Can we use GP73 as a biomarker for the detection of hepatocellular carcinoma?
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Background and aim
Hepatocellular carcinoma (HCC) is one of the top five leading causes of death in Egypt and its prevalence is increasing in the next 10–20 years. We aimed to detect the serum Golgi protein 73 (GP73) in patients with cirrhosis and with HCC, and to determine its sensitivity and specificity as a screening tool for the detection of HCC in this study.

Patients and methods
Serum GP73 was estimated in 93 participants (patients with HCC, patients with cirrhosis, and healthy controls).

Results
GP73 was elevated in patients with HCC and liver cirrhosis; serum level was very high in HCC patients ($P < 0.01$) when compared with the other studied groups. GP73 had sensitivity of 76%, specificity of 75%, at a cut-off value of 16.2 ng/ml with area under the receiver operator characteristic of 0.825 when compared with $\alpha$-fetoprotein that showed a sensitivity of 63%, specificity of 43% at a cut-off value of 16.5 ng/ml and area under the receiver operator characteristic of 0.611. By combining $\alpha$-fetoprotein and GP73 for the diagnosis of HCC, sensitivity and specificity were (93 and 25%), respectively. There is a significant positive correlation between diameter of the focal lesion and GP73 ($P = 0.01$ and $r = 0.071$). Nonsignificant positive correlation was detected as regards serum GP73 and the number of HCC.

Conclusion
GP73 can be used as a screening tool for the detection of HCC. Moreover, it shows a higher serum level with larger lesions.

Keywords:
$\alpha$-fetoprotein, GP73, hepatocellular carcinoma, screening

Introduction
Hepatocellular carcinoma (HCC) is the most common primary malignant tumor of the liver and the fifth most frequent malignant tumor in the world (third in terms of mortality) [1]. Its prevalence in the United States and in Western Europe is increasing. Cirrhosis is the strongest and the most commonly known risk factor for HCC, particularly, cirrhosis related to hepatitis C virus (HCV) and hepatitis B virus infections [2]. In Egypt, the rising trend of HCC incidence has been attributed to the high prevalence (14%) of HCV infection [3,4] among the general population [5]. The stage of cancer dictates the therapeutic choice, making early detection a primary objective. Early diagnosis of HCC is feasible. Many observational studies have reported that HCC is diagnosed at an earlier stage in patients who received surveillance [2]. Although HCC surveillance programs are controversial, most international societies – the European Association for the Study of the Liver and the American Association for the Study of Liver Diseases – recommend the use of ultrasound and $\alpha$-fetoprotein (AFP) in patients with a high risk of developing the condition, at 6–12 months frequency [6]. The use of AFP alone is strongly discouraged, and its use, in addition to ultrasound, is controversial. Patients with abnormal screening tests require additional investigations. Although the optimal methods of screening and the cost effectiveness of surveillance for HCC remain to be established, systematic screening still offers the best hope for early diagnosis, treatment eligibility, and improved survival [7–12]. Therefore, there is a strong demand by clinicians for new HCC-specific biomarkers. GP73 is a resident Golgi protein, shown to be upregulated in hepatocytes of patients with acute, chronic hepatitis and cirrhosis [13,14] with a significant increase in the sera of patients with hepatitis B virus and HCV-related HCC [15–17], providing a marker for its early detection [18,19]. In this study, we aimed to evaluate the presence of GP73 in the serum of all patients with cirrhosis and to determine its sensitivity and specificity as a screening tool for the detection of HCC.
Patients and methods
A total of 93 patients, 59 with cirrhosis with HCC, 20 with cirrhosis but no HCC, and 14 healthy controls, were enrolled. They were recruited from the Hepatology and General Internal Medicine outpatient clinics, Kasr Al Aini Hospital, Cairo University. A questionnaire was completed for every patient. The groups of patients with cirrhosis with HCC and no HCC were similar in age, race, and ethnicity. HCC was diagnosed by histopathology and/or two imaging modalities [ultrasound, triphasic computed tomography (CT) showing vascular enhancement]. Triphasic CT findings were typical in 40 cases; ultrasound-guided liver biopsy was performed for 19 patients. Diagnosis of cirrhosis was based on clinical, laboratory, and imaging evidence of hepatic decompensation or portal hypertension. Patients with cirrhosis and elevatedAFP, but no evident focal hepatic lesion on ultrasound, were subjected to triphasic CT performed within 3 months before and 6 months after the enrollment in the study. They have been followed up for up to 12 months with no evidence of HCC. The group of healthy controls had no history of liver disease, no risk factors of viral hepatitis, and normal liver biochemistry. Ultrasound performed proved to be normal and hepatitis markers (hepatitis B surface antigen, hepatitis B core antibody, total and anti-HCV/antibody) were negative. A blood sample was drawn from each patient at the time of imaging. Serum aminotransferases (alanine transaminase and aspartate aminotransferase), serum total and direct bilirubin, complete blood count, albumin, and prothrombin time were measured in all patients using standards methods. Serum AFP was quantitatively assessed using a CanAg AFP enzyme immunoassay enzyme immunoassay assay kit. An immunoblot analysis for GP73 was performed. Equal volumes of patient sera (0.5 µl per lane) were resolved by SDS/polyacrylamide gel electrophoresis on 4–20% polyacrylamide gradient gels, and GP73 was detected [20], followed by densitometric analysis. Informed consent before liver biopsy was taken and the specimen was examined by a hepatopathologist.

The institutional ethics committee approved the study and an informed consent form was obtained from all participants.

Statistical analysis
Quantitative variables were expressed as mean and standard deviation for parametric data and median for nonparametric data. Qualitative variables were expressed as frequency and percentage. The χ² (goodness-of-fit) test compares the observed and expected frequencies in each category to test whether all categories contain the same proportion of values. Descriptive statistics for HCC and non-HCC [liver cirrhosis (LC) and normal] groups as regards GP73 were compared using box plots. Sensitivity, specificity, and positive predictive value (PPV) and negative predictive value (NPV) were calculated for predicting GP73, and for combined GP73 and AFP, receiver operating characteristic curves were constructed to assess the power of the GP73 and AFP in predicting HCC by calculating the area under the curve, which was considered if more than or equal to 0.825 and 0.611, respectively. Pearson's correlation (r) was performed to detect the relationship between different parameters studied.

Results
Our study showed that there was a male predominance (88.1%) in the HCC group. The demographic, clinical, and laboratory data of the studied patients are shown in (Table 1).

Serum level of GP73 ranged from 1.1 to 51 ng/ml with a mean of 2.919 ± 1.2102 ng/ml in healthy participants. In patients with liver cirrhosis, it ranged from 2.6 to 38.2 ng/ml, whereas in patients with HCC, serum level of GP73 ranged from 8.7 to 58.1 ng/ml. Serum GP73 was detected in all participants with highest levels in the HCC group (Table 1). Mean serum GP73 in the HCC group shows significant statistical difference (P < 0.01) (Fig. 1). Moreover, the median level of GP73

<table>
<thead>
<tr>
<th>Table 1 Patients’ characteristics</th>
<th>HCC (n = 59)</th>
<th>Cirrhosis (n = 20)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>55.81 ± 5.307</td>
<td>53.65 ± 6.098</td>
<td>0.137</td>
</tr>
<tr>
<td>Sex (male : female)</td>
<td>52 : 7</td>
<td>17 : 3</td>
<td>0.033</td>
</tr>
<tr>
<td>ALT (IU/l)</td>
<td>65.59 ± 38.130</td>
<td>90.15 ± 30.308</td>
<td>0.011</td>
</tr>
<tr>
<td>AST (IU/l)</td>
<td>77.05 ± 40.587</td>
<td>94.05 ± 17.071</td>
<td>0.011</td>
</tr>
<tr>
<td>Total bilirubin (mg/dl)</td>
<td>3.097 ± 5.4599</td>
<td>2.635 ± 1.4698</td>
<td>0.710</td>
</tr>
<tr>
<td>Albumin (g/dl)</td>
<td>3.244 ± 0.5998</td>
<td>2.970 ± 0.2251</td>
<td>0.227</td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>1.20 ± 0.419</td>
<td>1.48 ± 0.496</td>
<td>0.033</td>
</tr>
<tr>
<td>INR</td>
<td>1.466 ± 0.3688</td>
<td>1.390 ± 0.3194</td>
<td>0.413</td>
</tr>
<tr>
<td>Child–Pugh class (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>20.3</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>54.4</td>
<td>70.3</td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>15.3</td>
<td>15.7</td>
<td></td>
</tr>
<tr>
<td>AFP (ng/ml)</td>
<td>59.93 ± 72.194</td>
<td>19.51 ± 9.126</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td>GP73 (ng/ml)</td>
<td>26.027 ± 12.26</td>
<td>13.23 ± 7.97</td>
<td>&lt;0.01*</td>
</tr>
<tr>
<td>Liver texture</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cirrhotic/heterogenous (%)</td>
<td>94.9/5.1</td>
<td>0/20/0</td>
<td>0.049</td>
</tr>
<tr>
<td>Ascites (absent/present)</td>
<td>47.5/52.5</td>
<td>80/20</td>
<td>0.01</td>
</tr>
</tbody>
</table>

AFP, α-fetoprotein; ALT, alanine transaminase; AST, aspartate aminotransferase; GP73, golgi protein 73; HCC, hepatocellular carcinoma; INR, international normalized ratio. *Statistically significant (P < 0.05, P < 0.01 highly significant).
was 24.5 ng/ml in patients with HCC, whereas it was 11.3 ng/ml in the LC groups. AFP median level was 23 in the patients with HCC and 16.7 ng/ml for the patients with LC.

Receiver operating characteristic curves were plotted to define the optimal cut-off values and to identify the sensitivity and specificity for serum GP73 and AFP in differentiating patients with HCC from patients with cirrhosis without HCC. The area under the receiver operator characteristic (AUROC) curve for GP73 was 0.825 (95% confidence interval: 0.72–0.93), with a sensitivity of 76%, specificity of 75%, NPV of 0.76, PPV of 0.90, and a cut-off value of 16.2 ng/ml. The AUROC curve for AFP was 0.611 (95% confidence interval: 0.49–0.73), with a sensitivity of 63%, specificity of 43%, NPV of 0.29, PPV of 0.77, and a cut-off value of 16.5 ng/ml (Fig. 2). By combining serum GP73 and AFP for the diagnosis of HCC, it was found that sensitivity rises to 93%, specificity was 25%, PPV was 79%, and NPV was 55.6%.

The size of HCC foci ranged from 1 to 4 cm with a mean of 2.05 ± 1.30. In this study, there is a significant positive correlation between the diameter of the hepatic focal lesion and GP73 ($P=0.01$ and $r=0.071$) (Fig. 3). Non-significant positive correlation was detected as regards serum GP73 and the number of HCC ($P=0.60$).

**Discussion**

In Egypt, chronic HCV is the main cause of liver cirrhosis and liver cancer, which is one of the top five leading causes of death [21]. However, Egypt is one of the developing countries with limited resources and a screening program using low cost with high-accuracy tools for early detection of HCC is needed. The lack of AFP sensitivity of 39–65% and specificity of 76–97% [22] has elucidated the need for a new tumor marker, which should be sensitive, specific, and can be used as a single tool.

In this study, GP73 was elevated in all patients with the HCC group showing the highest serum levels when compared with the patients with cirrhosis and healthy controls with a statistically significant difference. This is in agreement with other studies [16,23]. Gu et al. [23] had shown that a serum level of GP73 is higher in patients with liver diseases than in healthy control.
GP73 is better than AFP in differentiating patients with HCC from patients with cirrhosis without HCC. The AUROC curve for GP73 was 0.825 with a sensitivity of 76%, specificity of 75%, at a cut-off level of 16.2 ng/ml, whereas AFP showed an AUROC of 0.611 with a sensitivity of 63%, specificity of 43% at a cut-off level of 16.5 ng/ml, which is similar to the results by Marrero et al. [16], showing that AUROC for GP73 was 0.79 with a sensitivity of 69% and specificity of 86% at a cut-off value of 10 ng/ml, whereas AUROC for AFP was 0.61 with sensitivity of 30%, specificity of 96%, and a cut-off value of 99 ng/ml.

The median level of GP73 was 24.5 ng/ml in patients with HCC, whereas it was 11.3 ng/ml in the LC groups. AFP median level was 23 in the patients with HCC and 16.7 ng/ml for the patients with LC. Marrero et al. [16] reported a rise in the median serum level of GP73 from 5.1 (1.2–23.1 range) for the HCV-related cirrhosis group to 19.4 (range 1–33.1) relative units in those with HCV-related HCC and a significant rise in AFP in the comparative groups, but not as high as the GP73 rise. This is close to the results of our study.

The diameter of HCC foci positively correlated with GP73 serum level. This is in not accordance with another study that stated that there was no correlation between GP73 levels and other prognostic parameters including tumor size [24].

Serum AFP is the most widely studied screening test for detecting HCC. The normal range for serum AFP levels is 10–20 ng/ml and a level more than or equal to 400 ng/ml is usually regarded as diagnostic. Furthermore, some studies have indicated that the high serum concentration of AFP correlates with poor prognosis of patients with HCC. However, two thirds of patients with HCC and a tumor size \( \geq 5 \) cm have serum AFP levels less than 200 ng/ml and up to 20% patients with HCC do not produce AFP [25,26]. The need for a novel tumor marker with higher sensitivity and specificity for the detection of early HCC lesions (T1-T2) is in demand. GP73 had showed higher sensitivity and specificity for the detection of HCC than AFP and the combination of both markers had higher sensitivity, but poorer specificity. In conclusion, GP73 can be used as a diagnostic marker for HCC; however, more studies are needed to verify its role as a prognostic marker.

No conflict of interest to declare.

References