Insulin growth factor-1 and insulin growth factor binding protein-3 in Egyptian patients with chronic hepatitis C

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Submitted: 13 January 2007
Accepted: 14 March 2007

Arch Med Sci 2007; 3, 1: 46-51

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Abstract

Introduction: The liver is the major source of insulin growth factor-1 (IGF-1) and its main binding protein, insulin growth factor binding protein-3 (IGFBP-3), which modify its bioavailability, and their concentrations might reflect liver synthetic capacity. The aim of the study was to evaluate serum levels of IGF-1 and IGFBP-3 in patients with chronic hepatitis C and their potential use as a marker of hepatic synthetic capacity.

Material and methods: Thirty patients with chronic hepatitis C virus infection were included in the study: 16 patients with chronic hepatitis and 14 patients with liver cirrhosis. Thirteen healthy volunteers, age and sex matched with the cases, were used as a control group. Serum IGF-1 and IGFBP-3 were measured in all patients and controls by enzyme-linked immunosorbent assay (ELISA).

Results: Serum levels of IGF-1 in patients with liver cirrhosis were significantly lower than those in patients with chronic hepatitis and those in healthy controls (110.4±57 ng/ml, 308.1±188.5 ng/ml and 274.1±81.6 ng/ml respectively, $p<0.001$). IGF-1 correlated negatively with age and AST (r=-0.467 and -0.393 respectively, $p \leq 0.05$), and positively with prothrombin concentration (r=0.461, p=0.05). Serum levels of IGFBP-3 were significantly lower in patients with chronic hepatitis and liver cirrhosis than those in healthy controls (3576.8±743.5 ng/ml, 2323.1±1073.1 ng/ml and 4675.1±1274.2 ng/ml respectively, $p<0.001$) with a significant difference between patients with liver cirrhosis and patients with chronic hepatitis ($p<0.001$). Also IGFBP-3 was significantly lower in patients with schistosoma infection than in patients without schistosoma (2648.3±838.1 ng/ml and 4058±1513.4 ng/ml respectively, p=0.002). IGFPP-3 correlated negatively with age and AST (r=-0.485 and -0.619 respectively, $p<0.001$) and positively with serum albumin and prothrombin concentration (r=0.509 and 0.617 respectively, p=0.02 and 0.006 respectively).

Conclusions: IGF-1 and IGFBP-3 may be useful parameters for assessment of liver function. IGFBP-3 is an early predictor of hepatic dysfunction and can be used as a marker for the severity of liver disease.

Key words: IGF-1, IGFBP-3, chronic hepatitis, liver cirrhosis.

Introduction

Insulin growth factor-1 (IGF-1) is a single chain molecule with three intrachain disulphide bridges consisting of 70 amino acid residues. It is considered an important anabolic hormone which is active throughout one's life, inducing anabolic metabolism and stimulating DNA synthesis, cell proliferation and meiotic division [1]. It is secreted mainly by the liver and circulates in two states, free and bound to binding proteins [2].

Six insulin growth factor binding proteins (IGFBP) have been isolated and chemically characterised and they are mainly secreted by the liver [3]. The main IGFBP in serum is IGFBP-3, which consists of 264 amino acid residues. It binds nearly 95% of circulating IGF in the human body, forming a stable ternary complex with IGF and the acid labile subunit. The complex is believed to serve as a reservoir in circulation to prolong the half lives of both IGF-1 and IGF-2 and it restricts their extravascular transit [4].

Chronic liver disease is associated with marked changes in body composition. These changes are accompanied with impaired generation of IGF-1 and altered production of IGFBP-3. Several studies reported that serum IGFBP-3 levels were abnormally low in patients with liver cirrhosis and correlated with severity, and that the levels were significantly lower than normal mean values in the healthy population. The close correlation of IGFBP-3 levels with hepatic functions indicates a dominant regulatory role of hepatocytes [5].

Factors that modulate serum levels of IGF-1 and IGFBP-3 include age, sexual development, nutrition, thyroid hormones, hepatic or renal function and growth hormone (GH). Growth hormone is certainly the major regulator of both peptides, and the progression of chronic liver disease to cirrhosis results in a state of acquired GH resistance with high GH and low IGF-1 levels [6, 7].

The aim of the study is to evaluate serum levels of IGF-1 and IGFBP-3 in patients with chronic liver diseases and their potential use as a marker of hepatic synthetic capacity.

Material and Methods

Patients

After receiving ethical committee approval, we started recruiting patients who signed written informed consent from the hepatology and internal medicine outpatient clinics of the Medical Services Unit at the National Research Centre. Thirty patients with chronic hepatitis C virus infection were selected for the study; they were 18 men and 12 women, their ages ranging from 27 to 67 years. They consisted of 16 patients with chronic hepatitis (8 men, 8 women, age range: 27 to 51 years), and 14 patients with liver cirrhosis (10 men, 4 women, age range: 38 to 67 years). We excluded any patients with extrahepatic failure (cardiac, respiratory or renal failure), metabolic disease (diabetes mellitus or thyroid dysfunction), evidence of recent systemic infection or active variceal bleeding. Patients with recent alcohol intake or corticosteroid therapy were also excluded.

Thirteen healthy volunteers, age and sex matched with the cases, were used as a control group.

All patients and controls were subjected to full history taking including history of alcohol or drug abuse and past history of schistosomiasis infection, thorough clinical examination with special attention to general signs suggestive of liver disease (jaundice, spider navi, palmar erythema, lower limb oedema), and careful abdominal examination. Chest, heart and neurological examinations were also done. Height and body weight were assessed and body mass index was calculated by dividing body weight in kilograms per height in square metres. Patients were subjected to laboratory investigations in the form of: complete blood count, conventional liver biochemical tests (aspartate aminotransferase, alanine aminotransferase, bilirubin, serum albumin, prothrombin time and concentration) and schistosoma antibody. Abdominal ultrasound was done for all patients. IGF-1 and IGFBP-3 were assessed for all patients and controls. HCV was diagnosed by hepatitis C virus antibody detected by third generation test (ELISA) with elevated transaminase level more than twice the upper limit of normal for more than six months duration. Liver cirrhosis was diagnosed clinically, in the laboratory and by ultrasonographic finding of shrunken liver and/or portal hypertension and/or ascites.

Laboratory methods

Fasting blood samples were obtained after an overnight fast, and were immediately centrifuged and stored at -20°C until analysis. At the time of the analysis blood was allowed to thaw at room temperature.

Determination of IGF-1 by enzyme-linked immunosorbent assay (ELISA) using kit manufactured by Biosource Europe S.A., catalogue number: KAPB2010.

Principle. As IGFBP-3 interferes with the determination of IGF-1, an extraction test is done to separate IGF-1 from its binding protein by adding extraction solution (ethanol + HCl) and incubating for 30 minutes then centrifuging at ≥10000 rpm for 2 minutes at 4°C. The supernatent is transferred and a neutralizing solution is added with a sample diluent.

IGF-1 in the extracted samples is determined by ELISA technique using antibodies with high affinity and specificity for two different epitopes on IGF-1. A first monoclonal anti IGF-1 antibody bound to a polystyrene well will capture the IGF-1 of the sample in the presence of a second alkaline phosphatase conjugated monoclonal anti-IGF-1 antibody. Following the incubation and the one-step formation of the solid phase IGF-1 conjugated monoclonal antibody sandwich, the well is washed to remove excess unbound conjugated antibody. The chromogen/substrate is added and a yellow colour develops. The colour intensity is proportional to the IGF-1 concentration in the patient's sample. The intensity of the yellow colour is measured using a spectrophotometer with a 405 nm filter. IGF-1 concentrations in patients are read from a calibration curve. Each laboratory establishes its own reference values [8].

Determination of IGFBP-3 by ELISA using kit manufactured by Biosource Europe S.A., catalogue number 2014.

Principle the same as that of IGF-1 [8].

Table I. Mean serum levels of IGF-1 and IGFBP-3 in patients and healthy controls

Variable (mean±SD)	Chronic hepatitis (n=16)	Liver cirrhosis (n=14)	Healthy controls (n=13)	p value (ANOVA)
IGF-1 (ng/ml)	308.1±188.5*	110.4±57*	274.1±81.6	0.001
IGFBP-3 (ng/ml)	3576.8±743.5*	2323.1±1073.1**	4675.1±1274.2	0.001

** significant difference with controls(t test between two groups)*
*** significant difference with chronic hepatitis and control (t test between two groups)*

Table II. Mean serum levels of IGF-1 and IGFBP-3 in patients with and without schistosoma infection

Variable (mean±SD)	HCV patients with schistosoma infection (n=13)	HCV patients without schistosoma infection (n=17)	p value
IGF-1 (ng/ml)	199.9±168.4	245.4±156.9	0.407
IGFBP-3 (ng/ml)	2648.3±838.1	4058±1513.4	0.002*

** p is significant*

Table III. Correlation coefficient between IGF-1 and age and conventional liver biochemical tests

Variables	r	p value
Age	-0.467	0.004*
Bilirubin	-0.446	0.375
AST	-0.393	0.05*
ALT	-0.312	0.128
Albumin	-0.021	0.93
Prothrombin concentration	0.461	0.05*

** p is significant*

Statistical methods

Data are presented as means ±SD and percentages. The compiled data were computerized and analyzed by EPI Info version 6.2 produced through the collaboration between CDC/WHO and by SPSS PC+, version 7.5. The following tests of significance were used: analysis of variance (ANOVA) test between more than two means, t test between means to analyze mean difference. A level of significance with $p \leq 0.05$ was considered significant, $p<0.001$ was considered highly significant and $p>0.05$ was considered insignificant. Multiple correlation coefficients {r} were used to determine the correlation of the studied parameters to each other.

Results

IGF-1 in patients with chronic hepatitis, cirrhosis and control group

The results show that mean serum levels of IGF-1 in patients with liver cirrhosis were significantly lower those that in patients with chronic hepatitis ($p<0.001$) and healthy controls ($p=0.001$) and no significant difference between patients with chronic hepatitis and healthy controls (Table I). Also there was significant difference between patients with and without schistosoma infection (Table II). IGF-1 correlated negatively with age and AST and positively with prothrombin concentration ($p=0.05$) (Table III, Figures 1, 2, 3).

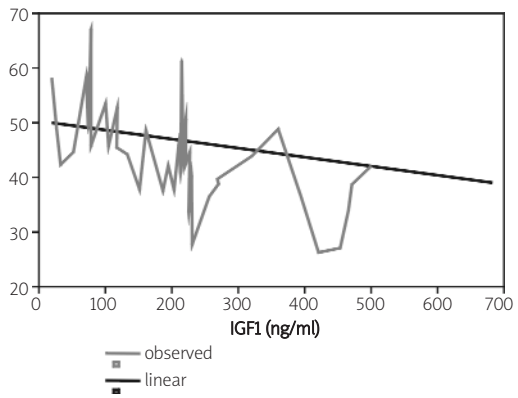

Figure 1. Regression line for the correlation between serum IGF-1 and age of patients

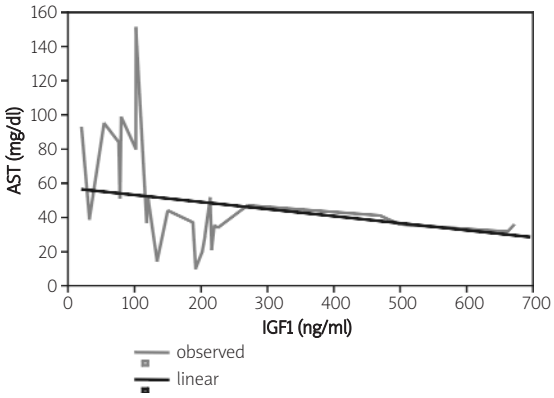

Figure 2. Regression line for the correlation between serum IGF-1 and AST

Table IV. Correlation coefficient between IGFBP-3 and age and conventional liver biochemical tests

Variables	r	p value
Age	-0.485	0.001*
Bilirubin	-0.720	0.106
AST	-0.619	0.001*
ALT	-0.283	0.171
Albumin	0.509	0.022*
Prothrombin concentration	0.617	0.006*

** p is significant*

IGFBP-3 in patients with chronic hepatitis, cirrhosis and control group

We found a significant difference in mean serum concentrations of IGFBP-3 between patients with chronic hepatitis, liver cirrhosis and the control group ($p<0.001$) (Table I). In patients with schistosoma infection, mean serum concentrations of IGFBP-3 were significantly lower than in patients without schistosoma infection (Table II). IGFBP-3 correlated negatively with age and AST, and positively with serum albumin and prothrombin concentration (Table IV, Figures 4, 5, 6, 7).

Discussion

The liver is the central organ of the endocrine growth hormone/insulin-like growth factor (GH/ILGF) axis. GH is secreted by the anterior pituitary gland and acts through the hepatic GH receptor to regulate the production of the potent mitogenic growth factor, IGF-1 [9]. The availability of IGF-1 to its tissue receptors is further regulated by the high affinity IGF binding proteins (IGFBP1 to 6) of which the liver is also a significant source. It has been established that more than 95% of IGF in serum circulate bound to IGFBP-3, which has the highest serum concentration among all IGFBP [10].

In our study, we demonstrated low serum IGF-1 and IGFBP-3 levels in patients with liver cirrhosis compared to healthy controls. Several previous studies reported similar results [3, 5, 11, 12, 13]. Vyzantiadis et al. (2003) [14] and Ottensen et al. (2001) [15] reported in their studies that the decrease of IGF-1 in liver cirrhosis correlated with the severity of liver disease graded according to Child-Pugh classification [16]. Shaarawy et al. (1998) [17] reported in their studies low serum IGFBP-3 levels in patients with early hepatic cirrhosis compared to healthy controls, and patients with more advanced cirrhosis without or with ascites showed more pronounced low levels of serum IGFBP-3 than those of patients with early hepatic cirrhosis. In our study we did not study the difference in serum levels of IGF-1 and IGFBP-3 in different grades of liver cirrhosis due to

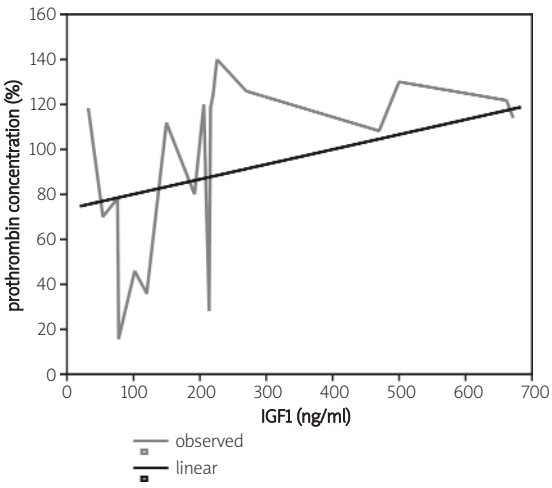

Figure 3. Regression line for the correlation between serum IGF-1 and prothrombin concentration

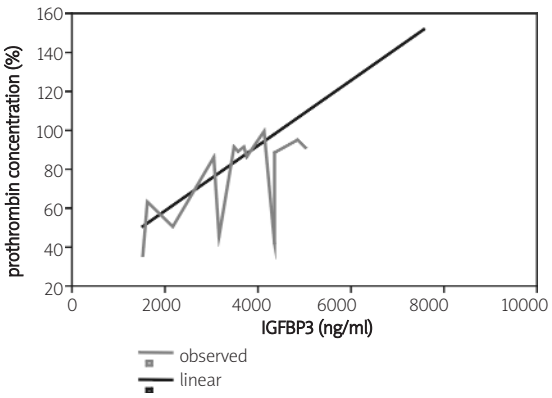

Figure 4. Regression line for the correlation between IGFBP-3 and age of patients

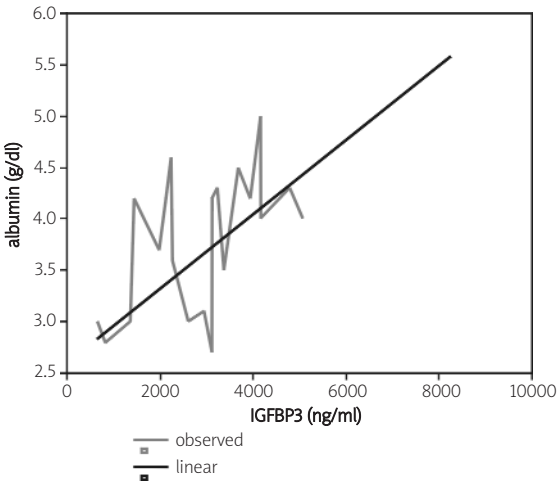

Figure 5. Regression line for the correlation between IGFBP-3 and serum albumin

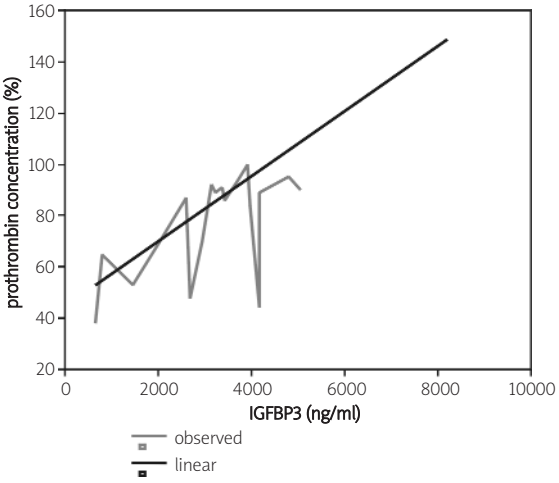

Figure 6. Regression line for the correlation between IGFBP-3 and prothrombin concentration

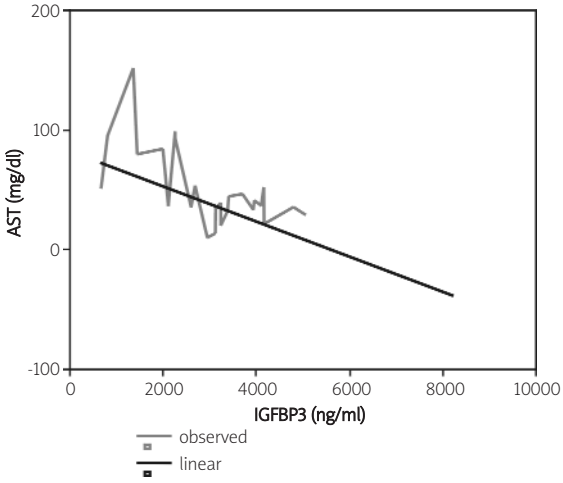

Figure 7. Regression line for the correlation between IGFBP-3 and AST

the smaller number of patients with liver cirrhosis. However, serum IGFBP-3 was lower in patients with liver cirrhosis than in patients with chronic hepatitis, which indicates that it may be a marker for the severity of liver disease, but there was no significant difference in IGF-1 levels between patients with chronic hepatitis and patients with liver cirrhosis.

We studied the correlation of serum levels of IGF-1 and IGFBP-3 with the commonly used liver biochemical tests, and found that IGF-1 and IGFBP-3 correlated negatively with AST and positively with prothrombin concentration, but the correlation with IGFBP-3 is stronger and also it had an additional positive correlation with serum albumin. In agreement with our results, Shaarawy et al. (1998) [17] reported that serum IGFBP-3 level is a good marker for hepatic synthetic capacity as it correlated negatively with transaminases and prothrombin time and positively with serum albumin. They also found that 50% reduction in serum IGFBP-3 levels concords with no percent reduction in serum albumin levels in early liver cirrhosis. They reported that the sensitivity and specificity of the serum IGFBP-3 test in cases of liver cirrhosis was found to be 76% and 100% respectively.

Donaghy et al. (2002) [9], reported that basal IGF-1 and IGFBP-3 levels dropped markedly in liver cirrhosis due to severe GH resistance in these patients, caused by feedback maladjustment of the GH–IGF-1–IGFBP-3 axis. They considered that the fact of impaired IGF-1 and GFBP-3 production and the severity of GH resistance seen in cirrhosis likely reflected the effect of injury to the liver, the central organ of the endocrine GH–IGF-1–IGFBP-3 axis.

The progression of chronic liver disease to liver cirrhosis results in a state of acquired GH resistance with high GH and low IGF-1 and IGFBP-3 levels [5, 18]. In a study done by Assy et al. (1997) [19], IGF-1 and IGFBP-3 were lower in patients with liver cirrhosis before and after stimulation with recombinant human GH. Similar results were also obtained by Scharf et al. (1996) [12].

In our study IGF-1 and IGFBP-3 were correlated negatively with age, in agreement with Wu et al. (2004) [13]. Similar results regarding IGF-1 have been reported in previous studies in a Mediterranean [20], an American [21] and a Northern European population [22]. Although the underlying mechanism remains unknown, it has been suggested that the association between age and IGF-1 could be related to age-induced changes of GH, the main regulator of IGF-1 synthesis and secretion [2].

In agreement with previous studies [20, 21, 22], IGF-1 and IGFBP-3 were not correlated with BMI, which suggests that IGF-1 and IGFBP-3 levels reflect liver dysfunction rather than malnutrition. However, experimental administration of therapeutic doses of GH that increase IGF-1 levels significantly affects body composition. It is possible that therapeutic doses of IGF-1 may have an effect on BMI whereas IGF-1 within the normal range has no effect on BMI [2].

Patients with schistosoma infection showed lower IGFBP-3 levels than patients without schistosoma infection, which may indicate that schistosoma infection had an additional harmful effect on hepatic function.

Conclusions

IGF-1 and IGFBP-3 may be useful parameters for assessment of liver function. IGFBP-3 is an early predictor of hepatic dysfunction as it decreases even

in chronic hepatitis, can be used as a marker for the severity of liver disease and is a more useful parameter than IGF-1 for assessment of hepatic functional reserve as it has a stronger correlation with liver biochemical tests.

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