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Original article

Hepatocellular Carcinoma Detection in Hepatitis C Virus-Related Cirrhosis by Glypican-3

Alaa Kandil [a], Seham G. Ameen [b]

[a] Department of Hepatology, Gastroenterology and Infectious Diseases, , Benha faculty of medicine, Benha University, Egypt. [b] Department of Clinical and Chemical Pathology , Benha faculty of medicine, Benha University, Egypt.

Correspondence to: Seham G. Ameen, Department of Clinical and Chemical Pathology, Benha faculty of medicine, Benha University, Egypt.

Email:

seham.ameen@fmed.bu.edu.eg

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Abstract

Background: Globally, hepatocellular carcinoma (HCC) is the second-leading cause of cancer-related fatalities and is a highly prevalent tumor. A brand-new molecule with a significant connection to the pathogenesis and detection of hepatocellular carcinoma has emerged: glypican-3, a heparan sulfate proteoglycan expressed on the surface of hepatocellular carcinoma cells. Therefore, the purpose of this study is to determine whether Glypican-3 is used alone or in conjunction with α-fetoprotein (AFP) to diagnose hepatocellular carcinoma. **Patients and Methods:** This study was conducted on 75 patients with a history of chronic hepatitis C-related cirrhosis, Patients were divided into three equal groups. Group I including cirrhotic patients without HCC, group II cirrhosis with HCC, and group III normal individuals serve as a control group. Serum levels of glypican-3 and α-fetoprotein (AFP) were measured, abdominal ultrasound, and triphasic computed tomography were done for all the study members. Serum alpha-fetoprotein measured by chemiluminescence and Glypican-3 was measured by enzyme linked immunosorbent assay (ELISA) kits. **Results:** The study included 40 male and 35 female arranged in the 3 groups. The age ranged between 43 and 65 with a mean of 41.98 in group 1, 55.4 in group 2, and 39.32 in group 3 which is statically significant. Glypican 3 (GLP3) with a cut-off>0.52 show a sensitivity of 96% and specificity of 94% while AFP with a cut-off>45 shows a sensitivity of 92% and specificity of 88%. GLP3 was more sensitive in the detection of the single focal lesion. **Conclusions:** Glypican-3 can be a pivotal diagnostic serum marker for HCC and may add value to alpha-fetoprotein increasing the overall detection of HCC.

Keywords: Hepatocellular carcinoma, HCC, Glypican 3, AFP.

Introduction

Hepatocellular carcinoma (HCC) is a challenging global health concern as it presents the sixth most common cancer worldwide and the third leading cause of cancer death globally with increasing incidence worldwide. Estimations reveal that by the year 2025, more than a million persons will be affected with liver cancer annually [(1)].

While in Egypt, it represents the fourth most common cancer. Also, HCC is conceded as a national challenging problem as Egypt comes in the 3rd and 15th place in Africa and worldwide, respectively in HCC incidence [(2)].

Hepatocellular carcinoma (HCC) prognosis remains poor, with high mortality index, despite the novel treatment methods [(3)].

HCC may be diagnosed with non-invasive methods such as ultrasound, computed tomography (CT), magnetic resonance imaging (MRI), and serum biomarkers while invasive methods such as biopsy not recommended for all lesions for fear of increasing the risk of tumor seeding and bleeding, and false-negative result due to obtain tissue from an inappropriate site, AASLD recommends biopsy only in lesions not typical for HCC on contrast-enhanced imaging [(4)].

The burden of HCC is arising from most of the cases diagnosed in the advanced stage so curative treatment options are missing. Thus, early diagnosis would help the patient and prevent the global burden [(5)].

Advanced imaging studies or Biopsy are not reliable methods for screening and surveillance of HCC due to their cost-effectiveness, time consumption, not being available at every center and intraoperative error, so serum tumor markers such as AFP are the most common method used in the surveillance and early detection of HCC in cirrhotic patients [(4)].

Alpha-fetoprotein (AFP) is an oncofetal glycoprotein produced by the yolk sac, fetal liver, and gastrointestinal tract in fetal life. Only a trace amount of AFP can be measured in adults due to its rapid decline in adulthood. The utility of AFP as surveillance and the diagnostic test has been criticized due to both low sensitivity and specificity [(6)].

The core of this criticism is that Serum AFP levels are normal in 30–40% of patients with HCC, with good specificity AFP lacks sensitivity as it is also released by the normal liver in the set of the inflammatory process and hepatocyte damage and most of HCCs develop in cases of long-standing liver disease, to distinguish HCC from background liver pathology as hepatitis requires a high diagnostic cut-off level of AFP about 400–500 ng/mL range. Thus, many HCCs did not achieve these diagnostic levels until the late stages which magnitude the burden of HCC [(7)].

The American Association for the Study of Liver Diseases recommended that AFP is optional for the screening of HCC with ultrasonography, while the European Association for the Study of the Liver recommends ultrasonography alone [(8,9)].

In Egypt, there has been a remarkable increase in the proportion of hepatocellular carcinoma (HCC) among chronic liver disease patients. This could be explained by the presence of the main risk factors such as hepatitis C virus (HCV) infection, hepatitis B virus (HBV) infection, the presence of national follow-up programs for cirrhotic patients which increase the detection rate and the awareness among HCC, also the eradication of hepatitis virus that share in the increase of cirrhotic patients survival rate which gives some of them a time to develop HCC [(10)].

The urge for Biomarkers that could be used in screening, and early detection of HCC and can distinguish HCC from inflammation and cirrhosis has become a major challenge globally and locally, especially in groups suffering from cirrhosis, whose numbers are constantly increasing [(5)].

In general, a biomarker valuable for clinical use must have an acceptable level of sensitivity and specificity, be minimal or non-invasive, cost-effective, tumor-specific, and easily detectable in bodily fluids. From this general view, we conclude that the ideal HCC biomarker is the one that can diagnose asymptomatic patients, is tumor-specific, and can be widely used in surveillance programs. Also, a combination of one or more tumor markers may increase the sensitivity [(11).]

Glypican-3 (GPC3) is a membrane-associated proteoglycan that is specifically up-regulated in hepatocellular carcinoma (HCC) although rarely or not expressed in normal liver tissues making it a perfect diagnostic marker and could be used as a targeted therapy for HCC (12), another advantage for GPC3 was noted by Aydin et al., 2021 as the serum level of GPC3 decreases post-treatment correlated with response to locoregional chemotherapy compared to change in serum AFP in HCC patients awaiting liver transplantation [(13)].

Aim of the work: This study aims to determine the role of Glypican-3 in the diagnosis of HCC.

Patients and methods

Study design: Comparative cross-sectional study.

Study settings: The study was conducted on 75 subjects whose ages ranged from 43 to 65 years in the period between November 2021 and July 2022 obtained from the hepatology, gastroenterology, and infectious diseases department, Benha University hospital. The study was approved by local ethical committee of Benha faculty of Medicine. (REC-FOMBU 41-10-2022)

Study population: Patients were divided into three groups.

Group I: including 25 Patients with HCV-related cirrhosis without HCC.

Group II: including 25 Patients with HCV-related cirrhosis with HCC.

Group III: including 25 normal individuals serve as a control

Liver cirrhosis: determined by elevated liver stiffness by transient elastography

HCC: detected by US and CT

Inclusion Criteria

- Cirrhotic patients (more than 7KPa with elastography).

- with one or more imaging evidence of HCC.

Exclusion Criteria

- Age less than 18 years.
- Patients with significant alcohol consumption.
- Patients with symptoms and signs suggest:
  a. Hemochromatosis.
  b. Wilson's disease.
  c. Alpha-one anti-trypsin deficiency.
  d. Autoimmune hepatitis.
- Patients with a history of chemotherapy.
- Severe heart failure.
- Severe renal failure.
- Pregnancy.
- Previous loco-regional /systemic therapy for HCC.
- Autoimmune and collagenic diseases.
- Other non-hepatic malignancies and metastasis.
- Cryptogenic cirrhosis.
- Budd-Chiari syndrome or other vascular disorders.

All patients were subjected to the following:

1. Clinical assessment including history taking and clinical examination.
2. Blood sampling and biochemical assays

Fasting venous blood samples (5 ml) were collected. 3 ml of blood was allowed to clot and then centrifuged at 3500g for 5 min to separate the serum used for assessment of aspartate aminotransferase (AST), alanine aminotransferase (ALT), Alkaline phosphatase (ALP)) , total bilirubin, direct bilirubin, albumin, creatnine and glucose concentrations were assayed using Beckman CX4 chemistry analyzer (NY, USA, supplied by the Eastern Co. For Eng. & Trade-Giza, Egypt). Viral infection status (HCV Ab and HBS Ag) were measured by ELISA, Serum AFP level was determined using chemiluminescence (Abbott USA) and serum Glypican 3 was measured by enzyme linked immunosorbent assay (ELISA; BioMosaics company, USA).. 2 ml blood was collected in vacutainer tubes containing EDTA for CBC.

3. Abdominal ultrasonography.
4. Fibroscan [10 valid measures of liver stiffness are indicated with at least 60% success rate and interquartile range < 30% of the median value with results expressed in Kilopascals (KPa).
6. Triphasic CT abdomen.

Statistical analysis

The Statistical Package for Social Science SPSS software version 20.0. (Armonk, NY: IBM Corp) was used to collect, edit, code, and input the data. Quantitative data were reported as means, standard deviations, and ranges wherever parametric, but qualitative data were expressed as numbers and percentages.

The Chi-square test was used to compare the groups in terms of qualitative data.

The independent t-test was used to compare quantitative data with parametric distribution between the two groups.

Using Repeated Measures ANOVA, it was possible to compare more than two paired groups of quantitative data with parametric distribution.

The allowable margin of error was set at 5%, while the confidence interval was set at 95%. The p-value was therefore deemed significant as follows:

P > 0.05 denotes non significance, P 0.05 denotes significance, while P 0.01 Highly denotes significance.

The diagnostic value for each marker was assessed using Sensitivity, specificity, positive (PPV) and negative (NPV) predictive values. Receiver operating characteristic curves (ROC) were constructed to assess the validity of the markers in predicting HCC by calculating the area under the curve (AUC). Pearson correlation test was used to identify the correlation between Glypican-3 and different clinicopathological variables.

Results

Between November 2021 and July 2022, 75 participants with ages ranging from 43 to 65 were recruited from the hepatology, gastrointestinal, and infectious diseases department at the Benha University hospital. Three groups of individuals were formed. **Group I:** 25 Patients with HCV-related cirrhosis without HCC. **Group II:** 25 Patients with HCV-related cirrhosis with HCC. **Group III:** 25 normal individuals serve as a control group.

p: p value for comparing between the three studied groups
p1: p value for comparing between group 1 and group 2
p2: p value for comparing between group 1 and group 3
p3: p value for comparing between group 2and group 3

Demographic features in the studied groups are illustrated in **table 1**, Regarding sex, this study includes 40 male and 35 female arranged in the 3 groups as follow 14 male and 11 female in group 1, 13 male and 12 female in group 2, and 13 male and 12 female in the control group 3. The age ranged between 43 and 65 with a mean of 41.98 in group 1, 55.4 in group 2, and 39.32 in group 3 which is statically significant. Regarding smoking, diabetes millets, and hypertension group 1 shows 52% smokers, 44% diabetic, and 52% hypertensive, Group 2 shows 72% smokers, 31% diabetic, and 40% hypertensive while group 3 which serve as a control group there was no one smoker, diabetic or hypertensive.

Regarding Child Pugh score, three groups arranged as follow Child A 0% in groups 1 and 2 and 100% for group 3, Child B 68%,56% for groups 1 and 2 while 0% for group 3 and Child C 32%, 11% in groups 1 and 2 while 0% for group 3 **table 1.**

As regard, laboratory findings between study groups **table 2** shows, complete blood picture there were statically significant differences between the three groups regarding hemoglobin, WBCs, and platelets while there were no significant differences between group 1 and 2 as regard hemoglobin and there were no differences between group 1 and 3 regarding WBCs. Also, there were significant differences between the study groups as regards ALT, AST, Albumin, Bilirubin, INR, and GGT while there were no statically differences between group 1 and group 2 as regards ALT and AST P1 0.072 and 0.747 for each. The renal function shows significant

differences between the study groups were noticed but there were no differences regarding creatinine between groups 1 and 2 P1 equals 0.218 and between groups 2 and 3 P3 equals 0.055.

Regarding tumor markers in the studied groups (table 2) :

AFP there was a significant difference between the study groups as <0.001 and these differences continue between group 1 and group 2, group 1 and group 3, and group 2 and group 3 as P1, P,2, and P3 equal <0.001 for each. Also, there were significant differences between the study groups regarding GLP3 as P <0.001 and these differences continue between group 1 and group 2, group 1 and group 3, and group 2 and group 3 as P1 and P3 equal <0.001 and P2 equal 0.004.

Regarding ascites detected by ultrasound, there were no ascites in 36% and 44%, mild to moderate in 28% and 20%, and moderate to severe ascites in 36% and 36% of cases in group 1 and 2 respectively which was non-significant between the two groups **table 3**.

As regards Fibro scan illustrated in **table 4** there were statically differences between the study groups regarding Fibro scan results as the mean for each group was 13.12,18.74 and 4.90 respectively.

Regarding the distribution of HCC Group II:

Table 5 shows the classified patients according to the following parameters tumor size <2cm 24%, 2-5cm 48% and >5cm 28% of cases, Malignant portal vein thrombosis 28% of cases show malignant thrombosis, regarding the number of lesions 32% of cases show multiple lesions and 40% of cases show regional lymphadenopathy.

Also, **Table 5** shows the Relation between AFP, GLP3, and different parameters in HCC group 3.

Table (6): Validity (AUC, sensitivity, specificity) for Glp3 and AFP to discriminate HCC from Cirrhosis:

Glypican 3 (GLP3) with a cut-off>0.52 shows a sensitivity of 96% and specificity of 94% while AFP with a cut-off>45 shows a sensitivity of 92% and specificity of 88%. GLP3 was more sensitive in the detection of the single focal lesion.

Table (1) Demographic features and characteristics of the studied patient groups.

	Group 1 cirrhotic (n = 25)		Group 2 HCC (n = 25)		Group 3 Control (n = 25)		
	No.	**%**	**No.**	**%**	**No.**	**%**	
Sex							
Male	14	56.0	13	52.0	13	52.0	
Female	11	44.0	12	48.0	12	48.0	
smoking							
nonsmoker	12	48	7	28	25	100	
smoker	13	52	18	72	0	0	
Diabetes							
Non diabetic	14	56	17	68	25	100	
Diabetic	11	44	8	32	0	0	
Hypertension							
Non hypertensive	12	48	15	60	25	100	
Hypertensive	13	52	10	40	0	0	
Age (years)							
Min. – Max.	43.0 – 60.0		49.0 – 66.0		48.0 – 65.0		
Mean ± SD.	41.98 ± 10.70		55.44 ± 5.39		39.32 ± 8.72		
Child-Pugh score							**p**
A	0	0.0	0	0.0	25	100.0	
B	17	68.0	14	56.0	0	0.0	<0.001*
C	8	32.0	11	44.0	0	0.0	

p: p value for comparing between the three studied groups
*: Statistically significant at $p \leq 0.05$

Table (2) Laboratory finding in the studied groups

CBC	Group1	Group 2	Group 3	p
hemoglobin				
Min. – Max.	8.40 – 12.1	7.1 – 11.20	9.3 – 15.20	<0.001*
Mean ± SD.	10.11 ± 0.79	9.85 ± 0.91	12.89 ± 1.55	
Sig. bet. Grps.		p_1=0.568,p_2<0.001*,p_3<0.001*		
WBCs				
Min. – Max.	4.00 – 9.95	3.12 – 5.85	4.52 – 9.1	<0.001*
Mean ± SD.	6.88 ± 1.72	4.63 ± 0.73	6.32 ± 1.05	
Sig. bet. Grps.		p_1<0.001*,p_2=0.229,p_3<0.001*		
Platelets				
Min. – Max.	49.5– 300.0	55.3 – 115.0	160.2 – 372.0	<0.001*
Mean ± SD.	152.9±65.01	80.72 ± 17.94	243.24 ± 54.94	
Sig. bet. Grps.		p_1<0.001*,p_2<0.001*,p_3<0.001*		
ALT (U/L)				
Min. – Max.	15.4 – 90.0	15.3– 90.0	11.5– 35.0	<0.001*
Mean ± SD.	34.94 ± 16.13	45.28 ± 19.33	20.0 ± 5.86	
Median (IQR)	30.0(25.0 – 36.0)	45.0(32.0 – 60.0)	18.0(16.0 – 24.0)	
Sig. bet. Grps.		p_1=0.072,p_2<0.001*,p_3<0.001*		
AST (U/L)				
Min. – Max.	15.0 – 70.0	13.0 – 70.0	8.0 – 40.0	<0.001*
Mean ± SD.	31.28 ± 10.50	36.08 ± 17.20	19.24 ± 7.72	
Median (IQR)	28.50(24.0–34.0)	37.0(18.0 – 50.0)	19.0(13.0 – 22.0)	
Sig. bet. Grps.		p_1=0.747,p_2<0.001*,p_3<0.001*		
Albumin (gm/dl)				
Min. – Max.	2.25– 4.55	2.12– 4.0	4.42 – 5.0	<0.001*
Mean ± SD.	3.67 ± 0.49	2.71 ± 0.51	4.77 ± 0.22	
Median (IQR)	3.80 (3.50 – 4.0)	2.50(2.30 – 3.10)	4.80 (4.50 – 5.0)	
Sig. bet. Grps.		p_1<0.001*,p_2<0.001*,p_3<0.001*		
Bilirubin				
Min. – Max.	0.80 – 2.50	1.10 – 4.50	0.90 – 1.10	<0.001*
Mean ± SD.	1.34 ± 0.45	2.14 ± 0.83	0.99 ± 0.07	
Median (IQR)	1.20 (1.0 – 1.60)	1.90(1.70 – 2.50)	1.0 (0.90 – 1.0)	
Sig. bet. Grps.		p_1<0.001*,p_2<0.001*,p_3<0.001*		
INR				
Min. – Max.	1.0 – 1.79	1.24 – 1.79	0.90 – 1.20	<0.001*

Mean ± SD.	1.33 ± 0.25	1.50 ± 0.16	1.01 ± 0.05	
Median (IQR)	1.21(1.15 – 1.55)	1.45(1.38 – 1.63)	1.0 (1.0 – 1.0)	
Sig. bet. Grps.	$p_1=0.019^*, p_2<0.001^*, p_3<0.001^*$			
GGT				
Min. – Max.	12.8 – 113.0	49.0 – 75.0	6.0 – 28.0	$<0.001^*$
Mean ± SD.	34.14 ± 16.38	56.52 ± 6.97	15.96 ± 5.72	
Median (IQR)	34.0(20.0 – 42.0)	55.0(52.0 – 60.0)	16.0(11.0 – 19.0)	
Sig. bet. Grps.	$p_1<0.001^*, p_2<0.001^*, p_3<0.001^*$			
Renal function				
Urea (mg/dl)				
Min. – Max.	15.0 – 40.0	15.0 – 70.0	18.0 – 33.0	$<0.001^*$
Mean ± SD.	22.20 ± 4.44	44.0 ± 16.18	25.92 ± 4.65	
Median (IQR)	22.0(20.0 – 24.0)	50.0(30.0 – 55.0)	26.0(23.0 – 29.0)	
Sig. bet. Grps.	$p_1<0.001^*, p_2=0.008^*, p_3=0.007^*$			
Creatinine				
Min. – Max.	0.80 – 1.60	0.67 – 1.31	0.56 – 1.30	$<0.001^*$
Mean ± SD.	1.08 ± 0.17	1.0 ± 0.19	0.89 ± 0.17	
Median (IQR)	1.0 (1.0 – 1.20)	1.02(0.86 – 1.12)	0.89 (0.77 – 1.0)	
Sig. bet. Grps.	$p_1=0.218, p_2<0.001^*, p_3=0.055$			
AFP (ng/dl)				
Min. – Max.	5.0 – 70.0	30.0 – 1400.0	1.0 – 11.0	$<0.001^*$
Mean ± SD.	21.50 ± 17.86	445.32 ± 481.97	4.84 ± 2.76	
Median (IQR)	12.0 (10.0 – 30.0)	200.0 (90.0 –1000.0)	4.0 (3.0 – 6.0)	
Sig. bet. Grps.	$p_1<0.001^*, p_2<0.001^*, p_3<0.001^*$			
GLP3 (ng/dl)				
Min. – Max.	0.41 – 0.56	0.51 – 0.67	0.39 – 0.47	$<0.001^*$
Mean ± SD.	0.47 ± 0.04	0.58 ± 0.04	0.44 ± 0.02	
Median (IQR)	0.47 (0.44–0.50)	0.56 (0.55–0.59)	0.44 (0.43–0.46)	
Sig. bet. Grps.	$p_1<0.001^*, p_2=0.004^*, p_3<0.001^*$			

IQR: **Inter quartile range** SD: **Standard deviation**

p: p value for comparing between the three studied groups

p_1: p value for comparing between **group 1** and **group 2**

p_2: p value for comparing between **group 1** and **group 3**

p_3: p value for comparing between **group 2**and **group 3**

*: Statistically significant at $p \leq 0.05$

Table (3) Ascites diagnosed by ultrasound in the studied groups

Ascites	Group 1 (n = 25)	Group 2 (n = 25)	p
No ascites	9/25 36%	11/25 44%	
Mild to moderate	7/25 28%	5/25 20%	0.676
Moderate to severe	9/25 36%	9/25 36%	

Table (4) Fibro scan in the studied groups.

Fibro scan (Kpa)	Cirrhosis (n = 25)	HCC (n = 25)	Control (n = 25)	p
Min. – Max.	6.32 – 22.34	15.24 – 22.37	4.0 – 6.0	
Mean ± SD.	13.12 ± 4.90	18.74 ± 2.48	4.90 ± 0.43	<0.001*
Median (IQR)	13.01(8.24 – 16.87)	19.25(16.40–20.62)	5.0 (4.60 – 5.10)	
Sig. bet. Grps.	p_1=0.001*,p_2<0.001*,p_3<0.001*			

IQR: **Inter quartile range** SD: **Standard deviation**
p: p value for comparing between the three studied groups
p_1: p value for comparing between **group 1** and **group 2**
p_2: p value for comparing between **group 1** and **group 3**
p_3: p value for comparing between **group 2**and **group 3**
*: Statistically significant at p ≤ 0.05

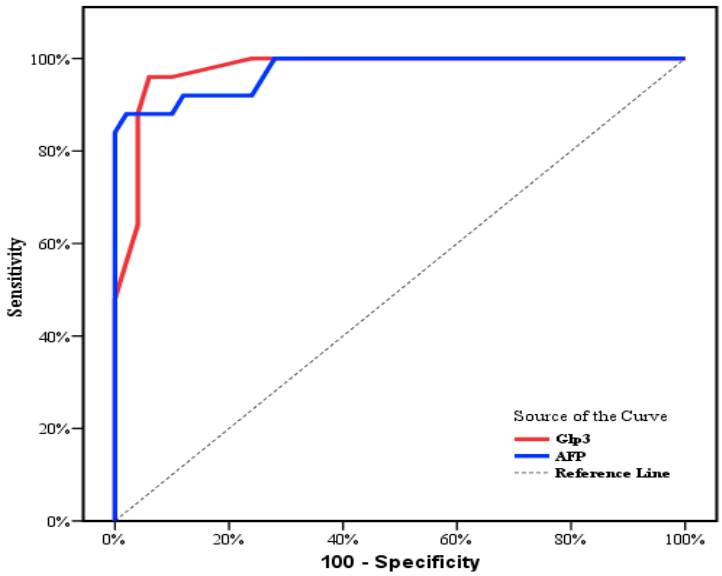

Fig. (1): ROC curve for Glp3 and AFP to discriminate HCC

Table (5): Relation between serum markers (GLP3 - AFP) and triphasic CT findings distribution in HCC group 3

			GLP3				AFP			
			Min. – Max.	**Mean ± SD.**	**Median**	**p**	**Min. – Max.**	**Mean ± SD.**	**Median**	**p**
Tumor size (cm)	**N**	**%**								
<2	**6**	24	0.51 – 0.63	0.56 ± 0.04	0.56		30.0 – 300.0	93.0 ± 103.33	60.0	
2 – 5	**12**	48	0.53 – 0.67	0.58 ± 0.04	0.56	0.544	80.0 – 1000.0	231.25 ± 249.71	165.0	<0.001*
>5	**7**	28	0.53 – 0.64	0.59 ± 0.04	0.59		600.0 – 1400.0	1114.29± 260.95	1200.0	
Malignant portal thrombosis										
No	**18**	**72**	0.51 – 0.65	0.57 ± 0.04	0.57	0.053	30.0 – 1400.0	505.72 ± 504.31	215.0	0.317
Yes	**7**	**28**	0.56 – 0.61	0.58 ± 0.03	0.59		50.0 – 1450.0	420.0 ± 526.56	235.0	
Single or multiple										
Single	**18**	**68**	0.51 – 0.67	0.56 ± 0.04	0.56	0.017*	30.0 – 1100.0	190.72 ± 241.10	125.0	<0.001*
Multiple	**7**	**32**	0.55 – 0.65	0.61 ± 0.03	0.61		600.0 – 1400.0	1100.0 ± 264.58	1200.0	
Lymphadenopathy (regional)										
No	**15**	**60**	0.53 – 0.67	0.57 ± 0.04	0.56	0.371	30.0 – 1300.0	276.87 ± 382.63	130.0	0.080
Yes	**10**	**40**	0.51 – 0.65	0.59 ± 0.04	0.59		30.0 – 1400.0	698.0 ± 522.96	800.0	

t: Student t-test **F**: **F for One way ANOVA test**

p: p value for comparison between the studied categories

*: Statistically significant at $p \leq 0.05$

Table (6): Validity (AUC, sensitivity, specificity) for Glp3 and AFP to discriminate HCC from Cirrhosis

	AUC	**P**	**95% C.I**	**Cut off**	**Sensitivity**	**Specificity**	**PPV**	**NPV**
Glp3	0.976	<0.001*	0.948 – 1.0	>0.52	96.0	94.0	88.9	97.9
AFP	0.974	<0.001*	0.943 – 1.0	>45	92.0	88.0	79.3	95.7

AUC: Area Under a Curve p value: Probability value

CI: Confidence Intervals

NPV: Negative predictive value PPV: Positive predictive value

*: Statistically significant at $p \leq 0.05$

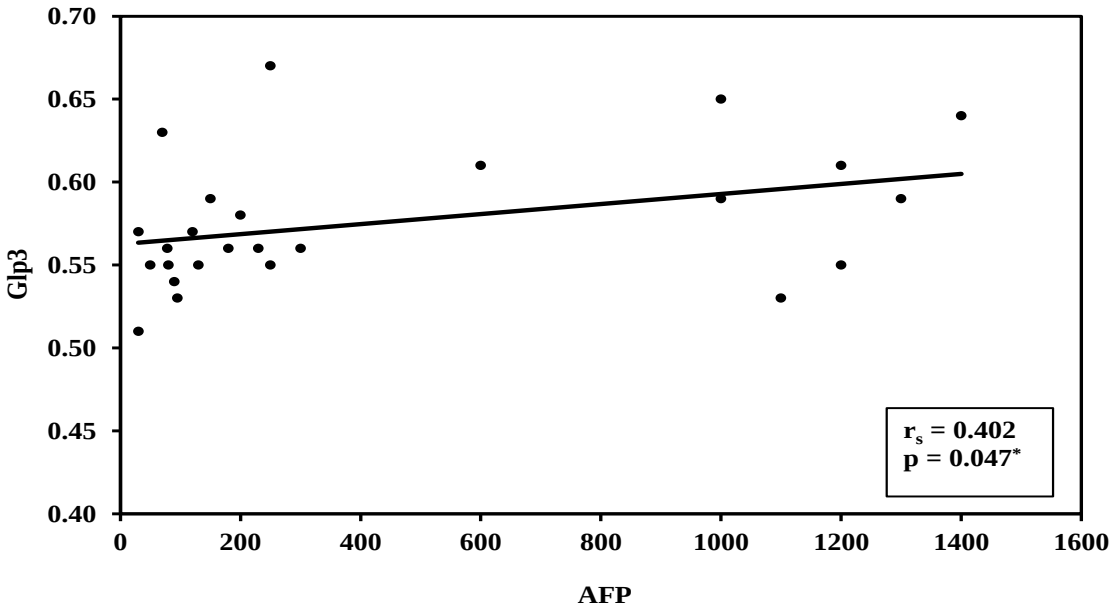

Fig. (2): Correlation between AFP and Glp3 in in HCC group

Discussion

To minimize the impacts of HCC, screening for it has significantly improved in Egypt during the past few years.

HCC is viewed as the sixth most common cause of malignancy-related morbidity. Also, HCC incidence is in alarming rising rate, and it has become a major health problem world-wide [(1)].

AFP is widely used to identify and follow HCC. However, it is not usually elevated to a diagnostic level, particularly in small and solitary HCC [(14)]. Additionally, it may be high in patients with chronic HCV with no signs of HCC. As a result, a novel biomarker with better diagnostic precision than AFP is highly desired [(15)]. Glypican-3 (GPC3) is a membrane-associated proteoglycan that is specifically up-regulated in hepatocellular carcinoma (HCC) although rarely or not expressed in normal liver tissues making it a perfect diagnostic marker and could be used as a targeted therapy for HCC [(12)], another advantage for GPC3 was noted as the serum level of GPC3 decreases post-treatment correlated with response to locoregional chemotherapy compared to change in serum AFP in HCC patients awaiting liver transplantation [(13)].

The present study was conducted on 75 individuals with male predominance as there was 40 males and 35 females, The age ranged between 43 and 65 years with a mean of 41.98 years in group 1, 55.4 years in group 2, and 39.32 years in group 3. Those results were similar of the study done in 2017 which proved HCC is an age-dependent tumor with peak age around 50 year which is more common in males support the hypothesis of a protective role of estrogens [(16)]. Regarding smoking, diabetes, and hypertension group 1 shows 52%

smokers, 44% diabetic, and 52% hypertensive, Group 2 shows 72% smokers, 31% diabetic, and 40% hypertensive while group 3 which serve as a control. A group of researchers run a study to determine the relation between smoking and hepatocellular carcinoma in HCV patients and mentioned that in chronic hepatitis C patients with severe fibrosis, continuing smoking after achieving SVR could be a risk factor for post-SVR HCC which goes with our study [(17)]. Regarding diabetes mellitus it was mentioned that diabetes may be a predisposing factor for HCC which is against our study [(18)].

Regarding complete blood picture hemoglobin, WBC and platelets were decreased in HCC group which runs parallels with ***(Pavlovic et al., 2019)*** but these may be unsignificant as these results also appears cirrhotic patients with or without HCC because of cirrhotic complications development course [(19)].

In our study, there was a statistically significant difference regarding ALT and AST between the studied groups, this agrees with others who linked that to the progression of fibrosis in the set of HCC which affect AST and ALT [(20)].

Regarding GGT and serum albumin, serum GGT was significantly elevated and serum albumin was significantly decreased in the hepatocellular group. This was in accordance to a study performed in 2021 which concluded that serum GGT levels and especially high GGT plus low serum albumin levels, were significantly associated both with HCC patients' survival and tumor aggressiveness characteristics, regardless of AFP levels in a large Turkish cohort [(21)]. This might be especially useful since most HCC patients do not have elevated levels of AFP, [(21 and 22)].

In the current study regarding ascites detected by ultrasound, there was no ascites in 36% and 44%, mild to moderate in 28% and 20%, and moderate to severe ascites in 36% and 36% of cases in group 1 and 2 respectively which was non-significant between the two groups. This was mentioned in the study done in 2012 which concluded that there were no statistically significant differences between HCC group and cirrhotic group regarding ascites as ascites is one of cardinal signs of cirrhosis and portal hypertension but also severity of ascites linked with portal invasion and tumor size [(23)]. This was in accordance to our current study results.

Regarding Child-Pugh score, Child score C is increased in HCC group which is a logic finding as HCC deteriorate the liver status and Child score is linked to measure liver function and predict outcomes [(9)].

In this study, fibro scan results show statistically significant differences between the three groups with higher liver stiffness in HCC group that was typically mentioned before that high liver stiffness was an important risk factor for developing new liver cancer in HCV patients [(24)].

Glypican 3 was found to be higher in HCC group with mean (0.58 ± 0.04 ng/dl) than cirrhotic group (0.47 ± 0.04 ng/dl) and control group (0.44± 0.02 ng/dl) that goes

with a group of researchers who mentioned that GPC3 is mildly affected by cirrhosis and there are no differences between its level in cirrhosis and in healthy group but markedly elevated in HCC group [25], In another group of researchers claimed that GPC3 levels are the same in both cirrhotic and HCC [26]. In continuation of this controversies, it was mentioned that GPC3 has no value at all in screening of HCC in patients with steatohepatitis-related cirrhosis [26 and,27]. But ***(Yang et al., 2014)*** stand in front of these thoughts and conducted a meta-analysis which consists of 22 studies to assess the role of GPC3 in HCC and found that serum GPC3 was a reliable biomarker for detection of HCC, with a fair sensitivity and specificity which runs with our study results [28].

In our study Glypican 3 (GLP3) with a cut-off >0.52 shows a sensitivity of 96% and specificity of 94% while AFP with a cut-off >45 shows a sensitivity of 92% and specificity of 88%. GLP3 was more sensitive in the detection of the single focal lesion.

These findings agree with ***(Mahmoud and Mahgoub 2020)*** who revealed that serum GPC3 has sensitivity (93%) and specificity (94 %) in HCC detection and also, while for AFP cutoff value of >100 ng/mL had sensitivity of 70% and specificity of 93.33% [29].

These findings are consistent with earlier research which found that GPC3 is more sensitive and specific than AFP in detecting HCC, with a sensitivity of 91.7% compared to AFP's 41.7% and a specificity of 100% compared to AFP's 80.4%, respectively [30].

Conclusions

Glypican-3 can be a pivotal diagnostic serum marker for HCC and may add value to alpha fetoprotein increasing the overall detection for HCC.

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