Diagnostic and Prognostic Role of Serum Glypican 3 in Patients With Hepatocellular Carcinoma

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α -Feto protein (AFP) is the widely used tumor marker in the diagnosis of hepatocellular carcinoma (HCC). The aim of this study was to assess the diagnostic and prognostic validity of a novel marker, serum Glypican-3 (GPC3) and to compare AFP in patients with HCC. One hundred and twenty-eight patients (75 patients with HCC, 55 patients with cirrhosis, and 28 healthy controls) were included in this study. Cut-off value of GPC3 was 3.9 pg/ml. AFP was divided into four subgroups, according to cut-off values with 13, 20, 100, and 200 ng/ml. Sensitivity, specificity, and positive and negative predictive values of GPC3 and AFP₁₃, AFP₂₀, AFP₁₀₀, AFP₂₀₀ subgroups and also GPC3+AFP₁₃, GPC3+AFP₂₀, GPC3+AFP₁₀₀, GPC3+AFP₂₀₀ combinations were compared. Serum

GPC3 levels were significantly higher in patients with HCC and cirrhosis compared with control subjects ($P < 0.05$). The median serum GPC3 levels were 3.9 pg/ml in controls, 5.51 pg/ml in patients with cirrhosis, and 5.13 pg/ml in those with HCC. The median serum AFP levels were 1.37 ng/ml in controls, 2.32 ng/ml in cirrhotics, and 50.65 ng/ml in HCC patients. The sensitivity, specificity, and positive and negative predictive values of GPC3 was 61.33, 41.82, 58.97, and 44.43%, respectively. The values for AFP were 68.57, 94.55, 94.12, and 70.27%, respectively. There was no correlation between GPC3 levels and prognostic parameters. GPC3 is not a useful diagnostic and prognostic marker for HCC. *J. Clin. Lab. Anal.* 25:350–353, 2011. © 2011 Wiley-Liss, Inc.

Key words: glypican-3; α -fetoprotein; hepatocellular carcinoma

INTRODUCTION

Hepatocellular carcinoma (HCC) is the third most frequent cancer and also the fourth leading cause of cancer-related death worldwide. The diagnosis can be difficult and often requires the use of serum markers, one or more imaging modalities, and histologic confirmation. Ideally, tumors should be detected when they are small in patients who are able to withstand standard therapy. However, HCC is frequently diagnosed late in its course because of the absence of pathognomonic symptoms and the large functional reserve of the liver (1,2). As a result, many patients have untreatable cancer at the time of diagnosis. The median survival of the patients with HCC is approximately 6–20 months (3). Development of more sensitive and specific serum markers for the early detection of HCC may lead to improved survival of patients.

Glypican-3 (GPC3) belongs to the glypican family of glycosyl-phosphatidylinositol-anchored heparan sulfate proteoglycans, which plays an important role in cellular growth, cell differentiation, and cell migration (4). GPC3 has been reported to be increased in HCC in comparison with preneoplastic lesions and cirrhotic tissues at the mRNA and protein levels (5–9). Interestingly, GPC3 mRNA levels are more frequently elevated than those of AFP, with the difference even greater in small HCC (10). These findings suggest the potential

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value of GPC3 as a novel tumor marker for the diagnosis of HCC.

In this study, we aimed to evaluate the diagnostic value of serum GPC3 levels in patients with HCC and to compare the clinical significance of serum GPC3 levels with AFP in the diagnosis of HCC based on the diagnostic criteria, such as diagnostic sensitivity, specificity, and positive and negative predictive values.

MATERIAL AND METHODS

The study protocol was approved by the Ankara University School of Medicine. Patients and controls gave written informed consent before the investigation.

One hundred and fifty-eight subjects were enrolled in the study between May and October 2009. The patients were divided into three groups: 75 cirrhotic patients with HCC (Group 1), 55 cirrhotic patients without HCC (Group 2), and 28 healthy controls (Group 3). Demographic and clinical information was gathered and a blood sample was collected from each subject.

The control group was enrolled from healthy subjects without a history of liver disease, alcohol consumption, or risk factors for viral hepatitis. All subjects in the control group were documented to have normal liver biochemistry.

The diagnosis of HCC was made by histopathology. If histopathology was not available, the diagnosis was reached by two imaging modalities (ultrasound, magnetic resonance imaging or computed tomography) showing a vascular-enhancing mass. Diagnosis of cirrhosis was based on liver histology or clinical, laboratory, and imaging evidence of hepatic decompensation or portal hypertension. Tumor staging was determined using the United Network of Organ Sharing-modified tumor node metastasis (TNM) staging system for HCC (11). Early HCC was defined as T1 (single lesion <2 cm in diameter) and T2 (single lesion between 2 and 5 cm in diameter or <3 lesions each <3 cm in diameter) lesions. A 10 ml blood sample was drawn from each subject, spun, aliquoted, and serum was stored at -80°C until testing.

AFP was tested using commercially available immunoassays utilizing enhanced chemiluminescence at our hospital central laboratory. The upper limit of the normal level was 13 ng/ml.

GP73 test is commercially available (Wuhan EIAab Science Co., Wuhan, China). The coating antibody for the kit is mouse-derived monoclonal and the detecting antibody is rabbit-derived polyclonal. The microtiter plate provided in this kit has been precoated with an antibody specific to GPC3. The Standard is 20 ng/ml after adding 0.5 ml standard diluent. Standards or samples are then added to the appropriate microtiter plate wells with a

biotin-conjugated antibody preparation specific for GPC3, and avidin conjugated to horseradish peroxidase is added to each microplate well and incubated. Then, a TMB substrate solution is added to each well. Each well in the plate adds 100 μl of standard solution or samples, but TMB only adds 90 μl per well. Only the wells that contain GPC3, biotin-conjugated antibody, and enzyme-conjugated avidin will exhibit a change in color. The enzyme-substrate reaction is terminated by the addition of a sulphuric acid solution, and the color change is measured spectrophotometrically at a wavelength of $450 \pm 2 \text{ nm}$. The concentration of GPC3 in the samples is then determined by comparing the optical density of the samples to the standard curve. The performance characteristics of human GPC3 (GPC3 ELISA kit) were as follows: intra-assay coefficient of variation <4.5, inter-assay coefficient of variation <7.2, spike recovery = 97%.

Descriptive statistics for AFP and GPC3 were compared by box plots, including the outliers. All values were reported as mean $\pm$ SD. Mann-Whitney *U* test was used to determine group differences in medians because of the nonparametric distribution of AFP and GPC3. For binary variables, the χ^2 test was utilized to compare groups. Multivariate analysis and multiple logistic regression tests were utilized to determine the difference in proportions of HCC patients with AFP and GPC3 levels above or below the cut-off values. Log transformation was carried out on AFP and GPC3 before performing the receiver operating characteristic (ROC) curves. To determine the optimal cut-off value for GPC3 and AFP in the diagnosis of HCC, ROC curves were constructed using all possible cut-offs for each assay. A two-tailed *P*-value of 0.05 was used to determine statistical significance. All analyses were performed with SPSS 15.0.

RESULTS

The demographic and clinical features of the patients are summarized in Table 1. Serum GPC3 levels were significantly higher in patients with HCC and cirrhosis compared with control subjects ($P < 0.05$). The median serum GPC3 levels were 3.9 pg/ml (range = 3.9–7.7) in controls, 5.51 pg/ml (range = 3.9–236.2) in patients with cirrhosis, and 5.13 pg/ml (range = 3.9–93.2) in those with HCC. The median serum AFP levels were 1.37 ng/ml (range = 0.61–6.89) in controls, 2.32 ng/ml (range = 0.61–85.24) in cirrhotics, and 50.65 ng/ml (range = 0.8–37.642) in HCC patients ($P < 0.0001$ for HCC vs. controls without liver disease and cirrhotics). ROC curve analysis were plotted to define the optimal cut-off values, and to identify the sensitivity and specificity for serum GPC3 and AFP in differentiating patients with HCC from those with cirrhosis. The AUROC for GPC3

TABLE 1. Demographic and Clinical Characteristics of the Three Groups

	HCC (<i>n</i> = 75) group I	Cirrhosis (<i>n</i> = 55) group II	Control group III	<i>P</i> value
Age (years)	63+9.9	58+12.7	62.2+12.3	0.06
Gender (M/F)	54/21	33/22	16/12	0.2
HBV	39	16		
HBV+HDV	3	5		
HCV	17	9		
HCV+HBV	2	1		
Alcoholic liver disease	2	6		
Cryptogenic liver disease	12	14		
Autoimmune liver disease	0	2		
Wilson disease	0	2		

HBV, Hepatitis B virus; HCV, Hepatitis C virus; HDV, Hepatitis D; M, male; F, female.

TABLE 2. The Sensitivity, Specificity, Positive, and Negative Predictive Values for the Serum Levels of GPC3, AFP Subgroups and Combinations of GPC3 and AFP Subgroups

	GPC3	AFP ₁₃	AFP ₂₀	AFP ₁₀₀	AFP ₂₀₀
Sensitivity (%)	61.33	68.57	60	45.71	38.57
Specifity (%)	41.82	94.55	98.08	100	100
Positive predictive value (%)	58.97	94.12	97.67	100	100
Negative predictive value (%)	44.43	70.27	64.56	59.14	56.12
	GPC3+AFP ₁₃	GPC3+AFP ₂₀	GPC3+AFP ₁₀₀	GPC3+AFP ₂₀₀	
Sensitivity (%)	67.35	66.67	64.84	64.04	
Specifity (%)	71.88	67.65	58.97	56.1	
Positive predictive value (%)	88	85.33	78.67	76	
Negative predictive value (%)	41.82	41.82	41.82	41.82	

was 0.743 (95% CI = 0.645–0.84), with a sensitivity of 61.33%, a specificity of 41.82%, positive predictive value of 58.97%, negative predictive value of 44.43%, and an optimal cut-off point of 3.9 pg/ml, whereas the AUROC for AFP was 0.94 (95% CI = 0.895–0.985), with a sensitivity of 68.57%, a specificity of 94.55%, and a cut-off of 13 ng/ml. GPC3 had a worse AUROC compared with AFP ($P < 0.001$).

Table 2 presents the calculated sensitivities, specificities, diagnostic accuracy, and positive and negative predictive values for the serum levels of GPC3, AFP subgroups, and GPC3+AFP subgroups.

No correlation was found between GPC3 and AFP levels ($P > 0.05$). GPC3 levels were comparable among patients with HCC and cirrhosis with respect to the Child–Pugh score, portal vein thrombosis, extrahepatic metastasis, and tumor size and early HCC.

DISCUSSION

In this study, we found that serum GPC3 levels were higher in patients with HCC and cirrhosis than healthy

controls. However, it had a lower sensitivity and specificity for the diagnosis and determining prognosis in patients with HCC. Although, the sensitivity and specificity of combined use of serum GPC3 and AFP was superior to AFP alone, the differences were not statistically significant.

The most commonly used marker for HCC is serum AFP. It is generally accepted that serum levels greater than 500 mcg/l (in most laboratories, normal is between 10 and 20 mcg/l) in a high-risk patient is diagnostic of HCC (12). However, HCC is often diagnosed at a lower AFP level in patients undergoing screening (13,14). In addition, serum concentrations are normal in up to 40% of HCC (15). This means that 40% of examined HCC patients may be considered false negatives. Thus, there is a need for the enhancement of the detection of HCC using new tumor markers.

GPC3 inhibits cell proliferation and induces apoptosis in some types of tumor cells (16). Therefore, down-regulation of the expression of GPC3 is not surprising in some of the different origin tumors. Embryonic liver and intestine showed high expression; however, in a normal

adult, tissues remain silent. Hence, GPC3 acts as an oncofetal protein, such as AFP. Similarly, although the expression of GPC3 in the colon is absent, a high expression in colorectal cancer has been observed. GPC3 is only detected in HCC cells, but not in benign liver tissues (5,16,17). Reviewing the literature, a small number of studies have been reported on the potential diagnostic role of GPC3 in patients with HCC.

A recent study suggested that serum GPC3 was superior to AFP in detecting small HCC and was not correlated with tumor size and stage of HCC (18). In another study, the simultaneous determination of GPC3 and AFP (at a cut-off value of 20 ng/ml) significantly increased the sensitivity of the diagnosis to 80%. The combined use of serum GPC3 and AFP may significantly increase the sensitivity for differentiating HCC from chronic liver disease (19). In our study, the diagnostic value of serum GPC3 was not better than AFP in patients with HCC. Furthermore, the combination of GPC3 and AFP was superior to AFP alone; however, this difference was not statistically significant.

In this study, the prognostic parameters of HCC, including tumor diameter, number of lesions, Child-Pugh score, TNM stage, CLIP score, portal vein thrombosis, and extrahepatic metastases, were also evaluated. We did not observe a statistically significant correlation between those parameters and GPC3, and we also obtained similar results for AFP13 and AFP20. However, we found a significant correlation between CLIP score and lesion diameter with AFP100 and lesion diameter with AFP200.

Most of the studies revealed that GPC3 is an important tumor marker in the diagnosis of HCC. In contrast, in this study, GPC3 alone and combination with AFP subgroups were not superior to AFP. Hence, we suggested the use of serum GPC3 alone and also combined with AFP is not a good choice even for the cost-effective approach.

In conclusion, GPC3 has a lower diagnostic and prognostic value for HCC and cirrhosis.

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