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Serum Levels of HGF, IGF-I, and IGF-II in Patients with Cirrhosis: Clinical Implications

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ABSTRACT

Objective: The aim of this study was to determine the serum levels of hepatocyte growth factor (HGF), insulin-like growth factor-I (IGF-I) and insulin-like growth factor-II (IGF-II) in patients with cirrhosis, and to investigate whether measurement of these cytokine levels could reflect the severity of cirrhosis assessed by Child-Pugh staging.

Patients and Methods: Sera levels for HGF, IGF-I and IGF-II obtained from 48 patients with cirrhosis, 50 patients with chronic hepatitis, and 14 healthy controls were measured by ELISA assay.

Results: Mean serum HGF level was significantly higher in cirrhotic patients than in patients with chronic hepatitis and healthy controls. In contrast, the mean values for IGF-I and IGF-II in cirrhotic patients were significantly lower than in the other groups. The mean levels for HGF in Child B and C were significantly higher than those in Child A. No significant difference of HGF level was observed between patients with Child B and C. The mean levels for IGF-I and IGF-II in Child C were significantly lower than those in Child B and Child A. There were significant correlation between the levels of HGF, IGF-I or IGF-II and Child-Pugh staging

Conclusion: Serum HGF, IGF-I and IGF-II levels reflect the degree of liver dysfunction, and may be used as complementary prognostic markers to Child-Pugh staging in assessment patients with cirrhosis.

Key words : cirrhosis, HGF, IGF-I, IGF-II, Child-Pugh score

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Cirrhosis, a pathological condition defined by deranged hepatic architecture resulting from progressive fibrosis, represents the final common pathway through which nearly all chronic liver diseases produce morbidity and mortality. These wound-healing processes include production and secretion of chemokines and cytokines, recruitment of inflammatory cells to the injured sites, activation of fibroblasts to produce extracellular matrix (ECM), regeneration of damaged tissue through cell proliferation and differentiation, and, finally, matrix remodeling[1]. The progressive accumulation of ECM distorts liver architecture and, consequently compromises hepatocyte function, causing life-threatening complications such as variceal bleeding, ascites, and progressive liver failure. It has been shown that several growth factors including transforming growth factor-alpha (TGF-α), hepatocyte growth factor (HGF) and insulin-like growth factor-I and II (IGF-I, IGF-II) modulate and play an important role in the hepatic wound-healing process[2].

HGF is a multifunctional polypeptide, originally discovered as a unique cytokine that promotes hepatocyte proliferation and liver regeneration[3,4]. The mature form of HGF is a heparin-binding, heterodimeric glycoprotein consisting of a 69-kDa -chain and a 34-kDa -chain held together by a disulfide bond[5]. In the liver, HGF is produced by nonparenchymal cells, such as Kupffer cells, sinusoidal endothelial cells, and hepatic stellate cells. Extensive studies have demonstrated that HGF regulates diverse cellular processes such as cell survival, proliferation, migration, and differentiation[6]. These biological actions are mediated by a single c-met receptor, the product of a c-met proto-oncogene, which is a member of the receptor tyrosine kinase superfamily. In liver diseases, levels of circulating HGF may vary due to several factors including enhanced production, decreased hepatic clearance, or both because the liver is the major organ through which HGF is eliminated from the circulation. Previous studies have suggested that serum HGF levels may reflect the degree of liver dysfunction in chronic liver diseases[7].

IGF-I, IGF-II, two major forms of insulin-like growth factors (IGFs) family, are single-chain molecules with three intrachain disulfide bridges consisting of 70 and 67 amino acid residues respectively[8]. These growth factors are considered as essential anabolic hormones, which regulate DNA synthesis, cell proliferation and meiotic division[9,10]. Hepatic IGF-I production is principally regulated by growth hormone (GH), whereas the synthesis of IGF-II is relatively GH independent[11]. The availability of IGF-I and IGF-II to their tissue receptors is regulated by the high affinity specific binding proteins (IGFBPs) of which the liver is an important source. Since most circulating IGF-I and IGF-II are synthesized by hepatocytes, lower levels of these polypeptides are found in patients with chronic liver diseases, and may reflect the severity of liver dysfunction[12,13].

The aim of this study was to determine circulating HGF, IGF-I and IGF-II levels in patients with cirrhosis and compared to those with chronic hepatitis and healthy individuals. We also investigated whether measurement of these cytokine levels could reflect the severity of cirrhosis assessed by Child-Pugh grading.

Materials and Methods

Patients

Sera for HGF, IGF-I and IGF-II level measurements were obtained from 3 groups of subjects attending King Chulalongkorn Memorial Hospital (Bangkok, Thailand) between January 2003 and December 2005. The samples were collected from each subject at the time of their clinical evaluation and stored at -70 ˚C until tested. The study was approved by the Ethical Committee of the Faculty of Medicine, Chulalongkorn University. Informed consent was obtained according to the regulations of the committee.

Group 1 consisted of 48 patients with cirrhosis. There were 34 males and 14 females, with the mean age of 46.2 ± 11.9 years. Cirrhosis was diagnosed based on histological examinations and/or imaging studies. The severity of cirrhosis was classified according to Child-Pugh grading. In this study, there were 23 (47.9%), 15 (31.3%), and 10 (20.8%) patients in Child A, B, and C grading, respectively.

Group 2 consisted of 50 patients with chronic hepatitis. There were 33 males and 17 females, with the mean age of 34.1 ± 9.3 years. The diagnosis of this group was based on persistent elevation of serum alanine aminotransferase (ALT) level, and confirmed by histological examinations.

Group 3 consisted of 14 adult healthy individuals. There were 8 males and 6 females, with the mean age of 31.4 ± 9.4 years.

Measurement of serum HGF, IGF-I and IGF-II levels

Serum HGF was determined by an ELISA kit (IMMUNIS HGF EIA, Institute of Immunology Co., Ltd., Japan). Serum IGF-I and IGF-II were determined by ELISA kits (IGF-I; R & D systems, Inc., Minneapolis, MN, and IGF-II non-extraction DSL-2600, Diagnostic system Lab, Webster, Tex). The determinations of these growth factors were performed according to the manufacturer' instructions.

Statistical analysis

The data were expressed as mean values ± standard deviation. Comparisons between groups were analyzed by a two-tail ANOVA analysis. Spearman correlation coefficients were used to determine the correlation between the levels of HGF, IGF-I, IGF-II and Child-Pugh score. P values below 0.05 were considered statistically significant. All statistical analyses were performed using the SPSS software for windows 10.0 (SPSS Inc., Chicago, IL).

RESULTS

Serum HGF, IGF-I and IGF-II and in patients with cirrhosis, chronic liver diseases and healthy controls

The average level of serum HGF in patients with cirrhosis (1.8 ± 1.5 ng/ml) was significantly higher than those of patients with chronic hepatitis (0.7 ± 0.7 ng/ml) and healthy controls (0.3 ± 0.2 ng/ml) (P <0.001). Similarly, the mean serum HGF level in patients with chronic hepatitis was also significantly higher than that of the control group (P = 0.036).

Conversely, the mean values for IGF-I and IGF-II in cirrhotic patients (127.3 ± 57.1 and 971.4 ± 392.5 ng/ml, respectively) were significantly lower than those in patients with chronic hepatitis (168.3 ± 64.2 and 1306.6 ± 444.8 ng/ml, respectively, P = 0.001) and healthy subjects (219.3 ± 77.9 and 1635.1 ± 399.7 ng/ml, respectively, P <0.001). The mean serum IGF-I and IGF-II levels in patients with chronic hepatitis were also significantly lower than those of healthy controls (P = 0.037 and P = 0.014, respectively). (Table 1)

Serum HGF, IGF-I and IGF-II levels in cirrhotic patients with different Child-Pugh scores

The mean values for HGF, IGF-I and IGF-II were 0.9 ± 0.7, 165.0 ± 57.0 and 1218.4 ± 371.1 ng/ml, respectively, in patients with Child A; 2.2 ± 1.5, 107.6 ± 24.9 and 858.2 ± 234.2 ng/ml, respectively, in Child B. The mean values for HGF, IGF-I and IGF-II in patients with Child C were 3.2 ± 1.6, 70.3 ± 16.1 and 573.0 ± 170.0 ng/ml, respectively. Significant difference of these three parameters was found between control group and any stage of cirrhosis (P <0.001). Statistic analysis also showed that the mean levels for HGF in Child B and C were significantly higher than those in Child A (P = 0.007 and P = 0.001, respectively). However, no significant difference of HGF level was observed between patients with Child B and C (P = 0.124). In contrast, the mean levels for IGF-I and IGF-II in Child C were significantly lower than those in Child B (P <0.001 and P = 0.002, respectively) and Child A (P < 0.001). The mean levels for IGF-I and IGF-II in Child B were also significantly lower than those in Child A (P < 0.001, and P = 0.001, respectively) (Figure 1A-1C).

Correlation of serum HGF, IGF-I and IGF-II levels with Child-Pugh score

In cirrhotic patients, the correlation between the levels of HGF, IGF-I or IGF-II and Child-Pugh staging were observed (r = 0.618, P <0.001; r = -0.675, P <0.001; and r = -0.662, P <0.001, respectively). Also, there were negative correlation between serum HGF level and serum IGF-I or IGF-II levels (r = -0.367, P <0.001; and r = -0.490, P <0.001). In addition, the correlation between the level of IGF-I and IGF-II was observed (r = 0.708, P <0.001).

Table 1 Serum concentrations of HGF, IGF-I and IGF-II in patients with cirrhosis, chronic hepatitis and healthy subjects

Group	n	HGF (ng/ml)	IGF-I (ng/ml)	IGF-II (ng/ml)
Cirrhosis	48	1.8 ± 1.5	127.3 ± 57.1	971.4 ± 392.5
Chronic hepatitis	50	0.7 ± 0.7	168.3 ± 64.2	1306.6 ± 444.8
Controls	14	0.3 ± 0.2	219.3 ± 77.9	1635.1 ± 399.7

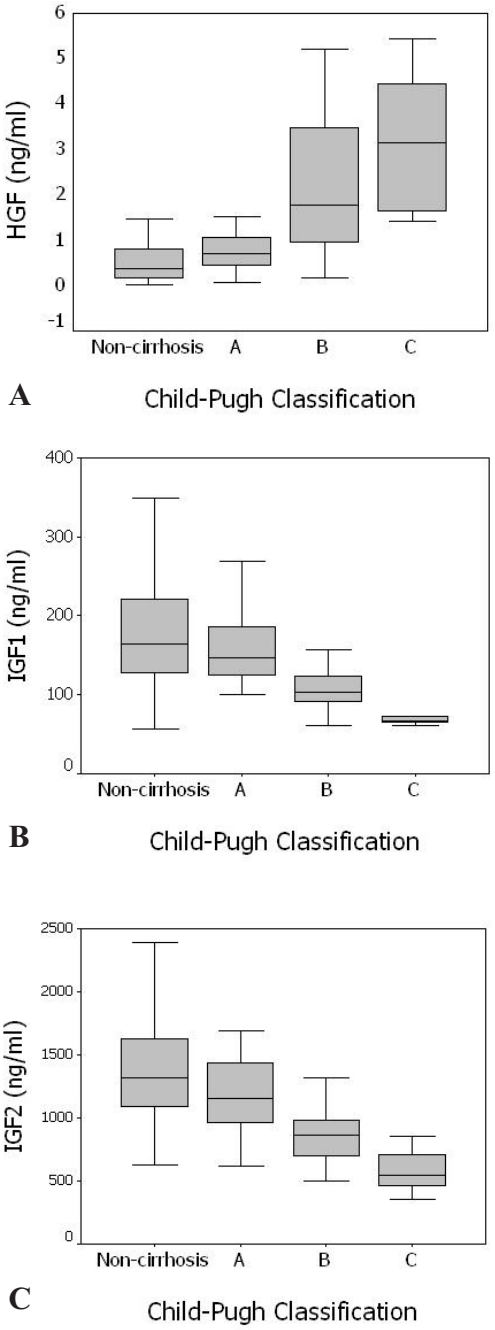

Figure 1 Serum concentrations in patients with cirrhosis according to Child-Pugh grading A) HGF B) IGF-I C) IGF-II

DISCUSSION

Among several growth factors, HGF, IGF-I and IGF-II are implicated in hepatic wound healing process. HGF is well known as pleiotropic substance with mitogenic, motogenic, morphogenic, and tumor suppressor activities[3,6]. HGF achieves its effect on epithelial cells primarily in a paracrine fashion[14]. The role of HGF, especially in liver diseases, has been widely investigated, including its relation to regeneration, antifibrosis, cytoprotection, and differentiation. Accumulated evidence has indicated that levels of circulating HGF correlate with the prognoses of various liver diseases. For instance, among patients with acute hepatitis, increased levels of HGF were observed in non-survivors with fulminant hepatic failure[15]. A correlation between serum HGF levels and survival of patients with alcoholic hepatitis was also reported[16]. Previous data have shown that serum HGF levels of patients with chronic hepatitis, cirrhosis, or hepatocellular carcinoma (HCC) are significantly higher than in controls. Recently, we have demonstrated an association between serum HGF and patients'survival in untreated HCC[17]. Thus, in agreement with previous data, this study confirmed a strong positive correlation between serum HGF level and the degree of liver dysfunction assessed by Child-Pugh staging.

The mechanism by which cirrhosis induces the elevation of circulating HGF has remained unclear[4]. Nonetheless, it could be speculated that the induction of serum HGF may probably due to the increased production of this cytokine from distant organs, such as the lung and kidney. Also, with the progression of liver damage, clearance of HGF in the liver progressively diminishes. Moreover, as the molecule of HGF is processed from a biologically inactive single-chain precursor into the two-chain active form in the liver, levels of active HGF may be significantly disturbed in the damaged liver, and a single-chain precursor may become a major form in the serum. As a result, in patients with fulminant hepatitis or decompensated cirrhosis, extremely high serum levels of HGF were detected. In such cases, the majority of HGF may exist in the single-chain precursor instead of the active form.

The IGF system exhibits a fundamental role in the regulation of cellular proliferation, differentiation, and apoptosis. Thus, disruptions in the balance of IGF system components may lead to excessive proliferation and survival signals[8]. Since the liver is the major site of IGF-I/II synthesis and metabolism, it is anticipated that chronic liver disease might alter the circulating IGF-I/II levels[12,18]. In consistent with previous data, our results confirmed that serum IGF-I and IGF-II levels were significantly lower in patients with cirrhosis than those with chronic hepatitis and healthy individuals. Moreover, circulating concentrations of IGF-I and IGF-II were gradually decreased and correlated well with the degree of liver dysfunction. These

lines of evidence indicate that impaired hepatic IGF-I and IGF-II levels may be the potential indicators for evaluation of liver dysfunction and clinical outcome of the patients.

Our data showed that serum IGF-I level was highly correlated with serum IGF-II concentration. Moreover, both IGF-I and IGF-II levels were equally correlated with Child-Pugh scores. This observation, however, was not in agreement with previous data that serum IGF-II was more sensitive and effective than serum IGF-I in terms of predicting liver dysfunction and clinical prognosis[12,13]. For example, previous data showed that serum IGF-II responded comparably lower in patients with Child C than with Child B, and the range of serum IGF-II concentrations was much more clearly delineated from normal to excessively low in patients with severe dysfunction than the case for serum IGF-I[12,13]. Moreover, specific IGF-II mRNA was mainly found in hepatocytes, which indicated the impaired serum IGF-II production was the direct effect of decreased liver function. In contrast, baseline IGF-I level was also decreased under other circumstances besides liver dysfunction, such as low serum albumin, malnutrition and glucose metabolic abnormality, most of which are the complications of advanced cirrhosis[19,20].

In conclusion, our study demonstrated that serum HGF level increased in cirrhotic patients, and raised its serum level was closely associated with disease progression. Conversely, serum levels of IGF-I and IGF-II were significantly lower in cirrhotic patients, particularly those who had advanced liver dysfunction. Signal cooperation between these cytokine pathways may be an important factor in the progression of cirrhosis. Thus, assessment of these cytokine levels may improve the prognostic prediction in cirrhotic patients than using Child-Pugh score alone.

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