Association of Circulating Insulin-Like Growth Factor 1 With Hepatocellular Carcinoma: One Cross-Sectional Correlation Study

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Deregulation of insulin-like growth factor-1 (IGF-1) has been implicated in the pathogenesis of several malignancies. This study aimed to investigate the association of changes in circulating IGF-1 with hepatocellular carcinoma (HCC). The radioimmunoassay was used to analyze serum IGF-1 levels of 65 HCC patients and 165 healthy subjects. Serum IGF-1 levels were significantly decreased in the HCC patients as compared with the healthy subjects (158.46 ± 105.07 vs. 247.63 ± 149.96 ng/mL, $P < 0.001$). Furthermore, insulin resistance was significantly higher in the HCC patients than the healthy subjects ($P = 0.027$). In addition, the significant correlations of serum IGF-1 levels with

age and insulin resistance in the healthy subjects were not noted in the HCC patients. Intriguingly, individuals with hepatitis C virus (HCV), not hepatitis B virus, had remarkably decreased IGF-1 levels in both groups of the HCC patients and healthy subjects. Moreover, in the HCV subgroup, serum IGF-1 levels were significantly reduced in the HCC patients than the healthy subjects (113.14 ± 71.28 vs. 172.42 ± 74.02 ng/mL, $P = 0.003$). In conclusion, decreased serum IGF-1 levels were associated with HCC and the decrease was remarkably noted in those patients concomitant with chronic hepatitis C. *J. Clin. Lab. Anal.* 24:195–200, 2010. © 2009 Wiley-Liss, Inc.

Key words: IGF-1; hepatocellular carcinoma; hepatitis C virus; hepatitis B virus; insulin resistance

INTRODUCTION

Insulin-like growth factor-1 (IGF-1) is a single chain peptide that has structural homology with pro-insulin (1). Although IGF-1 is synthesized widely, the majority of circulating IGF-1 is derived from the liver (2–3). Its synthesis is influenced by several factors (e.g., growth hormone and nutritional intake) (4,5). In addition to the metabolic effects (e.g., glucose homeostasis), IGF-1 has profound effects on the regulation of proliferation, survival, differentiation, and transformation of many cell types (6–9). Thus, a growing body of literature has been exploring the potential role of

circulating IGF-1 in a variety of diseases in an autocrine/paracrine manner (10–14). As we have known, the potential to develop cancers is influenced by a combination of genetic and environmental factors. There is increasing evidence that the inappropriate expression of IGF-1, which has appeared to contribute

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Received 11 July 2008; Accepted 1 April 2009

DOI 10.1002/jcla.20320

Published online in Wiley InterScience (www.interscience.wiley.com).

to progression and behaviors of cancers, might provide a link between these factors and the development of cancers.

Hepatocellular carcinoma (HCC), primarily originating from the liver, is the fifth most common malignancy in the world and estimated to cause approximately half a million deaths annually (15). The established major risk factors for HCC include hepatitis B virus (HBV), hepatitis C virus (HCV), diabetes and so on (16–18). Although many studies have mentioned the involvement of deregulated circulating IGF-1 in several cancers, its possible association with HCC is seldom presented so far (19). Furthermore, although the liver is the main source of IGF-1, it needs to be specified if the same effects by which IGF-1 exerts on other cancers may also happen in HCC. In addition, the possible interplay between IGF-1 and those risk factors of HCC is mostly undetermined.

In this study, the authors evaluated the levels of serum IGF-1 of the HCC patients, as compared with those of the healthy subjects, and further correlated the results with other clinical characteristics as well as risk factors (HBV, HCV, and insulin resistance). This study provides some important information regarding the possible association of circulating IGF-1 with HCC.

METHODS

Subjects

One hundred and twelve HCC patients were prospectively recruited from the Surgical Department of Kaohsiung Medical University Hospital between November 2005 and October 2006. Patients, who had undergone hepatectomy, trans-arterial chemoembolization (TACE), radiofrequency ablation or alcohol injection, were excluded in this study. A total of 65 naïve patients, including 47 males (72.3%) and 18 females (27.7%), entered this study. The average age of the HCC group was 58.83 ± 12.93 years (mean $\pm$ standard deviation (SD)), ranging from 20 to 83 years. Among them, 51 patients were admitted for surgical resection and the other 14 patients for TACE. All were established with HCC through surgical pathology or a fine needle aspiration biopsy.

Healthy participants admitted for an annular medical checkup at the Center of Health Examination in Kaohsiung Medical University Hospital were enrolled during the same period. Notably, only the healthy participant without evidence of HCC, based on normal AFP level and negative ultrasound result, was eligible. A total of 165 healthy subjects, including 112 males (67.9%) and 53 females (32.1%), were randomly selected from these eligible participants. The average age of the healthy subject group was 47.67 ± 10.81 years, ranging from 24 to 81 years. Informed consent was

obtained from each individual and the protocol was approved by the Institute Review Board of the Kaohsiung Medical University Hospital.

Blood Sampling and Biochemical Assays

Fasting blood samples of 65 HCC patients and 165 healthy subjects were taken in the morning before surgery and fine needle aspiration as well as medical checkups, and the serum was immediately separated by centrifugation and stored at -20°C until further analysis. Serum levels of IGF-1 and insulin were measured by radioimmunoassay (RIA) in the Department of Nuclear Medicine of the Kaohsiung Medical University Hospital, using commercially available human RIA kits of IGF-1 (Adaltis Italia, Casalecchio di Reno, Italy) and insulin (Diagnostic Products Corporation, Los Angeles, CA). All samples were run in triplicate, and the three measurements were averaged for statistical analysis. The sensitivities of the IGF-1 and insulin were 2.06 ng/mL and 1.2 mU/L. The interassay and intraassay coefficients of variation were <7.4 and $<7.0\%$ for IGF-1, and <10.0 and 7.3% for insulin. Serum levels of fasting glucose, total cholesterol, triglyceride, AST, ALT, alkaline phosphatase, and bilirubin were determined according to standard laboratory procedures in the Department of Clinical Biochemistry of the Kaohsiung Medical University Hospital.

Hepatitis Virus Markers and Determination of Insulin Resistance

The patients with chronic hepatitis B were seropositive for hepatitis B surface antigen (HBsAg) for more than 6 months. HBsAg was analyzed by RIA (General Biological Cooperation, Sinchu, Taiwan). The presence of anti-HCV was tested by using third generation enzyme-linked immunosorbent assay kits (Abbott, North Chicago, IL). Insulin resistance index was determined using homeostasis model assessment ($\text{HOMA-IR} = \text{fasting insulin (mU/L)} \times \text{fasting glucose (mg/dL)} \times 0.05551/22.5$).

Statistical Analysis

All the statistical analyses were performed by the SPSS software package for Windows; version 10.0. Values were presented as mean $\pm$ SD. Univariate analyses between HCC patients and healthy subjects were performed using two-tailed *Student's t test* and χ^2 test, and confirmed by *Fisher's exact tests*. Correlation of serum IGF-1 levels with clinical and biochemical characteristics in HCC patients and healthy subjects were analyzed by *Spearman's ρ coefficient test*. $P < 0.05$ was regarded as statistically significant.

RESULTS

The clinical and biochemical characteristics of 65 HCC patients and 165 healthy subjects are shown in Table 1. No significant difference was noted between the two groups in gender, BMI, and serum levels of triglyceride, ALT as well as AP. The positive rate of viral hepatitis, average age, and serum levels of AST and total bilirubin were significantly higher in the HCC patients than in the healthy subjects (Table 1). Furthermore, the mean value of HOMA-IR was significantly increased in the HCC patients in comparison with the healthy subjects (5.35 ± 8.82 vs. 2.53 ± 6.90 , $P = 0.027$) (Table 1). In contrast, in addition to serum cholesterol levels, serum IGF-1 levels were significantly decreased in the HCC patients as compared with the healthy subjects (158.46 ± 105.07 vs. 247.63 ± 149.96 ng/mL, $P < 0.001$) (Table 1).

The correlations of serum IGF-1 levels with the clinical and biochemical characteristics between these two groups are shown in Table 2. In the healthy subjects, serum IGF-1 levels were positively correlated with HOMA-IR and serum AP levels ($r = 0.224$, $P = 0.005$; $r = 0.223$, $P = 0.014$, respectively), but were inversely correlated with age and serum AST levels ($r = -0.358$, $P < 0.001$; $r = -0.383$, $P < 0.001$, respectively) (Table 2). However, in the HCC patients, the significant correlation was only noted between serum IGF-1 and AP levels ($r = -0.267$, $P = 0.047$) (Table 2). The significant correlation of serum IGF-1 with age and HOMA-IR found in the healthy subjects did not exist in the HCC patients.

All the subjects of these two groups were further divided into age ≥ 55 years and < 55 years, HOMA-IR ≥ 2.53 and < 2.53 , and two hepatitis virus types. The average IGF-1 levels of those subgroups were shown in

Table 3. Serum IGF-1 levels of the HCC patients were significantly lower than those of the healthy subjects in the age < 55 years and HOMA-IR < 2.53 subgroups (176.01 ± 123.74 vs. 273.00 ± 153.39 ng/mL, $P = 0.003$; 136.15 ± 80.39 vs. 246.77 ± 154.50 ng/mL, $P < 0.001$, respectively) (Table 3). Intriguingly, as to viral hepatitis type, it was noticed that individuals with HCV, not HBV, had remarkably decreased IGF-1 levels in both groups of the HCC patients and healthy subjects. In addition, serum IGF-1 levels were significantly reduced in the HCC group than in the healthy group in both subgroups of HBV and HCV (180.14 ± 105.10 vs. 263.08 ± 164.33 ng/mL, $P = 0.02$; 113.14 ± 71.28 vs. 172.42 ± 74.02 ng/mL, $P = 0.003$, respectively) (Table 3).

TABLE 2. Correlation of Circulating IGF-1 Levels With Clinical and Biochemical Characteristics in HCC Patients and Healthy Subjects

Variable	HCC patients		Healthy subjects	
	r^a	P^a	r^a	P^a
Age	-0.183	0.144	-0.358	<0.001
BMI	0.221	0.077	-0.146	0.085
HOMA-IR	0.210	0.102	0.224	0.005
Cholesterol	0.010	0.937	0.031	0.712
Triglycerides	0.075	0.559	0.063	0.453
AST	-0.191	0.127	-0.383	<0.001
ALT	0.120	0.342	-0.245	0.002
AP	-0.267	0.047	0.223	0.014
Bilirubin	-0.118	0.348	-0.095	0.284

^aSpearman's ρ coefficient test.

HCC, hepatocellular carcinoma; BMI, body mass index; HOMA-IR, Homeostasis model of assessment—insulin resistance; AST, aspartate aminotransferase; ALT, alanine aminotransferase; AP, alkaline phosphatase.

TABLE 1. Clinical and Biochemical Characteristics of HCC Patients and Healthy Subjects

	HCC patients ($n = 65$)	Healthy subjects ($n = 165$)	P -values
Gender (M/F)	47/18	112/53	NS
BMI (kg/m^2)	24.72 ± 3.75	24.36 ± 3.43	NS
Age (years)	58.83 ± 12.93	47.67 ± 10.81	<0.001
Viral hepatitis (P/N)	59/6	69/96	<0.001
HOMA-IR	5.35 ± 8.82	2.53 ± 6.90	0.027
Cholesterol (mg/dL)	171.71 ± 38.97	185.79 ± 35.24	0.011
Triglycerides (mg/dL)	110.14 ± 80.65	117.06 ± 70.43	NS
AST (IU/L)	58.25 ± 38.71	35.32 ± 45.97	<0.001
ALT (IU/L)	60.42 ± 45.87	50.29 ± 77.21	NS
AP (mg/dL)	101.41 ± 83.34	112.13 ± 41.87	NS
Bilirubin (mg/dL)	1.00 ± 0.52	0.79 ± 0.33	0.005
IGF-1 (ng/mL)	158.46 ± 105.07	247.63 ± 149.96	<0.001

Values are presented as mean $\pm$ SD. HCC, hepatocellular carcinoma; M, male; F, female; BMI, body mass index; P/N, positive/negative; IGF-1, insulin-like growth factor-1; HOMA-IR, Homeostasis model of assessment—insulin resistance; AST, aspartate aminotransferase; ALT, alanine aminotransferase; AP, alkaline phosphatase; NS, no significance.

TABLE 3. Serum IGF-1 Levels (ng/mL) in HCC Patients and Healthy Subjects Categorized by Age, HOMA-IR and Hepatitis Virus Type

	HCC patients (n)	Healthy subjects (n)	P-values
Age			
≥ 55 yrs	150.09 ± 95.33 (44)	165.66 ± 103.08 (39)	NS
< 55 yrs	176.01 ± 123.74 (21)	273.00 ± 153.39 (126)	0.003
HOMA-IR			
≥ 2.53	199.21 ± 131.86 (23)	251.66 ± 128.86 (29)	NS
< 2.53	136.15 ± 80.39 (42)	246.77 ± 154.50 (136)	< 0.001
Virus type			
None	184.48 ± 172.82 (6)	278.07 ± 160.93 (96)	NS
Hepatitis B	180.14 ± 105.10 (35)	263.08 ± 164.33 (25)	0.020
Hepatitis C	113.14 ± 71.28 (21)	172.42 ± 74.02 (44)	0.003

HCC, hepatocellular carcinoma; HOMA-IR, Homeostasis model of assessment—insulin resistance; NS, no significance.

DISCUSSION

IGF-1, one circulating peptide hormone, has been implicated in several oncogenic processes. High serum IGF-1 levels are associated with an increased risk for breast, prostate, colorectal, pancreatic, and lung cancer (20–23). The hyperproliferative state of IGF-1 is in keeping with its known mitogenic and anti-apoptotic effects (24,25). Intriguingly, this study showed that serum IGF-1 levels were significantly decreased in the patients with HCC, when compared with the healthy subjects. This interesting result was in accordance with other previous studies (26–28). However, in comparison with other cancers, little is known about the reasons that could lead to this decrease in HCC.

Previous studies have pointed out the fact that there is a physiological decline of circulating IGF-1 with ageing (29). In this study, we found that serum IGF-1 levels were inversely correlated with age in the healthy subjects, but this correlation was not noted in the HCC patients (Table 1). The significant decrease of serum IGF-1 levels with HCC was still noted in the younger subgroup (age < 55 years), but not in the older subgroup (age ≥ 55 years), implied that age might not be the main cause resulting in the significant decrease of serum IGF-1 levels in HCC.

Davila et al. suggest that diabetes is an independent risk factor for HCC (30). This study also found that serum glucose and insulin levels were significantly higher in the HCC patients as compared with the healthy subjects in our study (data not shown). Moreover, the HCC group had significantly increased HOMA-IR than the healthy subject group (Table 1). Therefore, it is rational to speculate whether there is some association between decreased concentrations of serum IGF-1 and increased insulin resistance in HCC. Data from Clemmons et al. demonstrate that IGF-1 is necessary for

normal insulin sensitivity and impairment of IGF-1 synthesis results in a worsening state of insulin resistance (31). Furthermore, studies have shown that animals will become insulin-resistant when IGF-1 synthesis by the liver is deleted and this is improved after IGF-1 administration (32). In addition, recent data indicate that IGF-1 synthesis is disturbed in liver cirrhosis and reflects the severity of the clinical stage (33). In fact, the majority of our HCC patients were diagnosed with liver cirrhosis by their pathology in this study. Taken together, it was suggested that impaired liver function caused by liver cirrhosis in the HCC patients might result in the decrease of IGF-1 production by the liver and the subsequent increase of insulin resistance.

HCC has ranked the first among all the cancers that cause the death of Taiwanese in the past decade. The main causes of HCC in Taiwan are chronic hepatitis B and C infections, with >90% of patients positive for HBsAg or antibody to hepatitis C virus (anti-HCV) (34,35). After categorized by the hepatitis virus type, this study showed that the decrease of serum IGF-1 levels in the HCC patients compared with the healthy subjects reached a significant difference in both of the HBV and HCV subgroups (Table 3). Therefore, in addition to the oncogenic roles of HBV and HCV, the possible interplay between circulating IGF-1 and these two viruses may take part in the development of HCC (36,37).

In accordance with Mahdy et al., this study found that serum IGF-1 levels of the HBV subgroups were lower than those of the subgroups without any viral infection, regardless of the HCC patients and healthy subjects (Table 3) (38). However, both decreases did not reach the significant difference in its respective group. In fact, previous study suggested that only IGF-II was involved in the altered autocrine mechanisms of cell growth control, which were clearly implicated in HBV-associated HCC (39). In contrast, the role of IGF-1 in HBV-associated HCC is presently not clear.

On the other hand, the individuals with HCV, compared with those with no viral hepatitis, had remarkably decreased IGF-1 levels in both groups of the HCC patients and healthy subjects. Furthermore, in the HCV subgroup, serum IGF-1 levels continued to decrease significantly after HCC development (Table 3). This finding is compatible with the result from Mazziotti et al., who demonstrate that the development of HCC is accompanied by a significant reduction of serum IGF-I levels in the patients with HCV-related liver disease (40). Taken together, in spite of the fact that chronic hepatitis C will decrease circulating IGF-1 by itself, there indeed exists some unknown relationship between decreased IGF-1 and

HCC development. It should deserve further studies whether a decrease in circulating IGF-1 will contribute to HCC.

In conclusion, this study demonstrated that serum IGF-1 concentrations were significantly decreased in the HCC patients. Moreover, age-related change and increased insulin resistance might not contribute to it. Further efforts are warranted to explore the possible interplay of circulating IGF-1 and HCV in the development of HCC.

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