Significance of hepatocyte growth factor concentrations in serum of patients with liver cirrhosis and patients with hepatocellular carcinoma
Heba S. El Deen\textsuperscript{a}, Hemmat E. El Haddad\textsuperscript{b}

Objective
Egypt has the highest prevalence of chronic hepatitis C virus (HCV) infection worldwide with the development of cirrhosis and hepatocellular carcinoma (HCC). The hepatocyte growth factor (HGF)–cMET axis promotes cell survival, proliferation, migration, and invasion. This study aimed to evaluate the role of serum HGF as a noninvasive biomarker in the diagnosis of liver cirrhosis and HCC.

Patients and methods
This study included 80 individuals. They were divided into three groups: group 1 included 20 healthy volunteers as a control group, group 2 included 30 patients with liver cirrhosis, and group 3 included 30 patients with HCC.

Results
HGF was highly significantly elevated in the HCC group (median 3709 pg/ml) and the cirrhotic group (median 2843.5 pg/ml) compared with the control group (median 913 pg/ml). \textalpha-Fetoprotein (AFP) was highly significantly elevated in the HCC group (median 128.5 ng/ml) than both the cirrhotic (median 4.9 ng/ml) and the control (median 3.15 ng/ml) group. Results of aspartate aminotransferase/alanine aminotransferase, APRI, fibroindex, and model 3 in the cirrhotic group were highly significantly different from those in the control group. We found a positive significant correlation between HGF and AFP for all the participants studied. There were direct correlations between HGF and aspartate aminotransferase/platelet ratio index (APRI), fibroindex, and model 3. The sensitivity and specificity of HGF for selective detection of the HCC group over the non-HCC group (HCV group and healthy control group) were 93.3 and 46%, respectively, at a cut-off value of 1415 pg/ml, whereas that of AFP were 100 and 92%, respectively, at a cut-off value of 10 ng/ml, and area under the curve of HGF and AFP were 0.787 and 0.999, respectively. The sensitivity and specificity of both AFP and HGF together were 100 and 66%, respectively, at the same cut-off values. The odds ratio of occurrence of HCC in patients with elevated HGF levels was 11.926 and 95% confidence interval 2.56–55.55.

Conclusion
We conclude from this study that both HGF and AFP can be used as noninvasive biomarkers for early detection of HCC in HCV cirrhotic patients, especially if their values match the cut-off levels detected in our study.

Keywords: \textalpha-fetoprotein, APRI, fibroindex, hepatocellular carcinoma, hepatitis C virus, hepatocyte growth factor, model 3

Introduction
Liver cirrhosis represents the final stage of several chronic hepatic diseases [1]. It is a diffuse process of architectural disorganization characterized by fibrosis and the formation of structurally abnormal parenchymal nodules [2]. This results in portal hypertension, portosystemic shunting, and a decrease in the effective parenchymal mass [3]. Cirrhosis is a dynamic condition, where two extreme processes occur: fibrogenesis and fibrolysis [4]. Progressive accumulation of collagen as well as other proteins in the extracellular matrix eventually results in disrupted liver morphology, parenchymal function impairment, and ultimately portal hypertension and its related sequelae [5].

Hepatocellular carcinoma (HCC) ranks as the fifth most common cancer worldwide and the third most frequent cause of cancer-related death [6]. It is one of the fastest tumors resulting from chronic infection by hepatitis B and C viruses [7]. It represents the most common primary malignant tumor of the liver and is one of the major causes of death among patients with cirrhosis [6,8].
In 2001, HCC in Egypt was reported to account for about 4.7% of chronic liver disease patients. In another study, in 2005, a marked increase from 4 to 7.2% was reported over a decade. Patients with advanced liver disease, particularly cirrhosis, are those at risk for HCC and should be screened every 6 months for its development [9].

HCC generally develops following an orderly progression from cirrhosis to dysplastic nodules to early cancer development, which can be cured reliably if discovered before the development of vascular invasion [10].

Among patients with chronic hepatitis C, serum α-fetoprotein (AFP) values are frequently elevated, even in the absence of HCC. Factors associated with elevated AFP include severity of liver disease, female sex, and Black race [11].

Hepatocyte growth factor (HGF) is a multifunctional factor that is produced in various body organs and can affect mitogenesis, cell motility, matrix invasion, and epithelial carcinogenesis [12].

HGF exerts its actions through tyrosine phosphorylation of its receptor, cMET, which is a proto-oncogene product. HGF exerts protective effects on epithelial and nonepithelial organs (including the heart and brain) through antiapoptotic and anti-inflammatory signals. During organ diseases, plasma HGF levels increase significantly. Thus, endogenous HGF is required for minimization of diseases, whereas insufficient production of HGF leads to organ failure. Moreover, emerging studies have delineated key roles of HGF during tumor metastasis, whereas HGF antagonism leads to antitumor outcomes [13].

Under normal physiological conditions, the HGF and its receptor, the MET transmembrane tyrosine kinase (cMET), are involved in embryogenesis, morphogenesis, and wound healing. The HGF–cMET axis promotes cell survival, proliferation, migration, and invasion through modulation of epithelial–mesenchymal interactions. HGF transcription is upregulated by inflammatory modulators such as tumor necrosis factor α, IL-1, IL-6, transforming growth factor-β, and vascular endothelial growth factor (VEGF) [14].

HGF suppresses the increase in transforming growth factor-β1, which plays an essential role in the progression of liver cirrhosis with a decrease in profibrogenic markers such as collagen expression and α-smooth muscle actin and inhibition of fibrogenesis. HGF has also been shown to prevent tissue fibrosis in the kidneys by increasing MMP-9 and suppressing expression of TIMP-1. It also upregulates the antiapoptotic protein Bcl-xL. It has been shown previously that the intrahepatic expression of HGF-specific receptor cMET decreases at an early stage of cirrhosis development and significantly decreases at the time of cirrhosis manifestation, leading to decreased antifibrotic effects despite elevated levels of HGF [15].

HGF (also known as the scattering factor) activates the MET signaling pathway, thereby increasing the invasive and metastatic potential of the cells and allowing the survival of cancer cells in the blood stream and facilitating cancer progression [16]. Dysregulated cMET signaling upregulates protease production (plasminogen dependent and independent) and increased cell dissociation through extracellular matrix degradation, facilitating tumor invasiveness and metastasis. Cross-talk between cMET and epidermal growth factor receptor and cMET and VEGF signaling pathways is also implicated in promoting tumor survival. HGF–cMET signaling induces upregulation of VEGF expression and endothelial VEGFR2 expression and downregulation of endogenous inhibitors of angiogenesis [14]. In HCC, the mRNA levels of HGF and the MET receptor are markedly increased compared with those in normal liver. A high serum HGF concentration is associated with a poor prognosis for overall survival after hepatic resection, and the serum level of HGF represents the degree of the carcinogenic state in the livers of patients with C-viral chronic hepatitis and cirrhosis [16].

**Patients and methods**

This is a cross-sectional observational comparative study that was carried out in Kasr Al Ainy Internal Medicine Clinic on 80 individuals. All patients were informed about their inclusion in the study. The study was approved by the ethical committee of chemical pathology department. The participants were divided into three groups: group 1 included 20 healthy volunteers as a control group, group 2 included 30 patients with liver cirrhosis, and group 3 included 30 patients with HCC. All patients were diagnosed by clinical examination, biochemical investigations, abdominal ultrasonography, and abdominal triphasic computed tomography.

All patients were positive for hepatitis C virus (HCV) as evidenced by positive HCV IgG antibody detected by the ELISA technique. Written informed consents were obtained from all participants.
Exclusion criteria were as follows: spontaneous bacterial peritonitis, inflammatory bowel disease, systemic sepsis, other types of malignancy, diabetes mellitus, chronic renal failure, lung diseases, and vascular or cardiac disorders.

**Methods**

**Blood sample**

Ten milliliters of venous blood was withdrawn from each participant. The samples were divided as follows:

1. Two milliliters collected in an EDTA tube for complete blood count.
2. 1.8 ml collected in 0.4 sodium citrate for prothrombin time (PT), prothrombin concentration (PC), and international normalized ratio (INR).
3. The rest of the sample was collected in a plain tube, left to clot, and then serum was separated and divided into three aliquots: one for blood chemistry, which was assayed on the same day as sample collection, and the second and the third for AFP and HGF, which were stored at -20°C until the time of assay.

All patients and controls in this study were subjected to the following:

Liver function tests including serum alanine aminotransferase (ALT), serum aspartate aminotransferase (AST), serum alkaline phosphatase (ALP), serum γ-glutamyl transferase (GGT), and serum total bilirubin, serum albumin, and serum total protein. The liver functions were assayed using the chemistry autoanalyzer (Dimension from Dade Behring RXL, version 5.2; Siemens Healthcare Diagnostic Inc., Newark, Delaware, USA).

Red blood cells, white blood cells, and platelet counts, hemoglobin concentration, PT, PC, and INR were also determined.

To determine the stage of hepatic fibrosis in our cases, the following equations were used:

**APRI:** AST level (in IU/l, divided by the upper limit of normal) and platelet count (in 10⁹ cells/l) were measured, respectively [17].

**Fibroindex:** this was measured using the following equation [18]:

\[
\text{Fibroindex} = 1.738 - 0.064 \times \left( \frac{\text{platelet count}}{\text{mm}^3/10^9} \right) \\
+ 0.005 \times \text{AST} (\text{IU}/1) + 0.463 \\
[\gamma\text{-globulin} (\text{g/dl})].
\]

**Model 3:** this index may be derived from the following regression formula [19]:

\[
\log \text{odds} = -5.56 - 0.0089 \times \text{platelet} (\times 10^9/1) \\
+ 1.26 \times \frac{\text{AST}}{\text{ALT}} \text{ ratio} + 5.27 \times \text{INR}.
\]

Serum AFP was assayed using the electrochemiluminescence technique (Bayer Healthcare LLC, Tarry Town, New York, USA) [20].

Serum HGF was assayed by the quantitative sandwich ELISA technique using HGF ELISA kit (catalog no. DHG00; R&D Systems Inc., Minneapolis, Minnesota, USA) [21].

**Statistical analysis**

The results were analyzed using the SPSS computer software package (version 13; SPSS Inc., Chicago, Illinois, USA). Quantitative data were presented as mean ± SD for normally distributed data and as medians and percentiles for skewed data. Qualitative data were presented in the form of frequencies and percentiles. Differences among groups were determined using one-way analysis of variance with post-hoc test and Kruskal–Wallis analysis of variance, and/or the Mann–Whitney test for normally distributed and skewed data, respectively. The Pearson χ²-test and/or Fisher’s exact test were used to detect associations between two variables. For correlations between variables, Pearson’s and/or Spearman’s correlation coefficients were calculated. All tests were two tailed and considered statistically significant at P value less than 0.05. Multiple receiver operating characteristic curves were constructed by calculating sensitivities and specificities of the studied analytes at different cut-off points.

**Results**

Comparison between the three groups, normal control participants, patients with liver cirrhosis, and patients with HCC, showed no significant difference in terms of age and sex, but the hematological data were significantly different. The indices of fibrosis, AFP, and HGF were significantly higher in the HCC group compared with the other two groups (Table 1).

The radiological data of the cirrhosis group and the HCC group for detection of cirrhosis, splenomegaly, ascites, and portal hypertension were not significant. The number of masses in the HCC group showed one mass in 22 patients, two masses in five patients, and three masses in three patients (Table 2).

HGF is significantly correlated with all parameters, except AST/ALT, number of masses, mss size, and total.
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proteins. Upon correlation of HGF with the number of masses in the HCC group, we found that the value of HGF tended to be higher with the number of masses, but this was not statistically significant because of the small number of patients (Tables 3 and 4 and Figs 1–21).

The area under the receiver operating characteristics curve of HGF and AFP for the HCC group versus both the cirrhotic and the control groups were 0.787 and 0.999, respectively.

The odds ratio and 95% confidence interval for HGF were 11.926 and 2.56–55.55, respectively.

<table>
<thead>
<tr>
<th>Patients data</th>
<th>Control group (n = 20)</th>
<th>Cirrhosis group (n = 30)</th>
<th>HCC group (n = 30)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sex (male/female) [n (%)]</strong></td>
<td>65/35 [13 (7)]</td>
<td>63.3/36.7 [19 (11)]</td>
<td>70/30 [21 (9)]</td>
<td>0.852</td>
</tr>
<tr>
<td><strong>Age (years)</strong></td>
<td>50.4 ± 4.89</td>
<td>54.3 ± 7.87</td>
<td>53.7 ± 7.3</td>
<td>0.139</td>
</tr>
<tr>
<td><strong>WBCs (×10^3 cell/μl)</strong></td>
<td>7.39 ± 1.46</td>
<td>8.17 ± 3.78</td>
<td>5.64 ± 3.1**</td>
<td>0.001</td>
</tr>
<tr>
<td><strong>RBCs (×10^6 cell/μl)</strong></td>
<td>4.77 ± 0.45</td>
<td>2.99 ± 0.57**</td>
<td>3.62 ± 0.58**</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Platelet (×10^3/mm³)</strong></td>
<td>271.5 (230.25–316)</td>
<td>121.5 (66–162.25)**</td>
<td>117.5 (81.5–151)**</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Hb (g/dl)</strong></td>
<td>13.52 ± 1.75</td>
<td>8.84 ± 1.97**</td>
<td>10.5 ± 1.95**</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>PC%</strong></td>
<td>97.22 ± 8.56</td>
<td>52.45 ± 13.09**</td>
<td>59.48 ± 14.72**</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>PT (s)</strong></td>
<td>11.87 ± 0.85</td>
<td>18.18 ± 3.57**</td>
<td>16.62 ± 2.94**</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>INR</strong></td>
<td>0.96 ± 0.21</td>
<td>1.59 ± 0.39**</td>
<td>1.49 ± 0.24**</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Albumin (g/dl)</strong></td>
<td>20 (16–23.75)</td>
<td>58 (40–80.25)**</td>
<td>60 (44.75–119.5)**</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Protein (g/dl)</strong></td>
<td>21.5 (17–36)</td>
<td>43.5 (27.5–54)**</td>
<td>50 (31–71)**</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>AST (IU/l)</strong></td>
<td>3.06 (1.97–4.55)</td>
<td>4.9 (3.65–8.55)**</td>
<td>128.5 (81.75–239.5)**</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>ALT (IU/l)</strong></td>
<td>70 (57.25–82.5)</td>
<td>98 (60.5–137)</td>
<td>150 (108–210)**</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>ALP (IU/l)</strong></td>
<td>28.5 (19–37.5)</td>
<td>33.5 (24.75–53.5)</td>
<td>54 (30.25–82.75)**</td>
<td>0.002</td>
</tr>
<tr>
<td><strong>GGT (IU/l)</strong></td>
<td>1.44 ± 0.41</td>
<td>1.35 (1.15–2)**</td>
<td>1.4 (0.83–12.06)**</td>
<td>0.006</td>
</tr>
<tr>
<td><strong>Model 3</strong></td>
<td>1.64 (~2.16 to -0.65)</td>
<td>3.21 (2.01–5.08)**</td>
<td>3.05 (1.52–5.25)**</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>APRI</strong></td>
<td>0.17 (0.14–0.28)</td>
<td>1.34 (0.61–3.07)**</td>
<td>1.64 (0.97–3.01)**</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Fibroindex</strong></td>
<td>0.78 (0.64–1.28)</td>
<td>1.35 (1.15–2)**</td>
<td>1.4 (0.83–12.06)**</td>
<td>0.006</td>
</tr>
<tr>
<td><strong>HGF (pg/ml)</strong></td>
<td>913 (770.7–1166.5)</td>
<td>2843.5 (2119–3721)**</td>
<td>3709 (2574.5–5128.75)**</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Data presented as mean ± SD or median (interquartile range); AFP, α-fetoprotein; ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, γ-glutamyl transferase; Hb, hemoglobin; HCC, hepatocellular carcinoma; HGF, hepatocyte growth factor; INR, international normalized ratio; PT and PC, prothrombin time and concentration; RBPS, red blood cells; WBCS, white blood cells; *Significant difference from the control group; †Significant difference from the cirrhotic group.

| Table 2 Radiological data (ultrasonography and triphasic computed tomography) of the patients studied | | |
| Radiological findings | Liver cirrhosis group (n = 30) | HCC group (n = 30) | P value |
| Cirrhosis | 30 (100) | 30 (100) | 1.0 |
| Splenomegaly | 24 (80) | 21 (70) | 0.552 |
| Ascites | 20 (66.7) | 13 (43.3) | 0.119 |
| Portal hypertension | 13 (43.3) | 11 (36.7) | 0.793 |
| **Number of masses** | | | |
| One mass | — | 22 (73.3) | — |
| Two masses | — | 5 (16.7) | — |
| Three masses | — | 3 (10) | — |

HCC, hepatocellular carcinoma.

| Table 3 Correlations between hepatocyte growth factor and aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, γ-glutamyl transferase, total bilirubin, albumin, total protein, α-fetoprotein, and noninvasive indirect biochemical markers for all participants studied | Variables | HGF | R | P |
| AST | 0.653 | <0.001 |
| ALT | 0.458 | <0.001 |
| AST/ALT | 0.169 | 0.133 |
| ALP | 0.501 | <0.001 |
| GGT | 0.289 | 0.009 |
| Total bilirubin | 0.487 | <0.001 |
| APRI | 0.574 | <0.001 |
| Fibroindex | 0.503 | <0.001 |
| Model 3 | 0.537 | <0.001 |
| AFP | 0.561 | <0.001 |
| Number of masses | 0.250 | 0.185 |
| PT | 0.571 | <0.001 |
| PC | −0.607 | <0.001 |
| INR | 0.611 | <0.001 |
| WBCs | 0.012 | 0.914 |
| RBCs | −0.443 | <0.001 |
| Hb | −0.327 | 0.003 |
| Platelet | −0.424 | <0.001 |
| Albumin | −0.508 | <0.001 |
| Protein | −0.031 | 0.785 |
| Mass size | 0.108 | 0.573 |

AFP, α-fetoprotein; ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, γ-glutamyl transferase; Hb, hemoglobin; HGF, hepatocyte growth factor; INR, international normalized ratio; PC, prothrombin concentration; PT, prothrombin time; RBC, red blood cell; WBC, white blood cell.
Discussion
HCC represents the fifth most common cancer in the world and the third most frequent cause of mortality among oncological patients [10]. Egypt has the highest prevalence of chronic HCV infection worldwide, ranging from 6% to more than 40% among regions and demographic groups, leading to increasing rates of HCC [22].

HCV is a major cause of liver disease and it is considered the most common cause of chronic liver disease in Egypt. Although HCV infection is often asymptomatic, it is likely to progress to cirrhosis and HCC; 20% of chronic hepatitis C patients develop cirrhosis after 10–20 years [23]. Early detection of patients with HCC is useful because it yields a better prognosis as HCC tends to grow slowly and remains confined to the liver. Early detection is possible with ultrasound scanning and AFP monitoring, although the use of AFP as a screening test is complicated by frequent false-positive and false-negative results [24].

HGF, or scatter factor, was first identified as a factor

| Table 4 Diagnostic sensitivity, specificity, accuracy, predictive value of negative, and predictive value of positive of serum hepatocyte growth factor level of hepatocellular carcinoma patients in comparison with α-fetoprotein for selective detection of hepatocellular carcinoma |
|-------------------------------------------------|-----|-----|-----|
| Diagnostic data                               | HGF (%) | AFP (%) | Both HGF and AFP (%) |
| Sensitivity                                   | 93.3 | 100.0 | 100.0 |
| Specificity                                   | 46.0 | 92.0  | 66.0  |
| PPV                                           | 50.9 | 88.2  | 62.2  |
| NPV                                           | 92.0 | 100.0 | 100.0 |
| Accuracy                                      | 63.7 | 95.0  | 78.2  |
| Cut-off                                       | 1451 pg/ml | 10 ng/ml |

AFP, α-fetoprotein; HGF, hepatocyte growth factor; NPV, negative predictive value; PPV, positive predictive value.

Correlations between hepatocyte growth factor (HGF) and α-fetoprotein (AFP) for all the participants studied. There is a positive correlation between HGF and AFP for all patients (groups 2 and 3) ($P = 0.000$ and $r = 0.561$).

Correlation between hepatocyte growth factor (HGF) and aspartate aminotransferase (AST).

Correlation between hepatocyte growth factor (HGF) and alanine aminotransferase (ALT).

Correlation between aspartate aminotransferase (AST)/alanine aminotransferase (ALT).
from plasma from humans and rabbits, and also rat platelets that could induce the proliferation of hepatocytes in culture. Following its initial discovery, HGF was shown to be produced primarily by mesenchymal cell types, especially fibroblasts, in a...
variety of tissues including the lung, heart, kidney, liver, skin, and brain [25].

The aim of this study was to assess the serum level of HGF and AFP in patients with liver cirrhosis.
with HCV infection and those of HCC, in addition to HCV, and to compare them with normal individuals to evaluate the role of serum HGF as a noninvasive biomarker in the diagnosis of liver cirrhosis and HCC. This study was carried out on 80 individuals divided into three groups: group 1 included 20 healthy control participants, group 2 included 30 patients with HCV infection and liver cirrhosis, and finally, group 3 included 30 patients with HCC, in addition to HCV infection and liver cirrhosis.

In our study, the serum HGF levels were 535–1529, 925–7481, and 1369–8717 pg/ml in the control, cirrhotic, and HCC groups, respectively. HGF was highly significantly elevated in the HCC group [median 3709 (2574.5–5128.75) pg/ml] and the cirrhotic group [median 2843.5 (2119–3721) pg/ml] compared with that of the control group [median 913 (770.7–1166.5) pg/ml] (P = 0.000).

Several researchers have confirmed similar results of increased HGF levels in patients with liver cirrhosis and HCC compared with normal control participants such as the studies of Shiota et al. [26] and Vejchapipat et al. [27]. In our study, the level of HGF was higher in the HCC group than that in the cirrhotic group, but this difference was not statistically significant.

However, Yamagami et al. [28], found that the mean serum HGF concentration was significantly higher in patients with HCC than in patients with chronic hepatitis or cirrhosis.

Karabulut et al. [29] found that the baseline serum HGF levels were significantly higher in patients with HCC than in the control group (P < 0.001).

The serum AFP levels in our study were 1.2–8.4, 2.6–30, and 25.9–745 ng/ml in the control, the cirrhotic, and the HCC group, respectively.
The AFP level was highly significantly increased in the HCC group [median 128.5 (81.75–239.5) ng/ml] than both the cirrhotic group [median 4.9 (3.65–8.55) ng/ml] and the control group [median 3.15 (1.97–4.55) ng/ml] (P = 0.000). The AFP level of the cirrhotic group was still higher than that of the control group, but this difference was not statistically significant. Similar to our results, Jia et al. [30] found that serum AFP level was significantly higher in HCC patients than in liver cirrhosis patients and healthy individuals. The results from the studies of Hussein et al. [9] and Cheng et al. [31] yielded the same results. Gadelhak et al. [32] found a significantly elevated AFP level in the HCC group than that in the healthy group.

El Badrawy et al. [33], in their study in 2013, reported that tissue expression of AFP showed positivity in cirrhosis and high expression in HCC and that of HGF was higher in the liver cirrhosis and HCC groups compared with the control group.

Our study showed that the fibrotic indices AST/ALT, APRI, fibroindex, and model 3 were significantly higher in the cirrhotic group compared with the control group (P = 0.006 for all), confirming their role as noninvasive markers for liver fibrosis. These results were in agreement with those of Lackner et al. [34] as these fibrotic indices can enable the diagnosis of liver cirrhosis.

In our study, we found a positive significant correlation between HGF and AFP for all the participants studied (r = 0.561, P = 0.000); however, we did not find this correlation in each group separately. The same results were reported by Yamagamim et al. [28].

In our study, there were direct correlations between HGF and APRI, fibroindex, and model 3 (Table 3).

Our study showed direct significant correlations between HGF and each of AST, ALT, ALP, GGT, and total bilirubin in all the participants studied (r = 0.653, P = 0.000; r = 0.458, P = 0.000; r = 0.501, P = 0.000; r = 0.289, P = 0.009; and r = 0.487, P = 0.000, respectively). The same positive correlations were reported in the study by Shiota et al. [26], but Yamagamim et al. [28] showed that serum HGF level was not significantly correlated with other indicators of liver functions, such as the AST level, the ALP level, and the GGT level.

Karabulut et al. [29] found that male patients had higher serum HGF levels compared with female patients (P = 0.01). Serum HGF levels were significantly higher in the patients with elevated serum ALT levels than others with normal serum ALT levels (P = 0.05) [29].

Our study also showed indirect significant correlations between HGF and albumin in all the participants studied (r = -0.508, P = 0.000) as shown in Table 3 and direct significant correlations between HGF and each of PT and INR in all the participants studied (r = 0.571, P = 0.000 and r = 0.611, P = 0.000, respectively) as shown in Table 5. Shiota et al. [26], found the same correlation with albumin, but in their study, serum HGF levels showed a negative correlation with PT.

Upon correlation of HGF with the number of masses in the HCC group, we found that the value of HGF tends to be higher with the number of masses, but this was not statistically significant because of the small number of patients.

The sensitivity and specificity of HGF have been shown to vary with the different cut-off values used. According to our results, at a cut-off 1451 pg/ml (1.451 ng/ml), the sensitivity was 93.3%, the specificity was 46%, and area under the curve (AUC) was 0.787.

The concentration of HGF (mean 3.69 vs. 5.58 ng/ml, AUC 0.71) was found in the study of Andersen et al. [35], and it was significantly higher (among other studied parameters) in patients with cirrhosis.

Yamagamim et al. [28] found in their study that the cut-off value of HGF for suspecting HCC in their cirrhotic patients was above 0.6 ng/ml and they concluded from their study that HGF may be a critical marker for emergence of HCC in their patients. Of course, they detected the HGF after only two days of storage of their samples, which is why their values were low and more specific [28]. In our study, our samples were stored till the end of collection; this explains the higher cut-off value and the decreased specificity.
The sensitivity and specificity of AFP have been shown to vary with the different cut-off values used. In our study, at a cut-off of 10 ng/ml, the sensitivity was 100%, the specificity was 92%, and AUC was 0.999.

Taketa et al. [36] reported a sensitivity of 95% and a specificity of 66% for AFP when the cut-off value was 10 ng/ml. Gad et al. [37] detected 99% sensitivity and 75% specificity for AFP for the diagnosis of HCC in Egyptian patients at a cut-off value of 10 ng/ml. Hussein et al. [9] found that the sensitivity and specificity of AFP at a cut-off value of 7.7 ng/ml were 89.8 and 93.3%, respectively. Stefanik et al. [38] reported that the sensitivity and specificity of AFP at a cut-off value of 20 ng/ml were 60 and 90%, respectively, Chen et al. [39] reported that the sensitivity and specificity of AFP were 72 and 78% when the cut-off value was 20 ng/ml, and Lok et al. [40] reported that the sensitivity and specificity of AFP were 61 and 81% at a cut-off value of 20 ng/ml. Sheng et al. [41] found that AUC for AFP was 0.771, Marrero et al. [42] found that AUC for AFP was 0.8, and Yamamoto et al. [43] found that AUC was 0.79 for AFP.

Zhou et al. [44] found that the serum AFP level is associated with more clinicopathological variables of HCC at a cut-off value of 400 ng/ml than 20 ng/ml. However, serum AFP level at a cut-off value of 20 ng/ml is of significant prognostic impact for both overall and tumor-free survival, whereas that under 400 ng/ml is not.

We conclude from this study that both HGF and AFP can be additional useful factors as noninvasive biomarkers for the early detection of HCC in HCV cirrhotic patients cause that the specificity of HGF is only 46% yet the specificity of AFP is 92%. Of course, this is highly premature to establish as a well-established fact because to confirm our findings, we need a large-scale study of Egyptian patients using the same parameters as well as the noninvasive markers for liver fibrosis instead of the invasive liver biopsy, especially as those markers were positively correlated with both HGF and AFP and delineated in a great way the liver pathological status of the patients in our study. We also need to evaluate the HGF in our samples within 48 h of the extraction of blood to avoid the spurious increase in HGF in stored samples, which may have altered the specificity and the cut-off value in our study. We also need to extend our research to include a large number of HCC patients with different numbers of masses and to correlate the number of these masses with the value of HGF. For the future, we must carry out genetic polymorphism studies of HGF.

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### Conflicts of interest
There are no conflicts of interest.

### References
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