

Serum insulin like growth factor-1 (IGF-1) and insulin like growth factor binding protein-3 (IGFBP-3) levels in liver cirrhosis

Karaciğer sirozunda serum insulin like growth factor-1 (IGF-1) ve insulin like growth factor binding protein-3 (IGFBP-3) düzeyleri

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Background/aims: Impaired growth hormone-insulin like growth factor system in hepatic cirrhosis leads to cirrhosis-related complications. In this study, we aimed to investigate whether serum levels of insulin like growth factor-1 and insulin like growth factor binding protein-3 are related to the level of hepatic dysfunction, clinical grade, and etiologic factors of the disease in patients with liver cirrhosis. **Methods:** Forty-two patients with liver cirrhosis who were diagnosed by means of clinical findings, endoscopy, imaging studies, or histopathology were enrolled in the study. An age- and sex-matched control group was comprised of 37 healthy controls with no signs of liver disease by clinical or laboratory findings. The demographic features (age, sex, height, and weight) and serum levels of liver function tests, urea, creatinine, sodium, potassium, insulin like growth factor-1, and insulin like growth factor binding protein-3 and hemogram values were recorded for each individual. The patients were grouped according to Child Pugh classification and etiology. **Results:** Insulin like growth factor-1 and insulin like growth factor binding protein-3 levels were significantly lower in the cirrhotic group in comparison to the control group ($p<0.005$). A statistically significant decrease in levels of insulin like growth factor-1 and insulin like growth factor binding protein-3 was correlated with the degree of liver dysfunction, namely, lowest decrease in Child Pugh class A and highest decrease in class C. With respect to etiology, insulin like growth factor-1 levels of alcohol-related liver cirrhosis were significantly lower than those of hepatitis B-related cirrhosis. There was no relation between insulin like growth factor binding protein-3 level and etiology. In the cirrhotic group, insulin like growth factor-1 level was positively correlated with serum albumin and negatively correlated with serum creatinine and sodium levels and spleen size. Likewise, insulin like growth factor binding protein-3 level was positively correlated with serum albumin. There was a negative correlation between insulin like growth factor binding protein-3 level and serum bilirubin and spleen size. **Conclusions:** Insulin like growth factor-1 and insulin like growth factor binding protein-3 levels are related to the level of clinical impairment and were independent of the etiology. They may serve as novel markers of hepatocellular dysfunction.

Key words: Child class, liver cirrhosis, IGF-1, IGFBP-3

Amaç: Hepatik sirozda insülin benzeri büyüme faktörü sisteminde oluşan bozulma komplikasyonlara yol açabilir. Bu çalışmada sirozda serum insulin like growth factor-1 ve insulin like growth factor binding protein-3 düzeylerinin hepatic disfonksiyon, klinik durum ve etiyolojik faktörlerle ilişkili bulunup bulunmadığı araştırılmayı hedefledik. **Yöntem:** Klinik, görüntüleme, endoskopik veya histopatolojik olarak siroz tanısı konmuş 42 hasta çalışmaya alındı. Yaş ve cinsiyet açısından eşleştirilmiş, klinik ve biyokimyasal olarak karaciğer hastalığı tanısı dışlanmış 37 sağlıklı birey kontrol grubunu oluşturdu. Demografik özellikler (yaş, cinsiyet, boy ve kilo) ile karaciğer fonksiyon testleri, üre, kreatinin, sodyum, potasyum, insulin like growth factor-1 ve insulin like growth factor binding protein-3 ile hemogram verileri kaydedildi. Hastalar Child Pugh sınıflamasına ve etiyolojilerine göre tasnif edildi. **Bulgular:** Insulin like growth factor-1 ve insulin like growth factor binding protein-3 düzeyleri sirotik grupta kontrol grubuna göre belirgin düşük bulundu ($p<0.005$). Insulin like growth factor-1 ve insulin like growth factor binding protein-3'te azalma ile karaciğer disfonksiyonun derecesi arasındaki ilişki istatistiksel olarak anlamlı bulundu; diğer bir deyişle Child Pugh klas A'da en az düşme saptanırken en fazla düşüş klas C'de izlendi. Etiyoloji dikkate alındığında insulin like growth factor-1 düzeyi alkole bağlı sirozda hepatit B'ye bağlı sirozda gözlenen düzeylere göre belirgin olarak düşük bulundu. insulin like growth factor binding protein-3 ve etiyoloji arasında ilişki saptanmadı. Sirotik grupta insulin like growth factor-1, serum albumini ile pozitif, serum kreatinini, serum sodyumu ve dalak boyutuyla negatif ilişki saptandı. Insulin like growth factor binding protein-3 ise sadece serum albumini ile pozitif yönde ilişkili bulundu. Insulin like growth factor binding protein-3 ve serum bilirubini ve dalak boyutu arasındaki ilişki ise negatif yöndeydi. **Sonuç:** Insulin like growth factor-1 ve IGF-3 düzeyleri sirozda klinik evreyle ilişkilidir. Düzeyler etiyolojiden bağımsızdır. Hepatoselüler disfonksiyonun yeni belirteçleri olarak kullanılabilirler.

Anahtar kelimeler: Child sınıfı, karaciğer sirozu, IGF-1, IGFBP-3

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INTRODUCTION

Growth hormone (GH) has multiple actions on bone, cartilage, adipose tissue, muscle, the heart, immune system, etc. GH is released from the anterior pituitary gland and binds to its receptors on the liver. The liver in turn synthesizes insulin like growth factor-1 (IGF-1) (1).

The IGF system is comprised of two growth factors, two receptors, and seven insulin-like growth factor binding proteins (IGFBP). This system operates in anabolism and cell proliferation (2,3). The liver is the predominant site of IGF production. More than 70% of IGFBP is IGFBP-3. IGFBP-3 binds nearly 95% of circulating IGFs. IGFBP-3 is known as the most important component of circulating binding proteins. It is primarily produced by the endothelial lining and Kupffer cells in the liver. Thus, any factor that impairs the liver parenchyme and resultant hepatocyte dysfunction may lead to decreased components of the IGF system in circulation.

The GH-IGF system is adversely affected in liver cirrhosis. Low IGF-1 and IGFBP-3 levels in cirrhosis are due to decreased hepatic synthesis (2-9).

This study was designed to determine the relationship between IGF-1 and IGFBP-3 levels and severity of liver dysfunction and etiology in cirrhosis, and whether these parameters could be used as novel markers in assessing liver function in addition to Child Pugh classification.

MATERIALS AND METHODS

Forty-two patients with liver cirrhosis were enrolled in the study (15 females and 27 males) in 2003-2004. They were diagnosed by clinical findings, histopathology, imaging studies, and endoscopy in our state hospital. Mean age of the cirrhotics was 52.0 (range: 19-78) and of the controls was 48.8 (range: 26-69) ($p=0.26$).

The patients were divided into three groups according to etiology: 14 alcohol related-cirrhosis, 14 hepatitis B-induced cirrhosis, and 14 hepatitis C-induced cirrhosis. They were also divided according to the Child Pugh class (CPC): 14 patients in A, 15 in B and 13 in C.

An age- and sex-matched control group was comprised of 37 healthy controls (16 females and 21 males). All the subjects met the inclusion criteria below.

Inclusion criteria:

Patient group:

Absence of (1) gastrointestinal system bleeding (variceal or nonvariceal) within the preceding week; (2) hepatic encephalopathy; (3) spontaneous bacterial peritonitis; (4) any kind of infectious disease within the preceding week; (5) suspicion of hepatocellular carcinoma or any other malignancy; (6) diabetes mellitus; (7) chronic renal failure; and (8) signs and symptoms suggestive of alcohol withdrawal during the time of the study.

Control group:

Absence of (1) any kind of infectious disease within the preceding week; (2) diabetes mellitus and other chronic illnesses; (3) history of malignancy; and (4) impaired liver function tests.

Each patient was measured for height and weight. Body mass index (BMI) was derived by calculating the weight in kilograms divided by the height in meters squared. Blood samples were collected for assessment of aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma glutamyl transferase (GGT), alkaline phosphatase (ALP), bilirubin (Bb), albumin, prothrombin time (PT), urea, creatinine (Cr), sodium (Na), potassium (K),

IGF-1, IGFBP-3, and hemogram. Samples were collected in fasting state in the morning. Serum IGF-1 and IGFBP-3 concentrations were quantified by KIP1588 IGF-1 coated tube radioimmunoassay (RIA-CT) kit and KIPB1014 immunoradiometric assay (IRMA) kit (Biosource Europe S.A.), respectively. The results were defined in nanogram/milliliter. Spleen size was determined by ultrasonography (USG). Sample collection, processing, and storage were done according to the instructions of the reference laboratory and the kits.

Statistical analysis:

All calculations were done with SPSS system 11.0 for Windows. Kruskal Wallis, Mann-Whitney U, chi-square, independent samples t test, and Pearson correlation analysis were used. Independent samples t test was used for comparison of age, IGF-1, and IGFBP-3 between the groups. Nonparametric test was used for the remaining data. Kruskal Wallis and Mann-Whitney U test were used for comparing IGF-1 and IGFBP-3 in terms of CPC and etiology. Pearson correlation was used for quantitative data. A probability value of $p<0.05$ was considered significant.

RESULTS

Serum IGF-1 and IGFBP-3 levels were significantly lower in the cirrhotics (Table 1) than in controls.

Table 1. IGF-1 (ng/ml) and IGFBP-3 (ng/ml) values in the patient and control groups

	Patient	Control	p
IGF-1	201±95	261±130	0.02
IGFBP-3	1765±636	3020±1059	<0.01

Alcohol-related cirrhosis was associated with lower IGF-1 when compared to hepatitis B-related cirrhosis in terms of etiology ($p=0.03$). This discrepancy was not observed between other etiologies (alcohol vs. hepatitis C and hepatitis B vs. C).

IGF-1 and IGFBP-3 concentrations decreased progressively from CPC A to CPC C (Figure 1) ($p=0.003$ vs. $p=0.001$, respectively). The difference was more pronounced between CPC A and B-C in IGF-1 and between each group in IGFBP-3 (Table 2).

In the cirrhotic group, IGF-1 level was positively correlated with serum albumin ($r=0.436$, $p=0.004$)

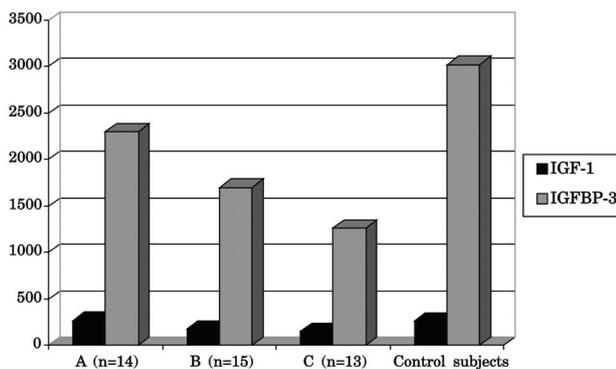


Figure 1. Relationship between Child Pugh class and serum IGF-1 (ng/ml) and IGFBP-3 (ng/ml)

Table 2. Statistical comparison of IGF-1 (ng/ml) and IGFBP-3 (ng/ml) according to CPC

	IGF-1	IGFBP-3
CPC A	270±97	2300±523
CPC B	180±84	1702±548
CPC C	152±63	1264±352
CPC A vs. B	$p=0.014$	$p=0.011$
CPC A vs. C	$p=0.001$	$p=0.001$
CPC B vs. C	$p=0.549$	$p=0.028$

CPC: Child Pugh class

and negatively correlated with serum Cr ($r= -0.382$, $p=0.008$), Na ($r=0.349$, $p=0.024$), and spleen size ($r= -0.308$, $p=0.047$) on USG. Likewise, IGFBP-3 level was positively correlated with serum albumin ($r=0.388$, $p=0.011$). There was a negative correlation between IGFBP-3 level and serum Bb ($r= -0.312$, $p=0.044$) and spleen size ($r= -0.434$, $p=0.004$). There was no correlation between serum IGF-1 and IGFBP-3 levels and serum transaminases, ALP, GGT, platelet count, PT, and BMI (Table 3).

Table 3. Relationship between IGF and IGFBP-3 and quantitative data

	r	p
IGF-1 and albumin	0.436	0.004
IGF-1 and Cr	-0.382	0.008
IGF-1 and Na	0.349	0.024
IGF-1 and spleen size	-0.308	0.047
IGFBP-3 and albumin	0.388	0.011
IGFBP-3 and spleen size	-0.434	0.004
IGFBP-3 and total bilirubin	-0.312	0.044

Cr: Creatinine, Na: Sodium

DISCUSSION

Low IGF-1 levels may be involved in the development of cirrhotic complications including malnutrition, insulin resistance, impaired immunity, and osteoporosis (10-12). The IGF system also has a role in cellular growth and differentiation. Thus, it may be operational in hepatocarcinogenesis as well as in other types of carcinomas. Transformation of hepatocytes to neoplastic cells demands these growth factors. Many products of oncogenes are similar to IGF receptors in terms of transmembranous tyrosine kinase activity. IGFs also promote transcription of protooncogenes that modulates transcription of other genes stimulating cellular growth (13).

Poor nutritional status may be responsible for low levels of IGF-1, IGFBP-3, and albumin. IGF-1 level decreases even in nutritional problems of short duration. Therefore, lower IGF-1 is not unexpected in liver cirrhosis, where malnutrition is a serious problem. In an animal study, IGF-1 is increased with alcohol abstinence even further with high-calorie diet and IGF-1 is infusion (10,12).

IGF-1 and IGFBP-3 were inversely correlated with insulin resistance determined by hyperinsulinemic euglycemic clamp test; and vice versa for GH (14). Hepatic mRNA levels for GH receptor

and IGF-1 were significantly decreased in a study done by Donaghy *et al.* (4).

In our study, serum levels of both IGF-1 and IGFBP-3 were significantly lower in the cirrhotics when compared to controls. These results were compatible with those of the other reports in the literature (3-8,15). Low-normal limit of IGFBP-3 by IRMA is 1577 ng/ml in adults over 20 years of age. In the cirrhotics, the lowest level was 1129 ng/ml, while it was 1961 ng/ml in the control subjects. Mean values were 1765 ng/ml and 3020 ng/ml, respectively. Low-normal limit of IGF-1 by RIA is 144 ng/ml in adults over 20 years of age. In the cirrhotics, the lowest level was 106 ng/ml, while it was 131 ng/ml in the control subjects.

There are conflicting reports about an association between etiology and the IGF system. Vyzantiadis *et al.* (16) found no relation with etiology. Wu *et al.* (9) noted in their study that all subjects with hepatitis B-induced cirrhosis had low levels of IGF-1 and IGFBP-3.

Mendenhall *et al.* (12) found a relation between alcohol and IGF-1 in rats. Recovery was better for combined treatment modality (IGF-1 therapy, high-calorie diet, and alcohol abstinence) than each one alone. Alcohol negated benefits of IGF-1 therapy and high-calorie diet. We found that IGF-1 levels were significantly lower only in alcohol-related liver cirrhosis when compared to hepatitis B-related liver cirrhosis. IGFBP-3 level was not associated with etiology.

Since the liver is the abundant site of IGF-1 and IGFBP-3 production and their production is under the control of GH, several studies were designed to show whether they may serve as surrogate markers of hepatocyte dysfunction and severity of liver impairment. Our data revealed a progressive decrement in IGF-1 and IGFBP-3 from CPC A to CPC C, which was statistically significant. Since the liver is the major production site of the IGF system, the more severe the cirrhosis, the lower the levels of IGF-1 and IGFBP-3. Therefore, it was not surprising to see that albumin was positively related with IGF-1 and IGFBP-3.

In our study, IGF-1 and IGFBP-3 were not correlated with BMI even when weight was corrected for ascites. We think that increased weight due to ascites in some of the cirrhotics contributed to this result. In a previous report where nutritional status and IGF-1 relationship was evaluated, triceps skin fold was not correlated with IGF-1 level (17).

We believe that nutritional parameters including BMI may be compared to the clinical staging and IGF system.

Another issue in cirrhosis is hyponatremia. Hyponatremia in ascites and spontaneous bacterial peritonitis is a poor prognostic sign (18). Furthermore, severe hyponatremia is evident in highly mortal hepatorenal syndrome. Generally, hyponatremia is assumed to be a result of increased extracellular volume, namely dilutional hyponatremia.

There may be other factors adjusting Na level. IGF-1 increases renal plasma flow and glomerular filtration rate (GFR) in chronic renal failure as well as in healthy humans. IGF-1 operates in tubular sodium handling (19-22).

Intravenous IGF-1 causes antinatriuresis in healthy subjects and in patients with growth hormone insensitivity syndrome (23-25). This antinatriuretic effect may be a consequence of increased renin and decreased atrial natriuretic factor (24). The effect of IGF-1 on natriuresis is not uniform; antinatriuresis is observed in normovolemic healthy subjects but may lead to natriuresis in subjects with volume expansion. IGF-1 administration increases tubular sodium reabsorption in healthy subjects (19). Another proposed mechanism for the effect of IGF-1 on natriuresis is facilitation of its antinatriuretic effect via decreased IGFBP-3 and increased IGFBP-1. This proposal stands for the inverse correlation between the index of accessibility of IGF-1 to tissues (IGF-IGFBP index) and fractional sodium excretion (26). This effect was independent from the CPC. Peripheral arterial vasodilation and resultant decreased effective arterial blood volume in cirrhosis may facilitate the antinatriuretic effect of IGF-1 (27,28).

IGF-1 levels in our studied cirrhotic group were negatively correlated with serum Cr and Na levels. Hyponatremia in our patients may be a result of the antinatriuretic effect of IGF-1. Nevertheless, IGF-1 excess was not present; the level at which it induces antinatriuresis remains to be answered before reaching a final suggestion about its contribution to the pathogenesis of hyponatremia in cirrhosis.

Data of the studies outlined above and our study proved that the IGF system is an important indicator of liver function. IGF-1 and IGFBP-3 may serve as novel markers of hepatocellular dysfunction, albeit further prospective, large-scale studies are warranted to confirm these results. Experi-

mental models promise future therapies with IGF-1 for osteopenia, malnutrition, and recovery of

liver function and histology (29-31).

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