Glypican-3 as a tumor marker for hepatocellular carcinoma

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ABSTRACT

Hepatocellular carcinoma (HCC) is one of the most frequent cancers in the world. The burden of HCC has been increasing in Egypt with a doubling in the incidence in the past ten years. The prognosis of most patients is unsatisfactory due to rapid clinical deterioration after the initial diagnosis. Therefore, it is very important to detect HCC and the recurrence at its earlier period. Glypican-3 (GPC3) is a cell-surface protein, which is a member of the heparan sulfate proteoglycan family. GPC3 was identified in the serum of patients with HCC and can be used as a serological test for the diagnosis of HCC. The aim was to assess the value of serum GPC3 in Egyptian patients with HCC. This study was included 30 patients with HCC, 30 patients with liver cirrhosis and 20 healthy controls. For all groups we studied clinical data, image findings, serum alpha-fetoprotein (AFP) levels detected by enzyme immunoassay (EIA) kit and GPC3 gene expression was detected by real-time polymerase chain reaction (PCR). Tumour characteristics were assessed including size, number and site. Tumour staging was done using Okuda & Tokyo staging systems. The data showed that HCC patients had a significantly higher mean GPC3 values (p=0.000) than both cirrhotics and healthy control groups. GPC3 has a positive significant correlation with tumour size (p=0.015) and Tokyo staging system (p=0.047). GPC3 could be a useful diagnostic & prognostic marker for detection of HCC.

INTRODUCTION

Hepatocellular carcinoma (HCC) is one of the most frequent cancers in the world. The burden of HCC has been increasing in Egypt with a doubling in the incidence in the past ten years. The prognosis of most patients is unsatisfactory due to rapid clinical deterioration after the initial diagnosis. Therefore, it is very important to detect HCC and the recurrence at its earlier period. Glypican-3 (GPC3) is a cell-surface protein, which is a member of the heparan sulfate proteoglycan family. GPC3 was identified in the serum of patients with HCC and can be used as a serological test for the diagnosis of HCC. The aim was to assess the value of serum GPC3 in Egyptian patients with HCC. This study was included 30 patients with HCC, 30 patients with liver cirrhosis and 20 healthy controls. For all groups we studied clinical data, image findings, serum alpha-fetoprotein (AFP) levels detected by enzyme immunoassay (EIA) kit and GPC3 gene expression was detected by real-time polymerase chain reaction (PCR). Tumour characteristics were assessed including size, number and site. Tumour staging was done using Okuda & Tokyo staging systems. The data showed that HCC patients had a significantly higher mean GPC3 values (p=0.000) than both cirrhotics and healthy control groups. GPC3 has a positive significant correlation with tumour size (p=0.015) and Tokyo staging system (p=0.047). GPC3 could be a useful diagnostic & prognostic marker for detection of HCC.

HCC can only be cured if diagnosed at an early stage (Okochi et al., 2002), so there is a pressing need either to prevent the tumor or to diagnose it at a presymptomatic stage, when surgical interventions still possible (Kew, 2002).

Up to 20% of HCC do not produce alpha-fetoprotein (AFP) even when very large (Ryder, 2003) and slight increase are usual in acute hepatitis, chronic hepatitis and cirrhosis and overlaps can cause diagnostic difficulties (Sherlock and Dooley, 2002). Glypican-3 (GPC3), a heparin sulphate proteoglycan anchored to the plasma membrane and it is an oncofetal protein that is over expressed in HCC. Serum GPC3 has been shown to be significantly higher in HCC than in liver cirrhosis or in healthy controls thus a novel serological marker for the early detection of HCC (Lopez, 2005). GPC3 has been reported to be expressed in the majority (>70%) of hepatocellular carcinoma (HCC) as a diagnostic marker (Jia et al., 2007). This study was aimed at evaluating the diagnostic and prognostic value of Glypican-3 in comparison to the old established biomarker AFP levels in patients with HCC.
PATIENTS AND METHODS

Patients

This study was approved by the Ethics and Research Committee of the Benha faculty of Medicine, Benha University, Egypt. Serum samples were obtained from sixty patients with chronic liver disease, divided into two groups: Group (I) included thirty patients with HCC, Patients with cancers other than HCC or metastatic liver cancer were excluded. Group (II) included thirty patients with liver cirrhosis and without any evidence of HCC. Twenty healthy adults were recruited as controls (Group III). All patients included in this study had the procedure thoroughly explained to them. HCC was diagnosed by abdominal US and serum AFP, with or without triphasic CT scan and/or liver histopathology. AFP was assayed by an enzyme immunoassay (EIA) Kit (Roche Mannheim, Germany). The clinical/pathological data of the patients were recorded, including age, sex, viral infections {Hepatitis C Virus (HCV) and Hepatitis B Virus (HBV)}, alcohol intakes, biochemical liver function test results, and AFP levels. Severity of liver disease was assessed by and MELD (model for end stage liver disease) score (Kamath et al., 2001) and the updated MELD (uMELD) score (Sharma et al., 2008). Tumor characteristics were detected by Abdominal US with or without CT scan (including tumor size, number, site, halo sign and neovascularization). Tumor staging was done using Okuda (Okuda et al., 1985), and Tokyo (Tateishi et al., 2005) staging systems.

Blood sampling and biochemical assays

Fasting venous blood samples (5 ml) were collected by trained laboratory technicians. A portion of blood was allowed to clot and then centrifuged at 3500g for 5 min to separate the serum used for assessment of aspartate aminotransferase (AST), alanine aminotransferase (ALT), and γ-glutamyl transpeptidase (GGT), total bilirubin, direct bilirubin, albumin, creatinine and glucose concentrations were assayed using Beckman CX4 chemistry analyzer (NY, USA, supplied by the Eastern Co. For Eng. & Trade-Giza, Egypt). Viral infection status (HCV Ab and HBS Ag) were measured using Abbott, Axyam (USA, Supplied by al kamal company). Serum AFP level was determined using an enzyme-linked binding protein assay kit. All assays were performed in duplicate according to the manufacturer’s instructions. Serum aliquots were stored at -80°C until assayed and thawed immediately before the measurements levels. Another portion of blood was collected in vacutainer tubes containing EDTA to separate lymphocyte cells.

Quantification of GPC3 expression by real-time PCR

GPC3 gene expression was assayed for all samples and controls. Preparation of PBMCs obtained from peripheral blood of all patients and controls were isolated by Ficoll density centrifugation and sedimentation. RNA was extracted from PBMCs cells using QIAamp_viral RNA extraction kit. Quantification of was performed using TaqMan® Gene Expression assay (Applied Biosystems Inc, Foster City, CA, USA). β -actine was used as housekeeping gene (endogenous reference cDNA). Fractional threshold cycles (CT) were expressing the initial concentration of target sequence. Relative mRNA quantification was calculated using the arithmetic formula 2-ΔCT, where ΔCT is the difference between the CT of a given target cDNA and an endogenous reference cDNA. Thus, this value yields the amount of the target normalized to an endogenous reference.

Statistical Analysis

Statistical package (SPSS, version 10.0) was used for data management. Descriptive statistics was presented as mean±standard deviations for continuous variables, number and percentage for categorical variables (frequency distribution). Unpaired Student t-test (two sided) was used to test the significance of difference between the mean value of studied groups and chisquare test was used for comparison of categorical variables. The diagnostic value for each marker was assessed using Sensitivity, specificity, positive (PPV) and negative (NPV) predictive values. Receiver operating characteristic curves (ROC) were constructed to assess the validity of the markers in predicting HCC by calculating the area under the curve (AUC). Pearson correlation test was used to identify the correlation between Glypican-3 and different clinicopathological variables. The significance level was set at p<0.05.

RESULTS

The demographic features and characteristics of the two patients’ groups were summarized in Table 1. A total of 60 adults, which comprised 30 patients with HCC, 30 patients with liver cirrhosis and 20 apparently-normal control subjects were studied. The mean age of HCC patients was 57.6±9.7 years with a range between 40 and 73 years. In liver cirrhosis patients the mean age was 52.2±9.8 years with a range between 34 and 70 years. There was a significant difference in the mean ages of HCC patient group, liver cirrhosis (p=0.037). There was male predominance among the patients with HCC, 21 men (70.0%) versus 9 women (30.0%), with a male-to-female ratio of 2.3:1, in the liver cirrhosis patients there was 16 (53.3%) males versus 14 (46.7) females, with a male-to-female ratio of 1.1:1 but there was no significant difference in the sex ratios of HCC patients, liver cirrhosis patients (p=0.14) Regarding HCC etiology, the results showed that the frequency of HBV positivity had no statistically significant difference between the studied patients. It was detected only in 10.0% in HCC cases and in 13.3% in cirrhotic cases. HCV was present in 100.0% of HCC cases and 100.0% of cirrhotic cases with no statistically significant difference between the two groups. The severity of liver cirrhosis assessed by MELD, uMELD scores among the studied patients showed that both scores were significantly higher in HCC patients (p=0.000) (Table 1).
patients had the highest mean values (37725.87±54470.4 cycle threshold), with a significantly higher values (p=0.0001), than the mean of GPC3 values in both liver cirrhosis patients (8194.1±14893.86 cycle threshold) and healthy control (114±123.4 cycle threshold) groups. Figure 1.

Abdominal CT showed the dominant occurrence of HCC on top of cirrhosis (100%) and a higher percentage of portal vein thrombosis (PVT) (10%) and the higher incidence of the focal lesion to be single (66.7%), large (50%) and affecting the left lobe (63.3%). Metastasis was present in 3.3% of HCC cases.

Regarding Okuda, and Tokyo staging systems, most of HCC patients were relatively at advanced stage of the disease. As regards AFP levels, the data revealed that HCC patients had the highest mean value (296.7±213 ng/mL), with a significantly higher values (p=0.0001), than the mean of AFP values in both liver cirrhosis patients (39.6±33.4 ng/mL) and healthy control (5.8±2 ng/mL) groups. As regards GPC3 the data showed that HCC
In relation to prognosis, there was no significant correlation between AFP and the tumor number & size and Okuda& Tokyo staging among patients with HCC. There was significant positive correlation between GPC3 and the size (p=0.0015) and Okuda& Tokyo staging (p=0.047) among patients with HCC (Fig. 3&4).

Regarding AFP as a marker for HCC, our results supported by Huo et al., (2004) who concluded that serum AFP is a weak prognostic predictor in HCC patients and Guan et al., (2006) stated that the value of AFP is limited in the diagnosis and prognosis of HCC. Concerning Glypican-3 mean value, HCC cases had a mean value of (37725.87 ct) which was higher than that of patients with cirrhosis (8194.1 ct) and control (114 ct) subjects this results agree with El-Shenawy et al., 2012 who reported a higher mean values for HCC cases. ROC analysis of AFP used as a diagnostic test suggests that a value of about 17 ng/ml provides the optimal balance between sensitivity and specificity.

However at this level the sensitivity is only 63.3% and the specificity is 60% Bruix and Sherman (2005), Atta et al., (2008) reported exactly the same results. Concerning AFP, it was obvious from this study that there was no significant correlation between AFP and parameters of severity of liver disease as documented by Hou et al., (2004) and Atta et al., (2008). Also no significant correlation between AFP and tumour size, number or Okuda and Tokyo staging and this goes in agreement with Ryder, 2003 and Qin and Tang, 2002 respectively.

There was no significant correlation between Glypican-3 and severity of liver disease, this was in agreement with El-Shenawy et al., 2012. There was no significant correlation between Glypican-3 and size and Tokyo staging and this goes in agreement with El-Shenawy et al., 2012 who reported a significant correlation between Glypican-3 and tumour size and CLIP staging. Regarding Glypican-3 as a marker for HCC, our results supported by Liu et al., 2010 who concluded that Glypican-3 is a sensitive and specific marker for HCC diagnosis, also Yao et al., 2011 reported that Glypican-3 may be promising marker for diagnosis of HCC. Zou et al., 2010 concluded that Glypican-3 is a diagnostic and prognostic marker and expected to be an ideal target for the therapy of HCC.

CONCLUSION

Glypican-3 could be a sensitive, specific and accurate serum marker for diagnosing HCC.

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CONFLICT OF INTEREST

The author declare that they have no competing interests or conflict of interest.
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