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ORIGINAL ARTICLE

Utility of Alpha-Fetoprotein-L3 and Golgi Protein 73 for Diagnosis of Hepatocellular Carcinoma

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ABSTRACT

Background: *Hepatocellular carcinoma (HCC) is amongst the most common malignant tumors that carries a poor prognosis. Clinically, alpha-fetoprotein (AFP) is the most extensively used serum biomarker for diagnosing HCC.* ***Objectives:*** *The current study was conducted to explore the diagnostic value of serum levels of alpha-fetoprotein-L3 (AFP-L3) and Golgi protein 73 (GP73) regarding HCC, and to determine the diagnostic accuracy of these biomarkers when used individually as well as in combination with AFP.* ***Methodology:*** *Blood samples were collected from 50 patients with HCV-related cirrhosis (25 subjects with HCC and 25 without HCC) recruited from the outpatient clinics of the Specialized Internal Medicine Hospital, Mansoura University, Egypt. Serum concentrations of AFP-L3 and GP73 were evaluated using enzyme-linked immunosorbent assay (ELISA). Diagnostic performance of AFP-L3 and GP73 was determined by receiver operating characteristic (ROC) curve analysis.* ***Results:*** *Overall, the median serum level of AFP-L3 was higher in the HCC group compared to the cirrhotic group ($p=0.05$). Moreover, a statistically-significant difference was observed between the median serum value of GP73 in HCC patients compared to those with cirrhosis ($p < 0.001$). The ROC curve analysis showed that the area under the ROC curve (AUROC) values for AFP, AFP-L3 and GP73 were 0.88, 0.67 and 0.83, respectively. Of the 3 biomarkers, GP73 demonstrated the highest sensitivity (88%). The AUROC for AFP and AFP-L3 combination was 0.85, whereas that for AFP and GP73 was 0.90.* ***Conclusion:*** *Our findings indicate that GP73 is more sensitive than AFP and AFP-L3 in diagnosing HCC. Furthermore, the combined determination of GP73 and AFP could improve the diagnostic ability of HCC.*

INTRODUCTION

Universally, hepatocellular carcinoma (HCC) constitutes a substantial public health burden secondary to the outstandingly violent nature of this tumor. It ranks the fifth most common malignant tumor and the third leading cause of cancer-related mortality all over the world [1]. About 72% of the cases occur in Asia (more than 50% in China), 7.8% in Africa and 5.1% in North America [2].

It was estimated that HCC accounts for 70.48% of all liver tumors among Egyptians [3]. In the past decade, the incidence of HCC showed dramatic increase among Egyptians. This can be ascribed to several key elements including; hepatitis B, hepatitis C and aflatoxins. Other factors such as cigarette smoking, pesticides and endemic infections in the community, like schistosomiasis, may have further roles in the etiology or progression of the disease [4].

Because most patients with HCC are discovered at an advanced stage with a pre-existing liver disorder, the mortality rate of HCC is similar to the incidence rate. Accordingly, early detection of HCC is a paramount concern since HCC patients can receive effective treatment modalities including liver resection, percutaneous ablation and liver transplantation. Even though developments in imaging techniques, such as multi-detector computed tomography (MDCT) and magnetic resonance imaging (MRI), have improved the early radiologic diagnosis of HCC, atypical radiological findings in some patients can be cumbersome [5].

The current noninvasive tools for diagnosis of HCC include abdominal ultrasound (US) and the use of tumor markers such as serum alpha-fetoprotein (AFP) concentration [6]. Commonly, a serum AFP level of 20 ng/ml is used as a cutoff value to distinguish HCC from non-HCC [7]. Nevertheless, serum AFP testing has a low sensitivity, being normal in up to 40% of patients with HCC, particularly during the early stage of the disease [8]. Furthermore, it is often considerably high in patients with either cirrhosis or with aggravated chronic hepatitis without HCC [9]. Hence, there is a growing need for more

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reliable serum biomarkers that may improve the sensitivity for early detection of HCC.

Amongst these promising biomarkers, lens culinaris agglutinin-reactive fraction of AFP (AFP-L3) has been proposed. It represents a fucosylated variant of AFP that binds to a lectin and displays serum levels that are even with levels of AFP in human sera[10]. On the other hand, Golgi protein 73 (GP73) is a resident Golgi transmembrane glycoprotein that is constitutively expressed by biliary epithelial cells but minimally by hepatocytes. GP73 expression by hepatocytes is strikingly upregulated if there is a liver insult whatever the cause [11].

Many studies have investigated the usefulness of AFP-L3 and GP73 in diagnosing HCC. Nonetheless, results related to the diagnostic values of these biomarkers are still debatable. We, therefore, carried out this study to evaluate serum levels of AFP-L3 and GP73 in relation to HCC diagnosis and to determine the alone and joint diagnostic accuracy of these biomarkers when used with AFP.

METHODOLOGY

Study participants:

The subject cohort included 50 patients with HCV-related cirrhosis selected from the Hepatology Outpatient Clinics and the Early HCC Detection Clinic belonging to the Specialized Internal Medicine Hospital, Mansoura University, Egypt. All patients were enrolled in the study from June 2016 to May 2017 after signing an informed consent. The study design was approved by the local Institutional Review Board, Faculty of Medicine, Mansoura University.

Study groups:

The study cohort was further subdivided into 2 groups: group I; composed of 25 HCV-related cirrhotic patients with newly-developed HCC proved radiologically by abdominal US and triphasic abdominal CT (92% males and 8% females; age: 56.8 ± 6.6 years), and group II; composed of 25 HCV-related cirrhotic patients without HCC (60% males and 40% females; age: 58.8 ± 6.3 years). Demographic and clinical characteristics of the study population are shown in table 1.

The severity of liver cirrhosis was classified according to Child-Turcotte-Pugh classification into child A, B and C [12]. Among HCC group; Child A class was found in 2 patients, Child B in 8 patients and Child C in 15 patients. On the other hand, in the liver cirrhosis group; Child A class was detected in 9 patients, Child B in 11 patients and Child C in 5 patients. Tumor staging was determined by the Barcelona Clinic Liver Cancer (BCLC) staging system for HCC [13] as follows: BCLC stage 0 (very early HCC); no patients, BCLC stage A (early HCC); 8 patients (32%), BCLC stage B (intermediate HCC); 15 patients (60%) and BCLC stage C (advanced HCC); 2 patients (8%).

Exclusion criteria for the study group included all other conditions associated with elevated AFP rather than liver disease, patients with concomitant or past history of cancer, HCC patients who previously received specific treatment, patients with history of recent surgery, presence of severe co-morbidity such as advanced renal failure or decompensated heart failure and patients with any inflammatory conditions as spontaneous bacterial peritonitis (SBP) or chest infection.

Laboratory work-up:

Patients' fasting venous blood samples were obtained during their initial evaluation for HCC development. Liver function tests were determined using the commercially available chemiluminescence immunoassay analyzer (Roche Diagnostics Ltd., Shanghai, China). Bleeding profile including prothrombin time and international normalized ratio (INR) was investigated. Serum AFP concentrations were determined on the Elecsys 2010 using chemiluminescence immunoassay analyzer.

Measurement of serum levels of AFP-L3 and GP73:

Serum levels of AFP-L3 and GP73 were determined by enzyme-linked immunosorbent assay (ELISA, Sunred) using the commercially available ELISA kit provided by Sunred (sunredbio@msn.cn, Germany) according to the manufacturer's guidelines.

Statistical analysis:

Data were entered and statistically analyzed using the Statistical Package for Social Sciences (SPSS) version 22. Qualitative data were described as numbers and percentages with Chi-square (X^2) test used for comparison. Quantitative data were described as median and range after testing normality by Kolmogorov-Smirnov test. The Mann-Whitney test was used for comparison between groups. Receiver operating characteristic (ROC) curves were used to calculate validity (sensitivity and specificity) of continuous variables with calculation of best cutoff point. p values < 0.05 were considered to be statistically-significant.

RESULTS

Characteristics of the study cohort:

The demographic and clinical features of the study cohort are illustrated in table 1. No statistically-significant difference was observed between the HCC group and liver cirrhosis group regarding the age ($p > 0.05$). However, patients diagnosed with HCC showed a statistically-significant male preponderance (p=0.008).

Table 1: The demographic and clinical data of the study population

	HCC patients (n=25)	Liver cirrhosis patients (n=25)	Test of significance	*p* value
1) Age/years (Mean ± SD)	56.8 ± 6.6	58.8 ± 6.3	t=1.09	0.28
2) Gender	n (%)	n (%)		
• Male	23 (92.0)	15 (60.0)	χ^2=7.02	0.008*
• Female	2 (8.0)	10 (40.0)		
• Male : Female ratio	23 : 2	3 : 2		
3) Habits				
• Non smoker	15 (60.0)	19 (76.0)	χ^2=1.47	0.23
• Smoker	10 (40.0)	6 (24.0)		
4) Medical history				
• Diabetes mellitus	17 (68.0)	11 (44.0)	χ^2=2.9	0.08
• Hypertension	9 (36.0)	7 (28.0)	χ^2=0.37	0.54

***Abbreviations**: HCC: hepatocellular carcinoma; n: number of patients; t: Student t test; χ2: Chi-square test; p: probability and *statistically-significant (p <0.05).*

Radiologic findings of patients with HCC:

Ultrasonographic (US) examination revealed that out of 25 cases with HCC, 80% had a single focal lesion and 20% had multiple focal lesions. In addition, 34% of the patients had tumor size larger than 3 cm.

Liver function-related parameters:

For liver function tests, the serum levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were significantly higher in the HCC group compared to the liver cirrhosis group (66 *versus* 39 U/L; *p*=0.001, and 65 *versus* 43 U/L; *p*=0.003, respectively). Also, the serum level of total bilirubin was higher in the HCC group (2.7 *versus* 1.3 mg/dl; *p*=0.001), albeit no statistically-significant difference was detected among both groups in relation to serum albumin level (*p*=0.32). On the other hand, a considerable difference was found between the study groups regarding the INR (*p*=0.007). Data are presented in table 2.

Table 2: Laboratory parameters of patients with hepatocellular carcinoma *versus* patients with liver cirrhosis

	HCC patients (n=25)	Liver cirrhosis patients (n=25)	Test of significance	*p* value
ALT (U/L) Median (Min–Max)	66.0 (19.0–120.0)	39.0 (12.0–63.0)	Z = 3.3	0.001*
AST (U/L) Median (Min–Max)	65.0 (12.0–165.0)	43.0 (24.0–98.0)	Z = 2.98	0.003*
Total bilirubin (mg/dl) Median (Min–Max)	2.7 (1.1–33.3)	1.3 (0.7–9.6)	Z = 3.38	0.001*
Direct bilirubin (mg/dl) Median (Min–Max)	0.9 (0.2–23.7)	0.3 (0.1–5.2)	Z = 3.3	0.001*
Albumin (gm/dl) Mean ± SD	2.54 ± 0.59	2.71 ± 0.59	t = 1.00	0.32
INR Mean ± SD	1.59 ± 0.4	1.29 ± 0.32	t = 2.8	0.007*

Normally distributed data are expressed as mean ± SD. Other data are expressed as median (minimum, maximum).

***Abbreviations**: HCC: hepatocellular carcinoma; n: number of patients; ALT: alanine aminotransferase; AST: aspartate aminotransferase; Min: minimum; Max: maximum; SD: standard deviation; INR: international normalized ratio; t: Student t test; Z: Mann-Whitney U test; p: probability and *statistically-significant (p <0.05).*

Results of serum biomarkers' testing:

Among the 50 tested serum samples, the median serum level of AFP in the HCC cohort was greatly higher when compared to the liver cirrhosis cohort (109.5 *versus* 3.31 ng/ml; $p < 0.001$). Furthermore, the median serum value of AFP-L3 was higher in the HCC group than that in the liver cirrhosis group (3.54 ng/ml *versus* 2.82 ng/ml), but with a borderline significant difference (*p*=0.05). In addition, the median serum concentration of GP73 showed significantly different results in HCC patients compared to those with liver cirrhosis (25.2 *versus* 12.0 ng/ml; $p < 0.001$). Results are summarized in table 3.

Table 3: Serum concentrations of AFP, AFP-L3 and GP73 in the study groups

	HCC patients (n=25)	Cirrhotic patients (n=25)	Test of significance	*p* value
AFP (ng/ml) Median (Min –Max)	109.5 (2.2–2000.0)	3.31 (1.23–43.6)	Z = 4.3	<0.001*
AFP-L3 (ng/ml) Median (Min –Max)	3.54 (1.3–34.65)	2.82 (0.0–7.72)	Z = 1.95	0.05
GP73 (ng/ml) Median (Min –Max)	25.2 (10.3–161.0)	12.0 (10.0–26.7)	Z = 4.24	<0.001*

Abbreviations: *HCC: hepatocellular carcinoma; n: number of patients; AFP: alpha-fetoprotein; AFP-L3: alpha-fetoprotein-L3; GP73: Golgi protein 73; Min: minimum; Max: maximum; Z: Mann-Whitney U test; p: probability and *statistically-significant (p <0.05).*

Diagnostic performance of AFP, AFP-L3 and GP73 in HCC:

We assessed the diagnostic accuracy of AFP, AFP-L3 and GP73 using the receiver operating characteristic (ROC) curve analysis as shown in figure 1. For AFP, at a cutoff value of ≥ 5.14 ng/ml, the maximum area under the ROC curve (AUROC) in discriminating HCC from liver cirrhosis was 0.88 (95% confidence interval; 0.78–0.98) and the sensitivity, specificity and diagnostic accuracy were 80%, 83.3% and 81.8%, respectively. On the other hand, at a cutoff value of ≥ 2.88 ng/ml, the AUROC for AFP-L3 was 0.67 (95% confidence interval; 0.51–0.83) with a sensitivity of 60%, a specificity of 56% and a diagnostic accuracy of 58%. For GP73, at a cutoff value of ≥13.8 ng/ml, the maximum AUROC was 0.83 (95% confidence interval; 0.71–0.96) with 88% sensitivity, 64% specificity and 76% diagnostic accuracy.

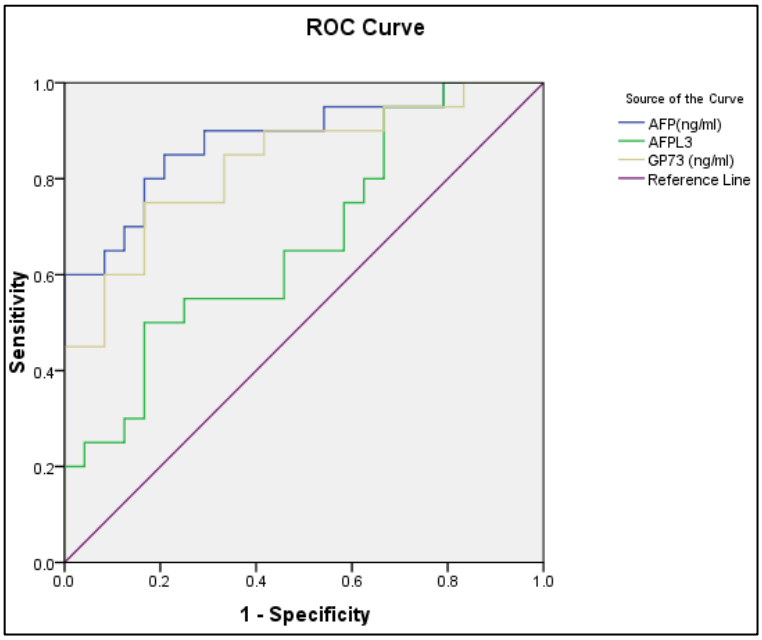

Fig. 1: Receiver operating characteristic (ROC) curve for alpha-fetoprotein (AFP), alpha- fetoprotein-L3 (AFP-L3) and Golgi protein 73 (GP73) in diagnosing hepatocellular carcinoma

The maximum area under the ROC curve (AUROC) values for AFP, AFP-L3 and GP73 in discriminating HCC from liver cirrhosis were 0.88, 0.67 and 0.83, respectively.

When we combined AFP and AFP-L3, the sensitivity, specificity and diagnostic accuracy were 80%, 76% and 78%, respectively (AUROC=0.85); meanwhile, AFP and GP73 combination had 89% sensitivity, 70% specificity and 84% diagnostic accuracy (AUROC=0.90). Of note, the combination of the 3 biomarkers (AFP, AFP-L3 and GP73) as a panel increased the sensitivity, specificity and diagnostic accuracy to 90%, 87.5% and 88.6%, respectively (AUROC=0.94). Data are demonstrated in table 4.

Table 4: Diagnostic performance of AFP, AFP-L3 and GP73 in distinguishing hepatocellular carcinoma from liver cirrhosis

	AUC (95% CI)	Cut off value	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Accuracy (%)
AFP (ng/ml)	0.88 (0.78–0.98)	≥ 5.14	80.0	83.3	80.0	83.3	81.8
AFP-L3 (ng/ml)	0.67 (0.51–0.83)	≥ 2.88	60.0	56.0	57.7	58.3	58.0
GP73 (ng/ml)	0.83 (0.71–0.96)	≥ 13.8	88.0	64.0	71.0	84.2	76.0
Combination of AFP, AFP-L3 and GP73	0.94 (0.87–1.02)		90	87.5	85.7	91.3	88.6
AFP and AFP-L3	0.85 (0.74–0.95)		80.0	76.0	76.9	79.2	78.0
AFP and GP73	0.90 (0.77–0.97)		89.0	70.0	71.0	81.0	84.0

Abbreviations: *AFP: alpha-fetoprotein; AFP-L3: alpha-fetoprotein-L3; GP73: Golgi protein 73; AUC: Area under curve; PPV: Positive predictive value and NPV: Negative predictive value.*

DISCUSSION

In the past 10 years, a remarkable growth in the prevalence of *HCC* among Egyptian patients with liver cirrhosis was perceived [14]. This could be attributed to the increasing exposure to many risk factors such as HCV and HBV infection. Advances in the diagnostic techniques of HCC along with prolonged survival of patients with cirrhosis may also be contributing explanations to such increase [15].

Despite the availability of different treatment options, the general prognosis of this aggressive tumor is still poor as most of the patients are diagnosed at a late stage [16]. Therefore, there is a necessity for evolving non-invasive reliable economic biomarkers for early detection of HCC to improve the overall survival rate of the patients. Accordingly, we conducted this research to assess the diagnostic performance of AFP-L3 and GP73 in this context.

In the existing study, serum levels of AFP-L3 and GP73 were checked in a cohort of 50 patients [25 HCV-related cirrhotic patients with newly-developed HCC (92% males and 8% females; age: 56.8 ± 6.6 years) and 25 HCV-related cirrhotic patients without HCC (60% males and 40% females; age: 58.8 ± 6.3 years)]. Our data demonstrated that at a cutoff value of ≥ 2.88 ng/ml, AFP-L3 had a sensitivity of 60%, a specificity of 56% and a diagnostic accuracy of 58% in differentiating HCC from hepatic cirrhosis. Though the median serum level of AFP-L3 was higher in the HCC group compared to the cirrhotic group (3.54 ng/ml *versus* 2.82 ng/ml), a marginal significant difference was recognized between both groups in this perspective (p=0.05).

Noteworthy, based on the results of this study, AFP-L3 had a lower sensitivity, specificity and diagnostic accuracy compared to AFP (60%, 56% and 58% *versus* 80%, 83.3% and 81.8%, respectively). In addition, analysis of the ROC curve showed that the AUROC for AFP-L3 is smaller than that of AFP (0.67 *versus* 0.88, p=0.01). This finding denotes that AFP-L3 has a lower discriminating power between HCC and liver cirrhosis.

In agreement with our results, Park et al. reported 59% sensitivity and 75% specificity for AFP-L3 in their cohort of HCC patients[17]. Similarly, Nouso and his colleagues concluded that the sensitivity of AFP-L3 was 51.5% in HCC patients [18]. In addition, Leerapun and his coworkers noticed that AFP-L3 had a sensitivity of 71% and a specificity of 63% for diagnosing HCC[19]. Nonetheless, Sterling et al. declared that AFP-L3 had only 37% sensitivity, but reached up to 92% specificity in their multicenter prospective study [20]. Alongside, Miura and his group concluded that AFP-L3 could not offer an exclusively reasonable key biomarker to detect HCC [21]. Consequently, results from our study and from previous studies denote that AFP-L3 has a limited usefulness as an independent diagnostic biomarker for HCC.

With reference to GP73, our study revealed that at a cutoff value of ≥ 13.8 ng/ml, this biomarker had 88% sensitivity, 64% specificity and 76% diagnostic accuracy. Moreover, the maximum AUROC was 0.83 with a statistically-significant difference between patients with HCC and those without HCC (p < 0.001). In line with these results, Marrero and his group affirmed that serum GP73 levels were considerably increased in patients with HCC compared to the cirrhotic group [22]. Likewise, our results are supported with the findings of Hou et al., where serum levels of GP73 were markedly higher in patients with HCC than those with cirrhosis (p < 0.001) with 73.4% sensitivity and 79 % specificity [23]. Recently, Jiao and his associates found that serum GP73 concentration had significant increase in patients with HCC in comparison with cirrhotic patients with AUROC=0.840 [24]. In contrast to

our results, Tian et al. found that serum levels of GP73 in HCC patients were amazingly lower than that in liver cirrhosis cohort. However, their assumptions may be attributed to sample selection biases in their study [25].

The analysis of serum biomarkers' combinations may deliver more precise and valuable evidence for future HCC diagnosis and prognosis [26]. In this work, our data proved that the combination of AFP, AFP-L3 and GP73 enhanced the sensitivity of HCC detection up to 90%. Besides, the combination of the 3 biomarkers significantly amended the diagnostic accuracy of HCC detection up to 88.6%, with good ability in discriminating HCC from liver cirrhosis (AUROC=0.94). On the other hand, AFP and AFP-L3 combination had 80% sensitivity, 76% specificity and 78% diagnostic accuracy; whereas, AFP and GP73 combination showed 89% sensitivity, 70% specificity and 84% diagnostic accuracy. These findings specify that AFP+GP73 combination is superior to AFP+AFP-L3 in diagnosing HCC. In accordance with these results, Morota et al., and Romeo et al. recorded 89% and 79% sensitivity to AFP+GP73 and AFP+AFP-L3 combinations, respectively, with respect to HCC diagnosis [27, 28].

CONCLUSION

The current data obtained denote that GP73 has a higher sensitivity than AFP and AFP-L3 in diagnosing HCC. Although GP73 and AFP combination increased the diagnostic performance, the combined effect of AFP and AFP-L3 testing did not improve the capability of AFP-L3 to discriminate between HCC and liver cirrhosis. The major limitations of this study are the relatively small number of newly-diagnosed HCC patients, as well as the short follow up interval. Thereby, results of this study need to be authenticated, in the future, in a larger cohort of patients with a longer follow up time frame to justify their application in clinical settings.

Conflicts of interest:
The authors declare that they have no financial or non financial conflicts of interest related to the work done in the manuscript.

- Each author listed in the manuscript had seen and approved the submission of this version of the manuscript and takes full responsibility for it.
- This article had not been published anywhere and is not currently under consideration by another journal or a publisher.

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