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Internal Medicine

Role Of Serum Glypican 3 As A Predictive Tool For Early Diagnosis Of Hepatocellular Carcinoma (HCC)

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Abstract

Background: Hepatocellular carcinoma (HCC) is one of the most aggressive malignancies worldwide and one of the main causes of cancer-related mortality globally. Serum alpha-fetoprotein (AFP) has insufficient sensitivity and specificity for detection of hepatocellular carcinoma (HCC). Estimation of serum level of certain up regulated proteins as glypican-3 (GPC-3) in HCC could be a promising biomarker for screening and early HCC detection. Recently glypican-3 (GPC-3) was suggested as novel biomarkers for the detection HCC.

Objective: To determine the role of serum GPC-3 in the early diagnosis of HCC

Methods: The study included eighty subjects: 30 patients with HCC and elevated AFP. ; 30 with liver cirrhosis in addition, 20 healthy subjects were included as a control group. Clinical and radiological features (abdominal ultrasonography and/or abdominal triphasic computed tomography) were recorded. Liver function tests, complete blood cell count, and serum AFP were measured. Serum GPC-3 values were determined by ELISA technique .

Results: This study revealed that: Serum levels of GPC-3 were significantly elevated in patients with HCC compared with liver cirrhosis and control groups ($p < 0.001$). Also, serum GPC-3 levels with cut-off value of 2.72 ug/L, had a sensitivity (93.0%) and specificity .(%٩٤)

Conclusion: Glypican-3 should be included in the screening programs for early HCC detection among cirrhotic patients as a rapid, sensitive, non-invasive and cost-effective diagnostic biomarker.

Key words: Glypican-3(GPC3) - Hepatocellular carcinoma (HCC) - Serum alpha-fetoprotein (AFP).

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Authorship: *All authors have contributed to the Article.*

INTRODUCTION

Hepatocellular carcinoma (HCC) is one of the most frequent and most common aggressive malignancies worldwide, with an increasing incidence globally, it ranks the third commonest cause of cancer-related mortality worldwide. Estimation of serum alpha-fetoprotein (AFP) level is a useful and widely used marker for the detection and monitoring of HCC, AFP levels may remain normal in the patients even with advanced HCC. So the overall AFP performance has been unsatisfactory in terms of its poor sensitivity and specificity.[2]

Glypican-3 (GPC-3) is an onco-foetal, heparin sulphate proteoglycan that is anchored to the plasma membrane by glycosylphosphatidyinositol.[3] Normally, GPC-3 is involved in the regulation of cell proliferation and survival during embryonic development and plays a crucial role as a tumor suppressor.[4] Furthermore, serum GPC3 level is detected to be higher in HCC patients than that in healthy individuals and hepatitis patients. Thus, it is suggested that serum GPC3 is a potential specific biomarker for HCC.[6] In addition, it was reported that the frequency of GPC3 expression in AFP-negative HCC patients is as high as 90%, suggesting that it can be used as a diagnostic tool for HCC.[5] In addition, GPC-3 is more sensitive marker than AFP for the detection of smaller HCC, with diameter of 3 cm or less.[7]

SUBJECTS AND METHODS

Subjects: This study was performed on a total of 80 subjects, selected from Tropical Medicine Department at Theodor Bilharz Research Institute (T.B.R.I) Hospital. They were divided into following groups: Group I (HCC): Comprised of 30 patients with HCC on top of cirrhotic liver with elevated serum AFP and typical imaging findings (abdominal

ultrasound, triphasic CT). Group II (LC): Comprised of 30 patients with liver cirrhosis (LC) on top of chronic hepatitis (CH), with normal serum AFP. Group III (Control): It included 20- age and sex matched appararently normal healthy volunteers. Informed verbal consents were obtained from all subjects. This study was approved by local ethical committee.All subjects were subjected to the following: Full History taking, examination for patients only and imaging techniques (abdominal ultrasonography and triphasic ct).

Laboratory investigations included a routine laboratory tests: complete blood count (cbc) whisch was done on automated cell counter beckman coulter act diff. and routine liver function tests (serum albumin, total bilirubin, direct bilirubin, alanine aminotranseferase (ALT), aspartate aminotransaminase (AST), total protein, alkaline phosphatase (ALP) and kidney function tests (serum urea and creatinine).

They were all assayed by the automatic chemical autoanalyzer Beckman Coulter Synchron CX5 Pro Special investigations included:1) Determination of serum HBsAg, and serum HCV Ab using ELISA method using kit purchased from Human (Cat. no. 51275) 2) Determination of serum AFP level was assayed by solid phase, chemiluminescent immunometric assay on automated analyzer-ADVIA centaur using kit supplied by Siemens Diagnostics. 3) Measurement of serum GPC3 level by enzyme linked immunosorbent assay "ELISA " method using kits provided from Uscn Life Science Inc.[8]

Specimen collection and storage: Eight ml venous blood was withdrawn from all subjects under complete aseptic conditions and then each was divided into 3 parts:

1) Six ml were collected on plain tubes , left for 10 minutes at room temperature to clot and then centrifuged at 800 x g (3000 rpm) for 5 minutes, then the separated serum was then divided into three separate aliquots .One aliquot used for routine biochemical laboratory tests(liver and kidney function tests) ,one for hepatitis (HBV,HCV) serological markers assay and the last one used for determination of serum AFP and GPC3. 2) Two ml were collected in a sterile vaccutainer containing EDTA for complete blood picture assay.

N.B: Prothrombin Time (PT), Prothrombin Concentration (PC) and International Normalized Ratio (INR) values were collected from patient's data sheets.

Methodology: Serum Glypican-3 level assay in serum using ELISA method.

The kit is a sandwich enzyme immunoassay for in vitro quantitative measurement of GPC3 in human serum, plasma, and other biological fluids. The microtiter plate provided in ELISA has been pre-coated with an antibody specific to GPC3. Standards or samples are then added to the appropriate microtiter plate wells with a biotin-conjugated polyclonal antibody preparation specific for GPC3 and Avidin conjugated to Horseradish Peroxidase (HRP) is added to each microplate well and incubated. Then a TMB (3,3'5, 5' tetramethyl-benzidine) substrate solution is added to each well. Only those wells that contain GPC3, biotin-conjugated antibody and enzyme-conjugated Avidin will exhibit a change in color. The enzyme-substrate reaction is terminated by the addition of a sulphuric acid solution and the color change is measured spectrophotometrically at a wavelength of 450nm. The concentration of GPC3 in the samples is then determined by comparing the optical density (O.D) of the samples to the standard curve.

Reagent preparation

All kit components and samples were brought to room temperature (18-25ºC) before use. The standard was reconstituted with 1.0mL of Standard Diluents, kept for 10 minutes at room temperature, shaked gently(not to foam). The concentration of the standard in the stock solution was 20ng/mL. Then stock solution was diluted to 10ng/mL and the diluted standard serves as the highest standard (10ng/mL). Then 7 tubes were prepared each containing 0.5mL Standard Diluents and the diluted standard was used to produce a double dilution series according to figure. (1) Then each tube was mixed thoroughly before the next transfer.

Seven points of diluted standard were done: 10ng/mL, 5ng/mL, 2.5ng/mL, 1.25ng/mL, 0.625ng/mL, 0.312ng/mL, 0.156ng/mL, and the last tube with Standard Diluent was the blank as 0ng/mL.The stock Detection A and Detection B were briefly centrifuged before use. Then was diluted with Assay, Diluent A and B, respectively (1:100). The wash buffer Solution (30×) was diluted with 580mL deionized water to prepare 600mL of Wash buffer (1×).

Assay procedure

Determination of wells for serially diluted standard, blank and samples was done and prepared. Seven wells for standards, 1 well for blank 100µLof each was added for each of corresponding dilutions of standard , blank and samples into the appropriate wells, then covered with the Plate sealer and incubated for 2 hours at 37 ^{0}C. Then the liquid of each well was removed. 100µL of Detection Reagent A working solution was added to each well. Incubated for 1 hour at 37 ºC after covering it with the Plate sealer. The solution was aspirated and washing with 350µL of 1× Wash buffer to each well using, multi-channel pipette. The remaining liquid from all wells was completely removed by snapping the plate onto absorbent paper. Totally washed 3 times.

After the last wash, any remaining Wash Buffer was removed by decanting. Then the plate was inverted and blotted against absorbent paper. 100µL of Detection Reagent B working solution was added to each well. Incubated for 30 minutes at 37 ºC after covering it with the Plate sealer. Aspiration/wash

process was repeated for 5 times as conducted in step 4. 90µL of Substrate Solution was added to each well. Then covered with a new Plate sealer. Incubated for 15 - 25 minutes at 37 ºC . The liquid was turned blue by the addition of Substrate Solution. 50µL of Stop Solution was added to each well. The liquid turned yellow by the addition of Stop solution.Then the liquid was mixed by tapping the side of the plate.Then, the microplate reader was run and the measurement was conducted at 450nm instantly . The concentration of GPC3 in the samples was determined by comparing the O.D. of the samples to the standard curve.

RESULTS

This study conducted on 80 subjects; divided into three groups: Group I (HCC): Included 30 patients with HCC on top of cirrhotic liver with elevated serum AFP, they were 26 males and 4 females patients. Their ages ranged between 46 and 53.3 years with mean age (48.80±1.36) years. Group II (LC): Included 30 patients with liver cirrhosis (LC) and normal serum AFP, they were 22 males and 8 females patients. Their ages ranged between 46 and 55 years, with mean age (49.7 ± 1.4) years. Group III (Control): Included 20 -age and sex matched - appararently healthy volunteers as the control group, They were 18 males and 2 females. Their ages ranged between 41.7 and 52.7 years, with mean age (47.6± 1.54) years.

Receiver- operator curves (ROC) curve for combination of GPC3, AFP to discriminate between HCC and LC (AUC for GPC3 was 0.993 at cut off 2.72 ng/ml and for AFP was 0.999 at cut off 54.5 ng/ml

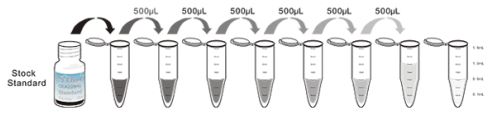

Fig. 1: serial dilution of GPC3 slandered

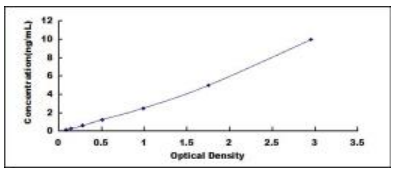

Fig. 2: Glypican-3 Standard Curve

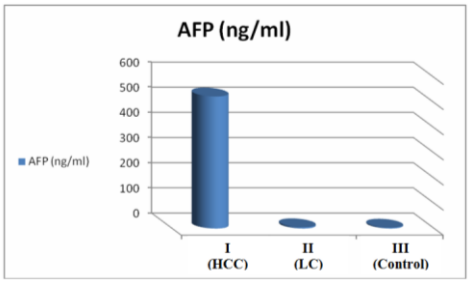

Fig. 3: AFP levels among the studied groups.

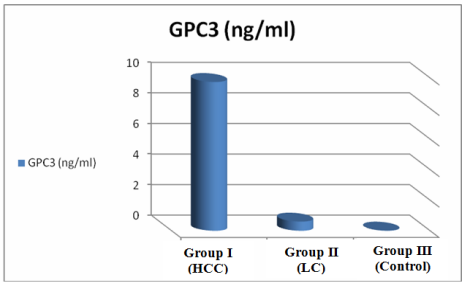

Fig. 4: Serum GPC3 levels among the studied groups

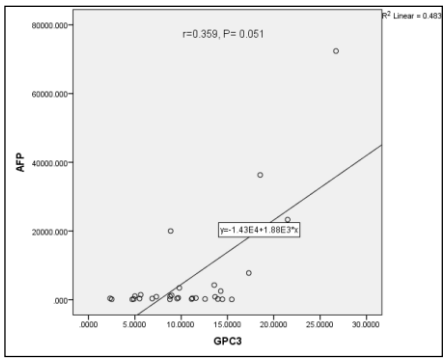

Fig. 5: Correlation AFP and GPC3 IN HCC

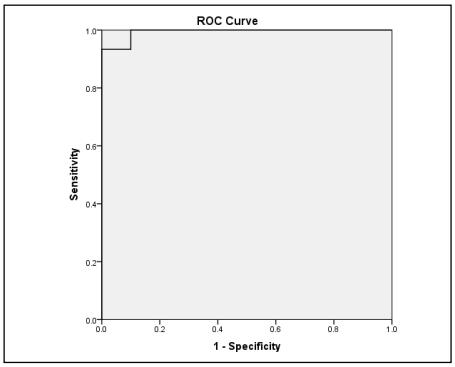

Fig . 6: ROC curve for glypican-3 and AFP for each group separately

Reagents	Quantity	Reagents	Quantity
Pre-coated, ready to use 96-well strip plate	1	Plate sealer for 96 wells	4
Standard	2	Standard Diluent	1×20mL
Detection Reagent A	1×120µL	Assay Diluent A	1×12mL
Detection Reagent B	1×120µL	Assay Diluent B	1×12mL
TMB Substrate	1×9mL	Stop Solution	1×6mL
Wash Buffer (30 × concentrate)	1×20mL	Instruction manual	1

Table 1: Reagents and materials provided

	Group I (HCC) (n =30)	Group II (LC) (n =30)	Group III (Control) (n =20)	P value
Age (years)	48.80±1.36	49.7 ± 1.4	47.6± 1.54	0.43
Gender Male , n=66 Female, n=14	 26/30 (86.7%) 4/30 (13.3%)\	 22/30 (73.3%) 8/30 (26.7%)	 18/20 (90%) 2/20 (10%)	0.236
Smoking +ve , n=36 -ve, n=44	 21/30 (70%) 9/30 (30%)	 15/30 (50%) 15 /30 (50%)	 0 / 20 (0%) 20/ 20 (100%)	0.000
Bilharziasis +ve, n=43 -ve, n=37	 22/30 (73.3%) 8/30 (26.7%)	 21 / 30 (70%) 9 / 30 (30%)	 0 / 20 (0%) 20 / 20 (100%)	 0.000
Diabetes +ve , n=16 -ve, n=64	 10 /30 (33.3%) 20/30 (66.7%)	 6 /30 (20%) 24 /30 (80%)	 0 / 20 (0%) 20 / 20 (100%)	 0.016

Table 2: Demographic data and principal characteristics of the studied groups.
**Data presented as number (%)*
***Data presented as mean ± standard deviation*
P value <0.05 is statistically significant

Ascites	HCC (n=30)	Cirrhosis (n=30)	P value
Absent, n=24	13/30 (43.3%)	11/30(36.7)	
Mild-moderate, n=11	4/30 (13.4)	7/30 (23.3)	0.599
Severe –refractory, n=25	13/30 (43.3)	12/30 (40)	

Table 3: Ascites findings among patients groups

Group / Test	Group I (HCC) (n =30)	Group II (LC) (n =30)	Group III (Control) (n =20)	P value
Bilirubin total (mg/dl)*	2.15 (1 – 3.9)a	2.55 (1.3 – 5.1)a	0.6 (0.5 – 0.9)b	0.000
Bilirubin direct (mg/dl)*	0.65 (0.27- 1.5)a	1.15 (0.47 – 2.9)a	0.1 (0.025 – 0.1)b	0.000)
AST (U/L)*	68 (46.75- 93.75) a	66 (46.75 – 98) a	18 (15 – 24.75) b	0.000
ALT (U/L)*	36.5 (19.5 – 51.25)a	36 (15 – 48.7) a	13 (10 – 19) b	0.000
Total protein (g/dl)*	6.6 (5.9 – 7.9) a	6.4 (5.9 – 6.9)a	7.2 (6.8 – 7.9)b	0.002
Albumin(g/dl)**	3.0 ± 0.7a	3.0 ± 0.8a	4 ± 0.3b	0.000
ALP (U/L)*	112 (74.7 – 163.5)a	100.5 (74 – 186) a	66 (48.5 – 79.5)b	0.000
Urea (mg/dl)*	33.6 (26 – 59.75)a	49.5 (28 – 107.25)a	26.9 (21.1 – 34.9)b	0.000
Creatinine (mg/dl)*	0.9 (0.8 – 1.4) a	1.2 (0.8 – 1.6) a	0.7 (0.6 – 0.8) b	0.000
AFP * (ng/ml)	525(206.7 – 2741.25)a	5.0 (3.75 – 8.0)b	3.5 (2.0 – 5.0)b	0.000
GPC3 * (ng/ml)	9.74 (6.6 – 14.03)a	0.6 (0.4 – 1.17)b	0.013 (0.01 0.03) c	0.000
Hb (g/dl)**	11.5 ± 2.25 a	10.6 ± 2.5 a	14± 0.9 b	0.000
TLC* ($x10^3$µl)	6.25 (4.95 – 7.87)	5.9 (3.8 – 8.1)	6.2 (4.67 – 8.0)	0.667
Platelets* ($x10^3$ µl)	135 (83.7 – 179.2) a	127.5 (83.25 – 178.75) a	286 (197.25–341.5)b	0.000
INR*	1.3 (1.16 – 1.56)a	1.6 (1.3 – 1.87)b	1.03 (1.0 – 1.13)c	0.000

Table 4: Comparison of laboratory tests among the studied groups. *Data expressed as Median, (25th % - 75 th-%-) percentiles. Data presented as mean ± SD. P value ≤ 0.05 is statistically different*
Groups bearing same initials are statistically indifferent at P value 0.05, while groups sharing different initials are statistically different at P value 0.05 (by ANOVA testing).

Group / Test	Group I (HCC) (n =30)	Group II (LC) (n =30)	Group III (Control)(n =20)	P value
HCV Ab -ve , n=8 +ve, n=52	 3/30 (10%) 27 /30 (90%)	 5/30 (16.7%) 25/30 (83.3%)	 20/20 (100%) 0/0	0.706
HBsAg -ve, n= 52 +ve, n=8	 27/30 (90%) 3/30 (10%)	 25/30 (83.3%) 5/30 (16.7%)	 20/20 (100%) -	0.706

Table 5: Descriptive comparison between the studied groups as regard HCV-Ab,and HBsAg.
Data presented as numbers (%)-P-Value ≤ 0.05, is statistically significant.

	Cut off point	Area under the curve	Sensitivity (%)	Specificity (%)	PPV (%)	NPV	Accuracy
Glypican3	2.72	0.993	93%	94%	93%	96%	95%

Table 6: Diagnostic role of GPC3 in detection of HCC
Results are calculated taking cut-off value of GPC3 = 2.72 ng/ml;
PPV: positive predictive value; NPV: negative predictive value; GPC3, glypican-3

DISCUSSION

HCC is considered to be the fifth commonest cause of cancer – related morbidity [9] Moreover, HCC incidence is rapidly rising at alarming rates, and it has become a major health concern globally. [10]

In Egypt, HCC is the second most frequent cancer among Egyptian males after bladder cancer and the sixth most frequent among Egyptian females.[12] Hospital-based studies from Egypt have reported an overall increase in the relative frequency of all liver-related cancers in Egypt.[13] This rising incidence may be due to high prevalence of HCV and its complications. [14]

HCC HAS poor prognosis of HCC patients when discovered[15], so simple, accurate and non-invasive diagnostic tools of HCC is a key goal for early diagnosis of the disease hence improving the poor prognosis of this cancer.[16]

AFP levels has been dropped from current surveillance guide-lines in Europe and the United States because of its low sensitivity and specificity.[3] Therefore, a novel biomarker with superior diagnostic accuracy than AFP is greatly desired.[6]

Glypican 3 is an oncofetal protein and a member of heparin sulfate proteoglycans family and functions as a tumor suppressor.[7] GPC3 can be detected in patients with nonmalignant liver disease, in about 50% of HCC patients and 33% of HCC patients seronegative for AFP. [17]

Investigation of usefulness of serum GPC3 level showed that GPC3 protein is an early sensitive and specific serum marker for initial diagnosis of HCC[7, 18] and for HCC recurrence after liver transplantation.[19]

In the present study, In group I (HCC), the age of the patients ranged between 46 and 53.3 years with mean age (48.80±1.36) years., This is consistent with another results[20], that found that the age of the patients with HCC ranged from 40 to 72 years. In addition, our finding is consistent with [13] findings, where patients of the age group 40-59 years were 3.7 times and of age group $\geq$ 60 years were 11 times more at risk to develop HCC.

The present study showed a male predominance of patients : 26/30 (86.7%) while female patients were 4/30 (13.3 %) in group I (HCC) These finding was in agreement with Egyptian study done by [13] who revealed that male patients were forming 85.4% while female patients were forming 14.6% among 1328 studied HCC patients and estimated that the calculated risk of development of HCC in Egypt was nearly three times higher in men than in women.

In addition to these findings are in concordance with the study of [13] who found that the males (85.8%) were more prevalent than females (14.2%) in HCC group. This finding may be explained by more exposure of males to risk factors, moreover sex hormones and other x-linked genetic factors may also, be considered.[23]

In this study smoking was significantly increased in group I (HCC) and in group II (LC) patient than group III (control) individuals. This coincides with a study by Koh et al. 2011[26,] in which they evaluated the association between smoking and risk of HCC, and it has suggested that there is a strong evidence to date that tobacco smoking is a causal contributing factor for HCC development in humans. Moreover, prediagnosis smoking and alcohol have a deleterious effect on HCC survival as reported by Shih, et al. [27]. This finding is in contradiction with Coady et al. [28] who reported that the effect of cigarette smoking is minimal and has been independently associated with HCC.

In this study, median serum alkaline phosphatase was also higher in group I (HCC) , in group II (LC), in comparison to group III (control). These findings are similar to results of Abu El Makarem et al.[3] who found that there was a statistically significant increase of ALT and AST when they compared between patients with HCC, patients with LC and control group. AST level was more elevated than ALT due to release of mitochondrial AST and the difference becomes greater as the disease progresses.

Chronic infections with HBV and/or HCV are the major etiological and risk factors for HCC worldwide, accounting for 80% of all HCC cases [33,34]. Hospital-based studies in Egypt have reported an overall increase in the relative frequency of all

liver related cancers in Egypt (>95% as HCC), from approximately 4% in 1993 to 7.3% in 2003 [13].

Investigations in Egypt have shown the increasing importance of considering HCV infection as the major risk factor in the etiology of liver cancer, estimated to account for 40-50% of cases, and the declining influence of HBV and HBV/HCV infection (25% and 15%, respectively) [35].

The mechanism of hepatocarcinogenesis by HBV was explained by Zhu et al.[36] who revealed that hepatitis B virus X protein (HBx) is a potential trigger of the epigenetic changes and can directly lead to cellular transformation, in addition to HCV encoded core proteins that cause disruption of regulatory pathways that control cell cycle [37].

In this study, HCV prevalence in HCC group (I) was 27/30 (90%) and was 25/30 (83.3%) in LC group (II). These results are in agreement with El-Zayadi et al.[13] who reported that HCV infection accounted for 86.9% of HCC cases during a single study over a decade for HCC in Egypt. Ahmed and colleagues [34]also reported a prevalence of HCV of 96% in HCC and 90% in LC.

In this study, HBV prevalence in HCC group it was 3/30 (10%), while in LC group was 5/30 (16.7%), these results were in concordance to, Ahmed and colleagues[34] who reported a prevalence of HBV in HCC 16 % and in LC 10%. These results were also, in agreement with El-Zayadi et al[13] who noticed a significant decline of HBV infection in HCC patients from 38.6% to 20.5% in their study for HCC in Egypt.

Alpha Feto protein is the most commonly used serum marker for routine surveillance of HCC. However there is a poor sensitivity and specificity of AFP has been reported in different studies and elevated serum AFP levels are noted in patients with chronic hepatitis C without HCC[38-41].

In this study , serum AFP , it was significantly increased in group I with median of 525 ng/ml (206.7 – 2741.25 ng/ml) and in group II with median of 5.0 ng/ml (3.75 – 8.0 ng/ml)than in group III with median of 3.5 ng/ml (2.0 – 5.0 ng/ml).

Wang et al. [19] reported that the AFP test has a sensitivity of 39 - 65%, a specificity of 76- 94%. So, because AFP diagnostic accuracy is unsatisfactory and questionable due to its low sensitivity. Therefore, HCC-specific biomarkers and more sensitive methods are urgently needed by clinicians to accurately diagnose HCC at an early stage [41].

In this study, it was found that the median GPC-3 level among patients with HCC (group I) was 9.74 ng/mL and ranging (6.6 - 14.03) ng/mL, which was higher than the cases with LC (group II): 0.6 ng/mL (0.4 - 1.17) ng/ml, and higher than those control (group III) subjects: 0.013 ng/mL (0.01 - 0.03) ng/mL.

These results are in agreement with Zakhary et al,[42] who reported that patients with liver cirrhosis had elevated levels of serum GPC3 with values ranging from 0.0 to 3.8 ng/ml and the level of serum GPC3 in patients with liver cirrhosis was significantly higher than those of healthy controls and compensated HCV.

Also these results come in agreement with Yang et al.[6] who had demonstrated that serum GPC3 level was significantly higher in HCC patients than those in control group subjects, and patients with hepatitis or liver cirrhosis. Moreover, these findings are in accordance with Badr et al.[7] who found that serum levels of GPC3 were significantly higher in patients with HCC compared with HCV cirrhosis group. Also, these levels were significantly higher in these two patients' groups versus controls. The same results also were reported by many other studies. [42,43,44]

In the present study the GPC3 was less than 2 ng/ml in sera of healthy subjects and patients with LC, but its level was significantly increased in 93.3%(28/30) of patients with HCC more than 4 ng/ml. In addition, only 3 (10%) patients with cirrhosis displayed elevated levels of serum GPC3 from 2-4 ng/ml. These results come in agreement with Capurro et al.[45] who demonstrated that GPC3 was undetectable in sera of healthy donors and patients with hepatitis, but its level was significantly increased in 53%(18/34) of patients with HCC. In addition, only one of 20 patients with cirrhosis displayed elevated levels of serum GPC3. Accordingly, the sensivity and the specificity of GPC3 were reported as 53% and 95%, respectively.

Tangkijvanich et al.[46] reported that the sensitivity and the specificity of GPC3 as serum maker for differentiating HCC from benign liver disease were 53% and 99%, respectively. Collectively, previous data and this study results confirm a very high sensitivity and specificity of GPC3 in differentiating HCC from non-malignant chronic liver disease.

In the present study, it should be mentioned that detectable serum GPC3 level in patients with HCC was influenced by the presence of viral hepatitis markers (anti-HCV positive). These data suggest that serum GPC3 might be more sensitive for detecting HCC in patients with pre-existing chronic viral hepatitis infection than those without viral markers. Thus, it appears that measurement of serum GPC3 may be advantageous for diagnosis of HCC, particularly in endemic areas of HCV or HBV infections [47, 48]

In the current study; sensitivity and specificity of serum GPC-3 were assessed and it was reported that: at a cut off value of 2.72ng/ml, serum GPC-3 has sensitivity (93.0%) and specificity (94 %).

These results are in agreement with Liu et al.[49] who reported that GPC3 protein is a sensitive and specific serum marker for diagnosis of early HCC. Liu et al. (2010) [49]observed that the serum GPC3 level was

high in 50% patients with HCC and its sensitivity and specificity for HCC were 47.0% and 93.5%, respectively.

Tsuchiya et al. and Liu et al.[10,49] reported that combined serum AFP and GPC-3 significantly increased the sensitivity to the diagnosis of HCC.

Many studies had demonstrated the efficacy of GPC-3 as a diagnostic tool in HCC and reported that the sensitivity and specificity ranged from 47-93.3%, and 41.8-100%, respectively [50-52].

On the other hand, four conflicting studies enrolled liver cirrhosis patients as control subjects, even though they had various complications, including alcoholic cirrhosis and HBV or HCV associated cirrhosis, all four studies observed that serum GPC3 level is higher in liver cirrhosis patients than that in HCC patients [47, 48, 53, 54]

This wide range of difference may be due to different patients' characteristics, the presence of HCV as an etiological factor for HCC or using different cut-off values for GPC-3 [10, 42.]

In this study, there was insignificant association between tumor size and the level of GPC3 and the level of AFP, these results come in agreement with Badr et al.[7] who found that serum GPC-3 levels were not correlated with tumor size.

Since serum GPC3 level estimation can be used as simple, rapid, non-invasive and a good diagnostic biomarker for early HCC detection, as GPC3 has very high sensitivity and specificity which can elevate the accuracy of diagnosis.

CONCLUSIONS

Serum GPC3 is highly sensitive and specific for detecting HCC. Moreover GPC3 is more sensitive in detection of small HCC than AFP. So GPC3 can be used for monitoring patients with cirrhosis that may develop HCC after many years, and it can be used as a useful biomarker for early detection of HCC when it is small, asymptomatic, and potentially curable.

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