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Study of Glypican-3 in Patients with Hepatitis C Virus-Related Hepatocellular Carcinoma

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Abstract

Background: Early diagnosis and treatment of HCC can greatly improve the efficiency of treatment and extend patient life.

Aim of Study: To investigate the expression of serum Glypican-3 (GPC3) in patients with Hepatocellular Carcinoma (HCC) and determine its efficacy as a screening test in early detection of HCC.

Patients and Methods: This case control study involved 30 HCC patients, 30 liver cirrhotic patients and 20 healthy controls. This study had been approved by local institutional research board in Menoufia Faculty of Medicine. All subjects participated in the study voluntarily and written informed consent was obtained from each participant. Clinical examination, abdominal ultrasonography and triphasic Computed Tomography (CT) for focal lesion were performed. Liver function tests were performed using clinical auto-analyzer, serum a-Fetoprotein (AFP) was measured using Enzyme-linked Immune-Sorbent Assay (ELISA) method and GPC3 was determined by ELISA kit for GPC3. Data were collected and statistically analyzed.

Results: GPC3 was highly significant higher in HCC group than cirrhotic and control groups. There was highly positive significant correlation between GPC3 and child score, size of focal lesions and number of focal lesions. The sensitivity of GPC3 in diagnosis of HCC was (68.5%) and the specificity was (83.3%) at cut off point (58.2ng/ml) that elicited from the Receiver Operator Characteristic (ROC) curve with very good Area Under Curve (AUC) (0.814), whereas that the sensitivity of AFP was (66.7%) and the specificity was (66.7%) at cut off point (380ng/ml) that elicited from the ROC curve with very good AUC (0.679).

Conclusion: GPC3 is highly associated with HCC and is more sensitive than AFP for early detection of HCC.

Key Words: *Alpha -fetoprotein – Enzyme-linked immune-sorbent assay – Glypican-3 – Hepatocellular carcinoma.*

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Introduction

HCC is the dominant variety of liver cancer; it represents the fourth leading cause of cancer deaths worldwide [1]. It constitutes about 70% of all liver tumors among Egyptians. The Hepatitis C Virus (HCV) infection is the most common risk factor of HCC in Egypt which leads to cirrhosis and severe liver damage [2]. About 60-80% of HCV infection leads to chronic hepatitis in the patients, and 10-20% of those patients develop cirrhosis within 20-30 years. About 1-5% of patients with cirrhotic liver might develop HCC [3]. Abdominal ultrasonography is well established to be used with or without AFP every 6 months as the standard surveillance strategy [4]. Some tumors do not produce AFP while others produce it with high levels. So, the diagnostic levels of AFP is variable and not the same in many studies [5], however, it is accepted now that levels of more than 20ng/ml are diagnostic for HCC [6]. Glypican-3 (GPC3) is an oncofetal proteoglycan. It is attached to the hepatocyte cell wall. It is normally found in the embryonic hepatocyte but not in the normal mature liver. GPC3 is responsible of regulation, activation and depletion of various growth factors. This control depends on the power of glypicans to activate or suppress these growth factors and the reactions with their receptors [7]. A lot of studies have reported the involvement of GPC3 in many types of tumors, including HCC [8]. There is increasing evidence indicating that approximately 40% of HCC patients are positive for GPC3 and negative for AFP [9]. The aim of this study is to investigate the expression of serum GPC3 in patients with HCC and determine its efficacy as a screening test in early detection of HCC.

Patients and Methods

This case control study consisted of 80 subjects who visited the Oncology and Internal Medicine Department at Menoufia Liver Institute during the period from June 2019 till December 2019.

This study had been approved by Local Institutional Research board in Menoufia Faculty of Medicine. All subjects participated in the study voluntarily and written informed consent was obtained from each participant. Subjects were classified into three groups, group I included 30 HCC patients, group II included 30 liver cirrhosis patients and group III included 20 healthy subjects as control group.

Inclusion criteria included patients with HCV related liver cirrhosis and HCC, while patients with other malignancies or HCC distant metastasis were excluded.

Medical history taking and complete physical examination with particular emphasis on signs of chronic liver disease were done for all subjects.

Laboratory data included; Complete blood picture (CBC) analyzed in (an automated ADVIA-120 hematological analyzer), data on liver function tests (AST, ALT, serum bilirubin, International Normalized Ratio (INR) and serum albumin) analyzed in (AU480 BECK Man, USA analyzer), serum AFP was measured using ELISA method and GPC3 was determined by ELISA kit provided by (Chongqing Biospes Company, China), according to the recommendation of the manufacturer. The kit uses a double-antibody sandwich ELISA to assay the level of GPC3 samples.

Data were collected and statistically analyzed.

Radiological investigations were done like ultrasound and Triphasic CT on the abdomen and pelvis.

Clinical and Laboratory data of the cases were tabulated.

The study complied with the Faculty of Medicine, Menoufia University.

Statistical analysis:

The collected data was revised, coded and tabulated using Statistical package for Social Science (IBM Corp. Released 2017. IBM SPSS Statistics for Windows, Version 25.0 Armonk, NY: IBM Corp.). Data were presented and suitable analysis was done according to the type of data obtained for each parameter.

Mann Whitney Test (U-test) was done to test the normality of data distribution. Significant data was considered to be non parametric. Description of quantitative variables was in the form of mean and Standard Deviation (mean ± SD), description of qualitative variables was by frequency and percentage, chi square test was used to assess the relationship between two qualitative groups. Fisher's exact test was used to examine the relationship between two qualitative variables when the expected count is less than 5 in more than 20% of cells. Correlation analysis was used to assess the strength of association between two quantitative variables; Spearman's correlation coefficient defines the strength and direction of the linear relationship between two variables. p-value 0.05 was set to be statistically significant and p-value 0.001 was set to be highly significant.

Results

Regarding age, the mean was (57 ±7.3) years in HCC group, (53.7±6.6) years in Cirrhotic group and (51.3±7.3) years in Control group. So, there was no significant difference between HCC and cirrhotic groups (p_1-value 0.07). Also there was no significant difference between HCC group and control group (p_2-value 0.06) and there was no significant difference between cirrhotic group and control group (p_3-value 0.08). Regarding sex, HCC is more presented in males than in females. In HCC group there were 20 (66.7%) males and 10 (33.3%) females, in cirrhotic group there were 16 (53.3%) males and 14 (46.7%) females and in control group there were 13 (65%) males and 7 (35%) females. So, there was no significant difference among studied groups regarding sex (p-value more than 0.05 for each) (Table 1).

Regarding laboratory tests, the mean White Blood Cells (WBCs) count was (9.8 ±3.2 X 10^3/mm^3), (6.7±2.3 X 10^3/mm^3) and (8.7± 1.4 X 10^3/mm^3) for HCC, cirrhotic and control group respectively. So, there was no significant difference between studied groups regarding WBCs count (p-value 0.133). Regarding platelets, the mean platelets count was (105.1±32.9 X 10^3/mm^3), (93.60±30.6 X 10^3/mm^3) and (280.3±35.6 X 10^3/mm^3) for HCC, cirrhotic and control group respectively. So, the mean platelets count was highly significant lower in cirrhotic group than control group (p_1-value 0.001). The mean platelets count in HCC group was highly significant lower than control group (p_2-value 0.001). There was no significant difference between HCC and cirrhotic groups regarding platelets count (p_3-value 0.194). Regarding hemoglobin (Hgb) level, the mean Hgb level was

(10.8± 1.2gm/dl), (10.1 ± 1.6gm/dl) and (12.4±0.8 gm/dl) for HCC, cirrhotic and control groups respectively. So, the mean Hgb level in cirrhotic group was highly significant lower than control group (p_1-value 0.001). The mean Hgb level in HCC was highly significant lower than control group (p_2-value 0.001). There was no significant difference between HCC and cirrhotic groups regarding Hgb level (p_3-value 0.140). Regarding ALT, mean ALT level was (60.3 ± 19.3u/l), (55.5± 15.9u/l) and (21.5 ±4.2u/l) for HCC, cirrhotic and control group respectively. So, the mean ALT level in cirrhotic group was highly significant higher than control group (p_1-value 0.001), and the mean ALT level in HCC group was highly significant higher than control group (p_2-value 0.001) with no significant difference between HCC and cirrhotic groups regarding mean ALT level (p_3-value 0.394). Regarding AST, the mean AST value was (79.4 ± 26.8u/l), (71.5±21.2u/l) and (23.6±4.2u/l) for HCC, cirrhotic and control group respectively. So, the mean AST level in cirrhotic group was highly significant higher than control group (p_1-value 0.001), and the mean AST level in HCC group was highly significant higher than control group (p_2-value 0.001) with no significant difference between HCC and cirrhotic groups regarding mean AST level (p_3-value 0.527). Regarding Albumin, the mean Albumin level was (3.0 ±0.4g/dl), (2.9±0.3 g/dl) and (4.0±0.2g/dl) for HCC, cirrhotic and control group respectively. So, the mean Albumin level in cirrhotic group was highly significant lower than control group (p_1-value 0.001). And the mean Albumin level in HCC group was highly significant lower than control group (p_2-value 0.001). While, there was no significant difference between HCC and cirrhotic groups regarding mean Albumin level (p_3-value 0.297). Regarding total bilirubin, the mean total bilirubin level was (3.1 ±1.0 mg/dl), (1.6±0.5mg/dl) and (0.9 ±0.2mg/dl) for HCC, cirrhotic and control group respectively. So, there was no significant difference between cirrhotic and control groups regarding mean total bilirubin (p_1-value 0.053). While the mean total bilirubin level in HCC group was significant higher than control group (p_2-value 0.001) and the mean total bilirubin level in HCC group was significant higher than cirrhotic groups (p_3-value 0.001). Regarding direct bilirubin, the mean direct bilirubin level was (1.7±0.5mg/dl), (0.6±0.2mg/dl) and (0.2± 0.1mg/dl) for HCC, cirrhotic and control group respectively. So, there was no significant difference between cirrhotic and control groups regarding mean direct bilirubin (p_1-value 0.082). While the mean direct bilirubin level in HCC group was significant higher than control group (p_2-value 0.001), and the mean direct bilirubin level in HCC group was significant higher than cirrhotic groups (p_3-value 0.001). Regarding INR, the mean INR value was (1.5 ±0.3), (1.3±0.2) and (1.0±0.2) for HCC, cirrhotic and control group respectively. So, there was no significant difference between cirrhotic and control groups regarding mean INR value (p_1-value 0.465). While the mean INR value in HCC group was highly significant higher than control group (p_2-value 0.001). And, there was no significant difference between cirrhotic and HCC groups regarding mean INR value (p_3-value 0.176) (Table 2). Regarding AFP, mean AFP level was (451.5±97.3ng/ml), (10.7 ±23.9ng/ml) and (3.2 ± 1.6ng/ml) in HCC, cirrhotic and control groups respectively. So, the mean AFP level in cirrhotic group was highly significant higher than control group (p_1-value 0.001) and the mean AFP level in HCC group was highly significant higher than control group (p_2-value 0.001). And the mean AFP level in HCC group was significant higher than cirrhotic group (p_3-value 0.017). Regarding GPC3, the mean GPC3 level was (25.3 ±28.6 ■g/L■), (6.1 ± 10.9 ■g/L■) and (0.8±0.9 ■g/L■) in HCC, cirrhotic and control groups respectively. So, there was no significant difference between cirrhotic and control groups regarding serum GPC3 level (p_1-value 0.083) while the mean GPC3 level in HCC group was highly significant higher than control group (p_2-value 0.001). Also the mean GPC3 level in HCC group was highly significant higher than cirrhotic group (p_3-value 0.001) (Table 3). For GPC3 and other parameters in HCC group, our study showed that there was highly positive significant correlation between GPC3 and child score with (p-value 0.029), size of focal lesions with (p-value 0.011) and number of focal lesions with (p-value 0.001) in HCC group. There was negative significant between GPC3 and Albumin in HCC group with (p-value 0.676), but there was negative non significant correlation between GPC3 and age (p-value 0.065), WBCs (p-value 0.380), Platelets (p-value 0.927), Hgb (p-value 0.470), ALT (p-value 0.793), AST (p-value 0.907), total bilirubin (p-value 0.208), direct bilirubin (p-value 0.564), INR (p-value 0.462), urea (p-value 0.334), creatinine (p-value 0.914) and AFP (p-value 0.259) in HCC group (Table 4). For AFP, AFP showed excellent poor (AUC=0.679). At cut off value of 380ng/mL, sensitivity was 66.7%, specificity was 66.7%, PPV was 66.7%, NPV was 66.7%, and accuracy was 66.7%. For GPC3, GPC3 showed good AUC (AUC=0.814). At cut off value of 58.2 ■g/L■ sensitivity was 68.5%, specificity was 83.3%, PPV was 80%, NPV was 71.4%, and accuracy was 75%. For combination of 2 tests showed excellent

AUC (AUC=0.922). At cutoff point 380ng/ml for AFP and 58.2 ng/L for GPC3, sensitivity was 70%, specificity was 86.7%, PPV was 84%, NPV was 74.3%, and accuracy was 78.4% (Table 5).

Table (1): Statically comparison among studied groups regarding age and sex.

	Control N=20	LC N=30	HCC N=30	Statistical test	*p*-value	Post Hoc test
Age (years):						
Mean ± SD	51.3±7.3	53.7±6.6	57±7.3	F=1.7	0.190	p_1=0.08 p_2=0.06 p_3=0.070
Males:						
N	13	16	20	χ^2=1.3	0.527	p_1=0.413
%	65%	53.3%	66.7%			p_2=0.903 p_3=0.292
Females:						
N	7	14	10			
%	35%	46.7%	33.3%			

HCC : Hepatocellular-Carcinoma.
LC : Liver Cirrhosis.
SD : Standard Deviation.
p-value is significant if <0.05.
p : Comparison between control, LC and HCC.
p_1 : Comparison of LC versus control.
p_2 : Comparison of HCC versus control.
p_3 : Comparison between LC and HCC.
F : Fisher's exact test.
χ^2 : Chi-square test.

Table (2): Statically comparison among studied groups regarding laboratory tests.

Laboratory tests	Range	Control N=20	LC N=30	HCC N=30	Statistical test	*p*-value	Post Hoc test
WBCs (X 10^9/L):							
Mean ± SD	4000-11000	8.7±1.4	6.7±2.3	9.8±3.2	F=2.1	0.133	p_1=0.793 p_2=0.106 p_3=0.173
Platelets (X 10^9/L):							
Mean ± SD	150,000-450'000	280.3±35.6	93.6±30.6	105.1±32.9	F=214.1	0.001*	p_1=0.001 * p_2=0.001* p_3=0.194
Hemoglobin (g/dL):							
Mean ± SD	M: 13.5-17.5 F: 12.0-15.5	12.4±0.8	10.1±1.6	10.8±1.2	F=20.4	0.001*	p_1=0.001 * p_2=0.001* p_3=0.140
ALT (U/L):							
Mean ± SD	7-56	21.5±4.2	55.5±15.9	60.3±19.3	F=21.1	0.001*	p_1=0.001 * p_2=0.001* p_3=0.394
AST (U/L):							
Mean ± SD	10-40	23.6±4.2	71.5±21.2	79.4±26.8	F=9.1	0.001*	p_1=0.001 * p_2=0.001* p_3=0.527
Albumin (g/dL):							
Mean ± SD	3.5-5.5	4.0±0.2	2.9±0.3	3.0±0.4	F=78.8	0.001*	p_1=0.001 * p_2=0.001* p_3=0.297
Total bilirubin (mg/dL):							
Mean ± SD	0.1-1.2	0.9±0.2	1.6±0.5	3.1±1.0	F=20.9	0.001*	p_1=0.053 p_2=0.001* p_3=0.001*
Direct bilirubin (mg/dL):							
Mean ± SD	Less than 0.3	0.2±0.1	0.6±0.2	1.7±0.5	F=27.9	0.001*	p_1=0.082 p_2=0.001* p_3=0.001*
INR:							
Mean ± SD	0.8-1.2	1.1±0.1	1.3±0.2	1.5±0.2	F=36.2	0.231	p_1=0.465 p_2=0.001* p_3=0.176

HCC : Hepatocellular-Carcinoma.
LC : Liver Cirrhosis.
SD : Standard Deviation.
p-value is significant if <0.05.
p : Comparison between control, LC and HCC.
p_1 : Comparison of LC versus control.
p_2 : Comparison of HCC versus control.
p_3 : Comparison between LC and HCC.
WBCs : White Blood Cells.
ALT : Alanine Transaminase Enzyme.
AST : Aspartate Transaminase Enzyme.
INR : International Normalized Ratio.
F : Fisher's exact test.
* : Significant.
N : Number.

Table (3): Statically comparison among studied groups regarding AFP and GPC3 test.

	Norma range		Control N=20	LC N=30	HCC N=30	Statistical test	*p*-value	Post Hoc test
AFP (ng/mL):	*Less than 10:*	Mean	3.2	10.7	451.5	K=28.9	0.001 *	p_1=0.001 *
		SD	1.6	23.9	97.3			p_2=0.001 *
		Median	3.5	5	452.5			p_3=0.17
		Min	1	1	133.2			
		Max	7	108	663			
Glypican-3 (ng/mL):	*0.7-15:*	Mean	0.8	6.1	25.3	K=36.2	0.001 *	p_1=0.083
		SD	0.9	10.9	28.6			p_2=0.001 *
		Median	0.4	1	17.9			p_3=0.001 *
		Min	0.03	0.1	0.9			
		Max	3.6	48	93.3			

HCC : Hepatocellular-Carcinoma.
LC : Liver Cirrhosis.
AFP : Alfa Feto-Protein.
SD : Standard Deviation.
p-value is significant if <0.05.
p : Comparison between control, LC and HCC.
p_1 : Comparison of LC versus control.
p_2 : Comparison of HCC versus control.
p_3: Comparison between LC and HCC.
K : Kruskal-Wallis test.
* : Significant.
N : Number.

Table (4): Statically comparison between glypican-3 and other parameters in HCC group.

Parameters	Glypican-3	
	r	*p*
Age	–0.341	0.065
WBCs	–0.166	0.380
Platelets	–0.018	0.927
Hemoglobin	–0.137	0.470
ALT	–0.050	0.793
AST	–0.022	0.907
Albumin	0.080	0.676
Total bilirubin	–0.237	0.208
Direct bilirubin	–0.110	0.564
INR	0.139	0.462
Urea	0.183	0.334
Creatinine	0.213	0.259
AFP	0.020	0.914
CHILD score	0.399	0.029
Size of lesions	0.703	0.011
Number of lesions	0.819	0.001

r : Spearman's correlation coefficient.
WBCs : White Blood Cells.
ALT : Alanine Transaminase Enzyme.
AST : Aspartate Transaminase Enzyme.
INR : International Normalized Ratio.
AFP : Alpha-Fetoprotein.
HCC : Hepatocellular-Carcinoma.
p : Comparison between control, LC and HCC.

Table (5): Sensitivity and specificity of AFP and GPC3 for screening of HCC.

	AFP	Glypican-3	Combination of Glypican-3 and AFP
AUC	0.679	0.814	0.856
Cut off	380	58.2	
Sensitivity (%)	66.7	68.5	70
Specificity (%)	66.7	83.3	86.7
PPV (%)	66.7	80	84
NPV (%)	66.7	71.4	74.3
Accuracy (%)	66.7	75	78.4

AUC : Area Under Curve. PPV : Positive Predictive Value.
ROC : Receiver Operating Curve. NPV : Negative Predictive Value.
Rough AUC Guidelines: The area under the ROC curve (AUC) results were considered excellent for AUC values between 0.9-1, good for AUC values between 0.8-0.9, fair for AUC values between 0.7-0.8, poor for AUC values between 0.6-0.7 and failed for AUC values between 0.5-0.6).

Discussion

In the current study, the mean age was (57 ±7.3) years in HCC group, (53.7±6.6) years in cirrhotic group and (51.3±7.3) years in control group. So, there was no significant difference between HCC and cirrhotic groups. Also there was no significant difference between HCC group and control group and there was no significant difference between cirrhotic group and control group. Our study in agreement with Omar et al., **[10]** who reported that the mean age was 56.9±7.27 years in HCC group while was 52.89±9.24 years in cirrhotic group. Our results showed that HCC was highly prevalent in males, there were 20 (66.7%) males and 10 (33.3%) females in HCC group, in cirrhotic group there were 16 (53.3%) males and 14 (46.7%) females and in control group there were 13 (65%) males and 7 (35%) females. So, there was no significant difference among studied groups regarding sex. Our results agreed with Badr et al., **[2]** who reported that in HCC group they were 25 males and 5 females, in cirrhotic group they were 23 males and 7 females. Our study showed regarding platelets count the mean platelets count was highly significant lower in cirrhotic group than control group but there was no significant difference between HCC and cirrhotic groups regarding platelets count and regarding Hgb concentration showed no significant difference between HCC and cirrhotic group and highly significant difference between HCC and cirrhotic groups and control group being lower Hgb concentration in cirrhotic and HCC groups. Our results were agreed with Omar et al., **[10]** who showed that the mean Hgb concentration was (9.37± 1.57gm/dl) in cirrhotic group while in HCC group it was (9.98±1.28gm/dl) with no significant difference regarding the mean Hgb concentration. Also, the platelets count show no significant difference between HCC group (131.38 ±

74.7 10^3 X mm^3) and cirrhotic group (98.66±61.09 10^3 X mm^3). In contrary to our results, Paranaguá-Vezozzo et al., [11] who showed that the mean Platelets count was significant lower in HCC group (83.9 10^3 X mm^3) than cirrhotic group (118.5 10^3 X mm^3), the lower blood platelet count in HCC patients can be explained by a longer evolution of chronic liver disease with subsequent advanced portal hypertension and hypersplenism. Our study showed that the mean ALT and AST value were highly significant higher in HCC group and cirrhotic group than in control group with no significant difference between HCC group and cirrhotic group. Our study showed that there was highly significant difference regarding mean values of serum albumin, total and direct bilirubin and INR between HCC group and control group being lower albumin, higher total, direct bilirubin and INR in HCC group, also showed no significant difference regarding serum albumin, total bilirubin direct bilirubin and INR between HCC and cirrhotic groups. Also there was highly significant difference regarding mean values of serum albumin, but there was no significant difference regarding total, direct bilirubin and INR between cirrhotic and control groups. Our results agreed with Omar et al., [10] who showed that both ALT and AST mean values were highly significant higher in HCC group (59.4±37.1u/l for ALT and 85.4±65.8u/l for AST) and cirrhotic group (49.28±26.7u/l for ALT and 66.83±42.2u/l for AST) than in control group (23.77±6.5u/l for ALT and 24.07±6.9u/l for AST), while there was no significant difference between HCC group and cirrhotic group, also there were statistically highly significant low mean values of serum albumin in both HCC and cirrhotic groups being 2.24+0.48gm/dl and 2.7+0.54gm/dl respectively. Our results were in contrary to Paranaguá-Vezozzo et al., [11] who showed that both ALT and AST mean values were significant higher in HCC group (70u/l for ALT and 91u/l for AST) than in cirrhotic group (47u/l for ALT and 53u/l for AST). Our study showed that there was highly positive significant correlation between GPC3 and child score, size of focal lesions and number of focal lesions in HCC group. There was negative significant between GPC3 and Albumin in HCC group. But there was negative non significant correlation between GPC3 and age, WBCs, Platelets, Hgb, ALT, AST, total bilirubin, direct bilirubin, INR, and AFP in HCC group. Our study comes in agreement with Jia et al., [12] who reported that there was no significant correlation between serum levels of AFP and GPC3. Also Jia et al., [12] reported that there was no statistical association between serum GPC3 and age, gender, Child-Pugh score, AFP level, number of tumors, or tumor size. In contrast to our study Mohamed [13] reported that there was no significant association between tumor size and the level of GPC3 and the level of AFP. Although the test for AFP is widely available, inexpensive, and easy to perform, it has poor accuracy as a serological test for the early detection of HCC. Levels of AFP increase not only in people with HCC, but also in people with active hepatitis, cirrhosis without HCC, or exacerbation of the underlying liver disease, due to pathophysiological changes of inflammation and regeneration; this means the test can have low specificity in the population at risk Gopal et al., [14] . Our study showed that the mean AFP level in HCC group was significant higher than cirrhotic group while the mean AFP level in HCC group was highly significant higher than control group and the mean AFP level in cirrhotic group was highly significant higher than control group. Our results were close to Ismail et al., [15] who reported that AFP showed a significant elevation in the HCC group (4901.367 ±2185.800ng/ml) compared to the control group (4.033± 1.191ng/ml) and cirrhotic liver group (100.733±71.726ng/ml). Our study showed that the mean GPC3 level in HCC group was highly significant higher than cirrhotic and control groups and the mean GPC3 level in cirrhotic group was highly significant higher than control group. Also these results come in agreement with Yang et al., [16] 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. Our study showed that AFP showed excellent poor (AUC=0.679). At cut off value of 380ng/mL, sensitivity was 66.7%, specificity was 66.7%, PPV was 66.7%, NPV was 66.7%, and accuracy was 66.7%. Our results were close to also our results were close to Jia et al., [12] who reported that of the 102 samples from patients with HCC, 53 samples (51.96%) were positive for AFP at cut off point (400ng/ml). In our study GPC3 showed good AUC (AUC=0.814). At cut off value of 58.2 ng/L, sensitivity was 68.5%, specificity was 83.3%, PPV was 80%, NPV was 71.4%, and accuracy was 75%. Our result come in agreement with Mohamed [13] in which GPC3 was less than 2ng/ml in sera of healthy subjects and patients with liver cirrhosis, but its level was significantly increased in 93.3% (28/30) of patients with HCC more than 4ng/ml. In addition, only 3 (10%) patients with cirrhosis displayed elevated levels of serum GPC3 from 2-4ng/ml. At a cut off value of 2.72ng/ml, serum GPC3 has sensitivity (93.0%) and specificity (94%). Our study according to the combination of AFP and GPC3 tests, showed ex-

cellent AUC (AUC=0.922). At cutoff point 380 ng/ml for alpha-fetoprotein and 58.2 gg/L for GPC3, sensitivity was 70%, specificity was 86.7%, PPV was 84%, NPV was 74.3%, and accuracy was 78.4%. This comes in agreement with the study of Sun et al., **[8]** in which the sensitivity and specificity of combined detection of AFP and GPC3 reached 85.5% and 91.5%, respectively.

Conclusion:

We concluded that GPC3 is a valuable serum marker that can aid the early diagnosis of HCC. In combination, measurements of AFP and GPC3 have the advantage to improve the detection of one of the most common malignancies worldwide.

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دراسة جليبكان-٣ فى سرطان الكبد المرتبط بالإلتهاب الكبدى الفيروسى سى

يمكن للتشخيص المبكر وعلاج سرطان الكبد أن يحسن بشكل كبير من كفاءة العلاج ويطيل عمر المريض. حيث تهدف الدراسة إلى التحقيق من تعبير مصل جلبيكان-٣ (GPC3) فى مرضى سرطان الخلايا الكبدية وتحديد فعاليته كإختبار تحرى فى الكشف المبكر عن سرطان الخلايا الكبدية.

شملت الدراسة الحالية هذه ٣٠ مريضاً بسرطان الكبد، و٣٠ مريضاً بتليف الكبد، و٢٠ شخصاً من الأصحاء. تمت الموافقة على هذه الدراسة من قبل مجلس البحث المؤسسى المحلى بكلية الطب بالمنوفية. شارك جميع الأشخاص فى الدراسة طواعية وتم الحصول على موافقة مستنيرة مكتوبة من كل مشارك. تم إجراء الفحص السريرى وتصوير البطن بالموجات فوق الصوتية والتصوير المقطعى ثلاثى الأطوار للأفة البؤرية.

تم إجراء إختبارات وظائف الكبد بإستخدام محلل تلقائى إكلينيكى، وتم قياس مصل بروتين ألفا فيتوبروتين (AFP) ومصل (GPC3) بإستخدام طريقة مقايسة الممتز المناعى المرتبط بالإنزيم (ELISA) وتم جمع البيانات وتحليلها إحصائياً وأظهرت النتائج أن GPC3 أعلى معنوياً فى مجموعة سرطان الكبد مقارنة بمجموعات التليف الكبدى والأصحاء، وكان هناك إرتباط معنوى إيجابى للغاية بين GPC3 ومعامل (CHILD)، وحجم الأفات البؤرية وعدد الأفات البؤرية.

وخلصنا إلى أن مصل جليبكان-٣ يرتبط إرتباطاً وثيقاً بسرطان الكبد وهو أكثر حساسية من مصل بروتين ألفا فيتوبروتين للكشف المبكر عن سرطان الكبد.