Prednisolone mycophenolate mofetil</td>
</tr>
<tr>
<td>4</td>
<td>32</td>
<td>Male</td>
<td>Dilated cardiomyopathy</td>
<td>Trimethoprim/sulfamethoxazole valganciclovir</td>
<td>Prednisolone mycophenolate mofetil</td>
</tr>
<tr>
<td>5</td>
<td>26</td>
<td>Female</td>
<td>Dilated cardiomyopathy</td>
<td>Trimethoprim/sulfamethoxazole valganciclovir</td>
<td>Prednisolone mycophenolate mofetil</td>
</tr>
<tr>
<td>6</td>
<td>54</td>
<td>Male</td>
<td>Dilated cardiomyopathy</td>
<td>Trimethoprim/sulfamethoxazole valganciclovir</td>
<td>Prednisolone mycophenolate mofetil</td>
</tr>
<tr>
<td>7</td>
<td>61</td>
<td>Male</td>
<td>Dilated cardiomyopathy</td>
<td>Trimethoprim/sulfamethoxazole valganciclovir</td>
<td>Prednisolone mycophenolate mofetil</td>
</tr>
<tr>
<td>8</td>
<td>61</td>
<td>Male</td>
<td>Dilated cardiomyopathy</td>
<td>Trimethoprim/sulfamethoxazole valganciclovir</td>
<td>Prednisolone mycophenolate mofetil</td>
</tr>
<tr>
<td>9</td>
<td>43</td>
<td>Male</td>
<td>Myocarditis</td>
<td>Trimethoprim/sulfamethoxazole valganciclovir</td>
<td>Prednisolone mycophenolate mofetil</td>
</tr>
<tr>
<td>10</td>
<td>38</td>
<td>Male</td>
<td>Dilated cardiomyopathy</td>
<td>Trimethoprim/sulfamethoxazole valganciclovir</td>
<td>Prednisolone mycophenolate mofetil</td>
</tr>
<tr>
<td>11</td>
<td>58</td>
<td>Male</td>
<td>Dilated cardiomyopathy</td>
<td>Trimethoprim/sulfamethoxazole valganciclovir</td>
<td>Prednisolone mycophenolate mofetil</td>
</tr>
<tr>
<td>12</td>
<td>20</td>
<td>Female</td>
<td>Dilated cardiomyopathy</td>
<td>Trimethoprim/sulfamethoxazole valganciclovir</td>
<td>Prednisolone mycophenolate mofetil</td>
</tr>
<tr>
<td>13</td>
<td>61</td>
<td>Male</td>
<td>Dilated cardiomyopathy</td>
<td>Trimethoprim/sulfamethoxazole valganciclovir</td>
<td>Prednisolone mycophenolate mofetil</td>
</tr>
</tbody>
</table>

**Table 2. Clinical and Radiographic Manifestations, Treatment Regimens, and Follow-Up of Heart Transplant Recipients With Pulmonary Infections**

<table>
<thead>
<tr>
<th>Case</th>
<th>Symptoms</th>
<th>Thoracic Computed Tomography</th>
<th>Culture Result</th>
<th>Treatment</th>
<th>Treatment Duration (mo)</th>
<th>Follow-Up</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Fever Cough</td>
<td>Unilateral consolidation</td>
<td>No growth</td>
<td>Empiric antibiotic</td>
<td>&lt; 1</td>
<td>Died</td>
</tr>
<tr>
<td>2</td>
<td>Cough</td>
<td>Bilateral consolidation</td>
<td><em>Acinetobacter baumannii</em></td>
<td>Empiric antibiotic</td>
<td>1-3</td>
<td>Died</td>
</tr>
<tr>
<td>3</td>
<td>Cough</td>
<td>Unilateral consolidation</td>
<td><em>Acinetobacter baumannii</em></td>
<td>Empiric antibiotic + antifungal</td>
<td>&lt; 1</td>
<td>Died</td>
</tr>
<tr>
<td>4</td>
<td>Cough</td>
<td>Unilateral consolidation</td>
<td>No growth</td>
<td>Empiric antibiotic</td>
<td>&lt; 1</td>
<td>Improved</td>
</tr>
<tr>
<td>5</td>
<td>Cough</td>
<td>Unilateral consolidation</td>
<td>No growth</td>
<td>Empiric antibiotic</td>
<td>&lt; 1</td>
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<tr>
<td>6</td>
<td>Cough</td>
<td>Bilateral consolidation</td>
<td><em>Aspergillus fumigatus</em></td>
<td>Antifungal treatment</td>
<td>1-3</td>
<td>Still on antifungal treatment</td>
</tr>
<tr>
<td>7</td>
<td>Fever</td>
<td>Bilateral consolidation</td>
<td><em>Aspergillus fumigatus</em></td>
<td>Antifungal</td>
<td>1-3</td>
<td>Still on antifungal treatment</td>
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<tr>
<td>8</td>
<td>Fever Cough</td>
<td>Bilateral consolidation</td>
<td>No growth</td>
<td>Antifungal</td>
<td>3-6</td>
<td>Died</td>
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<tr>
<td>9</td>
<td>Fever</td>
<td>Unilateral consolidation</td>
<td><em>Aspergillus fumigatus</em></td>
<td>Antifungal</td>
<td>&lt; 1</td>
<td>Died</td>
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<tr>
<td>10</td>
<td>Fever Cough</td>
<td>Unilateral consolidation</td>
<td>No growth</td>
<td>Antifungal</td>
<td>1-3</td>
<td>Died</td>
</tr>
<tr>
<td>11</td>
<td>Cough</td>
<td>Unilateral consolidation</td>
<td>No growth</td>
<td>Empiric antibiotic + antifungal</td>
<td>1-3</td>
<td>Still on antifungal treatment</td>
</tr>
<tr>
<td>12</td>
<td>Fever</td>
<td>Unilateral consolidation</td>
<td>No growth</td>
<td>Empiric antibiotic</td>
<td>&lt; 1</td>
<td>Improved</td>
</tr>
<tr>
<td>13</td>
<td>Cough</td>
<td>Unilateral consolidation</td>
<td>No growth</td>
<td>Empiric antibiotic</td>
<td>&lt; 1</td>
<td>Improved</td>
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Abbreviation: MELD, model for end-stage liver disease.
suppressive agents used in transplant are composed of a triple regimen of cyclosporine or tacrolimus, azathioprine or mycophenolate mofetil, and corticosteroids. Higher doses of each drug are administered early postoperatively when the risk of acute rejection is greatest; therefore, infection is of major concern, especially during the first 1 to 3 months after heart transplantation. Both cyclosporine and tacrolimus inhibit the activation and proliferation of T cells by inhibiting interleukin-2 production. Azathioprine is a purine analog that prevents the proliferation of activated B and T lymphocytes; therefore, both cell mediated and humoral immunity are affected. Mycophenolate mofetil often is substituted for azathioprine because it inhibits de novo purine synthesis and lymphocyte proliferation. Corticosteroids are nonspecific in their effect and lead to a reduction in neutrophil chemotaxis, antigen presentation, T-cell activation and proliferation, and macrophage function. Induction therapy with antilymphocyte antibodies (cytolytic agents) or interleukin 2 receptor antagonists often may be administered perioperatively to prevent acute allograft rejection. Patients at risk for cytomegalovirus infection are predisposed to reactivation when cytolytic agents are used. Sirolimus, also known as rapamycin, inhibits the proliferative T-cell response to interleukin-2. Sirolimus is used as a substitute agent in patients intolerant or unresponsive to the calcineurin inhibitors or purine analogues.

The cause of pulmonary infections in heart transplant recipients is diverse. In a previous study, pulmonary infections were caused by opportunistic microorganisms (60%), nosocomial pathogens (25%), and community-acquired bacteria and mycobacteria (15%). Pulmonary infections in heart transplant recipients frequently are polymicrobial (24%-58%) in other studies. Aspergillus species were the most common cause of pulmonary infections in our population (23% isolates). The incidence of Aspergillus pneumonia was 3.6 cases per 100 heart transplants, similar to the incidence reported in recent studies. The changes in immunosuppressive treatment and the degree of environmental exposure, which are the main risk factors for the development of pulmonary aspergillosis, could explain the occurrence of Aspergillus infection.

Cytomegalovirus is the microorganism that most frequently causes pneumonia after heart transplantation and accounts for 23% to 32% isolates in various studies. In the present study, we did not observe cytomegalovirus infection in our heart transplant recipients, most probably due to our effective prophylaxis regimen.

Pneumocystis jiroveci pneumonia may be another cause of pulmonary infection in heart transplant recipients; with an incidence of 2% to 8% reported in the literature. Prophylactic cotrimoxazole is very effective and reduces the incidence of Pneumocystis jiroveci pneumonia almost to 0%, as observed in the present study.

Nosocomial bacteria are the leading cause of pneumonia after heart transplantation. In our study, the incidence was 2.4 episodes per 100 heart transplant recipients. Acinetobacter baumannii was the causative organism and resulted in death in 2 patients.

The timetable of infection in solid-organ transplantation has been useful for diagnosis. In the present study, 6 patients developed pulmonary infections between the first to third month, 5 patients after 6 months, 1 patient in the first month, and 1 patient between the third and sixth months after heart transplantation. In 50% of invasive pulmonary aspergillosis (IPA) patients, the infection occurred 3 months after heart transplant, which was consistent with data in the literature.

Most of the clinical manifestations of pneumonia after heart transplantation are nonspecific. Fever and cough were the most common symptoms in our study, and no hemoptysis was observed in any of the patients who had aspergillosis. An acute onset was observed in patients with nosocomial infection.

Some radiographic images are characteristic of specific causes of pneumonia after heart transplantation. Chest computed tomography is more sensitive than plain radiography for the diagnosis of acute pulmonary complications in immunosuppressed patients. In this study, unilateral infiltrates predominated and 1 cavitary lesion was observed in a patient who had aspergillosis.

However, the diagnostic use of clinical and radiographic manifestations in pneumonia is limited after a heart transplant. Only 46% empiric treatments indicated in this study were appropriate. For this reason, quick diagnostic procedures that guide antimicrobial treatment are necessary. The BAL is the main diagnostic procedure, and the diagnostic yield of BAL was 50% in our study. Other
bronchoscopic procedures are less sensitive. Examination of sputum should form part of the initial diagnostic study of pneumonia after heart transplantation, but this has limited diagnostic yield (15% in the present study).

Pulmonary infections are a leading cause of death after heart transplantation. In this study, there were 3.6 deaths due to pulmonary infections per 100 heart transplant recipients; 3.3%, 6%, and 7.6% patients died in other recent series. The overall mortality in heart transplant recipients with pneumonia is from 23% to 25% (23% in our study); this rate varies widely depending on the cause. Aspergillus pneumonia has the worst prognosis. The mortality rate associated with this pulmonary infection was 50% in a review involving 64 patients.

In conclusion, pulmonary infections are the most common cause of mortality in heart transplant recipients. It is suggested that heart transplant recipients with fever and cough should be evaluated for pulmonary infection, and IPA should be suspected if these symptoms occur within the first 3 months after heart transplant. Immediate empiric antibiotic therapy is important in treating pulmonary infections in heart transplant recipients.

References

**Abstract**

**Objectives:** Human epidermal keratinocytes are currently established as a treatment for burns and wounds and have laboratory applications. Keratinocyte culture contamination by unwanted cells and inhibition of cell proliferation are barriers in primary keratinocyte culture. According to the recent literature, these cells are hard to culture. The present study was conducted to evaluate the efficacy of gelatin-coated surfaces in keratinocyte cultures.

**Materials and Methods:** After enzymatic isolation of keratinocytes from normal epidermis by trypsin, the cells were cultured on gelatin-coated flasks in serum-free medium. Another group of cells were cultured as a control group without gelatin coating.

**Results:** We showed positive effects of surface coating with gelatin on the primary culture of keratinocytes. Culture of these cells on a gelatin-coated surface showed better proliferation with suitable morphology. By using gelatin, adhesion of these cells to the surface was more efficient and without contamination by small round cells.

**Conclusions:** Successful primary culture of keratinocytes on a gelatin-coated surface may provide better yield and optimal number of cells for research and clinical applications.

**Key words:** Cell culture, Dermatology, Laboratory technique, Skin

**Introduction**

The technique for culture of keratinocytes, the main epidermal cells, with production of epithelial sheets for grafting was reported in 1975.1 In vitro cultivation of keratinocytes has a wide range of applications in many aspects of clinical medicine and research, such as the treatment of burns, treatment of chronic wounds, and biological studies.2-4 Successful isolation of pure keratinocytes from human epidermis is important for expansion of these cells in tissue culture. Therefore, culture systems were established to support keratinocyte growth and proliferation.

Application of different techniques such as density gradient centrifugation, specific cell surface antibodies, and attachment to specific substrates have been proposed in previous studies for achievement of high homogeneous primary cultures of keratinocytes.5 Many studies of adherent cell cultures have been performed with 2-dimensional cell culture without extracellular matrix (ECM). However, the ECM has an important role in cell and tissue function and proper tissue development.6 Matrix leads to the formation of basement membrane and epithelial cell polarization.7

Some studies have been performed to find a suitable material as an ECM for culture of keratinocytes. Researchers indicated that collagen for murine keratinocyte adhesion and proliferation was better than gelatin and collagen hydrolysate.8 Proliferation of human keratinocytes is possible on fibronectin-coated dishes, and with this method, suitable cell growth was achieved with initial seeding rate 10% of the amount necessary for cells cultured on collagen-coated dishes and 5% for cells cultured on dishes not coated with collagen.9

Gelatin and collagen scaffolds have comparable properties.10 In this study, gelatin was selected to find...
an appropriate biomaterial for culture of keratinocytes on a coated surface. Collagen is one of the most abundant proteins of ECM, and gelatin is a collagen derivative. Gelatin has advantages such as biocompatibility and low cost.11

In response to these benefits and the limited number of studies on this topic, we selected gelatin to coat the substrate for keratinocyte culture. The purpose of this experiment was to evaluate the biological effects of a gelatin-coated surface on human epidermal adhesion, purification, and proliferation.

Materials and Methods

Keratinocyte isolation

Most materials were purchased (Gibco/BRL, Grand Island, NY, USA) except for specific items noted. According to the ethical guidelines of the 1975 Declaration of Helsinki, all studies involving human subjects were approved by the ethics committee of Shiraz University of Medical Sciences, and a consent form was obtained from patients.

Separation of the epidermis layer from the skin and cultivation of keratinocytes were performed according to published methods with slight variations.12 Skin pieces (n = 10) were provided by the plastic surgery department, with informed consent of patients who had reduction mammoplasty. The skin pieces were transferred to the cell culture laboratory in defined keratinocyte serum-free medium (DKSFM) supplemented with 5 μg/mL gentamicin. The skin was washed in 70% isopropanol and put in Dulbecco phosphate buffered saline (DPBS) containing 20 μg/mL gentamicin for 1 hour. Skin pieces (size, 0.5-1 cm²) were placed in Dispase solution (10 mg/mL) in DKSFM with 5 μg/mL overnight at 4ºC to 6ºC. On the next day, the epidermis was separated from the dermis, and the epidermal layer was digested with 0.05% trypsin/ethylene diamine tetraacetic acid for 5 to 10 minutes at 37ºC. After dissociation of cells, trypsin was inactivated with 10 mg/mL soybean trypsin inhibitor in DPBS. The cell suspension was centrifuged at 338 ×g for 5 minutes. The pellet was rinsed with DPBS and resuspended, and cells were counted with a hemocytometer and trypan blue.

Preparation of gelatin-coated surface and keratinocyte culture

Gelatin (1%) (Sigma-Aldrich, St. Louis, MO, USA) solution was prepared in distilled water. The gelatin solution was placed on the surface of flasks and incubated for 30 minutes at 37ºC. Excess solution was removed, and keratinocytes were cultured (density, 4-6 × 10⁴ cells/cm²) in flasks with coated and noncoated surfaces.

Cell features

After keratinocytes were seeded, cells were observed every day and evaluated for morphology, surface adhesion, proliferation, and homogeneity using an inverted microscope. We used Papanicolaou stain as a multichromatic staining cytologic technique for evaluation of keratinocytes. For this technique, keratinocytes were transferred to a slide and fixed in acetic acid-alcohol fixative for 15 minutes. The cells were stained with hematoxylin, Orange G, and Eosin Azure.

Results

Investigation of keratinocyte morphology showed epithelial morphology (also known as pavement stone) in keratinocytes, which were confirmed by Papanicolaou stain (Figure 1). The cultured cells in gelatin-coated flasks were compared with cells cultured in conventional polystyrene flasks (control...
The adhesion of cells in primary culture was analyzed every day. The cells cultured on gelatin showed a marked increase in adhesion rate compared with the control group. In the gelatin group, cells attached to the surface showed normal morphology of epithelial cells, but contamination with round attached cells was observed in the control group (Figure 2). Evaluation of proliferation after primary seeding showed that 7 of the 10 samples cultured on gelatin developed a monolayer appearance, but only 3 samples in the control group had this appearance (Figure 3).

Discussion

Cell culture is a complex process with cell proliferation under controlled conditions. Animal cell culture has become a frequent laboratory approach for maintaining fresh cells separated from their native tissue environment. There are many clinical indications for application of cells, and cell expansion methods made it possible to investigate the clinical potential of these methods. Therefore, cell therapy in skin problems has been considered. In the past decade, treatment of skin injuries by keratinocyte culture and tissue engineering has focused the attention of researchers.

The first development in skin tissue engineering was the in vitro culture of keratinocytes from separated epidermis. A major improvement in keratinocyte culture was made in the 1970s, when Rheinwald and Green cultivated human primary epidermal cells on murine fibroblasts (3T3 cells). Numerous researchers applied the method of cultured keratinocytes clinically for the treatment of severely burned patients. However, application of 3T3 cells for keratinocyte expansion is associated with the risk of contamination with infections and animal antigens. At present, the focus of research is to discover an optimal protocol for keratinocyte culture.

Despite the importance of human keratinocytes in cell therapy, limited studies have been conducted in the field of keratinocyte culture in Iran. Shokerzadeh and associates cultured rat keratinocytes in serum-free medium, and they applied an explant method for the culture of keratinocytes. Reiisi and coworkers reported the isolation and culture of newborn mouse epidermal stem cells by rapid adherence on a composite matrix made of type I collagen and fibronectin. In another experiment, researchers investigated different methods for separation of epidermal cells from skin, and concluded that the sodium bromide (4N) method...
yielded more live cells and had less toxicity than trypsin application.\textsuperscript{21}

Successful cultivation of keratinocytes and achievement of the appropriate number of cells are first steps toward application of these cells as a therapeutic approach for patients who have severe burn injuries and wounds. The purpose of this study was to isolate, culture, and improve the proliferation of human epidermal keratinocytes.

The skin epidermal layer is a stratified squamous epithelium composed of multiple layers of keratinocytes. There are difficulties in keratinocyte culture, including cell senescence, differentiation, apoptosis, and contamination with unwanted cells. Cultivation of keratinocytes under unsuitable conditions causes loss of proliferation potential and the occurrence of early differentiation.\textsuperscript{1, 22} In the present study, we applied coated tissue culture surfaces with gelatin for primary keratinocyte culture as a simple and rapid method.

The ECM provides a suitable microenvironment for cells and affects their behavior and function.\textsuperscript{23} Conventional adherent cell culture is performed on rigid surfaces. The differences between in vitro and in vivo conditions lead to the impossibility of generalizing the results of laboratory studies to the natural conditions in the body. Some synthetic matrices have been evaluated to mimic the ECM.\textsuperscript{11} Various compounds are present for coating the substrate with ECM proteins to aid cell attachment. To find a suitable biomaterial for coating the culture surface in this study, gelatin was selected because it is obtained by partial hydrolysis of collagen, which is the major protein of ECM in connective tissue. Gelatin is a soluble compound that does not have antigenicity and has lower cost than collagen.\textsuperscript{11, 24} Gelatin is widely used for pharmaceutical and clinical purposes. The biocompatibility of gelatin has been identified from its long-standing clinical applications in surgical biomaterials and as an ingredient in drugs.\textsuperscript{25} Researchers described that a gelatin hydrogel system prevents protein denaturation, and this interaction with ECM macromolecules leads to growth factors regulating its biological functions.\textsuperscript{26}

In this experiment, we observed positive effects of gelatin on primary culture of epidermal keratinocytes. In this method, contamination with round cells was removed. The results demonstrated that the ability of keratinocytes to reach confluence was significantly better when cultured on a gelatin-coated surface. Cell morphology, an important biomarker, was represented by a high nucleus-to-cytoplasm ratio that indicated less differentiated keratinocytes in early passages. However, with continued keratinocyte culture, it was observed that the morphology of keratinocytes was altered and the nucleus-to-cytoplasm ratio decreased.

Keratinocytes are a great source of epithelial cells for many cell biology research studies and medical applications.\textsuperscript{27} The requirement to achieve rapid wound dressing in patients who have skin injuries necessitated the development of cellular expansion of keratinocytes in vitro. In this study, it was concluded that gelatin, as a biological polymer, can be effective for expansion of keratinocytes for research and clinical purposes.

References


Considerations in the Improvement of Human Epidermal Keratinocyte Culture In Vitro

Maryam Kaviani,1 Bita Geramizadeh,1,2 Marjan Rahsaz,1 Saeed Marzban3

Abstract

Objectives: Large-scale expansion of epidermal keratinocytes is essential in the application of these cells for severe burn treatment in patients. Therefore, this study was designed to evaluate various conditions in the expansion of human epidermal keratinocytes.

Materials and Methods: The epidermis was separated from the dermis of skin samples using dispase. The epidermis was trypsinized for keratinocyte isolation. Keratinocytes were cultured in various conditions, with or without a human dermal fibroblast feeder layer, mitomycin C treatment, and different culture media.

Results: Our results suggest that keratinocytes cultured on a human dermal fibroblast feeder layer were grown for several passages. Extensive deformation and rapid deterioration were observed in the cultured cells without a feeder layer and in serum-free medium.

Conclusions: Human dermal fibroblasts treated with mitomycin C can provide optimal conditions for proliferation of keratinocytes.

Key words: Culture medium, Dermal fibroblasts, Feeder layer, Mitomycin C

Introduction

The epidermis is an excellent source of keratinocytes, and application of these cells is an invaluable strategy for skin engineering. Accessibility to appropriate keratinocytes is necessary for skin generation in the treatment of chronic ulcers and burn patients.1-3 The culture of epidermal keratinocytes under incompatible conditions leads to differentiation and growth arrest at an early passage in vitro.4,5 It has been possible to culture keratinocytes on feeder layers or by methods using defined serum-free and low-calcium media.5,6 To culture keratinocytes, xenotropic feeder layer cells were repeatedly used to improve cell survival, attachment, and proliferation.7

Extensive application of autologous keratinocytes in the fields of tissue engineering, burn treatment, and research necessitates successful cultivation of keratinocytes.8,9 There is evidence that stimulation of fibroblasts to produce growth factors affects keratinocyte-fibroblast interactions, and this may lead to keratinocyte growth by paracrine signaling.10 Coculture of keratinocytes with a feeder layer of 3T3 cells has been discussed for application in the treatment of skin wounds.11,12 The use of animal-derived feeder layers has disadvantages because of potential contamination with pathogens. Elimination of animal-derived materials in cell culture remains an important aim for epithelial remodeling.

Application of human feeder layers has been evaluated in a few studies.15-16 Irradiated human dermal fibroblasts and mouse fibroblasts (3T3 cells) can improve the life span of human keratinocytes. Therefore, the present study was performed to evaluate various conditions such as culture medium and feeder layer use on keratinocyte morphology and proliferation in our cell culture laboratory.

Materials and Methods

Separation of epidermis and dermis from skin samples

The separation of skin layers and cell culture were performed as previously described with a few
variations. All studies involving human subjects were approved by the ethics committee of Shiraz University of Medical Sciences in accordance with the ethical guidelines of the 1975 Declaration of Helsinki.

Twenty skin samples were collected from Plastic Surgery Department with informed consent, which were washed in 70% isopropanol. The skin was placed in Dulbecco phosphate buffered saline (DPBS) containing 20 μg/mL gentamicin for 1 hour. The skin was minced into small pieces and placed in 1.5 mg/mL dispase (Roche, Basel, Switzerland) in culture medium that contained 5 μg/mL gentamicin, overnight at 2°C to 8°C. The skin pieces were removed from the dispase solution, and the epidermis was separated from the dermis with fine forceps.

Isolation and culture of fibroblasts
The dermis was digested with 0.3% collagenase type I (Gibco/BRL, Grand Island, NY, USA) for 4 hours in a water bath at 37°C. The cell suspension was centrifuged at 1200 rpm for 5 minutes. The cell pellet was rinsed twice with DPBS. The cells were cultured in F12-Dulbecco Modified Eagle Medium (F12:DMEM, 1:1) (Gibco/BRL) supplemented with 15% fetal bovine serum (FBS) and 1 μg/mL gentamicin. The cells were incubated at 37°C in 5% carbon dioxide. The culture medium was replaced every 2 to 3 days.

Treatment of fibroblasts with mitomycin C as a feeder layer
The confluent fibroblasts were treated with mitomycin C for induction of the postmitotic state. To treat the cells, human dermal fibroblasts were incubated in DMEM containing 10% FBS and 8 μg/mL mitomycin C for 4 hours at 37°C. The cells were rinsed 3 times with DPBS and replated in new culture flasks in F12-DMEM containing 10% FBS.

Isolation and culture of keratinocytes
The epidermal pieces were digested with 0.05% trypsin/ethylenediaminetetraacetic acid (Gibco/BRL) for 5 to 10 minutes at 37°C. After cell dissociation, an equal volume of 1 mg/mL soybean trypsin inhibitor (Gibco/BRL) in DPBS was added to stop trypsin activity. The cell suspension was centrifuged at 1200 rpm for 5 minutes, and the pellet was washed with keratinocyte medium. The keratinocytes were counted using trypan blue and a hemocytometer and plated at a density of 4 to 8 x 10^4 cells/cm^2. Keratinocytes were cultured in different conditions, with or without the human dermal fibroblast feeder cells in keratinocyte medium (Sigma-Aldrich, St. Louis, MO, USA) with or without FBS.

Keratinocyte freezing and thawing
Keratinocytes from primary cultures were frozen. The cells were counted and 1 x 10^6 cells were suspended in cryopreservation medium containing 90% FBS and 10% dimethyl sulfoxide. Freezing was done with slow cooling.

For thawing, the cryovial was immersed in a water bath at 37°C. The cell suspension was diluted with 5 mL keratinocyte medium and centrifuged. The cell pellet was counted and cultured in 3 different conditions including (1) culture on feeder cells in keratinocyte serum-free medium, (2) culture without feeder cells in keratinocyte serum-free medium, and (3) keratinocyte medium supplemented with 10% FBS.

Analysis of morphology and expansion of cells in various conditions
During the culture of keratinocytes and fibroblasts, microscopic evaluation was done to inspect the morphologic and proliferative changes.

Results
Fibroblasts at primary culture in F12-DMEM and 15% FBS started attaching to the culture surface on day 2 and reached confluence on day 7 (Figure 1). The keratinocytes that were attached to the culture surface were visible 3 days after primary seeding in all groups, and the mean keratinocyte size and morphology were similar for all groups.

The cultured keratinocytes in conditions without serum or a feeder layer showed limited proliferation,
but supplementing the medium with 10% FBS led to increased proliferation rate. In the condition without serum and feeder layer, only 3 of 10 cases reached confluence. Keratinocytes in serum-free and serum-supplemented medium showed deformation with culture continuation (Figure 2).

The keratinocytes were cultured on feeder layer cells treated with mitomycin C with or without serum (Figure 3). With these conditions, colonies showed typical morphology of epithelial cells. In the application of serum-supplemented medium, the proliferation rate of cells was increased. Culture of keratinocytes after cryopreservation showed that growth of cells with normal morphology was possible on feeder cells in serum-free medium (Figure 4).

Discussion

Cultivation of human epidermal keratinocytes is an important method for skin engineering. Although keratinocytes can experience few passages, they provide an important source of epithelial cells with high proliferation rate and efficacy for many biological studies and wound therapies. It is important to obtain expansion of keratinocytes on a large scale and avoid the differentiation of these cells for clinical and experimental applications.

Culture of human keratinocytes from the epidermis has been evaluated by several researchers. The key to obtaining suitable keratinocytes is optimization of the culture system to support keratinocyte growth. We compared various conditions such as the presence of a feeder layer and type of medium on the growth of these cells. We also evaluated the growth of keratinocytes after cryopreservation in different conditions. We reported that application of feeder layer cells is effective for keratinocyte culture. Using serum in the culture medium led to increased proliferation rate, but with prolonged application of this medium, keratinocytes changed morphology. Keratinocytes showed normal size and morphology in conditions without serum or feeder layer cells, but growth rate was decreased and the cells had extensive apoptosis. Keratinocytes usually are cultured for 3 to 4 passages for clinical applications. We could culture these cells up to 3 passages. When the keratinocytes could be cultivated after cryopreservation, it was evident that growth of cells with normal morphology was possible on feeder cells in serum-free medium.

Figure 2. Keratinocyte Culture

(A) Keratinocyte culture in serum-free medium at the confluence stage (original magnification ×200).
(B) Keratinocyte culture in serum-supplemented medium at the confluence stage (original magnification ×100).
(C) Keratinocyte culture in serum-free medium at passage 3 (original magnification ×200).
(D) Keratinocyte culture in serum-supplemented medium at passage 3 (original magnification ×200).

Figure 3. Feeder Cells and Keratinocytes After Treatment With Mitomycin C

(A) Treated cells at second passage on day 2 (original magnification ×100).
(B) Keratinocytes cultured on feeder cells in serum-free medium (original magnification ×100).
(C) Keratinocytes cultured on feeder cells in serum-supplemented medium (original magnification ×200).
physiologic state.\textsuperscript{23} In addition, cocultures of keratinocytes with murine 3T3 cells are considered xenogenic products, but the use of autologous dermal fibroblasts as a feeder layer can be classified as an autologous product for clinical applications.\textsuperscript{24}

**References**


Human Leukocyte Antigen G and Renal Allograft Transplant

Eman Farid,1,2 Fatima Al-Wedaie,1,2 Khaled Tabbara,1 Amgad E. El-Agroudy,3 Sumaya M. Al-Ghareeb3

Abstract

Objectives: Studying immune tolerance induced by HLA-G in kidney allograft acceptance may help understanding of its mechanisms, hoping in the future to booster it and decrease the immunosuppressive drugs given that are well known to have serious adverse effects.

Materials and Methods: The current study sought to evaluate soluble HLA-G in 3 groups: kidney transplanted patients with no rejection episodes, transplanted patients with biopsy-proven renal rejection, and healthy age-matched nontransplanted individuals.

Three groups were studied: kidney transplanted patients with no rejection episodes (n = 43); transplanted patients with biopsy-proven renal rejection (n = 27); healthy, age-matched, non-transplanted individuals as controls (n = 42). Soluble HLA-G level was measured in the serum by a quantitative sandwich enzyme linked immuno-sorbent assay.

Results: sHLAG level was significantly higher in the transplanted patients compared with the control. Prograf and not cyclosporine or Rapamune had positive effects on sHLAG levels. Patients with chronic rejection had a significant lower level of sHLAG compared with a graft stable group. No effect of donor type, infection or duration post-transplant, on sHLAG levels was found.

Conclusions: The results of the current study are consistent with previous studies addressing the role of sHLAG in inducing immunotolerance postkidney transplant. The findings from the current study on the chronic rejection group, supports the on-going research of having a treatment with HLA-G/or derivate, which may constitute in the future a novel efficient anti-graft rejection therapy.

Key words: Immunotolerance, Kidney transplant, Tolerogenic molecule

Introduction

Human leukocytes antigen G (HLA-G) is an immune molecule also called tolerogenic molecule found to be associated with better graft acceptance.1 It was discovered in trophoblastic tissue,2 and is expressed at high levels by this tissue.3 The nonclassical HLA-G molecule is distinguished from classical HLA-class 1 by different features and properties, among them is its restricted tissue distribution and its biological properties leading to immune tolerance.4-6

Rejection of an allograft is a complex process with a cascade of events involving many cells, among them natural killer cells (NK) and T-cytotoxic cells, which play a prominent role in the cascade. Indeed, there is an immunologic effect of HLA-G on NK cells functions.7,8

While NK cells are involved mainly with innate immunity, the immunosuppressive properties of HLA-G also can be directed against adaptive immune responses. Impairment of CD4+ and CD8+ T-cell function has been well documented. Direct evidence is illustrated by the fact that HLA-G1, when transfected into target cells, blocks the cytotoxic response of CD8+ T cells needed for antigens expressed by these target cells.9,10 Soluble HLA-G
also has been shown to induce apoptosis in CD8+ T cells by interacting with CD8, leading to Fas ligand (FasL) upregulation, FasL secretion, Fas/FasL interaction then apoptotic signaling. Furthermore, in vitro studies have demonstrated that soluble HLA-G5 inhibits CD4+ and CD8+ T-cell proliferation after an allogeneic response induced by T-cell receptor activation, by binding to ILT2 receptors and arresting cell cycle progression. The effects of HLA-G also may be enhanced by up-regulation of inhibitory receptors in CD4+ cells via interactions with these inhibitory receptors and activation of a positive feedback loop.

In addition to protecting against NK cells and T cells, HLA-G has been shown to inhibit antigen presenting cell (APC) function, by a mechanism that mediates evasion of the adaptive immune response. Interactions between recombinant HLA-G complexes and the ILT4 receptor on human dendritic cells, in vitro, results in impaired dendritic cell maturation, characterized by reduced cell surface expression of MHC-II and costimulatory molecules, known to be induced by the maturation stimulus. The HLA-G/ILT4 interaction also has been shown to reduce the ability of dendritic cells in inducing allogeneic T-cell proliferation.

Human leukocyte antigen G has been reported to affect the transplant course by 2 mechanisms: First, HLA-G may inhibit both NK cell-and CD8+ T-cell-mediated cytolysis. Second, HLA-G may suppress the CD4+ T-cell-allo-proliferative response. Both mechanisms show that HLA-G molecules affect the main effector cells involved in graft rejection. The role of soluble (s)HLA-G in allograft tolerance may involve peripheral deletion of alloreactive T cells after allotransplant in vivo. In heart, liver, and combined liver-kidney transplant patients, increased sHLA-G has been associated with decreased acute rejection episodes, decreased chronic rejection, and better transplant outcomes. During a renal transplant, increased sHLA-G has been associated with a decreased frequency of HLA IgG antibodies.

According to Crispim and his colleagues treatment with tacrolimus (Prograf) but not with cyclosporine is associated with increased expression of HLA-G and absence of rejection. This indicates that increased HLA-G expression after kidney transplant may be related to the immunosuppressive treatment to prevent rejection. Glucocorticoids enhance the level of HLA-G transcripts in cultured trophoblast cells, which were one of the first cells reported to produce HLA-G. Besides treatment, the genetic background of donor and recipient and the presence of inflammatory mediators present in the graft milieu also may be responsible for HLA-G modulation. The significance of sHLA-G in immunotolerance is a subject of ongoing research.

Research objectives
The hypothesis of this study is that HLA-G is up-regulated in patients with a successful renal allograft. In contrast, in patients with frequent episodes of immunologically mediated (biopsy-proven) rejection, HLA-G is not up-regulated.

Studying immune tolerance induced by HLA-G in allograft acceptance may help to better understand the mechanisms involved. Understanding these immune suppressive mechanisms might allow us to manipulate them to boost immunosuppression and decrease the dosage of immunosuppressive drugs and their adverse events.

Materials and Methods

Study design
This prospective study was done on kidney transplanted patients in the Nephrology Transplant Unit, Salmaniya Medical Complex, Ministry of Health, Kingdom of Bahrain. This study is a population-based case-control study. This design basically included selected kidney transplanted subjects based on their graft stability status, either stable or having graft rejection, testing the role of sHLA-G in graft tolerance.

Subjects of the study

Exclusion criteria
Individuals having the following conditions were excluded from the study: autoimmune disease, malignancy, pregnancy, or allergy. In addition, pediatric transplanted cases (< 15 y) were excluded from this study.

Study groups
Three groups were studied:

• Group 1: kidney-transplanted patients with no rejection episodes (n = 56).

• Group 2: transplanted patients with biopsy-proven renal rejection (n = 27) Three of them had acute rejection while the rest had chronic rejection.
• Group 1 and 2 were further divided, according to duration of transplant into: less than 5 years, 5 to 10 years, and more than 10 years. Moreover, subdivision according to the immunosuppressive drug used, cyclosporine, rapamycin, or tacrolimus (Prograf), was made. Group 3: healthy age-matched nontransplanted individuals were used as controls (n = 43).

Methods
Five mL blood was collected from patients and controls, and the serum was separated within 15 minutes of collection. The serum samples were frozen at (-80°C) until the time of assay. A quantitative Sandwich enzyme linked immunosorbent (ELISA) assay (Uscn Life Science Inc., Wuhan, China), was used. Assay steps were followed according to the manufacturer’s instructions.

Statistical analyses
Descriptive statistics were performed to compare the various parameters between the different groups. Statistical analyses also were done with Microsoft Excel 2007 and Statistical analyses were performed with SPSS software (SPSS: An IBM Company, version 15.0, IBM Corporation, Chicago, IL, USA). Data are expressed as means ± SD, percentage, range and median. Statistical significance of differences was analyzed with the chi-square test.

Ethical/research approval
Transplanted patients and healthy controls recruited in the study were asked to complete and sign an informed consent form agreeing to participate. After explaining the purpose of the study and its implications, they were asked to fill a standard questionnaire form. Approval of the Salmaniya Medical Complex and Ministry of Health research committees was obtained. All of the protocols conformed to the ethical guidelines of the 1975 Helsinki Declaration.

Results
The study population included 3 groups; transplanted patients with stable graft, transplanted patients with graft rejection, and a healthy control group with no history of transplant. One hundred and twenty samples were investigated for sHLAG measurement. The age of subjects ranged from 17 to 70 years. Further subgrouping was done according to gender, immunosuppressive medication (rapamycin [RAPA], cyclosporine [CSA], Prograf), donor type (living related [LR], living nonrelated [LNR], deceased donor [DD]), posttransplant period (less than 5 years, from 5 to 10 years, and more than 10 years) and presence of infection (urinary tract infection [UTI], cytomegalovirus [CMV], hepatitis B virus [HBV], and hepatitis C virus [HCV]); details are shown in Table 1.

To explore a relation of sHLAG level with the graft stability or graft loss, the groups were compared simultaneously; the following results were found:
• Level of sHLAG was significantly higher (P < .001) in the total transplanted patients (n = 77) tested (19.6 ± 31.4 U/mL) compared to the control group (n = 43) (5 ± 10.2 U/mL) (Figure 1).
• Patients with chronic rejection (CR) (n = 18) (9.99 ± 9.67 U/mL) had a significant lower level of sHLAG (P = .047) compared with the graft stable group (n = 56) (20.3 ± 34.2 U/mL) (Figure 2).
• Patients with chronic rejection (CR) (n = 18) (9.99 ± 9.67 U/mL) had a lower level of sHLAG compared with patients with acute rejection (n = 3) (64.3 ± 23.9 U/mL), yet the difference was not significant (P = .061).

Table 1. Complications of Liver Biopsy After Liver Transplant

<table>
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<tr>
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<th>Rejection n = 21 (3AR)</th>
<th>Stable n = 56</th>
<th>Control n = 43</th>
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<td>F</td>
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<td>HBV</td>
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Abbreviations: CMV, cytomegalovirus; CSA, cyclosporine; DD, deceased donor; F, female; HBV, hepatitis B virus; HCV, hepatitis C virus; LNR, living-nonrelated; LR, living-related; M, male; RAPA, rapamycin; UTI, urinary tract infection
In an attempt to explore a relation of sHLAG level with the immunosuppressive medication used, whether RAPA, CSA or Prograf, a comparison was made in the graft stable group (n = 56), between the different groups.

No significant difference was found ($P = .350$) on sHLAG concentration, when comparing patients on RAPA (n = 16) (10.7 ± 15.7 U/mL) with patients on CSA (n = 16), (17.6 ± 29.9 U/mL). Similarly, no significant difference was found ($P = .123$), on sHLAG concentration, when comparing patients on RAPA (n = 16) (10.7 ± 15.7 U/mL) with patients on Prograf, (n = 16) (21.5 ± 22.2 U/mL). Also, no significant difference was found ($P = .647$) on sHLAG concentration, when comparing patients on CSA (n = 24), (17.6 ± 29.9 U/mL) with patients on Prograf (n = 16), (21.5 ± 22.2 U/mL).

Interestingly, the level of sHLAG was significantly higher ($P = .02$) in the graft stable group on Prograf (n = 16) (21.5 ± 22.2 U/mL) compared with the control group (n = 43) (5 ± 10.2 U/mL) (Figure 3).

On the contrary, no significant difference was found ($P = .099$) in sHLAG concentration, when comparing patients either CSA (n = 24) (17.6 ± 29.9 U/mL) or RAPA (n = 16) (10.7 ± 15.7 U/mL) with the control group (5 ± 10.2 U/mL)

Exploring the effect of each drug alone, a comparison was made for sHLAG level, for patients with stable graft and patients with chronic rejection, using the same immunosuppressive drug; the following results were found:

- Prograf in graft stable group (n = 16) versus CR group (n = 8)
  
  No significant difference was found ($P = .053$)

- CSA in graft stable group (n = 24) versus CR group (n = 6)
  
  No significant difference was found ($P = .232$)

- RAPA in graft stable group (n = 16) versus CR group (n = 3)
  
  No significant difference was found ($P = .094$).

In summary, Prograf, and not CSA or RAPA had a positive effect on sHLAG levels according to results of the current study.

No significant difference was found ($P = .384$) in sHLAG concentration according to the type of donor, whether LNR (n = 45; 21.4 ± 37.3 U/mL), or LR (n = 29; 15.5 ± 20.6 U/mL), in the examined transplant recipients.

Similarly, no significant difference was found ($P = .522$) in sHLAG concentration according to presence of infection, (n = 8; 15.4 ± 17 U/mL) or no infection (n = 48; 20.3 ± 34.2 U/mL).
infection (n = 69; 20.1 ± 32.7 U/mL), in the transplanted recipients [n = 77].

Regarding the effect of gender, no significant difference was found (P = .647) in sHLAG concentration when men (n = 39; 18.6 ± 27.3 U/mL) were compared with women (n = 17; 24.3 ± 47.1 U/mL), in the graft stable transplant recipients. Similarly nonsignificant findings were found in the CR group (n = 18) (P = .782), when sHLAG concentration of men (n = 9; 9.33 ± 9.79 U/mL) was compared with women (n = 9; 10.7 ± 10.1 U/mL).

Regarding sHLAG level according to posttransplant duration, no significant difference was found in sHLAG concentration when comparing the 3 periods less than 5 years (n = 28), between 5 to 10 years (n = 31), and more than 10 years n = 18 as shown below.

(A) Less than 5 years (n = 28) versus from 5 to 10 years (n = 31)
No significant difference in sHLAG concentration was found (P = .884) when comparing the period less than 5 years (19.8 ± 29.7 U/mL) and from 5 to 10 years (21.1 ± 38.8 U/mL).

(B) Less than 5 years (n = 28) versus more than 10 years (n = 18)
No significant difference in sHLAG concentration was found (P = .585) when comparing the period less than 5 years (19.8 ± 29.7 U/mL) and more than 10 years (15.9 ± 18.5 U/mL).

(C) From 5 to 10 years (n = 31) versus more than 10 years (n = 18)
No significant difference in sHLAG concentration was found (P = .528) when comparing the period from 5 to 10 years (21.1 ± 38.8 U/mL) and more than 10 years (15.9 ± 18.5 U/mL).

In summary the result of the current study did not show any effect of donor type; infection, or duration posttransplant, on sHLAG levels.

Discussion

The current study explores the role of soluble HLAG [sHLAG] in kidney transplant recipients as immunotolerant tools of the immune system. Three different groups were studied: patients with stable graft, those with graft rejection, and healthy controls with no history of kidney transplant. Soluble HLA-G concentration levels were measured in sera.

To evaluate whether clinical parameters affect sHLAG levels, we evaluated some transplant factors include whether the kidney was from (donor origin related [living related] vs nonrelated donor [nonliving related or deceased donor]), and infectious factors (BK virus, cytomegalovirus, hepatitis B virus, and hepatitis C virus). The results of our study revealed that neither the type of donor nor the presence of infection had an effect on serum sHLAG concentration; this was also reported by others.17-19 Regarding gender, we found no significant difference between male and female patients sHLAG concentrations.

To study the effect of duration posttransplant on HLAG, we divided the patients according to the period posttransplant into 3 subgroups: less than 5 years, from 5 to less than 10 years, and more than 10 years. No significant difference was found.

There was a significant higher sHLAG level found when comparing either of the transplanted group with the control group; this finding supports the role played by HLAG expression in graft immunotolerance as previously highlighted by others. Soluble HLA-G is expressed not only in patients with stable grafts, but in cases of acute or chronic graft rejection; this has been found in other studies.8,13 20,21 In addition, we found a significant difference between graft stable and graft rejection groups; same finding was previously reported by others.12 Nevertheless multifactor effects occur in the event of graft acceptance, like genetic background of donor and recipient and the presence of inflammatory mediators present in the graft milieu, which may affect HLA-G modulation. In the studied graft stable group, we observed 2 patients with high sHLAG concentration, [200 and 133 U/mL] while the mean for the whole group was 20.3 U/mL, 1 was exposed to an occupational chemical exposure accident while the other is an Indian hypertensive patient using herbal drinks; this raises the possibility of different environmental and nutritional factors that may affect HLAG expression; thus, future studies are required to understand this.

Moreover, we found no significant difference in the level of sHLAG between patients on different immunosuppressive drugs (RAPA, cyclosporine, and Prograf), yet when comparing patients on Prograf versus controls, a significantly higher level was observed; the role of Prograf in enhancing the HLAG expression was reported previously.12 Also
corticosteroids have been reported to enhance production of soluble HLA-G,13 yet none of our patients received it when tested.

Soluble HLA-G5 has been shown to suppress T-cell functions and induces regulatory T cells.22 Additionally,23 it was demonstrated that sHLA-G5 secreted by adult bone marrow-derived mesenchymal stem cells are responsible for the immunomodulatory effects; blocking experiments using neutralizing anti-HLA-G antibody demonstrate that HLA-G5 contributes first to the suppression of allogeneic T-cell proliferation and then to the expansion of CD4+CD25highFOXP3+ regulatory T cells.23 In the current study, the level of sHLAG in the peripheral blood appeared to be influenced by kidney transplant and by the immunosuppressive medications.

Conclusions

In conclusion, the results of the current study are consistent with previous studies addressing the role of sHLAG in inducing immunotolerance postkidney transplant.

In summary, the following were found:

- sHLAG was significantly higher in all transplanted patients compared to the control group.
- Prograf but not CSA or RAPA had a positive effect on the sHLAG level.
- Patients with CR had a significantly lower level of sHLAG compared with graft stable group.
- No effect of donor type; infection or duration posttransplant, on sHLAG levels.

The findings from the current study on the chronic rejection group, support the ongoing research of having treatment with HLA-G1 or HLA-G5 and T-reg, which may constitute a novel efficient antigraft rejection therapy in the future; yet sHLAG measurement must be improved and thus hopefully it may be used in the future to monitor progress of transplant patients. Indeed the recent article published 2014, addressing the role of HLA-G dimers in the prolongation of kidney allograft survival supports this concept.16

References

Protective Effect of *Artemisia Asiatica* Extract Against Renal Ischemia-Reperfusion Injury in Mice

Hyuk Jai Jang,1 Eui Kyun Jeong,2 Seong Su Kim,2 Ji Hwan Lee,1 Mi Young Oh,1 Ki Sung Kang,3 Hak Cheol Kwan,4 Kyung Il Song,5 Dae Woon Eom,6 Duck Jong Han7

Abstract

Objectives: An extract of *Artemisia asiatica* was reported to possess antioxidative and cytoprotective actions in various experiments. Ischemia-reperfusion injury remains a major problem in kidney transplant, and the inflammatory response to ischemia-reperfusion injury exacerbates the resultant renal injury. In the present study, we investigated whether an extract of *Artemisia asiatica* exhibits renoprotective effects against ischemia-reperfusion-induced acute kidney injury in mice.

Materials and Methods: Renal ischemia-reperfusion injury was induced in male C57BL/6 mice by bilateral renal pedicle occlusion for 30 minutes followed by reperfusion for 48 hours. An extract of *Artemisia asiatica* (100 mg/kg oral) was administered 4 days before ischemia-reperfusion injury. Sham operation and phosphate-buffered saline were used as controls. Blood and renal tissues were evaluated at 48 hours after ischemia-reperfusion injury.

Results: Treatment with an extract of *Artemisia asiatica* significantly decreased blood urea nitrogen, serum creatinine levels, and kidney tubular injury (*P* ≤ .05). Western blot showed that an extract of *Artemisia asiatica* significantly increased the level of heme oxygenase-1 and B-cell lymphoma 2 at 48 hours after ischemia-reperfusion injury and attenuated the level of inducible nitric oxide synthase.

Conclusions: An extract of *Artemisia asiatica* improves acute renal ischemia-reperfusion injury by reducing inflammation and apoptosis. These findings suggest that an extract of *Artemisia asiatica* is a potential therapeutic agent against acute ischemia-induced renal damage.

Key words: Heme oxygenase-1, Inducible nitric oxide synthase, Kidney transplant, Renal failure

Introduction

Renal ischemia-reperfusion injury (IRI) is a major problem in kidney transplant. The pathogenesis of IRI involves complex interactions between biochemical, cellular, vascular endothelial, and tissue-specific factors. Ischemia causes necrosis and apoptosis. Restoration of blood flow, which is important to prevent ongoing injury, paradoxically potentiates the inflammatory response exhausting the previous ischemic damages.1 Interruption of blood flow to the kidney and subsequent reperfusion causes an acute inflammatory response.2 Tubular cell apoptosis also contributes to the pathogenesis of renal IRI.3 Therapeutic approaches aimed at suppressing the inflammatory response and tubular apoptosis may be effective against renal injury and provide better prognosis after IRI.

The plant genus *Artemisia* includes > 300 species, many having medicinal value. Among these plants, *Artemisia asiatica* Nakai (*Artemisia asiatica*) has been used in traditional Oriental medicine for the treatment of microbial infections and inflammatory diseases. Various animal studies revealed that the extract of *Artemisia asiatica* has antioxidative and anti-inflammatory activities that contribute to its
protective effects against gastric damage, liver damage, and experimental pancreatitis.4-6

Based on these previous findings, it was hypothesized that the extract of Artemisia asiatica may suppress inflammatory activation and apoptosis and may confer renoprotection against renal IRI. The aim of the present study was to investigate whether the extract of Artemisia asiatica can abolish the harmful effects of renal injury in an animal model of renal IRI.

Materials and Methods

Experimental animals and renal ischemia-reperfusion injury

Male C57BL/6 mice (age, 8 wk; body weight, 22-25 g) were purchased from Dae Han Bio Link Co., Ltd., Eumseong, South Korea. The mice were housed in polycarbonate cages (Makrolon, Bayer Material Science LLC, Pittsburgh, PA, USA) under standard laboratory conditions (temperature, 22°C ± 2°C; relative humidity, 55%). The mice were given standard mouse chow and tap water ad libitum. The investigation conformed with the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health (publication No. 85-23, revised 1985) and was approved by the Ethics Committee of the Korea Institute of Science and Technology.

Mice were subjected to bilateral renal pedicle clamping for 30 minutes with microvascular clamps. Reperfusion commenced after the artery clamps were removed. Occlusion was verified visually by change in the color of the kidneys to a paler shade and reperfusion was verified by a blush appearance. During the procedure, mice were kept well hydrated with warm saline. The mice were maintained at a constant body temperature (37°C) using a warming pad. After the clamps were removed, reperfusion of the kidneys was observed. A similar sham operation was performed in control mice except that the renal pedicles were not clamped. Blood was sampled from the inferior vena cava and renal tissues were removed at 48 hours after reperfusion. Both kidneys were isolated, quick frozen in liquid nitrogen, and stored at -80°C until further analysis.

Artemisia asiatica administration

Isopropanol extracts of Artemisia asiatica (Richwood Trading Co., Ltd., Seoul, South Korea) were dissolved in vehicle (5% hydroxypropyl methylcellulose) at a concentration of 100 mg/kg body weight. A total of 30 mice were randomly divided into 3 groups (10 mice per group). The sham and IRI control groups were given a single dose of vehicle only. The drug-treated groups (Artemisia-treated) were given pretreatment with Artemisia extracts 4 days before bilateral IRI. The mice were given a single intragastric dose of vehicle with Artemisia extracts, except the sham and IRI groups.

Biochemical tests

Blood samples were obtained from the inferior vena cava. Serum blood urea nitrogen (BUN) and creatinine levels were measured using an analyzer (Cobas C 702 analyzer, Roche, Basel, Switzerland).

Histologic and immunohistochemical analysis

For histopathologic examination, kidneys were collected, cut coronally, fixed in 10% formaldehyde, and embedded in paraffin. Sections (thickness, 5 μm) were cut, stained with hematoxylin-eosin, and scored with a semiquantitative scale designed to evaluate changes in the kidney at 48 hours after IRI.7 The percentage of tubules in the corticomedullary junction that displayed cellular necrosis and a loss of brush border were counted and scored in a blinded fashion and graded from 1 to 4. One whole deep coronal section was examined under the microscope and graded according to extent of tubular necrosis, based on percentage of involvement of the kidney. Higher scores represented more severe damage (0, normal kidney; 1 [minimal necrosis], < 5% involvement; 2 [mild necrosis], 5% to 25% involvement; 3 [moderate necrosis], 25% to 75% involvement; and 4 [maximum score, severe], > 75% involvement). Immunohistochemical staining for monoclonal antibody against heme oxygenase-1 (anti-HO-1 antibody, Cell Signaling, Danvers, MA, USA) was performed for ethanol-fixed, paraffin-embedded tissue sections.

Western blot analysis

Kidneys were crushed in ice-cold 1 M tris(hydroxymethyl)aminomethane hydrochloride (Tris-HCl) buffer, pH 7.5, with protease inhibitors, 25 mM sodium fluoride, 10 mM Sodium Orthovanadate, 0.5 mol/L ethylenediaminetetraacetic acid (EDTA), and surfactant (1% Triton X-100; GenDEPOT, Barker, TX, USA), and centrifuged at
14,000 rpm for 20 minutes. Protein concentration was determined using a protein assay (Bradford Protein Assay, Bio-Rad, Hercules, CA, USA). Aliquots of 200 μg of protein extracts were separated on 10% to 15% sodium dodecyl sulfate polyacrylamide gels (SDS-PAGE, Bio-Rad) and transferred to nitrocellulose membranes (Bio-Rad). Membranes were blocked with 5% milk in buffer (TBST buffer: 10 mM Tris-base, 100 mM sodium chloride, 0.1% Tween-20, pH 8.0), and probed with HO-1, glyceraldehyde 3-phosphate dehydrogenase (GAPDH), B-cell lymphoma 2 (Bcl-2) (Cell Signaling, Danvers, MA, USA), and inducible nitric oxide synthase (iNOS) (1:200 dilution, Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA) overnight at 4°C. Membranes were probed with goat antirabbit (1:1000) or goat antimouse (1:1000) horseradish peroxidase-conjugated secondary antibody (Santa Cruz Biotechnology, Inc). Protein bands were detected using a chemiluminescent substrate (SuperSignal West Pico Chemiluminescent Substrate, Pierce, Rockford, IL, USA). Images of blots were acquired for quantification and analyzed.

Statistical analyses
All data are presented as mean ± SEM and were evaluated by 1-way analysis of variance with post hoc Bonferroni correction (SPSS for Windows, Version 16.0, SPSS Inc., Chicago, IL, USA). Statistical significance was defined by $P \leq .05$.

Results

Artemisia asiatica attenuated the renal functional deterioration that had been induced by ischemia-reperfusion injury

The IRI caused renal dysfunction in the vehicle-treated mice (IRI group), reflected by significant elevation of serum BUN and creatinine levels at 2 days after IRI (Figure 1). Renal dysfunction was attenuated in Artemisia-treated mice (Artemisia group), with serum BUN and creatinine levels lower than in the control mice at post-IRI day 2 (Artemisia vs IRI group, BUN and creatinine; $P \leq .05$) (Figure 1).

Treatment with Artemisia also attenuated histologic injury. The IRI group had severe tubular damage, evidenced by widespread tubular necrosis, loss of the brush border, cast formation, and tubular dilation at the corticomedullary junction at 2 days after IRI. However, the tissue injury score was significantly decreased in Artemisia-treated mice ($P \leq .05$) (Figure 1). Sham-operated mice incurred no tubular injury.

Artemisia asiatica treatment up-regulated the expression of heme oxygenase-1 proteins in the kidney

To elucidate the mechanism of Artemisia-induced cytoprotective effect, we examined protein expression using immunohistochemical staining and Western blot. The enzyme HO-1 has antioxidant and cytoprotective properties against oxidative stress. Expression of HO-1 was observed in the Artemisia-treated cells compared with cells from the IRI and sham groups (Figure 2). Treatment with Artemisia caused strong HO-1 immunostaining of the kidney compared with the sham and IRI groups (Figure 2).

Figure 1. Artemisia asiatica Protects Renal Function in Ischemia-Reperfusion Injury (IRI)

Artemisia asiatica (100 mg/kg) or vehicle was orally administered to mice 4 days before bilateral IRI. Blood samples were collected 2 days after IRI to determine levels of (A) serum creatinine and (B) blood urea nitrogen. Artemisia asiatica treatment markedly attenuated IRI-induced pathologic injury. Mouse kidneys were excised 2 days after IRI and assessed by tubular injury score. (C) The kidneys were sectioned and stained with hematoxylin-eosin to evaluate renal tubular damage. Data were reported as mean ± SEM (*$P \leq .05$, Artemisia vs vehicle-treated IRI groups).
Artemisia treatment significantly up-regulated the expression levels of HO-1 ($P \leq .01$) (Figure 2). This suggested that HO-1 induction by Artemisia correlated with its cytoprotective effect against IRI.

Artemisia asiatica treatment up-regulated the expression of B-cell lymphoma 2 protein and down-regulated expression of inducible nitric oxide synthase protein in the kidney. The IRI-induced iNOS protein expression was decreased by Artemisia asiatica extract ($P \leq .05$) (Figure 3). Early intracellular events that occur in the apoptotic process comprise mitochondrial changes mediated by protein members of the antiapoptotic Bcl-2 proteins. Therefore, Bcl-2 was evaluated by Western blot analysis to elucidate the mechanism by which Artemisia asiatica suppressed apoptosis after IRI. The immunoreactivity for Bcl-2 was markedly increased after treatment with Artemisia asiatica ($P \leq .05$) (Figure 3).

Discussion

In this paper, we showed that therapy with Artemisia asiatica extract protected against renal IRI in mice, and the protective effect was associated with suppression of the inflammatory response and tubular apoptosis. It did this by the antiapoptotic property that targets various components of the pathophysiologic pathway involved in IRI.


*Artemisia asiatica* extracts have been proven to possess anti-inflammatory, antioxidative, and cytoprotective effects and have shown effective protection in various models. In the current experiment, we administered extracts of *Artemisia asiatica*. Plant extracts have been used traditionally as herbal medicines to target inflammatory disease. The antioxidative and cytoprotective actions of *Artemisia asiatica* have been proven in gastric mucosal injury induced by nonsteroidal anti-inflammatory drugs, hepatic fibrosis and inflammation, and fibrosing chronic pancreatic lesions.4-6 We investigated whether *Artemisia asiatica* can abolish the harmful effects of renal injury in an animal model of renal IRI. The present study showed that treatment of mice with *Artemisia asiatica* resulted in better renal function than in mice not treated with *Artemisia asiatica*. Mice treated with *Artemisia asiatica* had lower plasma levels of BUN and creatinine caused by IRI and lower histopathologic scores. To our knowledge, this is the first study to explore the protective efficacy against renal IRI.

Inflammation after renal IRI is a major contributor to renal cell death. Inflammation is an important mechanism underlying the start and maintenance of renal cell injury because inflammation potentiates necrosis and apoptosis.8-10 Necrotic cells can potentiate the inflammatory process further via the release of toxic intracellular contents. In the present study, administration of *Artemisia asiatica* reduced IRI-induced expression of inflammatory genes such as iNOS. These data suggest that *Artemisia asiatica* has anti-inflammatory effects in renal IRI.

We investigated the possible mechanisms by our observations. Apoptosis is increasingly recognized as a major form of cell death during IRI and can affect the functional outcome independent of inflammation. There is evidence that renal tubular cell apoptosis plays an essential role in renal IRI and contributes to acute renal failure.3 Previous animal and human biopsy studies have shown that apoptosis is implicated in cell injury after renal IRI.9,11 Renal tubular apoptosis is a primary contributor to the pathophysiology of renal IRI.12 Abolition of early apoptosis improves renal IRI.9 *Artemisia asiatica* inhibits tubular cell apoptosis after renal ischemia with great efficacy. Our results on the effect of Bcl-2 expression raise the possibility of a direct effect on kidney epithelial cells at the mitochondrial level. Pharmacologic compounds were shown to improve renal injury by diminishing apoptosis.14-15 Our results provide evidence of the potent cytoprotective effect of *Artemisia asiatica* via the modulation of Bcl-2 gene expression. The Bcl-2 is induced by *Artemisia asiatica*. As a result, mitochondrial activity and cell integrity are maintained.

In the control of oxidative stress and antioxidant defense, HO-1 is an enzyme with antioxidant and cytoprotective properties against oxidative stress. The enzyme HO-1 is a cytoprotective enzyme involved in the response to oxidative stress, and its main function is associated with the degradation of heme to biliverdin, iron, and carbon monoxide.13 Overexpression of HO-1 in cells resulted in a marked reduction in injury and cytotoxicity induced by oxidative stress.15-16 The up-regulation of both HO-1 in the *Artemisia* group significantly improved renal IRI, which suggested that HO-1 may be a modulator of *Artemisia asiatica*-associated antioxidant properties.

In summary, *Artemisia asiatica* provided protection for mice against renal IRI, with suppression of inflammatory responses, reduction of tubular cell apoptosis, and cytoprotection. Therefore, *Artemisia asiatica* may be a potential therapeutic agent for renal IRI. The detailed mechanisms of protection should be explored in further studies.

References

Human Leukocyte Antigen Cw7-Mediated Protection Against Polyoma BK virus in Renal Transplant Recipients Who Received Grafts From Antigen-Positive Donors

Osama Gheith, Torki Al-Otaibi, Zakaria Zakaria, Medhat Abdel Halim, Naryanan Nampoory

Abstract

Objectives: Nephropathy from BK virus is an increasing problem in renal transplant recipients and has been correlated with newer immunosuppressive agents and the decline in acute rejection rates. We aimed to evaluate the effect of BK virus-positive kidney donors on the outcome of kidney transplant recipients after mean follow-up 21 months.

Materials and Methods: Among 18 kidney donors with BK virus in blood and urine, 5 donors were fit for donation. Clinical information was reviewed for the 5 kidney transplant recipients who received kidney allografts from these donors (mean donor age, 35 ± 3 y).

Results: All recipients except 1 were women (mean age, 49.4 ± 4.2 y; body weight, 68.2 ± 4 kg, follow-up, 21.6 ± 4 mo). All patients except 1 received antithymocyte globulin induction, and all 5 patients received steroids, tacrolimus, and mycophenolate mofetil as maintenance therapy. Ureter stenting was a routine procedure in each case. Human leukocyte antigen Cw7 was detected in 4 of 5 recipients, and the fifth case, the antigen was detected in the donor. At last follow-up, all patients were enjoying functioning grafts without recurrence of BK virus infection.

Conclusions: Polyoma BK virus-positive people can be accepted safely for kidney donation, especially with a possible protective role of human leukocyte antigen Cw7.

Key words: BK virus, End-stage renal disease, Immunosuppression, Outcome, Virology

Introduction

Polyoma BK viruses are widespread, double-stranded, nonencapsulated DNA viruses comprising BK and JC strains that are pathogenic in humans.1,2 Approximately 60% to 90% adult people worldwide are seropositive for BK virus. Primary infections usually occur early in childhood by oral and/or respiratory exposure.3,4 These infections usually are asymptomatic, but they have tropism for renal tubular and transitional cells and remain latent within the genitourinary tract.5-8 These viruses need immune modulation and host cell activation for replication with an impaired immune system, and this is prevalent in renal transplant patients (10%-60%).9 In more recent reports, reactivation occurs soon after transplant and is identified in the urine of 30% to 50% patients at 3 months after transplant.1,2

Progression from viruria to viremia and nephropathy is accepted as a stepwise transition.1,3,4 Such activation is determined by the detection of free viral particles in the urine by polymerase chain reaction (PCR), intranuclear viral-inclusion-bearing cells, decoy cells in urine cytology samples, and viremia by plasma PCR.10,11 Only 1% to 10% affected patients change from reactivated infection to histologically confirmed BK virus nephropathy, usually at mean 10 to 13 months after transplant (range, 6 days to 5 years).9,12-17 In other reports, it has been concluded that the median time to detect BK virus nephropathy...
after kidney transplant is 9.5 months, but the duration until graft failure is only 4 months after incidence of BK virus nephropathy.\textsuperscript{16}

Diagnosis of BK virus nephropathy is based on histologic appearance characterized by lymphocytic interstitial infiltrates, nuclear reaction to the anti-SV-40T antibody as evidence of viral replication, and positive PCR for BK virus DNA. It is difficult to make a clear differential diagnosis between BK virus nephropathy and acute cellular rejection. Moreover, it is well known that these diseases may coexist in the same patient, and the relation between their cause and effect remain controversial. We lack specific and effective antiviral treatment. Therefore, a decrease or discontinuation of immunosuppressive agents is essential as initial treatment for BK virus nephropathy.\textsuperscript{12}

Potent immunosuppression is believed to be the cause of the resurgence of BK virus in kidney transplant patients. Factors that may be associated with the risk of BK virus nephropathy include older age, male sex, white ethnicity, diabetes, renal tissue injury from ischemia, presence of cytomegalovirus, acute rejection, and treatment with high-dose steroid pulses.\textsuperscript{12,15}

The BK virus nephropathy is 1 of the major causes of allograft dysfunction or graft loss in patients in an overly immunosuppressed state. It has been showed that 45% patients with BK virus nephropathy progressed to irreversible graft failure with tubular atrophy and interstitial fibrosis.\textsuperscript{18} Although immunosuppressive agents are essential for therapy after organ transplant, these agents increase the incidence and deteriorate the severity of BK virus infection. Calcineurin inhibitors and mycophenolate mofetil are important in reactivation of latent BK virus infection.

There have been 10 cases in which the absence of human leukocyte antigen- (HLA-) C7 allele (a genetic trait) was observed in donors and recipients with sustained BK viremia.\textsuperscript{19} The precise role of the HLA-C7 gene is unknown, but its absence may increase the risk that infection will advance to sustained viremia, a preceding factor in BK virus nephropathy. Active BK virus infection has been detected in 35.4% renal transplant recipients.\textsuperscript{20} After studying pretransplant donor and recipient samples for BK virus antibody titer and HLA alleles, it was concluded that the data supported donor origin for early BK virus infection in kidney transplant recipients, and it was suggested that a specific HLA-C locus may be associated with failure to control BK virus infection.\textsuperscript{20}

Many (27%) kidney donors have positive PCR for BK virus.\textsuperscript{21} Based on preliminary results analyzing the molecular fingerprints of donor and recipient pairs, the donor may be the source of BK infection in some cases.\textsuperscript{21} The purpose of this study was to evaluate the effect of BK virus-positive kidney donors on the outcome of kidney transplant recipients after mean follow-up 21 months.

Materials and Methods

Patients

In 45 kidney donors screened for BK virus, 18 (40%) had positive BK virus in blood and urine (both qualitative and quantitative PCR), and only 5 donors (mean age, 35 ± 3 y) were fit for donation. We reviewed data about the 5 kidney transplant recipients who received kidney allografts from 2008 to 2009 at Hamed Al-Essa Organ Transplant Center in Kuwait. The management was approved by the local institutional ethical and scientific committees and is compliant with the principles laid down in the Helsinki’s declaration.

Induction and immunosuppression protocol

Immunologically low-risk patients were treated with 2 doses of basiliximab (first dose, 20 mg) on day 0 and second dose on day 4, but patients who had high risk received 5 doses of antithymocyte globulin as induction immunosuppression in addition to methylprednisolone (1000 mg intravenous) before transplant. Subsequently, tacrolimus was started (initial dose, 0.15 mg/kg; then twice daily to achieve plasma level 8-10 ng/mL during the first month and 5-8 ng/mL during the second and later months). Mycophenolate mofetil was prescribed (500 mg twice daily; increased to 750 mg twice daily in the first month). Methylprednisolone was given (500 mg intravenous 12 hours postoperative; then 250 mg for 3 successive days; then oral prednisolone in tapering doses aimed at 5 mg daily at the end of the sixth posttransplant month).

Quantitative polymerase chain reaction

Quantitative BK virus DNA was measured in serum at 2, 4, 6, 12, and 20 months after transplant date. This was performed by the National Virus Reference Laboratory using a qualitative assay (LightCycler,
Roche, Basel, Switzerland) and a quantitative real-time assay (TaqMan, Roche). Low viral load was defined as < 5000 viral copies/mL; intermediate viral load 5000-10 000 copies/mL, and high viral load > 10 000 copies/mL. Data collected included demographics, type of transplant, HLA match, type and dose of immunosuppression, use of indwelling ureter stents, history of delayed graft function, acute rejection episodes, pretransplant diabetes mellitus, and cold ischemia time. The serum creatinine level at baseline (2 weeks posttransplant) and at the most recent follow-up or October 2011 was evaluated.

Results

All recipients except 1 were female (mean age, 49.4 ± 4.2 y; body weight, 68.2 ± 4 kg; follow-up, 21.6 ± 4 mo) (Table 1). The original kidney disease was diagnosed in 1 patient each as autosomal dominant polycystic kidney disease, chronic interstitial nephritis, focal segmental glomerulosclerosis, diabetic nephropathy, and idiopathic. All patients except 1 receive antithymocyte globulin induction, and all patients were maintained on steroids, tacrolimus, and mycophenolate mofetil (Table 1).

Laparoscopic donor nephrectomy was a routine procedure in our center with average warm ischemia time 5 to 6 minutes and cold ischemia time 30 to 40 minutes, and ureter stenting was performed in all cases. There were 3 patients who had biopsied and acute tubular necrosis was the primary finding in all biopsies. Successful treatment in 1 patient was with plasma exchange for an episode of acute antibody mediated rejection (Table 1).

The mean creatinine level at baseline (2 weeks after transplant) was 108.8 μmol/L and at most recent follow-up was 116.4 μmol/L. At most recent follow-up, all patients had functioning grafts without evidence of recurrent BK virus infection.

Discussion

Nephropathy from BK virus is 1 of the major causes of allograft dysfunction or graft loss in patients with an overly immunosuppressed state. It has been reported that the median time to detect BK virus nephropathy after kidney transplant is 9.5 months with shorter time to graft failure.16 It was shown that 45% patients with BK virus nephropathy progressed to irreversible graft failure with tubular atrophy and interstitial fibrosis.21 Although immunosuppressive agents are essential for therapy after organ transplant, these agents increase the incidence and deteriorate the severity of infection. Calcineurin inhibitors and mycophenolate mofetil are important in reactivation of latent BK virus infection. Potent immunosuppression is believed to be responsible for BK virus in kidney transplant patients, especially in the presence of risk factors. The role of HLA-C7 allele is unknown, but its absence may increase the risk that infection will advance to sustained viremia, a preceding factor in BK virus nephropathy.12,15

In our series, we found that many risk factors were observed (3 males and 3 elderly in 5 patients) and all these patients received antithymocyte globulin induction and maintenance immunosuppression based on tacrolimus and mycophenolate mofetil. All were diabetics with ureter stenting. Moreover, all patients received their grafts from BK virus-positive kidney donors. The donor origin for early BK virus infection was noted in data about kidney transplant recipients,20 and similar data were reported by

| Table 1. Demographic Characteristics, Immunosuppressive Regimen, and Outcome of Renal Transplant Recipients |
|---------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Characteristics                 | Patient 1       | Patient 2       | Patient 3       | Patient 4       | Patient 5       |
| Nationality                     | Indian          | British         | Kuwaiti         | Kuwaiti         | Lebanese        |
| Sex                             | Female          | Female          | Female          | Female          | Male            |
| Age (y)                         | 40              | 57              | 62              | 62              | 56              |
| Original kidney disease         | ChTID           | ADPKD           | DN              | FSGS            | Idiopathic      |
| Immunosuppression               |                |                |                |                |                |
| Induction                       | Antithymocyte globulin STIM | Basiliximab STIM | Antithymocyte globulin STIM | Antithymocyte globulin STIM | Antithymocyte globulin STIM |
| Maintenance                     | STM             | STM             | STM             | STM             | STM             |
| Dialysis duration (mo)          | 7               | Present         | Preemptive      | Preemptive      | 12              |
| Ureter stent                    | Present         | Preemptive      | Present         | Present         | Present         |
| Rejection/ATN                   | no/yes          | no/no           | no/yes          | no/yes          | no/yes          |
| Baseline/last creatinine (μmol/L) | 92/192          | 98/95           | 132/78          | 113/89          | 109/100         |
| Baseline/last hemoglobin (g/L)  | 127/136         | 136/135         | 123/105         | 151/130         | 154/121         |
| Patient outcome                 | Living          | Living          | Living          | Living          | Living          |

Abbreviations: ADPKD, autosomal dominant polycystic kidney disease; ATN, acute tubular necrosis; ChTID, chronic tubulointerstitial disease; DN, diabetic nephropathy; FSGS, focal segmental glomerulosclerosis; STIM, steroid, tacrolimus, and mycophenolate mofetil
others.21 Despite these risk factors, none of our patients developed BK viremia or viruria (confirmed by qualitative and quantitative PCR) after follow-up >12 months, an observation that could be explained by the presence of the protective HLA-Cw7 observed in 4 patients and in the donor of the remaining fifth case. These HLA antigens are a well-known group of genes that produce interferon. It also has been suggested that a specific HLA-C locus may be associated with failure to control BK virus infection.21

Since natural killer (NK) cells are involved in the protection from viral infection, a reduced size of a ligand-specific NK subset in individuals homozygous for some HLA-B/C haplotypes may help explain their increased susceptibility to virus-induced diseases.22 In the same direction, others suggested a link between the product of a gene in the HLA-A1-Cw7-B8-DR3 haplotype and disease related to human immunodeficiency virus 1 (HIV-1).23

It is worthwhile to mention that cytotoxic T lymphocytes (CTLs) constitute a major immune defence mechanism for sustained recovery from viral infections. A previous study supported the role of the HLA-C locus in generating CTL responses and constituted the first report of an HLA-Cw7-restricted HIV-1 envelope-specific CTL response in HIV-positive patients, which may be important in the control of HIV replication in vivo.24 Moreover, another study concluded that, in Croatian patients with chronic hepatitis C, HLA-Cw7 was the predictor of sustained virologic response to interferon α therapy.25 It is possible that this HLA allele may be involved in presenting a BK virus antigen and initiating a CTL-mediated immune response, as shown for Hantavirus.26 The HLA-C alleles may play a role as determinants of the level of NK cell activity. This effect has been postulated to relate to the finding that HLA-C molecules are ligands for inhibitory and ligand-specific NK subset in individuals homozygous.

In conclusion, Polyoma BK virus-positive people can be accepted safely for kidney donation especially with the possible protective role of HLA-Cw7. Further long-term and larger randomized studies are required to evaluate this issue.

References


Index to Volume 13 Supplement 1

Author Index

A
Aarnink A 201
Abdel Halim M 117, 242, 383
Abdelfattah MR 95
Abou-Youssef HS 23
Abuelmagd MM 111
Afshari A 83
Ahmadinejad Z 127
Ahmed MF 111
Aikawa A 18
Akar Özkan E 177, 183, 263
Akdur A 59, 71, 108, 124, 133, 145, 247, 276, 280, 312, 315
Akkurt ES 356
Aldar A 59, 71, 108, 124, 133, 145, 247, 276, 280, 312, 315
Akkurt ES 356
Alam T 37
Al-Ghareeb SM 170, 371
Ali MH 111
Aliabadi N 306
Allal A 165, 201
Al-Otaibi T 117, 242, 383
Alqahtani S 30
Al-Sayed Z 242
Amer K 318
Amin A 64
Anish TSN 197
Aoun Bahous S 55
Arslan H 280
Arslan S 59, 108, 145, 301, 335
Asaie S 306
Aydın C 286
Aytekin C 312
B
Babayev F 266
Bakr MA 111
Barsoum R 23
Baskin E 145, 247
Baştürk B 269
Batra R 9
Bayindir Y 290
Bayram Akkurt S 352
Ben Abdallah T 33
Benli S 323, 327
Bıçakçıoğlu M 286
Bircan HY 231
Börcek P 263
Boyvat F 71, 177
Bozbas H 235
Broering D 95
Broumand B 4, 90
C
Cameron AM 30
Camiran Firat A 301, 335
Can U 327
Çelik MR 290
Cevik H 231
Chacko M 30
Cho W 251
Christmas SE 207
Çolak T 193
Congy N 201
D
Daaboul Y 55
Darbouy M 83
Dashti H 127
Demirag A 231
Deniz E 346
Derle E 323, 327
Dhingra A 30
Djurj DJ 327
Diyil OM 290
Dogru M 214, 223
Dumenci N 165
Durukan E 219
Duvenci Birben O 214, 223
E
El Matri A 33
El Moghazy WM 100
El-Agroudy AE 170, 371
Elgedy H 75, 100
Elhindi YA 111
Elmaghrabi HM 111
Ensaroğlu F 133, 188, 193
Eom DW 377
Er Dedekarginoglu B 340
Erdil N 290
Ersoy MO 294
F
Faki S 159, 188
Falahzadeh ME 139
Farid E 170, 371
Faudel E 165
Fayad T 23
Fidan C 124, 247
G
Game X 201
Gedik E 286, 290, 294
George J 197
Geramizadeh B 83, 139, 306, 361, 366
Gerçekler F 177
Gheith O 117, 242, 383
Ghods AJ 13
Gholami S 139
Golubova TS 228
Gricious N 197
Gülleroglu K 247
Gurakar A 30
H
Habashy B 23
Haberal Reyhan AN 219, 312, 346
Hammad A 207
Han DJ 377
Handorf AM 37
Harmanci Ö 133, 159, 188, 193
Harrison TR 148
Hatata Y 64, 318
Hedt C 165
Hermelin M 165
Hewitt W 9
Hosny K 64, 318
Huseynov K 266
I
Işık B 286
Ismayilov H 266
Istrate MG 148
J
Jafarian A 127
Jamalidoust M 306
Jang HJ 377
Jeong EK 377
Jo SK 251
Jun H 251, 256
Jung CW 251, 256
K
Kamar N 165, 201
Kamble P 156
Kamel R 64, 318