RESEARCH ARTICLE

Predictive Value of Serum Insulin-like Growth Factor-1 in Hepatocellular Carcinoma

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Abstract

Background: Hepatocellular carcinoma (HCC) is the commonest primary malignant cancer of the liver in the world. Insulin-like growth factor-1 (IGF-1) levels reflect hepatic function and are inversely correlated with the severity of background chronic liver disease. Objective: This study evaluated whether basal serum IGF-1 levels can predict prognosis of HCC patients according to different risks of disease progression. Materials and Methods: A total of 89 patients with hepatocellular carcinoma (HCC) were recruited in 3 groups: Group I, 30 HCC patients receiving sorafinib; Group II, 30 HCC patients with best supportive care; and Group III include 29 patients undergoing transcatheter arterial chemoembolization (TACE). All patients were investigated for serum levels of AST, ALP, Bb, Cr, BUN, AFP and IGF-I. Results: Patients with disease control had significantly higher baseline IGF-1 levels 210 (185-232.5) ng/mL (p value<0.01) than did patients without disease control. Low basal IGF-1 levels were associated with advanced HCC, such as multiple tumors and advanced stage, and low IGF-1 levels predicted shorter TTP and overall survival in patients treated with TACE. Conclusions: The levels of serum IGF-1, expressed as continuous values, may be helpful for accurately assessing hepatic function and the prognostic stratification of patients with HCC.

Keywords: Hepatocellular carcinoma - IGF-1 - prognosis - serum factors

Introduction

Hepatocellular carcinoma (HCC) is the most common liver malignancy and rates fifth in incidence and third in mortality in the world. In Egypt, the incidence rate of HCC was doubled in the past 10 years (Abdelaziz et al., 2014). Multiple risk factors are associated with HCC disease etiology, with the highest incidence in patients with chronic hepatitis B virus (HBV) and hepatitis C virus (HCV) (Su et al., 2013). Hepatocellular carcinoma (HCC) is the most common form of liver cancer, usually triggered by chronic inflammation and continuous liver injury (Zhou et al., 2012). It is a highly vascular tumor characterized by fast infiltrating growth, early metastasis, high-grade malignancy and poor therapeutic efficacy.

The insulin-like growth factor (IGF) pathway has highly conserved function in mammals and plays a critical role in energy metabolism and cell renewal in response to nutrients (Steiner et al., 1985; De, 2004; Shaw et al., 2005; Dong et al., 2007; Pollak, 2008; Toyoshima et al., 2008). IGF pathway is not only involved in cell growth in tissue culture (Jones and Clemmons, 1995; Pollak et al., 2004), but it also promotes cell proliferation, migration and transformation into malignant clone (Khandwala et al., 2000; Pollak et al., 2004).

Insulin-like growth factor 1 (IGF-1), also called somatomedin C, is a protein that in humans is encoded by the IGF1 gene IGF-1 has also been referred to as a sulfation factor (Jansen et al., 1983; Hoppener et al., 1985). IGF-1 is produced primarily by the liver as an endocrine hormone as well as in target tissues in a paracrine/autocrine fashion (Miura et al., 1992). IGF-1) is a potent survival factor and implicated in the development and progression of various cancers (Maki, 2010). It has been shown to synergize with tissue hypoxia to enhance tumor growth and metastasis (Catrina et al., 2006). However, the relevance IGF-1 system to HCC is somewhat different from other malignancies. Because the majority of circulating IGF-1 is produced by the liver, IGF-1 levels reflect hepatic function and are inversely correlated with the severity of background chronic liver disease (Lorenzo-Zuniga et al., 2007).

Many studies have shown that a decrease in serum IGF-1 levels is associated with the development of HCC, regardless of the grade of hepatic dysfunction. So, the aim of present study was evaluated whether basal serum IGF-1 levels can predict prognosis of HCC patients according to different risks of disease progression.

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Materials and Methods

The study included 89 patients with hepatocellular carcinoma (HCC) attending the Clinical Oncology, Internal Medicine and Tropical Departments, Tanta University Hospitals as well as Alexandria clinical oncology department, Alexandria University. were recruited into 3 groups: Group I, 30 HCC patients received chemotherapy, Group II, 30 HCC patients with best supportive care, Group III include 29 patients underwent TACE.

All the patients were subjected to full history taking, through clinical examinations and receiving radiotherapy in Oncology department, Faculty of Medicine, Tanta University, Egypt. Laboratory assessment including aspartate aminotransferase (AST), alkaline phosphatase (ALP), bilirubin (Bb), albumin, prothrombin time (PT), creatinine (Cr), urea (BUN), alpha fetoprotein (AFP) and IGF-1 were done in Clinical Pathology Department, Faculty of Medicine, Tanta University, Egypt. Patients with HCC were diagnosed by triphasic CT with or without elevated AFP levels.

For first group whom received sorafinib (Tyrosine Kinase Inhibitor)as 400 mg PO q12hr, taken 1hr before or 2hr after meals

Samples were collected in the fasting state in the morning. Sample collection, processing and storage were done according to the instructions of the reference laboratory and the kits.

All samples is collected just before treatment, 3 months then 6 months interval.

Exclusion criteria: Any patient with diabetes mellitus, cardiac insufficiency, respiratory disorder, renal dysfunction, hepatic encephalopathy or spontaneous bacterial peritonitis was excluded. None of the control group was suffering from any other medical conditions. A written consent prior to participation in the study was taken from all patients and controls.

Laboratory investigations (Ng et al., 1998; Sanai et al., 2010).

Five ml fasting cubital vein blood sample of all patients were taken and 2 ml of blood will be collected in a plastic citrate vacutainers including 3.2% sodium citrate concentration to assess the prothrombin time. The residual volume of blood samples were collected in a sterile tube and centrifuged at 3000 rpm for 15 minutes for separating the serum to assess AST, ALP, Bb, Cr and BUN and residual serum were stored at -20°C for measuring AFP and IGF-I by using enzyme-linked immunosorbent sandwich assay (ELISA) following the manufacturer's guidance of Quantikine, R&D Systems China Co. Ltd. Kit.

Survival analysis

In this section we review the most commonly used survival analysis techniques for estimating distributions of lifetimes and the association between lifetimes and explanatory covariates. In classical survival analysis, interest focuses on the time to an event, most commonly a failure of some sort. Examples include time to death, treatment, failure or relapse Often it is not possible to observe all failures in the sample being studied, especially if the study terminates after a fixed follow-up period

The Kaplan-Meier curve

The Kaplan-Meier estimator KM t estimates the probability that the time-to Event or time to failure T exceeds any given value t Kaplan and Meier, 1958. It is typically plotted as a function of t over the range of times of interest and is decreasing curve with value 1 at time zero and other values given by:

$$KM(t) = \prod_{i:s<t}(1 - r_{si})$$

Where are the observed failure times and r is the estimated hazard or risk of failure at time s, among all individuals at risk of failure at time s.

it is clear that underestimating hazards will inflate the Kaplan-Meier curve and lead to overestimation of survival. The reverse will occur if low-risk individuals tend to be censored.

Cox regression

The Cox model is a description of the dependence of the risk of failure at anytime t on the covariates X. It is semi parametric in that no assumptions are made about how the hazard rates vary with time; however, the hazards for different covariate values are assumed to be proportional with a ratio that is constant over time.

Results

Baseline patient characteristics and treatment outcomes

The baseline characteristics of the study population are summarized in Table 1. Of the 89 patients, 74 (83.1%) were male. The median age at the time of diagnosis was 51 years (IQR, 48-58 years 76.8%). Only 10 (11.2%) patients had preserved Child-Pugh class (A) liver function and expressed high pre therapy IGFI level 185 (157-202) ng/ml. During a median follow-up period of 8 months; there were no complete responses, but 10(11.2%) patients had partial responses to treatment (Table 1). Another 15(16.9%) patients had stable disease, 64 patients (71.9%) experienced disease progression. The median time-to-progression (TTP) is 4 months 95% confidence interval (CI) (2-9.5). The overall cumulative death rate was 18.6% after 6months, 49.1% after 7months, and 71.1% after9 months and 94.9% after 10 months. The median OS was not reached (29 of 89 patients died (32.5% from all patients and 49.1 % from death rate).

The levels of serum IGF-1 according to clinical characteristics

The associations between clinical factors and the levels of pre therapy IGF-1 are described in Table 1. Patients with Child-Pugh class A (p value<0.001), absent cirrhosis (p value 0.016), absent vascular invasion (P value 0.027), A4 BCLC stage (p value<0.01) and partial tumor response (p value<0.01) had statistically significantly higher baseline IGF-1 levels.

On the other hand, they were not statistically significantly different according to age, gender, and Multifocality of liver lesion.

IGF-1 levels and treatment outcomes

Patients with disease control had significantly higher baseline IGF-1 levels 210 (185-232.5)ng/mL (p value<0.01) than did patients without disease control.

Serum IGF-1 levels as an independent prognostic factor for survival

IGF-1 was initially analyzed for its prognostic value as a continuous variable. Univariate Cox analyses showed that smaller tumor size (≤5 cm), INR level<1.7, absence of cirrhosis, absence of vascular invasion , PS≤0-1, stable disease and increasing levels of IGF-1>125 ng/ml were significantly associated with Longer OS. No significant differences were found according to age, gender, BCLC staging and child Pugh .

In the multivariate analysis, there was no independent risk factor for longer survival (Table 2).

Impact of IGF-1 on survival

According the levels of baseline serum IGF-1, a cut-off value of (125 ng/mL) was used, and P values were derived by log rank test. Patients with high pre therapy IGFI level had statistically significant longer overall survival (OS). The hazard ratio (HR) is 0.217 (95%CI 0.116-0.403), p

value=0.00 (Figure 1). Patients with TACE had longer OS and good disease control followed by patients received supportive care, lastly was the chemotherapy group but not reached statistically significant point. The Chi square is 3.860 p value=0.154 (Figure 2).

Figure 3 show that patients with high base line IGF-1 in chemotherapy group had statistically significant longer survival with good disease control. The Chi square is 8.149 P value = 0.004. Also, patients under best supportive care with high base line IGF-1 had statistically significant longer OS and good disease control. The Chi square is 11.474, p value=0.001 (Figure 4). Kaplan-Meier curve show that patients under TACE with high base line IGF-1 had statistically significant longer OS and good disease control. The Chi square is 15.202, p value=0.000 (Figure 5).

Serum IGF-1 levels as an independent prognostic factor for HCC progression

Univariate Cox analyses showed that absence of cirrhosis, smaller tumor size (≤5 cm), INR level<1.7, absence of vascular invasion, good PS, stable tumor and higher levels of IGF-1>125 ng/ml pre therapy and after
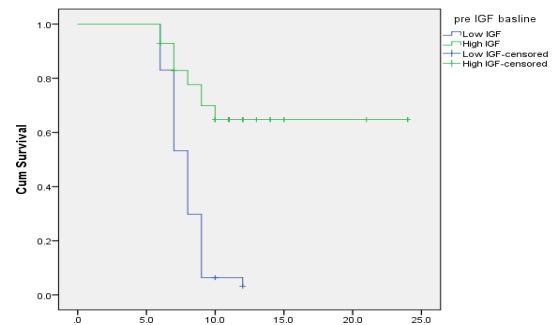

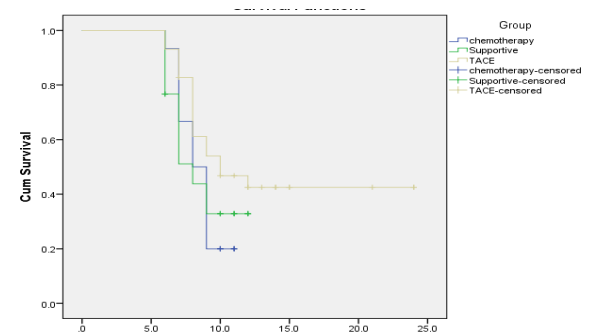**Figure 1. Overall Survival (OS) in All HCC Patients**

Table 1. The Levels of Pre Therapy Insulin-Like Growth Factor-1 (IGF-1) According to Clinical Characteristics

Variable	Patients (n= 89)	IGF-1(ng/ml) Median (IQR)	p
Age(years)			0.427
< 60	70(78.7%)	110(100-128.75)	
>=60	19(21.3%)	120(110-240)	
Gender			0.42
Female	15(16.9%)	120(100-210)	
Male	74(83.1%)	110(103.75-152.5)	
Cirrhosis			0.016
Absent	42(47.2%)	130(107.5-200)	
Present	47(52.8%)	110(100-120)	
Child-Pugh Class			<0.001
A	10(11.2%)	185(157-202)	
B	39(43.8%)	100(100-110)	
C	40(44.9%)	120(110-212.5)	
Tumor Multifocality			0.822
Single	40(44.9%)	110(106-185)	
Multiple	49(55.1%)	110(100-130)	
Tumor Response			<0.01
PR	10(11.2%)	210(185-232.5)	
PD	64(71.9%)	110(100-120)	
SD	15(16.9%)	120(110-240)	
BCLC staging			<0.01
A4	10(11.2%)	185(157-202.5)	
B	19(12.3%)	110(100-110)	
C	30(33.7%)	110(103-120)	
D	30(33.7%)	120(107-187.5)	
Vascular Invasion			0.027
Absent	43(48.3)	125(110-210)	
Present	46(51.7)	110(100-120)	

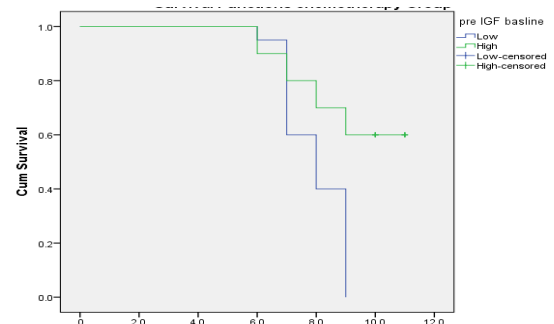**Figure 2. OS in HCC Patients Subgroups According to Line of treatment**

Figure 3. Overall Survival (OS) for Patients Received Chemotherapy

Table 2. Univariate and Multivariate Analysis of Factors Associated with Overall Survival (OS)

Variable	Univariate Analysis			Multivariate Analysis		
	Hazard Ratio	95%CI	p value	Hazard ratio	95%CI	p value
Age (< 60Vs≥ 60years)	1.308	0.679-2.522	0.422	-	-	-
Gender (Male Vs Female)	0.890	0.599-1.321	0.562	-	-	-
Cirrhosis	0.293	0.166-0.518	0.000	1.098	0.357-3.374	0.870
BCLC Staging System						
BCLC A4,B	0.000	0.00-0.0000015	0.969	-	-	-
BCLC C	1.162	0.597-2.264	0.659	-	-	-
BCLC D	1.059	0.578-1.942	0.853	-	-	-
Child-Pugh	1.385	0.953-2.022	0.088	0.862	0.333-2.229	0.759
INR	1.706	1.14-2.43	0.003	0.844	0.467-1.524	0.573
Tumor size(≤ 5cm Vs > 5cm)	0.015	0.00-0.132	0.000	0.895		
Vascular invasion	0.527	0.396-0.701	0.000	0.620	0.213-1.806	0.381
PS	1.607	1.2-2.152	0.001	1.535	0.855-2.757	0.151
Serum Albumin	0.767	0.542 -1.087	0.136	-	-	-
Response						
PR	1.314	0.846 -1.997	0.201	-	-	-
PD	0.000	0.00-0.00001	0.955	-	-	-
SD	2.15	1.018-4.553	0.045	1.055	0.444-2.509	0.904
Pre IGF level	0.217	0.116-0.403	0.00	0.838	0.420-1.672	0.616
IGF after 3 months	0.539	0.295-0.985	0.044	1.293	0.601-2.783	0.511
IGF after 6 months	0.104	0.043-0.249	0.000	0.849	0.319-2.261	0.743

Table 3. Univariate and Multivariate Analysis of Factors Associated with Time to Progression (TTP)

Variable	Univariate Analysis			Multivariate Analysis		
	Hazard Ratio	95%CI	p value	Hazard ratio	95%CI	p value
Age (< 60 *vs* ≥60years)	1.516	0.786-2.925	0.214	-	-	-
Gender(male *vs* Female)	0.896	0.604-1.330	0.214	-	-	-
Cirrhosis	0.567	0.428-0.753	0.000	0.905	0.510-1.604	0.732
BCLC staging system						
BCLC A4,B	0.00	0.00-0.00006	0.952	-	-	-
BCLC C	1.563	0.799-3.058	0.92	-	-	-
BCLC D	0.957	0.523-1.750	0.888	-	-	-
Child-Pugh	1.310	0.910-1.866	0.146	-	-	-
INR	1.667	1.155-2.408	0.006	0.755	0.401-1.420	0.383
Tumor size(≤ 5cm *vs* >5cm)	82.628	9.381-727.7	0.000	-	-	-
Vascular invasion	0.585	0.442-0.775	0.000	0.906	0.456-1.802	0.729
PS	1.6	1.187-2.155	0.002	1.778	1.114-2.84	0.016
Serum albumin<3.5	0.874	0.619-1.235	0.446	-	-	-
Tumor Response						
PR	0.921	0.395-2.145	0.84	-	-	-
PD	0.000	0.000 -0.00009	0.955	-	-	-
SD	2.414	1.139-5.115	0.021	1.055	0.444-2.509	0.904
Pre IGF1	0.184	0.097-0.346	0.000	0.559	0.276-1.132	0.106

3 and 6 months were statistically significantly associated with Longer TTP and good disease control. No significant differences were found according to age, BCLC stage, Child plug score or gender.

In the multivariate analysis, good PS was independent risk factors for longer TTP and better disease control. The hazard ratio (HR) for good PS was 1.778 (95%CI, 1.114-2.84) with p value=0.016 (Table 3). Figure 6 represent Kaplan-Meier which estimates of TTP in all HCC patients according the levels of baseline serum IGF-1. A cut-off value of125 ng/mL was used, and P values were derived by log rank test.

Patients with high pre therapy IGFI level had statistically significant longer TTP and good disease control. The hazard ratio (HR) is 0.184 (95%CI 0.097 - 0.346), p value=0.000. Patients with TACE had longer TTP and good disease control followed by patients received supportive care, Chi square is 1.468, p value=0.480 (Figure 7).

As regard line of treatment, patients who received chemotherapy with high base line IGF-1 had statistically significant longer TTP and good disease control. The Chi square is 9.424, p value=0.002 (Figure 8). Patients under best supportive care with high base line IGF-1 had statistically significant longer TTP and good disease control. The Chi square is 10.202, p value=0.001 (Figure 9). Patients who had done TACE with high base line IGF-1 had statistically significant longer TTP and good disease control. The Chi square is 24.087, p value=0.000 (Figure 10).
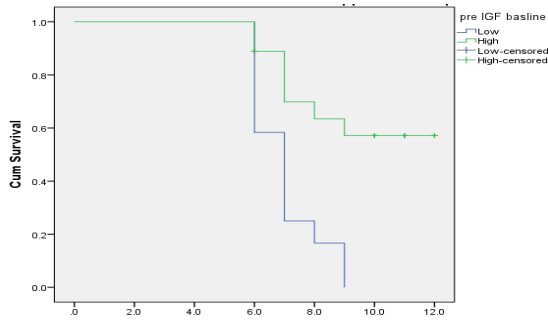
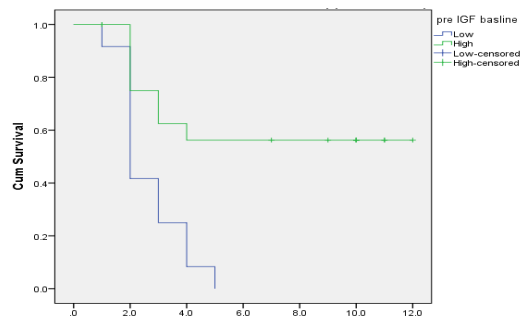**Figure 4. Overall Survival for Patients under Best Supportive Care**

Figure 5. Overall Survival for Patients with TACE

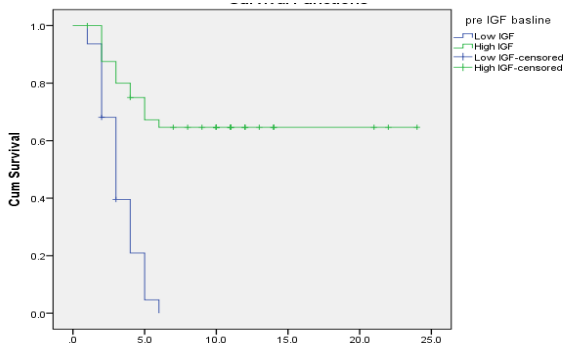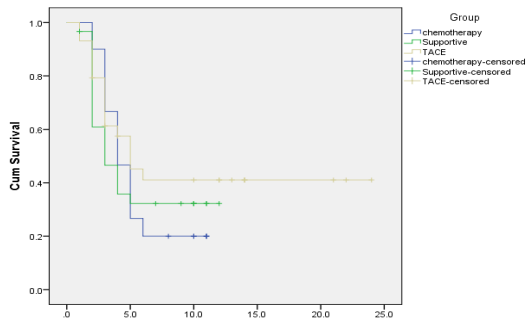**Figure 6. Time to Progression for all HCC Patients**

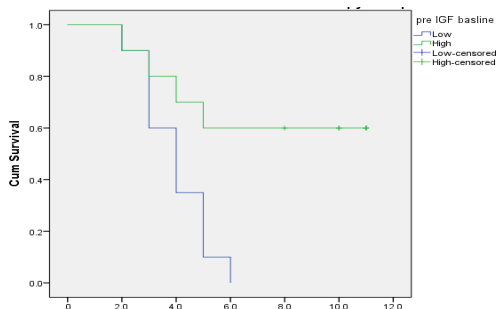**Figure 7. Kaplan-Meier Estimates of TTP in all HCC Patients Subgroups According to Treatment**

Figure 8. Kaplan-Meier Estimates Comparison of TTP in Patients Receiving Chemotherapy

Figure 9. Kaplan-Meier Estimates Comparison of TTP in Patients under Best Supportive Care

Figure 10. Kaplan-Meier Estimates Comparison of TTP in Patients Undergoing TACE

Discussion

In this study, we found that high pretreatment serum IGF-1 levels were statistically significant associated with better TTP and longer OS in HCC patients.

Although previous studies have shown that blood IGF-1 levels are a prognostic marker for HCC (Qian et al., 2010; Kaseb et al., 2011a; 2011b), this is the first study showing the potential association between serum IGF-1 levels and the efficacy of different treatment modalities for advanced HCC. The study was a prospective analysis which includes a control group of patients who received just supportive therapy. IGF-1 has been shown to stimulate hypoxia-inducible factor-1a activity in several cancer models (Page et al., 2002; Carroll and Ashcroft, 2006; Treiber et al., 2006; Sutton et al., 2007). Blockade of the IGF-1 pathway inhibited angiogenesis and tumor growth in experimental animals (Reinmuth et al., 2002; Beckert et al., 2006; Gariboldi et al., 2010; Bid et al., 2012).

The result that low serum IGF-1 levels were associated with poor prognosis appears to be paradoxical because high IGF-1 levels and subsequent activation of the IGF system have been known as relevant signaling alterations in various cancers (Moser et al., 2008). We found that the association of IGF-1 levels with PFS, and OS held true for patients receiving sorafenib based regimens. This implies that the mechanism underlying the predictive values of IGF-1 levels is possibly linked to antiangiogenesis, the common mechanism of action shared by sorafenib.

We measured free serum IGF-1 levels and found them also to be associated with good tumor control (SD). Patients with high levels of free serum IGF-1 had statistically significant longer OS and TTP. Nonetheless, our results maintain consistency with previous studies of the levels of circulating IGF-1 and prognosis in HCC patients. Kaseb et al showed that patients with low IGF-1

levels were more likely to exhibit advanced pathologic parameters of HCC, such as multinodularity, large tumor size, and vascular invasion, and had shorter OS (Maki, 2010).

Similarly, low baseline serum IGF-1 levels were associated with poor treatment response, PFS, and OS in patients who received anti-angiogenic therapy for advanced HCC and those underwent curative treatment for early-stage HCC (Shao et al., 2012).

The present study found that low basal IGF-1 levels were associated with advanced HCC, such as multiple tumors and advanced stage, and low IGF-1 levels predicted shorter TTP and OS in patients treated with TACE. Collectively, these findings suggest that the association between low circulating IGF-1 and unfavorable outcome may remain consistent across various stages of HCC and treatment modalities, and the oncogenic effects of circulating IGF-1 may not play a determinant role in the progression of HCC. However, the possibility that the autocrine/paracrine effects of IGF-1 may be more important than the systemic effects on HCC cannot be excluded.

The decrease of circulating IGF-1 in HCC patients has been attributed to a result of liver damage because hepatocytes are the main contributors of IGF-1 (Mazziotti et al., 2002).

Other studies showed that IGF-1 replacement or gene transfer therapy induced cytoprotective and anti-inflammatory effects leading to improvement of hepatic fibrosis in cirrhotic rats (Sobrevals et al., 2010) and IGF-1 treatment improved serum albumin levels in patients with cirrhosis (Conchillo et al., 2005).

So, low IGF-1 level is not only a result of liver cell damage, but may be also a contributor to the development of cirrhotic features by promoting pro-inflammatory and profibrogenic responses. Because inflammatory microenvironment and advanced hepatic fibrosis/cirrhosis are important in hepatocarcinogenesis (Yang et al., 2011) these may contribute to the higher recurrence rates in patients with low IGF-1 levels, although our hypothesis could not be directly tested in the present study because histological data were unavailable.

In our study, 47(52.8%) patients had cirrhosis; 39(43.8%) patients preserved Child Pugh class B and 10(11.2%) patients preserved Child Pugh class A, the levels of IGF-1 were still useful predictors of progression and death, independent of remnant liver function.

Therefore, the levels of serum IGF-1, expressed as continuous value, may be helpful for accurately assessing hepatic function and the prognostic stratification of patients with HCC in combination with traditional stepwise parameters, such as Child-Pugh class or BCLC stage.

The present study has some limitations. First, the study was performed with limited patients number and the cut-off level of IGF-1 (125 ng/mL) that was used to divide the patient population was different from previous studies (Kaseb et al., 20011b).IGF-1 level was significantly associated with disease progression and patient survival. Thus, its prognostic value appears to be consistent, regardless of the difference in the cut-off values. Further studies will be needed to develop multi-stage stratification that can discriminate subgroups with different prognoses.

Second, the influence of IGF-1 on OS was not fully investigated because of the relatively short follow-up period and small number of events. Further follow-up of our cohort will clarify this association.

In conclusion, the present prospective study found a statistically significant association between serum IGF-1 levels and treatment outcome in HCC patients who underwent TACE, received TKI sorafinib and best supportive care. Thus, serum IGF-1 levels may serve as an indicator of liver function and prognostic marker that reflects TTP and OS in HCC patients, which will be helpful for the precise risk stratification of patients

In conclusion, we have shown that high pretreatment serum IGF-1 levels were associated with better DCR, PFS, and OS of patients who received systemic therapy for advanced HCC. These findings warrant validation in large studies.

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