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Role of Serum Glypican-3 in the Diagnosis of Hepatocellular Carcinoma in the Upper Egypt

Ali Abdelrahman Ghweil[1], Ashraf Khodeary[2], Mohammed Tag-Adeen[3*], Marwa El-Sayed[4], Khaled Shahat[5], Ghada Abdelrazek[6]

[1]Department of Tropical Medicine and Gastroenterology, Faculty of Medicine, South Valley University, Qena, Egypt
[2]Department of Clinical Pathology, Faculty of Medicine, Sohag University, Sohag, Egypt
[3]Department of Internal Medicine, Faculty of Medicine, South Valley University, Qena, Egypt
[4]Department of Medical Microbiology and Immunology, Faculty of Medicine, South Valley University, Qena, Egypt
[5]Department of Internal Medicine, Faculty of Medicine, Al-Azhar University, Cairo, Egypt
[6]Department of Diagnostic Radiology, Faculty of Medicine, South Valley University, Qena, Egypt
Email: alimena1@yahoo.com, khodery11@gmail.com, tagmedicine@gmail.com, nooreleiman1@yahoo.com,
khaledshahat27@yahoo.com, drghadarad2016@yahoo.com, *tagmedicine@gmail.com

Abstract

Background and Aim: Early diagnosis of hepatocellular carcinoma (HCC) is essential for achieving good prognosis. Glypican 3 (GPC3) has been reported to be raised in HCC in comparison with non-neoplastic lesions. This work aimed to study the role of GPC3 in the early diagnosis of HCC in post-chronic hepatitis C (CHC) cirrhotic patients. **Patients and Methods:** A comparative study included 60 patients, 40 patients with HCC (HCC group) and 20 patients of CHC without HCC (control group). Diagnosis of HCC was based on abdominal ultrasound and triphasic CT, while biopsy was performed in debating cases. Serum samples for measurement of GPC3 and AFP levels were obtained from all participants. **Results:** The median levels of both AFP and GPC3 were significantly higher among HCC cases compared to controls. Analysis of the ROC curve showed that both AFP and GPC3 could be used to differentiate HCC cases from controls. AUROCs of GPC3 and AFP were 0.928 and 0.727 respectively, and both were statistically significant with p-values < 0.0001. The best cut-off value for GPC3 for HCC detection was 3.15 ng/ml with 82% sensitivity (95%CI: 67 - 93) and 95% specificity (95%CI: 75 - 99), and for AFP it was 257 ng/ml with 77% sensitivity (95%CI: 61 - 89) and 85% specificity (95%CI: 62 - 97). **Conclusion:** Serum GLP-3 is highly sensitive and specific for detecting HCC, more than AFP for the early detection of HCC, and the combination of both yielded improved sensitivity.

Keywords

Hepatocellular Carcinoma, Alpha Fetoprotein, Glypican-3

1. Introduction

Globally, hepatocellular carcinoma (HCC) is one of the most common cancers and represents the third most common cause of death worldwide [1] [2] [3]. The most important causes of HCC are liver cirrhosis, chronic hepatitis C (CHC) and chronic hepatitis B (CHB) infections, as well as fungal carcinogens, such as aflatoxin B1 [4].

According to the World Health Organization (WHO), around 3% of the world's population are infected with hepatitis C virus (HCV), with its highest prevalence in Egypt ranging from 10% - 20% of population; therefore, liver cancer represents around 12% of gastro intestinal malignancies [5] [6].

The HCC prognosis depends on number and size of tumors [7], which in turn depends on the lag time between the onset of HCC and the time of its diagnosis. However, HCC is usually asymptomatic or incidentally discovered during routine abdominal ultrasound (US) examination of cirrhosis, and most of detected lesions are locally advanced with limited therapeutic options [8] [9] [10].

As early HCC detection plays a crucial role for its proper management, regular screening for high-risk patients, including serum tumor markers as α-fetoprotein, and imaging modalities, such as US, computed tomography (CT) or magnetic resonance imaging (MRI), is highly advised [11].

Alpha fetoprotein (AFP) is the most widely used serum tumor marker for the recognition and monitoring of HCC. The American Association for the Study of Liver Diseases (AASLD) guidelines recommended the use of AFP level $\geq$ 200 ng/ml instead of fine-needle cytology for HCC diagnosis especially in cirrhosis [12] [13] [14] [15]. However, serum AFP is not always elevated in all HCC patients, especially in lesions smaller than 5 cm in diameter. Moreover, its level may be elevated in non-malignant chronic liver diseases, such as chronic hepatitis and cirrhosis, and in other types of cancers, such as germ cell, stomach, biliary tract and pancreatic tumors, or even normally during pregnancy [14].

The glypican-3 (GPC3) is one of the glypican families of glycosyl phosphatidyl inositol (GPI)-anchored heparan sulfate proteoglycans [16], which plays an important role in cellular growth, differentiation and migration [17]. Higher GPC3 levels at both the mRNA and protein have been reported in HCC compared to pre-neoplastic and non-neoplastic lesions. The mRNA level of GPC3 was found to be much more elevated than AFP. This advantage of GPC3 over AFP was specially detected in the diagnosis of small HCC tumors [18]. The aim of the work was to evaluate the role of GPC3 in the early diagnosis of HCC in CHC-related cirrhotic patients.

2. Ethical Clearance

The study protocol was approved by the ethical committee of Qena Faculty of Medicine, South Valley University. A written informed consent was obtained from each participant in the study.

3. Patients and Methods

3.1. Patients Selection

A total of 40 post-CHC cirrhotics with HCC were included in HCC group and 20 post-CHC cirrhotics without HCC were included as a control group. All patients were consecutively selected during their routine follow up in HCV clinic, Gastroenterology Department, South Valley University Hospital, Qena, Egypt between January 2017 and January 2018. Serum samples were obtained from all patients for routine investigations and GPC3 and AFP measurements.

3.2. HCC Diagnosis

Abdominal ultrasonography using Philips, ClearVue-650 system (Philips Electronics, Eindhoven, Netherlands) and triphasic CT using GE Healthcare-Brivo system (GE Healthcare, Chicago, IL, USA) were performed for all patients in order to diagnose or exclude HCC. Abdominal MRI using GE Healthcare–Signa Open Speed (GE Healthcare, Chicago, IL, USA) and biopsy were requested in debating cases with atypical lesions.

3.3. AFP Measurement

Serum AFP level was assessed by Mini Vidas system (Biomirieux, France) which is a compact automated immunoassay system based on the Enzyme Linked Fluorescent Assay (ELFA) principles.

3.4. GPC3 Measurement

Serum GPC3 level was measured using a (Quantikine ELISA kit) which is a solid phase sandwich enzyme linked immunosorbent assay done according to the manufacture pamphlet.

3.5. Statistical Analysis

Continuous variables were presented as means and standard deviations (SD), and t test was used for comparison between means. Non-parametric variables were expressed as medians and ranges, and Mann Whitney test was used for comparison in-between medians. The percentages of positive AFP and GPC3 subjects in each group was calculated and compared to each other using Chi square test. ROC curve analysis was done to estimate the most relevant cut off values for both AFP and GPC3 to differentiate HCC cases from controls. Finally, univariate binary logistic regression analysis was done to estimate the predictive value of AFP and GPC3 for the diagnosis of HCC. The data were analyzed using the IBM-SPSS software version 24 (IBM corporation, 2016, Chicago, USA).

4. Results

The HCC group has included 40 patients, 9 females (22.5%), with mean age of 59 ± 9 years (range: 46 - 72 years) while the control group has included 20 patients, 7 females (35%), with mean age of 61 ± 7 years (ranged: 49 - 70 years).

Stages of HCC according to BCLC staging were very early HCC in 8, early HCC in 21, intermediate stage in 7 and advanced HCC in 4 patients.

Our result showed statistically significant differences between the levels of GPC3 and AFP in the study groups. The median GPC3 and AFP were significantly higherin the HCC group than in the control group (4.6 ng/ml versus 1.9, p-value < 0.001) and (1406 ng/ml versus 90.5, p-value < 0.001) respectively, Table 1.

In ROC curve analysis, GPC3 was an excellent HCC predictor with AUROC of 0.928 (p < 0.001), while AFP was an average predictor with AUROC of 0.727 (p < 0.001), Figure 1 and Table 2. Univariate regression analysis showed both markers were statistically significant HCC predictors with p-values < 0.001 and odds ratios: 5.895 (95% CI: 2.226 - 15.616) for GPC3 and 0.03 and 1.004 (95% CI: 1.000 - 1.008) for AFP respectively, Table 3. The most relevant cut off value of GPC3 for HCC detection was 3.15 ng/ml with 82% sensitivity (95%CI: 67 - 93) and 95% specificity (95%CI: 75 - 99), and for AFP it was 257 ng/ml with 77% sensitivity (95%CI: 61 - 89) and 85% specificity (95%CI: 62 - 97), Table 4. Adding GPC3 to AFP has improved specificity of both markers to 100% as all non-HCC patients in the control group had GPC3 and AFP levels lower than the determined cut off values, while the sensitivity showed little decrease to 72% (95%CI: 56 - 85) as 29/40 HCC patients had GPC3 and AFP levels higher than the determined cut off values.

5. Discussions

HCC has bad clinical outcome with high mortality and a 5-year survival rate of less than 10% from the time of clinical diagnosis [19]. In Egypt, HCC represents a major health problem because of its rising incidence and late detection which contributes to limited therapeutic options and unfavorable prognosis in most of cases. Therefore, it is highly recommended to find a new marker for screening and early detection of HCC [20] [21].

AASLD has recommended the use of GPC3, heat shock protein-70 and glutamine synthetase, because the co-positivity of any two of these three markers confirms the diagnosis of HCC [22]. Also, the Clinical Practice Guidelines of the European Association for the Study of the Liver (EASL) recommend these three markers for confirming HCC diagnosis [23] [24].

The aim of this study was to estimate the value of GPC3 as a diagnostic tool for early detection of HCC with 85% sensitivity and 100% specificity. Our study revealed that GPC3 level was significantly higher among HCC patients compared to controls. Several studies have demonstrated the efficacy of GPC3 ASA diagnostic tool in HCC, with a sensitivity ranged between 47% - 90% and a specificity between 40% - 100% [25] [26] [27]. This wide range of difference among studies may be attributed to different patients' demographic data and the use of different cut-off values for GPC-3 [28].

In a previous study by Zakhary *et al.*, 2012 [29], among Egyptian patients with HCC on top of CHC, the sensitivity and specificity of AFP for differentiating

Table 1. Comparison between GPC3 and AFP levels in HCC group and controls.

Marker		HCC Group	Control Group	Mann Whitney	p value
GPC3 (ng/ml)	Mean ± SD	5.95 ± 3.51	1.95 ± 0.60	58	<0.001
	Median (range)	4.6 (1.2 - 14.3)	1.9(0.9 - 3.1)		
AFP (ng/ml)	Mean ± SD	1307.1 ± 1040.0	117.55 ± 74.67	170.5	<0.001
	Median (range)	1406 (11 - 3581)	90.5 (30 - 235)		

Table 2. Area under the ROC curve of GPC3 and AFP.

Test Result Variable (s)	Area	Std. Error	P value	Asymptotic 95% Confidence Interval	
				Lower Bound	Upper Bound
GPC3	0.928	0.035	<0.001	0.859	0.996
AFP	0.787	0.063	<0.001	0.664	0.910

Table 3. Univariate regression analysis of GPC3 and AFP.

	p value	Odd's ratio	CI of Odd's ratio
AFP	0.035 (S)	1.004	1.000 - 1.008
GPC3	<0.001 (HS)	5.895	2.226 - 15.616
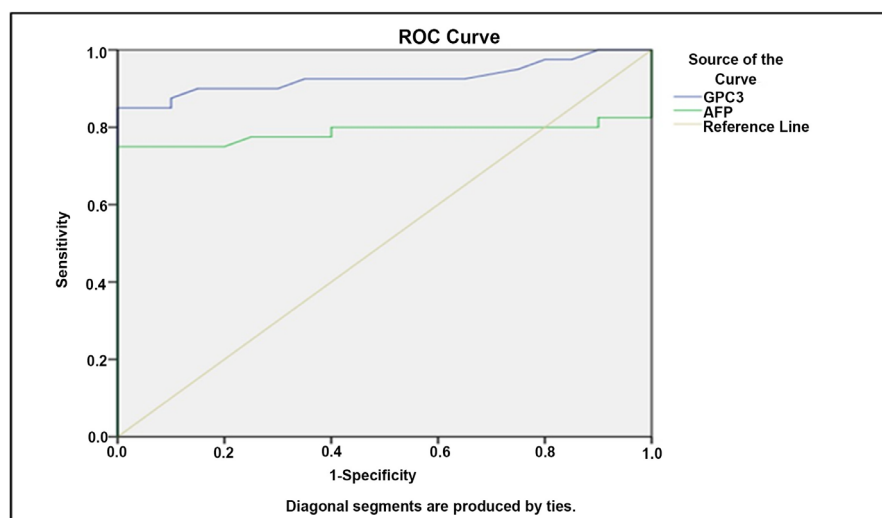
Figure 1. ROC curve analysis shows higher AUROC of GPC3 for prediction of HCC than that of AFP (0.92 vs 0.78).

HCC from non-malignant liver disease at a cut-off level of 20 ng/ml were 90% and 34.0%, respectively, and at a cut-off level of 100 ng/ml were 86.7% and 80%, respectively.

Analysis of the ROC curve for both AFP and GPC3 in our study revealed that the most relevant cut-off value of GPC3 for HCC detection was 3.15 ng/ml and that of AFP was 257 ng/ml, while in Zakhary *et al.*, it was 3.8 ng/ml with 93.3%

Table 4. Validity of GPC3 and AFP for HCC Diagnosis.

Markers	HCC (n = 40)		Non-HCC (n = 20)		Sensitivity	Specificity	Positive Predictive Value	Negative Predictive Value	Accuracy
	True Positive	False Negative	True Negative	False Positive					
GPC3 (≥3.15 ng/ml)	33	7	19	1	82% (67 - 0.93)	95% (75 - 0.99)	97% (83 - 0.99)	73% (58 -0 .84)	87% (75 - 0.94)
AFP (≥257 ng/ml)	31	9	17	3	77% (61 - 0.89)	85% (62 - 0.97)	91% (78 - 0.97)	65% (51 - 0.77)	80% (68 - 0.89)
Both GPC3 & AFP	29	11	20	0	72% (56 - 0.85)	100% (83 - 0.100)	100%	64% (52 - .75)	82% (69 - 0.90)

sensitivity and 100% specificity for GPC3 and 151 ng/ml with 83.33% sensitivity and 100% specificity for AFP. The higher AFP cut-off in our result may be explained by inclusion of CHC patients as a control group in our study while normal subjects have been included in Zakhary *et al*.

However, other studies stated that GPC3 is not a useful diagnostic marker for HCC. This may be due to different measuring procedures used in these studies or different population characteristics [30].

Our study has many limitations including small sample size, single center study, lack correlation between GPC3 level and stage of HCC. Also, as CHC is the most common cause of liver cirrhosis and subsequent HCC in Egypt, our study was confined only to CHC patients without inclusion of patients with chronic hepatitis B or other liver pathogens. In conclusion, Serum GLP-3 is sensitive and specific for detecting HCC. Also, it is more sensitive than AFP for the early detection of HCC. Moreover, a combination of both markers yielded an improved benefit.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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List of Abbreviations in the Order of Appearance

HCC	Hepatocellular Carcinoma	CHC	Chronic Hepatitis C
CHB	Chronic Hepatitis B	WHO	World Health Organization
HCV	Hepatitis C Virus	US	Abdominal Ultrasound
CT	Computed Tomography	MRI	Magnetic Resonance Imaging
AFP	Alpha Fetoprotein	GPC3	Glypican-3
AASLD	American Association for the Study of Liver Diseases		

Significance of the Study

Hepatocellular carcinoma is the third most common cause of death worldwide and its earlier detection might help in a better prognosis. This study was conducted to study the role of a novel serum marker (Glypican 3) in the early detection of liver cancer. We concluded that glypican 3 is sensitive and specific for the early detection of liver cancer.