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ORIGINAL RESEARCH

Significance of Glypican-3 in Early Detection of Hepatocellular Carcinoma in Cirrhotic Patients

Ahmed M. Tahon[1] · Magdy Z. El-Ghanam[2] · Samy Zaky[3] · Tarek Mostafa Emran[2] · Ali M. Bersy[2] · Fathiya El-Raey[3] · Elsayed A.Z.[4,5] · Amr M. El Kharsawy[2] · Dina Johar[6,7]

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Abstract

Background Egypt has high incidence of hepatocellular carcinoma (HCC). This is due to wide spread of hepatitis C virus (HCV) infection which is responsible for most of the cases of liver cirrhosis. The major diagnostic techniques for HCC include serum markers and various imaging modalities. Glypican 3 (GPC3) protein is highly expressed in HCC, but not in normal liver tissue. The significance of GPC3 as a predictor or diagnostic tool for human tumors other than HCC is unclear.

Aim To quantitatively assess the role of GPC3 in diagnosis of HCC in comparison to α-fetoprotein (AFP), ultrasonography (US), and computerized tomography (CT).

Patients and Methods This cross-sectional study enrolled 85 subjects: 40 cirrhotic patients with primary HCC, 30 cirrhotic patients without HCC, and 15 healthy individuals. All patients were recruited from the Gastroenterology and Tropical Departments and outpatient clinics of New Damietta Hospital during the period from November 2010 to August 2012.

Results GPC3 is positive in some HCC patients with normal levels of AFP. AFP has lower sensitivity (67.5%) compared to higher sensitivity of GPC3 (82.5%), and near specificity (61.2%) to GPC3 (57.8%).

Conclusion and Significance The combined serum AFP and GPC3 significantly increased the sensitivity of HCC diagnosis. Although GPC3 is better than AFP in diagnosis of HCC, it still lacks the 100% sensitivity and specificity because some patients have negative or normal level of GPC3 (below the cutoff point 1.5 ng/ml) despite being diagnosed by triphasic CT.

Keywords α-Fetoprotein · Ultrasonography · Triphasic CT · Glypican-3 · Hepatocellular carcinoma · Cirrhosis · Viral hepatitis

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✉ Dina Johar
dinajohar@gmail.com; umjohar@myumanitoba.ca

Ahmed M. Tahon
ahmedtahoun@gmail.com

Magdy Z. El-Ghanam
GamalGhannam@gmail.com

Samy Zaky
samyzs55@yahoo.com

Tarek Mostafa Emran
drtarekemran@yahoo.com

Ali M. Bersy
mohmed_ali0@hotmail.com

Fathiya El-Raey
faresmd76@yahoo.com

Elsayed A.Z.
ashrafzs1958@hotmail.com

Amr M. El Kharsawy
ahmedamr1982@yahoo.com

[1] Department of Clinical Pathology, Faculty of Medicine, Al-Azhar University, Nasr City, Cairo, Egypt

[2] Department of Clinical Pathology, Faculty of Medicine, Al-Azhar University, Damietta, Egypt

[3] Department of Tropical Medicine, Faculty of Medicine, Al-Azhar University, Nasr City, Cairo, Egypt

[4] Faculty of Sciences, Jouf University, Jouf, Kingdom of Saudi Arabia

[5] Zoology Department, Faculty of Sciences, Al-Azhar University, Nasr City, Cairo, Egypt

[6] Department of Biochemistry and Nutrition, Faculty of Women for Arts, Sciences and Education, Ain Shams University, Heliopolis, Cairo, Egypt

[7] Department of Physiology and Pathophysiology, Max Rady College of Medicine, Rady Faculty of Health Sciences, University of Manitoba, Winnipeg, MB, Canada

Introduction

Hepatocellular carcinoma (HCC) represents the fifth most common cancer and the second leading cause of cancer-related deaths worldwide [1]. HCC typically develops in patients with chronic liver diseases, and cirrhosis, usually chronic hepatitis B virus (HBV) or HCV, as the strongest predisposing factors [2, 3]

Glypican 3 (GPC3) is a member of two gene families: glypicans and proteoglycans [4]. The distribution and functional significance of GPC3 isoforms are unknown. GPC3 is absent in hepatocytes of healthy subjects and patients with nonmalignant hepatopathy and can be detected in about 50% of HCC patients and 33% of HCC patients seronegative for both AFP and des-γ-carboxy prothrombin (DCP) [5]. GPC3 gene and mRNA are not detectable in normal tissues, except the placenta and fetal liver, and they are expressed in the majority of HCCs. The expression of GPC3 dynamically increases as hepatocytes change from normal to precancerous and cancerous lesions, indicating that the up-regulation of GPC3 mRNA may be a sensitive and specific biomarker of HCC, suggesting that over-expression of GPC3 is an early molecular event in the malignant transformation of hepatocytes and can distinguish it from other benign and malignant hepatic lesions [6].

GPC3 is also expressed to a lesser degree in melanoma, ovarian clear-cell carcinomas, yolk sac tumors, neuroblastoma, hepatoblastoma, Wilms' tumor cells, and other tumors. However, the significance of GPC3 as a diagnostic tool for human tumors other than HCC is unclear [7]. We aim to quantify the role of GPC3 in HCC diagnosis in comparison to AFP, US, and CT.

Methods

Patients

Study Design Cross-sectional study. The study is approved by the esearch and ethical committees of Al-Azhar Faculty of Medicine and Al-Azhar University Hospital, New Damietta, Egypt. All patients were recruited from the Gastroenterology and Tropical Departments and outpatient clinics of New Damietta Hospital during the period from November 2010 to August 2012. Written consents of participation are signed by all patients. A total number of 70 patients with liver cirrhosis and 15 healthy subjects matched for age and gender as a control group were included. Patients are divided into two groups:

Group 1 included 40 cirrhosis patients with primary HCC.
Group 2 included 30 cirrhosis patients without HCC, proved by history, clinical examination, laboratory, and US findings.
Group 3 included 15 healthy control individuals.

All subjects enrolled in the study were submitted to a clinical history taking.

Inclusion Criteria for HCC Group

A positive US and triphasic CT for malignant focal lesion, age > 50 years.

Inclusion Criteria for Cirrhosis Group

A confirmed clinical picture of cirrhosis, with positive US and routine laboratory tests for cirrhosis, age > 50 years.

Exclusion Criteria Subjects not fulfilling the above criteria, patients receiving chemotherapy, radiotherapy, local ablation of HCC, patients with extra-hepatic malignancies, pancreatic, and biliary diseases were excluded.

Methods

Imaging Procedures

We used ultrasonography (Ultrasonic, Sonix SP, Canada, 2010) and triphasic spiral CT scan (Spiral CT Siemens, Balance Somatome, 1999). Physical examination of the liver, spleen, and lymph nodes and abdominal US are demonstrated for all subjects. Abdominal triphasic spiral CT was done for patients in HCC group (Table 1).

Table 1 Demographic data and clinical history of patients and control groups

	Cirrhosis ($n = 30$)	HCC ($n = 40$)	Control ($n = 15$)
Age	53.03 ± 4.63	61.25 ± 4.17	59.93 ± 4.68
Gender			
Male	23 (76.7%)	33 (82.5%)	7 (46.7%)
Female	7 (23.3%)	7 (17.5%)	8 (53.3%)
Positive HCV-Ab (%)	30 (100%)	40 (100%)	0 (0%)
Positive HBs-Ag (%)	3 (10%)	2 (5%)	0 (0%)
Previous history of bilharziases (%)	30 (100%)	40 (100%)	0 (0%)
Ascites			
None	5 (16.7%)	14 (35%)	15 (100%)
Mild	4 (13.3%)	12 (30%)	0 (0%)
Moderate	14 (46.7%)	14 (35%)	0 (0%)
Marked	7 (23.3%)	0 (0%)	0 (0%)

Significant difference at alpha (P) < 0.05

Hematological Indices

Fresh blood samples are used for erythrocyte sedimentation rate (ESR) determination using Wintrobe method [8]. Complete blood count (CBC) is determined by Cell Dyne 1800 and peripheral blood smear examination. Blood hemoglobin (Hb) concentration was determined by photometric quantification of cyanmethemoglobin at 540 nm [9].

Prothrombin Time (PT) and Concentration

Fresh plasma is used for PT and concentration using blood coagulometer model SEAC S2, Biostec Liquiplstin, Egypt. Normal value (12–14 s), up to 75% in concentration, and an International Normalization Ratio (INR, that is the ratio of the patient's prothrombin time and the normal mean prothrombin time) is up to 1 [10].

Assessment of Liver Functions

The following indices are quantified spectrophotometrically using Hitachi 902 analyzer. Serum is used for the determination of albumin [11], and the reference range for adults is 3.5–5.5g/dl. Serum total bilirubin (TB) is determined after Webster [12], the reference range is 0.5–1 mg/dl. Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) are determined according to Bergemyer et al. [13], and the references range is 41 and 34 IU/l respectively. Alkaline phosphatase (ALP) is determined after Teitz [14], and the reference range is up to 290 IU/l. Criterion for exclusion from analysis was contamination of plasma by blood. Serum gamma glutamyl transferase (γ-GT) is determined according to [15].

Assessment of Kidney Functions

Serum creatinine is determined according to [16]. The reference range for adults is 0.5–1.0 mg/dl for women and 0.7–1.2 mg/dl for men. Blood urea is measured according to [17], and the reference range is 7 to 20 mg/dl.

GPC3 Assay

Serum GPC3 is quantified using ELISA immunoassay kit from CUABIO BIOTECH CO., LTD. The microtiter plate wells are pre-coated with GPC3-specific antibodies. Samples and standards are added to the wells. Biotin-conjugated polyclonal antibody and horseradish peroxidase-conjugated avidin are added, then TMB (3,3′,5,5′ tetramethyl-benzidine) substrate solution is added to plates. Only those wells that contain GPC3 will exhibit change in color. The enzyme-substrate reaction is terminated by the addition of a sulfuric acid solution, and the color change is determined within 30 min using a microplate reader Tecan Austria GmbH 5082 Grodig, Audtria, Model Sunrise-Basic Tecan, REF 16039400, at 450 nm [18, 19]. A standard curve was generated using Magellan 6 software. The detection range is 0.625–40 ng/ml.

AFP Assay

Serum AFP is quantified using chemiluminescence immunonoassay kit manufactured by SIEMENS Health Care Diagnostic Products, LTD. Chemiluminescence usually occurs when a highly oxidized molecule, such as peroxide, reacts with another molecule. The bond between the two oxygen atoms in a peroxide is weak, and when it breaks, the atoms reorganize, releasing energy in the form of light [20], without release of a temperature. IMMULITE SN automated quantitative immunoassay analyzer manufactured by DPC Cirrus Inc., Flandies, NT 07836, Los Angeles, CA, 90045, is used to measure the emitted light.

Statistical Analysis

Data is analyzed using Statistical Package for Social Sciences (SPSS) version 15. Qualitative data is presented as number and percent. Comparison between groups is done by Chi-square test. Quantitative data is tested for normality by Kolmogrov-Smirnov test. Normally distributed data is presented as mean ± SD. Student *t* test was used to compare between the three groups. Pearson's correlation coefficient is used to test the correlation between variables. Difference is considered significant at alpha (P) < 0.05.

Data Availability All data generated or analyzed during this study are included in this published article.

Results

Demographic Data and Clinical History of Patients and Control Groups

In this study, males represent higher percentage (82.5%) than female patients (17.5%). The mean age of HCC patients (years) is higher (61.25 ± 4.17) compared to mean age of cirrhosis patients (53.03 ± 4.63) and control subjects (59.93 ± 4.68). The background of chronic liver disease is HCV in 100% and HBV in 5% of patients. Bilharzial history is positive in 100% of patients. Ascites were found among 65% of HCC patients (Table 1). The distribution of patients in HCC stages is shown in supplementary Table 1, according to the Barcelona Clinic Liver Cancer (BCLC) staging system. The majority of HCC patients belong to advanced and end-stage HCC.

Liver Functions, Cholestatic Enzymes, and Hematological Indices in Patients and Control Groups

ALT, AST, TB and Direct bilirubin (DB), γ-GT, and INR increased significantly in HCC compared to cirrhosis and control subjects (Table 2).

Platelets' count is significantly lower in HCC and cirrhosis subjects compared to control. ESR1 and 2 increase significantly in HCC than cirrhosis and control subjects (Table 3). ESR1=ESR after one hour; ESR2=ESR after two hours.

AFP and GPC3 Levels in Patients and Control Groups

AFP and GPC3 levels increased significantly in HCC than cirrhosis patients and control subjects ($P < 0.001$). AFP and GPC3 showed significant difference between cirrhosis patients and control ($P = 0.006$) (Table 4).

Sensitivity and Specificity of AFP, GPC3, and Triphasic CT in Diagnosing Cirrhosis and HCC

The sensitivity and specificity of AFP combined with GPC3 is positively elevated in HCC and cirrhosis groups (Tables 5 and 6).

ROC curve was used for the best cutoff point of AFP and GPC3. GPC3 at a cutoff value of 1.5 ng/ml has higher sensitivity (82.5%) than AFP, and near specificity (57.8%) to AFP (67.5%) (Fig. 1). AFP at a cutoff value of 5.9 ng/ml has low sensitivity (67.5%) and low specificity (61.2%) (Fig. 2).

Discussion

The prognosis of HCC is poor because detection occurs at an advanced stage. Potentially curative treatment such as surgery is limited and is possible only for cases with small HCC malignancies. For this reason, more effective non-invasive surveillance strategies should be used to screen for early occurrence of HCC targeted to the population at risk [2].

Table 3 Hematological indices in patients and control groups

	Cirrhosis ($n = 30$)	HCC ($n = 40$)	Control ($n = 15$)	P
White blood cells	5.91 ± 3.65	4.94 ± 2.43	5.94 ± 1.21	0.277
Red blood cells	3.73 ± 1.08	3.82 ± 0.65	4.61 ± 0.38	0.002
Hb	10.23 ± 2.60	11.26 ± 1.59	13.54 ± 0.81	< 0.001
ESR1	24.87 ± 10.24	76.60 ± 17.35	6.13 ± 1.25	< 0.001
ESR2	52.00 ± 18.95	111.03 ± 11.63	13.13 ± 2.17	< 0.001
Platelet	73.73 ± 21.00	79.05 ± 32.08	276.27 ± 32.75	< 0.001

Significant difference at alpha (P) < 0.05

US is a sensitive gold standard for detecting small HCC (sensitivity < 1 cm), but very poor in the differentiation between nodules with respect to their nature. Triphasic spiral CT is an ideal gold standard imaging modality for the diagnosis of HCC. Serum AFP has poor sensitivity for the diagnosis of HCC, as small well-differentiated HCC is usually not associated with serum AFP elevation. Serum AFP elevation may be seen with inflammatory flares in chronic viral hepatitis [3].

Glypicans play important roles in embryonic development [21] and the regulation of cell proliferation and survival, particularly during development and malignant transformation [6]. In HCC, GPC3 interacts with the wingless-related integration site (Wnt) ligands and stimulates cell proliferation. Cellular proliferation induced by Wnt has recently been attributed to activation of both the extracellular signal-regulated kinase (ERK) and Wnt pathways, both of which are implicated in hepatocarcinogenesis associated with HBV and HCV infections [6].

Clinical studies have indicated that the simultaneous determination of GPC3 and AFP could significantly increase the sensitivity in HCC detection, without reduction in specificity [22]. GPC3 could be a putative marker in sensitizing AFP in HCC prognosis [23]. Liu et al. [4] reported that combined

Table 2 Liver functions and cholestatic enzymes in patients and control groups

	Cirrhosis ($n = 30$)	HCC ($n = 40$)	Control ($n = 15$)	P
ALT	41.00 ± 37.46	80.53 ± 26.96	23.47 ± 3.83	< 0.001
AST	53.37 ± 30.55	103.63 ± 32.18	23.80 ± 2.40	< 0.001
Total bilirubin (TB)	2.67 ± 3.19	3.24 ± 1.13	0.69 ± 0.14	< 0.001
Direct bilirubin (DB)	0.89 ± 1.87	1.49 ± 0.91	0.16 ± 0.03	0.003
Albumin	2.71 ± 0.51	2.65 ± 0.48	4.80 ± 0.11	< 0.001
INR	1.52 ± 0.49	1.61 ± 0.21	1.03 ± 0.05	< 0.001
ALP	88.90 ± 38.96	92.65 ± 35.09	68.27 ± 8.35	0.059
γ-GT	42.07 ± 22.50	49.95 ± 33.80	25.00 ± 6.09	0.012

Significant difference at alpha (P) < 0.05

Table 4 AFP and GPC3 levels in patients and control groups

	Cirrhosis ($n = 30$)	HCC ($n = 40$)	Control ($n = 15$)	P
AFP	7.51 ± 3.96	144.57 ± 21.82	4.55 ± 1.39	< 0.001
GPC3	2.07 ± 1.44	12.08 ± 14.11	1.25 ± 0.32*	< 0.001

Significant difference at alpha (P) < 0.05

*Significant difference between cirrhosis and control ($P = 0.006$)

serum AFP and GPC3 significantly increased the sensitivity of HCC diagnosis. Some other investigators have reported that GPC3 mRNA is up-regulated significantly in tumor tissues of HCC compared to paraneoplastic tissues of HCC, liver tissues of healthy adults, and liver tissues of patients with nonmalignant hepatic disease. Thus, GPC3 could also be a good non-invasive protein marker for HCC [24].

In the current study, the mean age of HCC group (61.25 ± 4.17) years was higher than cirrhosis (53.03 ± 4.63) and control groups (59.93 ± 4.68) ($P < 0.001$) as shown in Table 1. This result is similar to Wang et al [25] findings. Elzayadi et al. [26] reported that HCC in Egypt is more prevalent among older than younger age groups. This could be explained by the longer exposure to predisposing factors associated with aging, increasing the likelihood of malignant transformation [27].

Age-specific incidence rates are strongly affected by the etiology of the background liver disease. Old age is an independent risk factor for HCC, specially in areas where HCV infection is endemic. On the other hand, the incidence rates increase after 20 years of age in countries where HBV-related carcinogenesis is dominant [28]. Rodríguez-Díaz et al. [29] reported that the duration of the infection is directly related to HCC development.

In our study, male predominance in HCC and cirrhosis groups (Table 1) is similar to that found by Johnson [30]. Male to female ratio differs among countries, as greater ratios were noticed in the high incidence regions as Africa, China, Taiwan, and Japan [31]. HCC is more prevalent in males than females due to the difference of exposure to risk factors (HBV and HCV which are more prevalent in male patients). However, sex hormones and other X-linked genetic factors may also be important [32]. In Egypt, a study of the prevalence and epidemiological features of HCC was conducted and revealed that male patients were 85.4% while female patients were 14.6% among 1328 studied HCC patients [33], and this finding is close to our results, as the males represented 82.5% of HCC patients while females represented a markedly lower percentage of 17.5.

The rate of positive HBs-Ag in our HCC patients is 5% (Table 1), that is in accordance to Abdel-Wahab et al. [34] who found that positive HBs-Ag cases among their studied HCC patients were 5.9% due to widespread HBV vaccine. It has been estimated that 10–20% of the people infected with HBV developed HCC after 15–30 years [35]. The rate of positive HCV-Ab among our HCC patients was 100% (Table 1), that is slightly higher than the findings of El-Zayadi et al. [33], 85 and 87.9% respectively.

All HCC patients in this study had a higher past history of schistosomiasis (Table 1) than the results of El-Zayadi et al. [33] in 59.7% of their studied patients. Schistosomiasis was reported to cause an imbalance in HCV-specific T cell responses leading to increased viral load, a higher probability of HCV chronicity, and more rapid progression of complications in co-infected persons [36].

Ascites were found among 65% of our HCC patients (Table 1). Ascites among HCC patients are a result of hypoalbuminaemia and portal hypertension. Rarely, ascites may occur due to involvement of the peritoneum by the direct invasion or metastases spread. HCC should be suspected in any cirrhotic patients with refractory and/or bloody ascites [37].

The mean ALT (80.53 ± 26.96) and AST levels (103.63 ± 32.18) were elevated among our HCC patients (Table 2). AST increment was higher than that of the ALT. This finding is similar to the study of Brechot et. al. [37] who reported that the serum levels of AST and ALT was elevated in their HCC patients, the serum AST was higher than ALT and the difference builds up as the disease progresses.

Low serum albumin concentration in our HCC patients (Table 2) is similarly reported by Christopher and Smith [37] who explained that the onset of rapid deterioration in cirrhotic patients with decrease synthetic function of liver may signal the development of HCC with low serum albumin concentration and extenuating PT (unpublished data). This was identical to our study extended mean PT (15.07 ± 2.23) s (unpublished data) and INR (1.61 ± 0.21) compared to the standard value (11–13 s) (Table 2).

We found that the mean bilirubin level was (3.24 ± 1.13) mg/dl (Table 2). This agrees with the study of Saini et al. [38] who reported that 13% of their HCC patients were complaining of jaundice, and related this rise to tumor

Table 5 Comparing the sensitivity and specificity of AFP, GPC3, and triphasic CT applications in cirrhosis and HCC groups

	Cutoff point	Area under the curve	Sensitivity	Specificity	P
AFP	6.595	0.682	67.5%	61.2%	0.004
GPC3	1.575	0.830	82.5%	57.8%	< 0.001
Triphasic CT	N/A	N/A	100%	100%	N/A

Significant difference at alpha (P) < 0.05

Table 6 Synergistic effect of (AFP + GPC3) in positive elevation of cirrhosis and HCC sensitivity of diagnosis

	Cirrhosis ($n = 30$)	HCC ($n = 40$)	P value
AFP	6 (20%)	20 (50%)	< 0.001
GPC3	18 (60%)	35 (87.5%)	< 0.001
AFP + GPC3	27 (70%)	37 (92.5%)	< 0.001

Significant difference at alpha (P) < 0.05

infiltration of liver parenchyma, hilar invasion, or progressive terminal liver failure.

Hb, platelet, and RBC means were significantly different among our HCC, cirrhosis patients, and control groups. Significant lower levels of platelet and RBC count in HCC and cirrhosis group ($P < 0.001$) agrees with Gad et al. [39]. Our finding of WBC ($P = 0.277$) revealed insignificant difference among cirrhosis, HCC patients, and control groups. ESR1 and ESR2 ($P < 0.001$) presented significantly higher values in HCC than cirrhosis group (Table 3).

Previous studies demonstrated that AFP has a poor sensitivity of 39–65%, a poor specificity of 76–94% [3]. In this study, mean AFP level among patients with HCC (144.57 ± 21.82 ng/ml) was higher than that of cirrhosis group (7.51 ± 3.96 ng/ml). Our result shows similar trend to Suzuki et al. [40] who reported that patients with HCC had significantly higher serum AFP levels than patients with cirrhosis (mean 57 versus 12 ng/ml), $P < 0.001$ (Table 4).

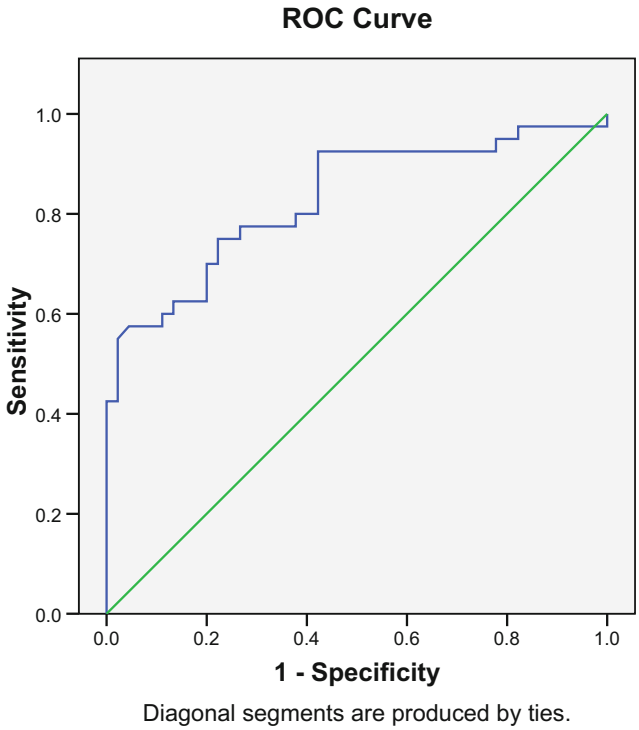

Fig. 1 Sensitivity and specificity of GPC3 in patients with cirrhosis, HCC, and control groups

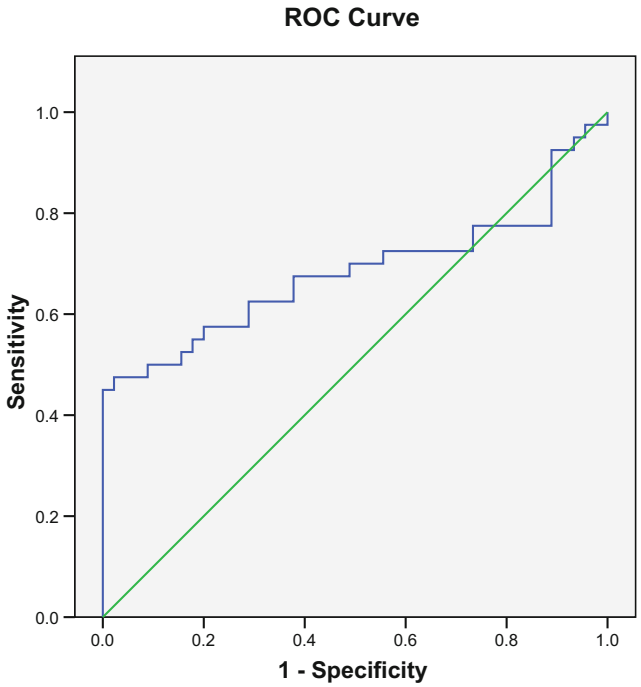

Fig. 2 Sensitivity and specificity of AFP in patients with cirrhosis, HCC, and control groups

AFP is poorly sensitive and poorly specific in diagnosing HCC and differentiating HCC from cirrhosis. AFP has low sensitivity (67.5%) and low specificity (61.2%) (Table 5). Our data agrees with Barletta et al. [41] who reported in HCC patients that sensitivity of AFP is only around (60%) and specificity was (50%), and with Wang et al. [3] who reported AFP sensitivity of 39–65% and specificity of 76–94%. Zhou et al. [42] observed that AFP has limited utility of differentiating HCC from benign hepatic disorders for the high false-positive and false-negative rates. Also, Chen et al. [43] found an elevated serum AFP levels in patients with chronic HCV without HCC. Moreover, Shirabe et al. [44] reported that AFP levels are elevated in benign liver diseases such as chronic hepatitis or cirrhosis, indicating low specificity.

Omata et al. [28] found that a significant proportion of small HCC (< 3 cm) does not secrete AFP to achieve a diagnostic level. Furthermore, the level of AFP is elevated in patients with both HCC and chronic liver disease, thus, there is a wide overlap between the two groups. Moreover, Stefaniuk et al. [2] reported significant higher AFP levels accompanying various liver diseases (viral hepatitis, liver cirrhosis, liver tumors: primary HCC and hepatoblastoma) and other neoplasms (mainly cancers of the digestive tract: pancreas, stomach, large intestine, and gallbladder). Huang et al. [43] found that reactivation of AFP production in adults occurs during liver regeneration and hepatocarcinogenesis. These results are confirmed by Stefaniuk et al. [2].

Bongiovanni et al. [45] found that elevations of AFP levels are often observed in acute and chronic viral hepatitis and are usually associated with hepatic cirrhosis. These elevations have correlated primarily to hepatic damage, with selective transcriptional activation of the AFP gene.

We found that mean GPC3 level among HCC patients (12.08 ± 14.11) ng/ml is higher than patients with cirrhosis (2.07 ± 1.44) ng/ml, $P < 0.001$ (Table 6). This result agrees with Hippo et al. [23] who reported that HCC patients had significantly GPC3 higher serum levels (4.8 ± 8.91) ng/ml than cases with cirrhosis without HCC (1.09 ± 0.74) ng/ml.

We find that GPC3 is higher than 1.5 ng/ml in (60%) of cirrhosis patients, and AFP is higher than 5.9 ng/ml in (20%) of patients. The combined serum AFP and GPC3 assay increased significantly the sensitivity in diagnosis of cirrhosis in (70%) of patients (Table 6).

Our finding revealed that GPC3 is higher than 1.5 ng/ml in (87.5%) of HCC patients and AFP is higher than 5.9 ng/ml in (50%) of HCC patients. Combined serum AFP and GPC3 significantly increased the sensitivity in diagnosis of HCC in 92.5% of patients (Table 6).

In this study, GPC3 is better than AFP in diagnosis of HCC. These results are in agreement with Liu et al. [4] who reported that GPC3 protein is a sensitive and specific serum marker for diagnosis of early HCC.

In this study, GPC3 is positive in some HCC patients with normal levels of AFP. This agrees with Nakatsura et al. [5] who reported that GPC3 can be detected in about 50% of HCC patients and 33% of HCC patients seronegative for AFP.

Although GPC3 is better than AFP in diagnosis of HCC, it lacks the absolute sensitivity and specificity because some patients have negative or normal level of GPC3 (< 1.5 ng/ml), despite being diagnosed by triphasic CT.

According to the BCLC staging system, we find that AFP is positive in 25% of patients with Grade A (early HCC). GPC3 is positive in 75% of patients with Grade A (early HCC). The combined serum AFP and GPC3 are positive in 87.5% of patients with Grade A HCC (supplementary Table 2).

Conclusion

AFP is a weak marker for HCC diagnosis and has poor ability in differentiating HCC from non-malignant hepatopathy. GPC3 is more sensitive than AFP in early diagnosis of HCC. The combined serum AFP and GPC3 significantly increases the sensitivity of HCC diagnosis. GPC3 still lacks the absolute sensitivity and specificity.

Authors' Contributions All authors contributed to conception, design, data analysis, and writing the manuscript. D.J. provided ENDNOTE X8 software required for the production of this manuscript.

Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

Ethics Approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the research committees of Al-Azher University Faculty of Medicine and Al-Azhar University Hospital in New Damietta, Egypt.

Informed Consent Written consent was obtained from all individual participants.

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