Evaluation of Renal Function By Cystatin C in Renal Transplant Recipients

Salma Ayub,1 Mirza Naqi Zafar,1 Tahir Aziz,2 Tanweer Iqbal,2 Sadia Khan,1 S. Adibul Hasan Rizvi3

Abstract

Objectives: We evaluated cystatin C concentration as a marker of glomerular filtration rate in renal transplant recipients, and its correlation with creatinine-based glomerular filtration rate by urinary creatinine clearance, and the Cockroft-Gault and Modification of Diet in Renal Disease formulas. Materials and Methods: In this cross-sectional study, we measured serum cystatin C levels and its correlation with serum creatinine, creatinine clearance, and glomerular filtration rate using the Cockroft-Gault formula and Modification of Diet in Renal Disease formulas. Results: One hundred two recipients between June and December 2012, were examined. The mean subject age was 31.87 ± 8.37 years; the male:female ratio was 4.3:1. Mean serum creatinine concentration was 141.44 ± 43.31 μmol/L (1.60 ± 0.49 mg/dL) and serum cystatin C 122.09 ± 38.95 nmol/L (1.63 ± 0.52 mg/L). Serum cystatin C was significantly correlated with serum creatinine (r=0.90; P < .001), creatinine clearance (r=0.77; P < .001), and the Cockroft-Gault (r=0.73; P < .001) and the Modification of Diet in Renal Disease formulas (r=0.82; P < .001). We assessed the correlation among serum cystatin C with serum creatinine, creatinine clearance, the Cockroft-Gault and Modification of Diet in Renal Disease at 1, 2-3, 4-5, and more than 5 years after transplant. The correlation between serum cystatin C and serum creatinine ranged from 0.8 to 1.0; cystatin C and creatinine clearance ranged from 0.8 to 0.85; serum cystatin C and the Cockroft-Gault formula ranged from 0.7 to 0.8; and serum cystatin C and the Modification of Diet in Renal Disease formulas ranged from 0.8 to 0.84.

Conclusions: Our results show that serum cystatin C is a reliable marker for estimating glomerular filtration rate among renal transplant recipients. This test can determine the glomerular filtration rate of renal transplant recipients on follow-up. Further studies are required to establish serum cystatin C as a standard test for monitoring glomerular filtration rate in transplanted patients.

Key words: Cystatin C, Kidney, Transplant

Introduction

Kidney transplant, because it improves the quality of life and survival in comparison to dialysis, is the treatment of choice for end-stage renal failure.1 Evaluating graft function is crucial for determining long-term graft and patient outcomes.2 Estimating glomerular filtration rate (GFR) is the best marker for assessing renal function after a kidney transplant.3 Glomerular filtration rate can be measured by exogenous substances such as inulin and radioisotopes 99mtechnetium, 51chromium-EDTA, and I-iothalamate. However, these methods are incompatible with routine monitoring because of their nephrotoxicity, expense, and procedural complexity.3

Clearance assays using creatinine as an endogenous marker currently are used to assess GFR in kidney transplant recipients.2-4 Unfortunately, in these recipients, GFR can be reduced by 50% before creatinine is elevated, and deterioration of graft function may not be shown exactly by slowly rising serum creatinine concentrations.5,6 Serum creatinin-
based GFR is unreliable owing to endogenous influences of sex, age, muscle mass, food ingested, or tubular secretion of creatinine. Creatinine clearance requires a 24-hour urine sample collection that can be affected by patient compliance and can substantially underestimate or overestimate the true GFR.

To overcome these problems, formulas for rapidly evaluating GFR based on serum creatinine have been developed. The most widely of these is the Cockcroft-Gault formula, which predicts GFR from serum creatinine, age, weight and sex; the other more accurate formula is the Modification of Diet in Renal Disease.

This backdrop leads to a search for an alternative endogenous marker of renal function. Cystatin C has proven to be a better marker of GFR than serum creatinine, because it estimates subtle changes in the GFR. Serum cystatin C has proven not only to be a better marker, but it has proven to be an ideal endogenous marker of kidney function because its constant value does not depend on sex and muscle weight.

We performed this study to assess renal function in renal transplant recipients by serum cystatin C. We assessed the correlation of serum cystatin C with markers of GFR including creatinine clearance, the Cockroft-Gault formula, and the Modification of Diet in Renal Disease. To the best of our knowledge, there are no studies that examine local data of serum cystatin C levels in kidney transplant recipients.

**Materials and Methods**

Between June 2012 to December 2012, one hundred two stable renal transplant recipients of either sex were studied (age, 18-50 y) who had maintained a stable serum creatinine level (standard deviation ± 8.84 μmol/L [0.1 mg/dL]) during the previous 3 months. All subjects were screened for compliance regarding urine collection in the study. The immunosuppressive regimen included cyclosporine or tacrolimus, with steroid and azathioprine, or mycophenolate mofetil in most cases. The study was approved by the Ethical Review Committee of the institute. All protocols conformed with the ethical guidelines of the 1975 Helsinki Declaration. Informed consent was obtained from all subjects. Each recipient underwent a series of biochemical evaluations including serum creatinine, serum cystatin C, and 24-hour urine collection for creatinine clearance.

Serum creatinine was measured by the Jaffe reaction using a Beckman Coulter Synchron CX3 Delta (Beckman Coulter, Inc., Fullerton, CA, USA). Serum cystatin C was measured by particle-based immunoturbidimetric assay (RANDOX) on a Vitalab Selectra E Clinical Chemistry analyzer (Dieren, The Netherlands). The methodology, auto analyzer, and reference values for serum cystatin C and serum creatinine were the same throughout the study. The equipment was calibrated daily, and a quality control check was carried out 3 times daily.

Completeness of collection was confirmed by (24-hour urinary volume [mL] × urinary creatinine [mg/dL] ÷ (100 × weight [kg])). Creatinine clearance measurements were performed by 24-hour urine collection. Twenty-four–hour creatinine clearance (mL/min/1.73 m²) was assessed by the formula (volume [mL/min] × urinary creatinine [mg/dL] × 1.73 m²) ÷ (urinary creatinine [mg/dL] × body surface area). Glomerular filtration rate was calculated by Cockroft-Gault formula based on creatinine: (mL/min/1.73 m²) = ([140-age] × weight) ÷ (72 × serum creatinine) × (0.85 if female). Glomerular filtration rate was calculated by the Modification of Diet in Renal Disease based on Creatinine: Modification of Diet in Renal Disease (mL/min/1.73 m²) = 186 × serum creatinine 1.154 × age 0.203 × (0.742 if female). Serum cystatin C reference range, 42.69 to 78.64 nmol/L (0.57-1.05 mg/L); and GFR (by creatinine clearance), 1.33 to 2.33 mL/sec/1.73 m² (80-140 mL/min/1.73 m²).

Statistical analyses were performed with SPSS software (SPSS: An IBM Company, version 17.0, IBM Corporation, Armonk, NY, USA). Means ± SD are expressed for quantitative variables (age, serum cystatin C, serum creatinine, creatinine clearance, GFR, Cockroft-Gault formula, and Modification of Diet in Renal Disease). Frequency is given in percentages for qualitative variable. P values < .05 were considered statistically significant. The correlation between variables was evaluated with Pearson product moment correlation analysis.

**Results**

Between June 2012 and December 2012, one hundred two renal transplants recipients were studied. Posttransplant times ranged from < 1 year to > 5 years. (Patient demographics are shown in Table 1). The mean age of recipients was 31.87 ± 8.37 years,
with male to female ratio of 4.3:1. Overall mean serum creatinine was 141.44 ± 43.31 μmol/L (1.60 ± 0.49 mg/dL), mean serum cystatin C level was 122.09 ± 38.95 nmol/L (1.63 ± 0.52 mg/L), mean creatinine clearance concentration was 0.96 ± 0.02 mL/sec/1.73 m² (57.85 ± 17.17 mL/min/1.73 m²), and mean GFR-Cockroft-Gault formula 0.95 ± 0.30 mL/sec/1.73 m² (57.21 ± 17.82 mL/min/1.73 m²), and mean GFR-Mofication of Diet in Renal Disease was 0.95 ± 0.32 mL/sec/1.73 m² (57.12 ± 19.28 mL/min/1.73 m²). No difference was seen between male and female recipients for all variables measured (Table 1). Serum cystatin C significantly correlated with serum creatinine, creatinine clearance, Cockroft-Gault formula and Modification of Diet in Renal Disease (Figure 1). Correlation of serum cystatin C with serum creatinine, creatinine clearance, Cockroft-Gault formula, and Modification of Diet in Renal Disease also were assessed at different times after transplant (Figure 2). Correlation of serum cystatin C with serum creatinine ranged from 0.8 to 1.0, serum cystatin C versus creatinine clearance ranged from 0.8 to 0.85, serum cystatin C versus the Cockroft-Gault formula ranged from 0.7 to 0.8, and serum cystatin C versus Modification of Diet in Renal Disease ranged from 0.8 to 0.84.

Figure 1. Correlation of Serum Cystatin C With Serum Creatinine, Creatinine Clearance, Cockroft-Gault Formula and the Modification of Diet in Renal Disease Formulas

Figure 2. Correlation Among Serum Cystatin C and Serum Creatinine, Creatinine Clearance, and the Cockroft-Gault and the Modification of Diet in Renal Disease formulas at Different Times After Transplant

Table 1. Renal Function Parameters in Kidney Transplant Recipients

<table>
<thead>
<tr>
<th>Parameters</th>
<th>All Recipients (n=103)</th>
<th>Male Recipients (n=67) 65%</th>
<th>Female Recipients (n=36) 35%</th>
<th>P Value Male vs Female</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>Mean 31.87 ± 8.37 (19.50)</td>
<td>31.89 ± 8.36 (19.50)</td>
<td>31.79 ± 8.66 (21.48)</td>
<td>.96</td>
</tr>
<tr>
<td>Range (19-50)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serum Creatinine μmol/L (mg/dL)</td>
<td>Mean 141.4 ± 43.3 (1.60 ± 0.49)</td>
<td>140.5 ± 35.3 (1.59 ± 0.40)</td>
<td>144.0 ± 68.6 (1.63 ± 0.77)</td>
<td>.85</td>
</tr>
<tr>
<td>Range (58.3-265.2 (0.66–3.00))</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serum Cystatin C nmol/L (mg/L)</td>
<td>Mean 122.0 ± 38.9 (1.63 ± 0.52)</td>
<td>119.8 ± 32.9 (1.60 ± 0.44)</td>
<td>134.0 ± 59.1 (1.79 ± 0.79)</td>
<td>.42</td>
</tr>
<tr>
<td>Range (59.9-232.1 (0.80-3.10))</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glomerular Filtration Rate-Creatinine Clearance mL/sec/1.73 m² (mL/min/1.73 m²)</td>
<td>Mean 0.96 ± 0.29 (57.85 ± 17.17)</td>
<td>0.98 ± 0.24 (58.77 ± 14.55)</td>
<td>0.75 ± 0.43 (54.05 ± 25.93)</td>
<td>.45</td>
</tr>
<tr>
<td>Range 0.35-1.87 (21-112)</td>
<td>0.43-1.67 (26-100)</td>
<td>0.35-1.87 (21-112)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glomerular Filtration Rate-Cockroft-Gault Formula mL/sec/1.73 m² (mL/min/1.73 m²)</td>
<td>Mean 0.95 ± 0.30 (57.21 ± 17.82)</td>
<td>0.96 ± 0.27 (57.69 ± 16.12)</td>
<td>0.92 ± 0.41 (55.11 ± 24.34)</td>
<td>.66</td>
</tr>
<tr>
<td>Range 0.32-1.83 (19-110)</td>
<td>0.40-1.83 (24-110)</td>
<td>0.32-1.75 (19-105)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glomerular Filtration Rate-Modification of Diet in Renal Disease Formula mL/sec/1.73 m² (mL/min/1.73 m²)</td>
<td>Mean 0.95 ± 0.32 (57.12 ± 19.28)</td>
<td>0.97 ± 0.27 (58.16 ± 16.04)</td>
<td>0.88 ± 0.50 (52.58 ± 29.77)</td>
<td>.43</td>
</tr>
<tr>
<td>Range 0.30-1.85 (18-111)</td>
<td>0.42-1.85 (25-111)</td>
<td>0.30-1.82 (18-109)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Discussion

Cystatin C is a low molecular weight, 132 amino acid, 13-kd protease inhibitor, produced by all nucleated cells at constant rate and existing in all body fluids.14 Because of its low molecular weight, cystatin C is freely filtered through renal glomeruli followed by tubular reabsorption, which catabolizes completely; it is not secreted by kidney tubules.15 Therefore, the urinary concentration of cystatin C is zero.16 During inflammation, its endogenous production is unaffected.17 The concentration of this protein is unaffected by sex, age, or body mass.13,17

Measuring GFR quickly is critical for estimating renal function in the renal transplant recipient. Glomerular filtration rate is a marker that recognizes early rejection of the kidney; therefore, is useful for following renal injury. Endogenous markers are of interest because of their speed and simplicity, and they have been proposed as alternate marker for assessing renal function in renal transplant recipients.2-4,17-19

We established a significant correlation among serum cystatin C with serum creatinine, creatinine clearance rate, Cockroft-Gault formula, and the Modification of Diet in Renal Disease as a marker for GFR. Our results suggest that serum C correlates well with serum creatinine (r=0.90; P < .001) and creatinine clearance (r=0.77; P < .001), which is similar to the observations made by Pöge and associates10 and Dharnidharka and associates.11 Also there was a good correlation among serum cystatin C and the GFR-Cockroft-Gault formula and the GFR-Modification of Diet in Renal Disease (r=0.73; P < .001, and r=0.82; P < .001). However, another study of 18 renal transplant recipients showed a lower correlation of serum cystatin C with serum creatinine (r=0.62; P < .05), creatinine clearance (r=0.76; P < .01), GFR-Cockroft-Gault formula (r=0.85; P < .01), and GFR-Modification of Diet in Renal Disease (r=0.60; P < .05).20 We also showed a statistically significant correlation among serum cystatin C and serum creatinine, creatinine clearance, the Cockroft-Gault formula, and the Modification of Diet in Renal Disease at different times after the transplant.

In conclusion, our results indicate that serum cystatin C is a reliable endogenous marker to estimate GFR among renal transplant recipients. An accurate estimate of GFR can be achieved by a blood test; thus avoiding the cumbersome and incorrect collection of 24-hour urine. This test has the potential to determine the GFR of renal transplant recipients. Further studies are required to establish serum cystatin C as a standard test for monitoring of glomerular filtration rate in transplanted patients.

References