Serum Golgi protein-73 in combination with α-fetoprotein for diagnosing hepatocellular carcinoma in Egyptian patients

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Background and aim
Hepatocellular carcinoma (HCC) is the third cause of cancer-related mortality. α-fetoprotein (AFP) is not a highly sensitive marker for predicting HCC despite its high specificity. Serum Golgi protein-73 (sGP73) seems to be promising new marker. This study aimed at evaluating the role of sGP73 alone and in combination with AFP for diagnosing HCC.

Patients and methods
This study was conducted on 90 Egyptian patients who were equally divided into two groups. Group 1 included 45 patients with hepatitis C virus-related liver cirrhosis without clinical or radiological evidence of HCC, and group 2 included 45 patients diagnosed as having HCC by triphasic abdominal computed tomography. Serum AFP and GP73 were measured using enzyme-linked immunosorbent assay.

Results
A cutoff value greater than or equal to 40 ng/ml for AFP had a sensitivity of 51.1%, specificity of 97.8%, and area under the curve (AUC) of 0.802. sGP73 with a cutoff value greater than or equal to 1.4 ng/ml yielded a sensitivity of 75.6%, specificity of 97.8%, diagnostic accuracy of 86.7%, and AUC of 0.908. The combined use of AFP and sGp73 led to an increase in sensitivity to 84.4%, specificity to 95.6%, diagnostic accuracy to 90%, and AUC to 0.947.

Conclusion
The combined use of AFP and sGp73 could increase the sensitivity and specificity for HCC diagnosis.

Keywords:
α-fetoprotein, golgi protein-73, hepatocellular carcinoma

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Introduction

Hepatocellular carcinoma (HCC) is the fifth predominant cancer worldwide, and the third cause of mortality related to cancers. In addition, it is the main cause of primary liver cancer, accounting for nearly 90% [1]. Chronic hepatitis C and B, aflatoxins exposure [1], hemochromatosis [2], and alcoholic hepatitis [3] are known predisposing factors of HCC.

Hepatitis C virus (HCV) is endemic in the Egyptian population, and HCC rate is estimated to be 14.7% [4]. HCC is ranked the second in men and the sixth in women as the leading cause of mortality related to carcinomas in Egypt [5].

Screening and early detection of HCC provide a hope for cure and increase survival of patients. Ultrasound is the most sensitive measure for screening at present, but it is highly operator dependent. α-fetoprotein (AFP) has been proven to be low in sensitivity and not cost-effective for screening and diagnosis [6–8].

HCC diagnosis depends mainly on radiological findings, where triphasic computed tomography (CT) or MRI show arterial enhancement and delayed venous washout, with only AFP as the blood marker [8]. AFP at a level of 16 ng/ml showed a sensitivity of 62.4% and a specificity of 89.4% in diagnosing HCC [9]. According to Ryder [10], a level greater than 400 ng/ml is usually regarded as diagnostic.

Recent studies have identified Golgi protein-73 (GP73 or Golgi phosphoprotein II) as a novel biomarker for HCC. GP73 is a 400-amino acid, 73-kDa transmembrane glycoprotein that normally resides within the cis-Golgi complex in the epithelial cells of certain human tissues [11]. It is expressed in the

binary epithelial cells of normal liver [12]. It has been previously shown that serum GP73 (sGP73) is significantly elevated in HCC patients [13].

GP73 has been found in a recent meta-analysis to have comparable sensitivity and specificity to that of AFP [10]. These values might be superior to those of AFP [13], and have an additional benefit of increasing AFP accuracy when included in the diagnosis [14].

This study aimed to evaluate the possible role of sGP73 in HCC diagnosis in Egyptian patients alone and in combination with AFP.

Patients and methods

The present case–control study was conducted at the Gastroenterology and Hepatology Unit, Internal Medicine and Tropical Medicine Departments, Ain Shams University Hospital, Cairo, Egypt from December 2014 to December 2015.

It included 90 patients who were divided into two groups.

Group 1 (control group)

This group included 45 patients with liver cirrhosis due to HCV infection. Liver cirrhosis was diagnosed clinically, biochemically, and radiologically. Chronic HCV infection was diagnosed by positive HCV antibody and positive quantitative HCV PCR.

Group II (patient group)

This group included 45 patients with HCC along with HCV-related cirrhosis diagnosed by two imaging modalities – abdominal ultrasound and the characteristic arterial enhancement and venous washout by triphasic abdominal CT.

Written informed consent was obtained from all patients before inclusion to the present study. The Research Ethical Committee of Faculty of Medicine, Ain Shams University, approved the study protocol according to the ethical guidelines of the 1975 Declaration of Helsinki.

Patients with hepatic focal lesions not due to HCC, such as hemangioma, hepatic cyst, or liver metastases, those infected with HIV, as well as those with any autoimmune diseases were excluded.

All participants were subjected to the following:

(1) Complete history taking and clinical examination.

(2) Laboratory investigations including the following:
   (a) Complete blood count was obtained using a Coulter Counter (Beckman Coulter, 250 South Kraemer Boulevard, Brea, California 92821, USA).
   (b) Serum alanine aminotransferase, aspartate aminotransferase (AST), serum bilirubin, albumin, serum creatinine, and blood urea nitrogen were determined using Synchron CX9 autoanalyzer (Scientific Instruments Division, Beckman Instruments Inc., Fullerton, California, USA).
   (c) International normalized ratio was obtained through Diagnostica Stago (Asnieres, France).
   (d) HCV-specific antibodies were detected using a third-generation enzyme-linked immunosorbent assay (ELISA) (Ortho Clinical Diagnostics, 1001 Route 202, Raritan, New Jersey, 08869, USA), and HCV RNA level was detected by HCV real-time reverse-transcription PCR (RT-PCR). HCV RNA was extracted from serum using a QIAamp Viral RNA Mini Kit (Qiagen, Hilden, Germany), the extract was added to Qiagen one-step RT-PCR Master Mix, and real-time RT-PCR was performed using Stratagene Mx3000P (Corbett Research, Mortlake NSW, Australia).
   (e) Serum AFP was assayed using solid-phase ELISA (BC-1009; Bio-Check, 323 Vintage Park Drive Foster City, California, 94404, USA) with a minimum sensitivity of 2.0 ng/ml.
   (f) GP73 was determined using a commercially available ELISA Kit supplied by Glory Science (Glory Science Co., Del Rio, Texas, USA). The kit is based on double-antibody sandwich ELISA to analyze the level of GP73 in samples. The chroma of the color and GP73 concentration of samples were positively correlated, and the concentrations of samples were determined using this standard curve.

(3) Radiological investigations included abdominal ultrasonography and triphasic abdominal CT. Ultrasound examinations were carried out for all recruited patients using an ultrasound machine (Hivision EUB-5500; Hitachi Medical Systems, Tokyo, Japan), a with convex-sector probe (3.57 MHz) for evaluating liver size, liver echogenicity, hepatic focal lesion(s), portal vein diameter and patency, and spleen size together with the detection of ascites and lymph nodes.

Dynamic contrast-enhanced CT scan (Brightspeed 16 MDCT, GE Healthcare, 5730 N Glen Park Rd, Milwaukee, WI 53209, USA) was performed for the diagnosis of HCC. Examination of the number, size, site, and shape of the focal lesion(s) was carried out. HCC was diagnosed when the lesion showed arterial contrast enhancement with delayed venous washout. Presence of liver echogenicity, splenomegaly, portal vein patency, and ascites was also confirmed by CT.

Statistical analysis

Data were analyzed using SPSS software computer program version 18 (SPSS, Chicago, Illinois, USA). Descriptive statistics are presented as means±SDs for continuous parametric variables, as medians and interquartile ranges for continuous nonparametric variables, and as numbers and percentages for categorical variables (frequency distributions). Independent *t*-test was used to test the significance of difference between the mean values of two studied groups with continuous parametric variables. The Mann–Whitney test was used to test the significance of differences regarding continuous nonparametric variables. The χ^2 test was used for comparing categorical variables. Significance level was set at a *P* value less than 0.05. Spearman's correlation test was used when indicated. The optimal cutoff level for diagnosis was obtained from the receiver operating characteristics curve. Predictive value test, likelihood ratio test, and diagnostic accuracy have been used for calculating the diagnostic characteristics of AFP and GP73 for differentiation between HCC and cirrhosis groups:

(1) Predictive value test: positive and negative predictive values, respectively were the proportions of positive and negative results of statistic and diagnostic tests that were true-positive and true-negative results.
(2) The likelihood ratio test was used for assessing the value of a diagnostic test. It used sensitivity and specificity to determine whether a test result usefully changed the probability of existence of a certain condition.
(3) Diagnostic accuracy was related to the ability of a test to discriminate between the target condition and health.

This study is registered at https://clinicaltrials.gov/show/NCT03039322. The registration identification number is (NCT03039322 Unique Protocol ID: 914).

Results

In the present study, HCC was more frequently observed in male patients than in females with a male-to-female ratio of 2.75 : 1. The HCC group included 33 (73.3%) males and 12 (26.7%) females, whereas the control group included 34 (75.6%) males and 11 (24.4%) females. There was a statistically nonsignificant difference between both groups regarding sex (*P*=0.8). Regarding age, patients with HCC had a mean age of 60.8±8.7 years, whereas cirrhotic patients had a mean age of 61.5±10.5 years, with a statistically nonsignificant difference between both groups regarding age (*P*=0.7).

The HCC group included seven (15.6%) patients in Child class A, 15 (33.3%) patients in Child class B, and 23 (51.1%) patients in Child class C. The control group included six (13.3%) patients in Child class A, 16 (35.5%) patients in Child class B, and 23 (51.1%) patients in Child class C, with nonsignificant statistical differences between both groups regarding Child class.

In the present study, 62.2% of HCC cases were from urban areas, 53.3% had a history of smoking, and 4.4% had a history of alcohol intake.

The presenting symptoms in the HCC group were abdominal pain in 68.9% of patients, abdominal distension in 64.4%, and weight loss in 42.2%. The

Table 1 Comparison between both groups regarding laboratory data

Variables	HCC (*N*=45)	Cirrhosis (*N*=45)	*P*
HB (g/dl) (mean±SD)	11.2±1.6	11.0±2.0	0.57^
WBCs (×10^3/ml) (mean±SD)	9.6±14.0	7.8±4.9	0.42^
Platelets (×10^3/ml) (mean±SD)	117.7±52.7	122.8±70.1	0.70^
Uric acid (N=3.4–7.0 mg/dl) (mean±SD)	10.0±2.2	6.1±2.2	<0.001*, ^
Creatinine (N=0.5–1.2 mg/dl) (mean±SD)	2.1±1.6	1.4±1.1	0.018*, ^
Albumin (N=3.5–5.3 g/dl) (mean±SD)	2.6±0.5	2.6±0.8	0.95^
INR (mean±SD)	1.5±0.3	1.6±1.0	0.314^
Total bilirubin (N=0.2–1.2 mg/dl) (mean±SD)	1.5±0.3	1.6±1.0	0.352^
ALT (N=7–40 IU/l) [Median (IQR)]	51.0 (39.0–71.5)	40.0 (28.5–57.5)	0.053#
AST (N=7–37 IU/l) [median (IQR)]	80.0 (56.0–139.5)	69.0 (43.5–102.0)	0.061#

ALT, alanine aminotransferase; AST, aspartate aminotransferase; HB, hemoglobin; HCC, hepatocellular carcinoma; INR, international normalized ratio; IQR, interquartile ranges; N, normal range; WBCs, white blood cells. ^Independent *t*-test. #Mann–Whitney test. *Significant.

most prominent physical features in HCC patients were splenomegaly in 75.6% and the presence of ascites in 64.4%.

There were nonsignificant differences between the HCC group and the cirrhosis group regarding serum alanine aminotransferase, aspartate aminotransferase, bilirubin, albumin, and international normalized ratio. There were significantly higher values of serum uric acid and creatinine in HCC patients than in cirrhotics as shown in Table 1.

Regarding radiological investigations, 33.3% of HCC patients showed heterogeneous hepatic echogenicity, 46.7% had shrunken liver, 64.4% had ascites, and 8.9% had portal vein thrombosis. Twenty-four (53.3%) patients in the HCC group had single hepatic focal lesion, and in 60% of them the lesion was in the right lobe. Five (11.1%) HCC patients had two focal lesions, 10 (22.2%) had three focal lesions, and six (13.3%) had more than three lesions. The median (interquartile range) size of focal lesions in the HCC group was 3.5 (2.5–5.0) cm.

There was a highly significant difference between both groups regarding AFP and GP73 serum levels (Table 2).

There was no significant correlation between sGP73 and other studied parameters in the HCC group. There was a significant negative correlation between sGP73 and age in the cirrhosis group (Table 3). There was no significant relationship between number of hepatic focal lesions and serum levels of AFP and GP73 among HCC patients (Table 4).

Using receiver operating characteristic curve analysis, the cutoff value of serum AFP greater than or equal

Table 2 Comparison between both groups regarding serum α-fetoprotein and Golgi protein-73

Variables	HCC (*N*=45)	Cirrhosis (*N*=45)	*P* value[#]
AFP (ng/ml) [median (IQR)]	56.0 (9.0–1000.0)	7.5 (3.1–12.0)	<0.001[*]
GP73 (ng/ml) [median (IQR)]	4.0 (1.3–10.8)	0.3 (0.2–0.8)	<0.001[*]

AFP, α-fetoprotein, HCC, hepatocellular carcinoma; GP73, Golgi protein-73; IQR, interquartile ranges. [#]Mann–Whitney test. [*]Significant.

Table 3 Correlation between serum Golgi protein-73 and other studied parameters in both groups

Variables	HCC (*N*=45)		Cirrhosis (*N*=45)	
	r	*P*	*r*	*P*
Age	–0.099	0.519	–0.312	0.037[*]
PV diameter	0.076	0.628	–0.272	0.071
HFL size	–0.110	0.471	–	–
Spleen size	–0.117	0.443	–0.004	0.981
HB (g/dl)	–0.040	0.796	0.061	0.690
WBCs (×10^3/ml)	0.005	0.973	0.096	0.532
Platelets (×10^3/ml)	–0.020	0.897	–0.083	0.588
Uric acid (mg/dl)	–0.299	0.066	0.129	0.397
Albumin (g/dl)	–0.108	0.482	0.112	0.464
INR	–0.033	0.830	–0.195	0.200
Total bilirubin (mg/dl)	0.012	0.936	0.030	0.844
ALT (IU/l)	0.017	0.914	–0.003	0.983
AST (IU/l)	0.096	0.531	0.011	0.945
AFP (ng/ml)	0.183	0.230	–0.192	0.206

AFP, α-fetoprotein; ALT, alanine aminotransferase; AST, aspartate aminotransferase; HB, hemoglobin; HCC, hepatocellular carcinoma; HFL, hepatic focal lesion; INR, international normalized ratio; *r*, Spearman's correlation; WBCs, white blood cells. [*]Significant.

Table 4 Relationship between number of hepatic focal lesions and serum levels of α-fetoprotein and Golgi protein-73 in the hepatocellular carcinoma group (*N*=45)

Number of hepatic focal lesions	Number of patients	GP73	AFP
One	24	6.8 (1.6–10.8)	19.3 (7.9–853.5)
Two	5	4.5 (1.3–10.8)	89.7 (17.3–153.5)
Three	10	3.3 (0.9–10.8)	281.5 (12.8–3213.8)
More than three	6	4.0 (0.8–10.8)	580.5 (11.3–8087.0)
P value		0.848	0.153

AFP, α-fetoprotein; GP73, Golgi protein-73.

to 40 ng/ml showed a sensitivity of 51.1%, a specificity of 97.8%, a diagnostic accuracy of 74.5%, and an area under the curve (AUC) of 0.802 in differentiating HCC from liver cirrhosis. The cutoff value of sGp73 greater than or equal to 1.4 ng/ml showed 75.6% sensitivity, 97.8% specificity, 86.7% diagnostic accuracy, and an AUC of 0.908 in differentiating HCC from liver cirrhosis. The combined use of the two markers (AFP and Gp73) increased the sensitivity, specificity, diagnostic accuracy, and AUC to 84.4, 95.6, 90, and 0.947, respectively (Tables 5–7 and Fig. 1).

Discussion

Screening for HCC is crucial in patients with liver cirrhosis, and the use of serological biomarkers in such patients may help early diagnosis and improve survival [8]. AFP has been considered as the only HCC marker for a long time, although its sensitivity of 39–65% is not satisfactory. In addition, it has low specificity because it can also be detected in patients with cirrhosis (up to 47%) and chronic hepatitis (up to 58%). Recent studies have identified sGP73 as a novel biomarker for HCC. The combined measurement of GP73 and AFP has been suggested to increase the sensitivity for HCC detection [15].

Table 5 Diagnostic characteristics of serum α-fetoprotein greater than or equal to 40.0 ng/ml in differentiating hepatocellular carcinoma and cirrhosis groups

	Value	95% CI
Sensitivity (%)	51.1	35.8–66.3
Specificity (%)	97.8	88.2–99.9
Positive predictive value (%)	95.8	78.9–99.9
Negative predictive value (%)	66.7	54.0–77.8
Positive likelihood ratio (LR+)	22.8	3.2–163.1
Negative likelihood ratio (LR–)	0.5	0.4–0.7
Diagnostic accuracy (%)	74.5	65.6–76.6

AFP, α-fetoprotein; CI, Confidence interval.

Table 6 Diagnostic characteristics of serum Golgi protein-73 greater than or equal to 1.4 ng/ml in differentiating hepatocellular carcinoma and cirrhosis groups

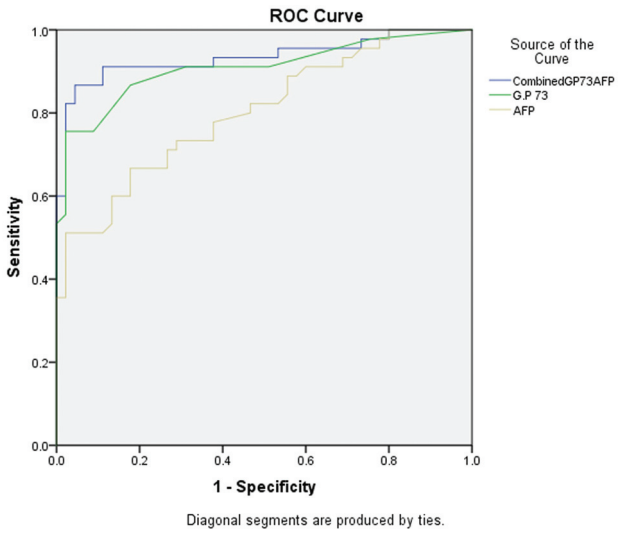
	Value	95% CI
Sensitivity (%)	75.6	60.5–87.1
Specificity (%)	97.8	88.2–99.9
Positive predictive value (%)	97.1	85.1–99.9
Negative predictive value (%)	80.0	76.0–89.6
Positive likelihood ratio (LR+)	33.5	4.7–237.8
Negative likelihood ratio (LR–)	0.3	0.2–0.4
Diagnostic accuracy (%)	86.7	78.0–88.8

CI, confidence interval.

In the present study, all HCC patients were HCV positive. HCV plays an important role in HCC pathogenesis in Egypt, because of its higher prevalence in this area, which is almost 15% [5,16,17].

There were significantly higher median levels of serum AFP and GP73 in the HCC group (56 and 4 ng/ml, respectively) than in the liver cirrhosis group (7 and 0.3 ng/ml, respectively). This is in agreement with the studies by Mao *et al.* [15], El-Awady *et al.* [18], and Dai *et al.* [19]. GP73, which is widely expressed in human epithelial cells, is not expressed in normal human hepatocytes, but is highly expressed in hepatocytes of liver disease patients, especially HCC patients [20]. The elevated levels of GP73 expression identified in HCC may be due to abnormal core fucosylation of GP73 by α1, 6-fucosyltransferase enzyme, which is increased in HCC. However, the precise mechanism of GP73 elevation in HCC is still unclear [21].

Figure 1



Receiver operating characteristic curves showing the sensitivity and specificity of serum a-fetoprotein, Golgi protein-73, and their combination in differentiating hepatocellular carcinoma and cirrhosis groups. The areas under the curves were 0.802, 0.908, and 0.947, respectively.

Table 7 Diagnostic characteristics of Golgi protein-73 greater than or equal to 1.4 ng/ml in combination with α-fetoprotein greater than or equal to 40.0 ng/ml in differentiating hepatocellular carcinoma and cirrhosis groups

	Value	95% CI
Sensitivity (%)	84.4	70.50–93.5
Specificity (%)	95.6	84.9–99.5
Positive predictive value (%)	95.0	83.1–99.4
Negative predictive value (%)	86.0	73.3–94.2
Positive likelihood ratio (LR+)	19.0	4.9–74.1
Negative likelihood ratio (LR–)	0.2	0.1–0.3
Diagnostic accuracy (%)	90.0	81.1–93.6

AFP, α-fetoprotein; CI, confidence interval.

The diagnostic range for measuring GP73 by ELISA produced by most companies lies between 0.6 and 40 ng/ml or between 6.25 and 200 ng/ml. Hence, the cutoff values were variable in previous studies. In the study by Hu *et al.* [13], a cutoff value of 7.4 ng/ml yielded a sensitivity of 77% and a specificity of 84%. In the study of Mao *et al.* [15], a cutoff value of 8.5 ng/ml had a sensitivity and specificity of 74.6 and 97.4%, respectively. However, in the study by Shi *et al.* [22], a cutoff value of 100 ng/ml had a sensitivity and specificity of 98 and 95%, respectively.In the present study, the used kit had a lower range (0.1–10.8 ng/ml), which proved that even in lower ranges of GP73 similar results can be obtained. With a cutoff value greater than or equal to 1.4 ng/ml, the sensitivity was 75.6%, specificity was 97.8%, diagnostic accuracy was 86.7%, and AUC was 0.908. On the other hand, AFP at a cutoff value greater than or equal to 40 ng/ml yielded a sensitivity of 51.1% and specificity of 97.8%, thus showing superior sensitivity and specificity of GP73 over AFP in differentiating HCC from cirrhosis. Similarly, multiple previous reports have shown that sGP73 is superior to AFP as a serum biomarker for HCC [13,15].

There was no correlation between the size or the number of HCC lesions and the levels of either GP73 or AFP, which is in agreement with previous studies performed by Mao *et al.* [15] and Tian *et al.* [23].

A recent systematic review addressed the issue of combining multiple biomarkers in increasing the accuracy of HCC diagnosis. Those biomarkers included AFP isoform (AFP-L3), desgamma carboxy prothrombin, GP73, and AFP. Of those, only the combined use of GP73 and AFP proved to be of useful for both HCC diagnosis and screening with the highest AUC=0.932 [24]. This was confirmed by another meta-analysis, which examined the effectiveness of GP73 in comparison with or in addition to AFP, and concluded that the best diagnostic accuracy was obtained by the combination of AFP and GP73 with an AUC of 0.91 [20]. This is similar to the results of the present study in which combined measurement of AFP and GP73 increased the sensitivity to 84.4% with adequate specificity reaching 95.6%, diagnostic accuracy 90%, and AUC 0.947 in differentiating HCC from liver cirrhosis.

Conclusion

GP73 could be a promising marker for diagnosing HCC, especially when combined with AFP. However, its addition to the HCC guidelines needs more validation on a wide-scale population with geographically variable backgrounds.

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Conflicts of interest

There are no conflicts of interest.

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