Objectives: Investigate the prognostic value of serum insulinlike growth factor-1 (IGF-1) and its binding protein 3 (IGFBP-3) in pediatric patients with liver cirrhosis, and investigate the correlation between these parameters and other available prognostic factors including Child-Pugh scoring, Pediatric End-stage Liver Disease, and Mayo End-stage Liver Disease scoring.

Materials and Methods: This prospective, case-controlled study was done at the Nemazee hospital for 12 months from August 2009 to August 2010. It included 45 pediatric patients (< 18 years) diagnosed with liver cirrhosis and 38 healthy age- and sex-matched controls. The extent and severity of the liver disease was evaluated by the Child-Pugh classification and Pediatric End-stage Liver Disease / Mayo End-stage Liver Disease scores. Serum levels of IGF-1 and IGFBP-3 were determined and were compared to controls and their correlation with Child-Pugh and Pediatric End-stage Liver Disease / Mayo End-stage Liver Disease scores were investigated.

Results: The most-common cause of liver cirrhosis was biliary atresia being found in 11 patients (24.4%) followed by tyrosinemia in 8 (17.8%). Serum levels of IGF-1 were significantly lower in cirrhotic patients compared with healthy controls (3.85 ± 3.69 nmol/L vs 41.79 ± 16.03 nmol/L; P < .001). Serum levels of IGFBP-3 also were significantly lower in patients with liver cirrhosis compared with healthy controls (46.66 ± 30.57 nmol/L vs 205.63 ± 25.52 nmol/L; P < .001). Serum levels of IGF-1 were significantly lower in patients with stage B (P = .047) and C (P = .036) of Child-Pugh classification compared with stage A. Serum levels of IGF-1 (r=0.227; P = .034) and IGFBP-3 (r=0.389; P = .008) were negatively correlated with Pediatric End-stage Liver Disease / Mayo End-stage Liver Disease scores.

Conclusions: The serum levels of IGF-1 and IGFBP-3 are decreased in children with liver cirrhosis. The stage of liver dysfunction is correlated to serum levels of IGF-1 and IGFBP-3 in children. Thus, these 2 factors can be used for assessing the prognosis and outcome in those children with liver cirrhosis.

Key words: Insulinlike growth factor-1, Insulinlike growth factor binding protein, Cirrhosis, Children, Prognosis

Introduction

Growth hormone (GH) is polypeptide hormone secreted from the anterior pituitary gland that plays an important role in metabolism and growth. Growth hormone has a receptor in almost all the body tissues including bone, cartilage, adipose tissue, muscle, the heart, and the immune system. In the liver, when GH attaches to its receptors, the liver synthesizes and secretes insulinlike growth factor-1 (IGF-1), which is a catabolic hormone responsible for protein synthesis.1 The GH system is responsible for several catabolic actions and consists of 2 growth factors and...
7 insulin-like growth factor (IGF) binding proteins (IGFBP).\textsuperscript{2, 3} The IGFBP-3 composes approximately 70\% of circulating IGFBP, which binds 95\% of circulating IGF-1. While hepatocytes are responsible for secreting IGF-1, IGFBP-3 is produced mainly by Kupffer cells and the endothelial lining of the liver.\textsuperscript{4} Thus, factors that damage hepatocytes result in a decrease in IGF-1 and factors damaging Kupffer cells decrease the IGFBP-3. Chronic conditions leading to chronic liver damage and decreased liver parenchyma can decrease IGF-1 and IGFBP-3 circulating levels through the aforementioned mechanism.\textsuperscript{5-11}

According to the above-mentioned evidences, we hypothesized that the degree and stage of liver failure in cirrhosis could be determined by measuring serum levels of IGF-1 and IGFBP-3. In the other words, the more severe and progressive the liver cirrhosis is, the lower the levels of detectable serum IGF-1 and IGFBP-3 are. Previous studies have shown that cirrhotic patients with lower levels of IGF-1 (< 10 nmol/L) have poor prognosis and survival.\textsuperscript{11}

However, no study has addressed this issue in pediatric patients with liver cirrhosis. We performed this study to investigate the prognostic value of IGF-1 and IGFBP-3 in pediatric patients with liver cirrhosis and to investigate the correlation between these parameters and other available prognostic factors including Child-Pugh classification, the Pediatric End-stage Liver Disease (PELD), and the Mayo End-stage Liver Disease (MELD) scoring.

\textbf{Materials and Methods}

This was a prospective, case-controlled study being performed in Nemazee Hospital, a tertiary health care center affiliated with Shiraz University of Medical Sciences, during a 12-month period from August 2009 to August 2010. We included 45 pediatric patients younger than 18 years old, diagnosed with liver cirrhosis and 38 healthy age- and sex-matched controls.

Cirrhosis was diagnosed by liver biopsy and/or by computerized tomography (CT) scan, ultrasonography, and clinical/biochemical examinations. Those with gastrointestinal tract bleeding (variceal or nonvariceal) within the preceding week, hepatic encephalopathy, spontaneous bacterial peritonitis, any kind of infectious disease within the preceding week, suspicion of hepatocellular carcinoma or any other malignancy, diabetes mellitus, and chronic renal failure were excluded.

We also included 38 healthy individuals being match for age and sex as the control group. These were selected from those referring to Motahari Clinic of Nemazee Hospital for routine check-ups. Those with any kind of infectious disease within the preceding week, diabetes mellitus, and other chronic illnesses, metabolic disorders, malignancies, and impaired liver function tests were excluded. The study protocol was approved by the institutional review board of Shiraz University of Medical Sciences and the approval of the Ethics Committee was achieved before beginning of the study. All the participants’ parents or legal guardian gave their informed written consent. All protocols conformed to the ethical guidelines of the 1975 Helsinki Declaration.

All patients and controls underwent a detailed medical history and physical examination. The demographic information including age and sex were recorded in all patients. The information was collected by means of a standard questionnaire. The weight and height of each individual was recorded by a physician, and the body mass index (BMI) was calculated according to its formula (weight in kilograms divided by height in meters squared). We also recorded the underlying diseases leading to liver cirrhosis and disease complications. Patients’ cirrhotic medications were recorded in the questionnaire.

The extent and severity of the liver disease was evaluated by the Child-Pugh classification, PELD score for those younger than 12 years old, and MELD score for those older than 12 years according to standard formulae, and were analyzed using the www.unos.org Web site.

Blood samples were drawn after an overnight fast to determine serum levels of IGF-1 and IGFBP-3. Liver function tests including serum total protein, albumin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), total and direct bilirubin, prothrombin time (PT), and international normalization ratio (INR). The levels of IGF-1 (IGF-1-IRMA, KIP0264; BIOSOURCE, Nivelles, Belgium) and IGFBP-3 (IGFBP-3-IRMA, KIP0264; BIOSOURCE, Nivelles, Belgium) were measured using standard enzyme-linked immunosorbent assay (ELISA). Sample collection, processing, and storage were done according to the instructions of the reference laboratory and the kits. The intra-assay and
interassay coefficients of variation (CV) were < 6% for all assays performed.

The information was prospectively entered into a computer database. Statistical analyses were performed with SPSS software (SPSS: An IBM Company, version 16.0, IBM Corporation, Armonk, New York, USA). Data are reported as means ± SD. An independent t test was used to compare parametric data between 2 study groups. The chi-square test was used to compare the proportions between groups. Correlations between the Child-Pugh and PELD/MELD scores, and the serum IGF-1 and IGFBP-3 levels were calculated using Pearson correlation analysis and the correlation coefficients were provided. A 2-sided P value < .05 was considered statistically significant.

**Results**

Overall, we included 45 children with liver cirrhosis and 38 healthy individuals. The mean age of the patients with cirrhosis and controls was 7.82 ± 6.22 years (range, 1 to 18 y) and 7.33 ± 5.92 years (range, 1.5 to 17 y) (P = .165). There were 28 boys (62.2%) and 17 girls (37.8%) among the patients. In the same way, there were 22 boys (57.8%) and 16 girls (42.2%) among the controls (P = .097). The mean BMI was found to be 14.9 ± 2.8 kg/m² in patients and 18.7 ± 2.5 kg/m² in controls (P = .035).

The most-common cause of liver cirrhosis in our series was biliary atresia being found in 11 patients (24.4%), followed by tyrosinemia in 8 (17.8%), cryptogenic in 8 (17.8%), and progressive familial intrahepatic cholestasis (PFIC) in 6 (13.3%). Other causes of cirrhosis included idiopathic neonatal hepatitis in 3 patients (6.7%), primary sclerosing cholangitis in 3 (6.7%), hepatitis C cirrhosis in 3 (6.7%), hepatitis B cirrhosis in 1 (2.2%), cardiac cirrhosis in 1 (2.2%), and Wilson disease in 1 (2.2%).

The most-common cirrhotic complication was jaundice in 27 patients (60%) followed by esophageal varices in 13 (28.9%), controlled ascites in 12 (26.7%), and gastrointestinal bleeding in 10 (22.2%).

Nutritional supplements were the most-commonly administered drugs in our series, being administered to 36 of the patients (80%). Among the patients, 5 (11.2%) consumed loop diuretics, and 2 (4.4%) received steroids.

According to the Child-Pugh classification for liver cirrhosis, 13 patients (28.9%) were stage A while 25 (55.6%) were stage B, and 7 (15.6%) were stage C. The mean PELD and MELD scores were 11.1 ± 6.8 and 12.4 ± 9.8. The mean values for liver function tests are given in Table 1.

### Table 1. Laboratory characteristics of 45 children with liver cirrhosis.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total protein (g/L)</td>
<td>74.6 ± 11.4</td>
</tr>
<tr>
<td>Albumin (g/L)</td>
<td>36.9 ± 7.7</td>
</tr>
<tr>
<td>Globulin (g/L)</td>
<td>37.2 ± 10.6</td>
</tr>
<tr>
<td>Alanine aminotransferase (U/L)</td>
<td>103.5 ± 83.7</td>
</tr>
<tr>
<td>Aspartate aminotransferase (U/L)</td>
<td>164.6 ± 113.8</td>
</tr>
<tr>
<td>Alkaline phosphatase (U/L)</td>
<td>1203.6 ± 758.7</td>
</tr>
<tr>
<td>Total bilirubin (µmol/L)</td>
<td>121.07 ± 137.83</td>
</tr>
<tr>
<td>Direct bilirubin (µmol/L)</td>
<td>58.48 ± 67.89</td>
</tr>
<tr>
<td>Prothrombin time (sec)</td>
<td>18.7 ± 18.5</td>
</tr>
<tr>
<td>INR</td>
<td>1.67 ± 0.66</td>
</tr>
</tbody>
</table>

**Abbreviations:** INR, international normalization ratio

Serum levels of IGF-1 were found to be 3.85 ± 3.69 nmol/L in those with liver cirrhosis and 41.79 ± 16.03 nmol/L in healthy individuals. The IGF-1 serum levels were significantly lower in cirrhotic patients compared with controls (P < .001). The serum levels of IGFBP-3 also were significantly lower in those patients with liver cirrhosis compared to healthy controls (46.66 ± 30.57 nmol/L vs 205.63 ± 25.52 nmol/L; P < .001). There was no significant difference between boys and girls regarding the IGF-1 (35.66 ± 3.30 nmol/L vs 43.22 ± 4.11 nmol/L; P = .512) and IGFBP-3 (44.27 ± 27.64 nmol/L vs 50.64 ± 35.43 nmol/L; P = .504) levels.

Serum levels of IGF-1 were significantly lower in patients with stage B (P = .047) and C (P = .036) of Child-Pugh classification compared with stage A. However, there was no significant difference between serum levels of IGF-1 in stage B and C of Child-Pugh classification (P = .986). Patients with stage A Child-Pugh classification had significantly higher levels of IGFBP-3 compared with stage B (P = .001) and C (P = .005). Again, there was no difference between stage B and C of the Child-Pugh classification (Table 2).

We performed a bivariate correlation analysis between the parametric variables including serum levels of IGF-1, IGFBP-3, liver function tests, Child-Pugh score, and PELD/MELD scores (Table 3 and 4).

### Table 2. Serum levels of IGF-1 and IGFBP-3 in different stages of liver cirrhosis according to the Child-Pugh classifications.

<table>
<thead>
<tr>
<th>Child-Pugh classification</th>
<th>IGF-1 (nmol/L)</th>
<th>IGFBP-3 (nmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stage A</td>
<td>5.90 ± 0.54</td>
<td>73.06 ± 35.87</td>
</tr>
<tr>
<td>Stage B</td>
<td>3.02 ± 0.25</td>
<td>36.96 ± 20.87</td>
</tr>
<tr>
<td>Stage C</td>
<td>2.78 ± 0.16</td>
<td>32.24 ± 19.88</td>
</tr>
</tbody>
</table>
The serum levels of IGF-1 were positively correlated with serum levels of IGFBP-3 and age, and negatively with Child-Pugh score, PELD/MELD scores (Figure 1), total and direct bilirubin. Serum levels of IGFBP-3 were positively correlated with serum levels of IGF-1 and age, and negatively with Child-Pugh score, PELD/MELD scores (Figure 2), total and direct bilirubin.

**Discussion**

The liver is the main source of IGF-1 secretion and protein synthesis. Thus, levels of serum IGF-1, as well as serum proteins, are decreased in those diseases in which liver function is diminished. Liver cirrhosis is considered the most-important reason for decreased liver function. In cirrhotic patients, serum albumin levels, as well as coagulative proteins, serum complements, and other proteins are decreased. The levels of direct bilirubin are also decreased because of diminished liver function in conjugating the bilirubin. In the same way, cirrhosis is accompanied by endocrine disturbances including decreased serum levels of IGF-1 and increased GH levels.

Data regarding the prognostic value of these 2 factors in pediatric patients with cirrhosis is scarce. Regarding this, we performed this study to determine levels of IGF-1 and IGFBP-3 in children with cirrhosis. We found that pediatric patients with cirrhosis have decreased levels of IGF-1 and IGFBP-3 compared to their healthy controls. We also tried to determine the correlation between the serum levels of IGF-1 and IGFBP-3 and the stage of the liver cirrhosis determined by 2 different scoring systems. We found that the serum levels of IGF-1 were significantly lower in patients with stage B and C of Child-Pugh classification compared to stage A.
Patients with stage A Child-Pugh classification had significantly higher levels of IGFBP-3 compared with stage B and stage C. Absence of statistically significant differences between stage B and C of Child-Pugh classification may result from an overlap between higher scores in stage B and lower scores in stage C, because the levels of these factors have negatively correlated with Child score (Figure 1). Serum levels of IGF-1 and IGFBP-3 were negatively correlated with PELD/MELD scores. These are consistent with previous studies.4, 5, 11, 14

Several mechanisms have been found responsible for the endocrine disturbances in patients with liver cirrhosis. Diminished IGF-1 synthesis secondary to derangement of hepatic cellular functions, defective growth hormone reserves, as well as disturbed feedback mechanisms of IGF-1 on the hypothalamic hypophyseal axis are among the most-important mechanisms of endocrine disturbance in cirrhotic patients.

There are several types of IGFs including IGF-1 and IGF-2. The IGF-1 is more abundant and more-potent than the other types of IGFs. The IGFBPs are also produced by the liver and are carriers of the IGF-1 in the serum. About 98% of the IGF-1 is bound to IGFBP-3 in the serum.11 Although the liver is believed to be the main source of IGFBP-3, it is shown that many cell types also produce IGFBP-3. It has been shown by Scott and associates that the IGFBP3 is also produced from nonparenchymal cells in vitro but not from isolated hepatocytes in primary culture.15

It was shown by Assy and associates11 that patients with cirrhosis have decreased basal levels of IGF-1 and IGFBP-3. That was a dynamic study measuring the levels of these 2 parameters before and after administering rhGH. The serum levels of IGF-1 and IGFBP-3 were measured before and 24 hours after administering a single subcutaneous injection of rhGH, 0.14 U/kg. They found that the mean serum IGF-1 levels 24 hours after rhGH injection predicted survival with 93% accuracy. The lower levels of IGF-1 were associated with poorer prognosis and a survival rate of 15%. Levels greater than 10 nmol/L were associated with a 100% survival at 1 year and a better prognosis. They also showed that serum levels of IGFBPs and IGF-1 alone are not good predictors of the disease’s natural course, prognosis, and survival rate. Thus, they concluded that stimulated IGF-1 levels are the best predictors of disease outcome in cirrhotic patients.11

In the same way, Donaghy and associates5 showed that basal GH resistance in patients with cirrhosis cause serum levels of IGF-1 and IGFBP-3 to decrease. It is caused by the feedback maladjustment of the GH/IGF-1/IGFBP-3 axis. They showed there is a direct relation between the severity of GH resistance, levels of IGF-1 and IGFBP-3, and severity of the liver injury. Thus, it is believed that the GH resistance is the main endocrine disturbance recognized in liver cirrhosis that is associated with the extent of liver injury and severity of liver mass loss. Some comorbidities and complications have been associated with severity of the liver failure and the endocrine disturbance including liver dysfunction, disorder of portosystemic shunting, and malnutrition of hepatic storage. As GH receptors are produced and secreted by hepatocytes, levels of these receptors are decreased in liver cirrhosis leading to resistance to GH and thus, decreased levels of IGF-1. This is a vicious cycle in which the more liver failure leads to more GH resistance and thus, decreased IGF-1 levels.5

We found that the serum levels of IGF-1 were positively correlated with serum levels of IGFBP-3 and age, and negatively with Child-Pugh score, PELD/MELD scores, total and direct bilirubin. Serum levels of IGFBP-3 were positively correlated with serum levels of IGF-1 and age, and negatively with Child-Pugh score, PELD/MELD scores, and total and direct bilirubin. These show that as the levels of IGF-1 and IGFBP-3 decrease in liver cirrhosis, prognosis, and survival decreases.

The interesting finding was the positive correlation between IGF-1 and IGFBP-3 with age. By increasing age, serum levels of these factors increase despite liver cirrhosis. These findings are in accord with previous studies.4, 11, 14, 16 Regarding this, Wu and associates10 found no significant difference regarding serum IGF-1 and IGFBP-3 between Child-Pugh classifications B and C. However, they found that serum IGF-2 was lower in patients with Child-Pugh classification C compared with B. This shows that fluctuation of serum IGF-2 is preserved even with severe liver dysfunction. They also found that the levels of IGF-2 are more decreased compared to serum levels of IGF-1 and IGFBP-3, and this makes it a better predictor for liver dysfunction in patients with liver cirrhosis. In the same way, Nikolic and associates17 showed that patients with cirrhosis have decreased levels of IGF-1, IGF-2, and IGFBP-3 compared with healthy individuals. They also found
a positive linear correlation between serum levels of IGF-1 and IGF-2, and with the stage of liver failure as assessed by the Child-Pugh classification. They suggested that IGF-2 serum levels would be a better factor for predicting the disease outcome in patients with liver cirrhosis compared to GH stimulated IGF-1 generation test and combined measurement of serum IGF-I, IGF-2, and IGFBP-3. Interpretation of IGF-1 level is complicated by pubertal stage; despite this, we included a control group that matched with our case group according to age and sex ($P > .05$), but we failed to check the pubertal stages of our cases according to the standard method, and this is the limitation of this study.

In conclusion, serum levels of IGF-1 and IGFBP-3 are decreased in children with liver cirrhosis. The stage of liver dysfunction is correlated to serum levels of IGF-1 and IGFBP-3 in children. Thus, these 2 factors can be used to assess the prognosis and outcome in those children with liver cirrhosis. Future studies should focus on treatment options of rhIGF-1 for liver cirrhosis.

References


