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ORIGINAL ARTICLE

Serum glypican-3 versus alpha-fetoprotein as diagnostic markers for hepatitis C-associated hepatocellular carcinoma: a comparative study

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ABSTRACT

Background: Globally, hepatocellular carcinoma (HCC) is ranked as the fourth most frequent cause of death from cancer. The creation of more precise and sensitive blood markers, like Glypican-3 (GPC-3), for the early identification of HCC may improve patient survival. So, we aimed to compare the clinical significance of serum GPC-3 levels with AFP in the diagnosis of HCC on top of HCV-related liver cirrhosis.

Methods: A case-control study was performed in the Hepatology and Gastroenterology Unit of the Internal Medicine Department at Zagazig University Hospitals. Ninety subjects were divided into Group I, which proved to be HCC patients. Group II: liver cirrhotic patients. Group III: Normal. All participants underwent a GPC-3 assessment.

Results: Alpha-fetoprotein (AFP) was superior on serum Glypican-3 in diagnosis of liver cirrhosis, whereas the best cutoff was (≥19.5 Vs. ≥2.25), AUC (0.945 vs. 0.859), sensitivity (93.3% vs. 83.3%), specificity (76.7% vs. 70 %), PPV (80% vs. 73.5 %), NPV (92% vs. 80.8 %), and overall accuracy (85% vs. 76.7 %) respectively. In diagnosis of HCC, Glypican-3 was superior on AFP, whereas the best cutoff of AFP vs. serum Glypican-3 was (≥32.5 Vs. ≥3.65), AUC (0.858 vs. 0.873), sensitivity (80% vs. 90%), specificity (75% vs. 76.7 %), PPV (61.5% vs. 65.9 %), NPV (88.2% vs. 93.9 %), and overall accuracy (76.7% vs. 81.1 %) respectively.

Conclusion: Serum GLP-3 could be a potential serum marker due to its high sensitivity and specificity in the detection of HCC.

Keywords: Glypican-3, alpha-fetoprotein, HCC, HCV, liver cirrhosis.

INTRODUCTION

As of right now, liver cancer is the second leading cause of cancer-related mortality. The most prevalent kind of liver cancer is HCC. Nevertheless, surgical interventions are the best treatment choice; not all patients are good candidates for surgery. Therefore, certain HCC biomarkers are clinically valuable for the evolution of HCC patients' early detection and therapy **[1].**

Similar to several developing nations, Egypt is undergoing an epidemiologic shift marked by a rise in urbanization, aging, environmental exposures, and the prevalence of smoking. Egypt leads the world in HCV prevalence, and rates of HCC are raising **[2].**

For men, it is the second most frequent cancer, while for women, it is the sixth most common one. Over the past ten years, HCC has become more prevalent in Egypt, and this trend is anticipated to continue **[3].**

The relative frequency of all liver-related malignancies increased overall, according to hospital-based research from Egypt, from roughly 4% in 1993 to 7.3% in 2003. According to another study, HCV infection now accounts for 50% of instances of HCC, indicating a growing significance in the disease's etiology. With respective rates of 25% and 15%, the effects of HBV and HBV/HCV infection have diminished **[4].**

Numerous studies have shown that GPC3 is an immune-therapeutic target for HCC in addition to being a highly specific tumor marker for diagnosis **[7].**

As a member of the glypican family, GPC3 is typically expressed in the kidney, lung, ovary, placenta, mammary gland, and embryonic tissues. There is no GPC3 expression in a healthy liver. HCCs had higher blood and tissue levels of GPC3 protein and gene expression than healthy or non-malignant livers despite the fact that overexpression of GPC3 is seen in HCCs **[8].**

Liver cancer is the second leading cause of cancer-related deaths worldwide, and hepatocellular carcinoma is the most common type. The pathogenesis of hepatocellular carcinoma is concealed, its progress is rapid, its prognosis is poor, and the mortality rate is high. Therefore, novel molecular targets for hepatocellular carcinoma early diagnosis and development of targeted therapy are critically needed. So, we aimed to compare the clinical significance of serum GPC-3 levels with AFP in the diagnosis of HCC on top of HCV-related liver cirrhosis.

METHODS

A case-control investigation was carried out from March 2020 to March 2021 on patients who were admitted to the Hepatology & Gastroenterology Unit in the Internal Medicine Department or who attended the outpatient clinics in Zagazig University Hospitals. Approval of the study design was obtained from the Institutional Review Board (IRB) unit #:5935-8-3-2020, Faculty of Medicine, Zagazig University. The IRB approved according to the ethical guidelines in the Declaration of Helsinki. Written informed consents were taken from the participants before sample collection.

This study has been conducted on 90 persons; their ages ranged from 30 to 75 years; all included subjects were divided into three groups:

Group I (HCC group): Thirty individuals had both HCV-related cirrhosis and radiographic indications of HCC, as shown by triphasic abdominal CT and abdominal US. These HCC patients were chosen among patients attending outpatient clinics or inpatients in the Internal Medicine Department with various stages (based on BCLC staging). Based on Child-Pugh categorization, they were assigned to Child A: 0, Child B: 9, and Child C: 21 categories.

Group II (liver cirrhosis group): Clinically compensated or decompensated cirrhosis was observed in thirty patients with HCV-related cirrhosis, laboratorial, and radiologically by abdominal US. Child-Pugh scoring was Child A: 0, Child B: 20, and Child C: 10.

Group III (controlled group): Thirty healthy participants were chosen from outpatient clinics who were matched for sex and age.

Patients who were excluded from the study with the following criteria: chronic viral hepatitis due to HBV, those receiving antiviral therapy for HCV, those with other malignancies such as pancreatic or colorectal carcinoma, those who had previously had a liver transplant, those who had previously received treatment for HCC (either surgical, interventional, or medical), and those who had severe comorbidities, such as advanced renal failure or decompensated heart failure.

Each participant underwent a detailed medical history either from himself or his relatives. Complete Clinical examination, including general and local abdominal examinations, including abdominal palpation of the liver and spleen, as well as manifestations of portal hypertension and liver cell failure, such as jaundice, ascites, ecchymosis, palmar erythema, and edema in the lower limbs.

Radiology: Abdominal ultrasound has been done to evaluate the liver, liver cirrhosis, HCC on top of cirrhosis, as well as the portal vein, spleen, and ascites. For the purpose of diagnosing the HCC group, triphasic CT abdomen was used; it adhered to particular HCC diagnostic radiological criteria (washout in the delayed and portal phases, and early augmentation in the hepatic arterial phase). The HCC group's BCLC staging score is then used to assess the HCC phases **[9].**

Biochemical investigations: Tests for liver function using the Roche Cobas Integra-800 autoanalyzer Hepatic enzymes [AST (Aspartate Amino Transferase) (u/1), ALT (Alanine Amino Transferase) (u/1)], serum albumin (gm/dl), and bilirubin (mg/dl). The bleeding profile was measured by autoanalyzer Sysmex, Japan, using PT (prothrombin time in seconds) and INR (international normalized ratio).

Biomarkers: ng/ml of serum alpha-fetoprotein utilizing the Roche Cobas Integra E 210 autoanalyzer. Glypican-3 (GPC-3) in humans ELISA A kit for measuring GPC3 levels in serum quantitatively.

Principle of the Assay

The technology used in the Glypican-3 kit was sandwich ELISA. Microwell plates were pre-coated with anti-GPC-3 Ab. Anti-GPC-3 Ab coupled with

biotin was used to detect antibodies. Microwells were filled with standards, samples, and biotin-conjugated detection antibodies. Wash buffer was then added and rinsed. After adding HRP-Streptavidin, unbound conjugates were eliminated using a wash buffer. To read the HRP enzymatic response, TMB substrates were utilized. HRP catalyzed TMB to yield a blue product, which became yellow upon the addition of an acidic stop solution. Yellow density in the sample taken in microwells is ∞ to GPC3. Using a microplate reader, measure the absorbance at 450 nm to quantify the GPC3 concentration.

Sample Collection and Storage:

Samples were isolated soon after collecting, then processed immediately, otherwise aliquoted and stored at -2O° C, avoiding several freezing and thawing. Following a 2-hour clotting period at room temperature or overnight centrifugation at 1000xg for 20 minutes, the supernatant was separated from the sample.

STATISTICAL ANALYSIS

SPSS, or the statistical program for the social sciences, was used to examine the data version 26 (IBM Corp., 2019). IBM Corp., Armonk, NY, Version 26.0 of IBM SPSS Statistics for Windows. Chi-square analysis, Fisher exact and Monte Carlo tests, Kruskal Wallis test, Spearman correlation coefficient, linear regression analysis, and ROC curve were among the tests that were employed. The level of statistical significance was set at $P<0.05$. A highly significant difference was present if $p\leq0.001$.

RESULTS

The studied groups did not differ statistically significantly in terms of gender, special habits, comorbidities, or age ($p>0.05$) (Table 1).

Regarding the existence of jaundice, lower limb edema, ascites, ecchymosis, pallor, flapping tremors, nausea, vomiting, weight loss, exhaustion, constipation, hematemesis and melena, and stomach pain, with the HCC group experiencing significantly more of it. This difference is statistically significant among the patients under study. There is a statistically significant difference ($p\leq0.001$) between the patients under study with regard to the size and texture of the liver, the spleen, the presence of localized lesions, portal vein thrombosis, and ascites. Focal lesions (hemangioma) were encountered in 3 non-HCC cirrhotic patients (Table 2).

All three of the analyzed groups' serum albumin, INR, and total bilirubin showed statistically significant differences. The least significant difference **$p\leq0.001$ (when comparing any two of the groups separately, the differences are significant). Regarding AST and ALT, there is a statistically significant difference between the study groups. When comparing the control group to the other groups pairwise, there is a substantial difference (both are significantly lower in the control group).

Regarding alpha-feto protein, there is a statistically significant difference between the groups under study (when pairwise comparison is performed, the difference is significant between each pair of groups). The highest amount among the groups with hepatic cirrhosis, HCC, and control. Regarding Serum Glypican-3, there is a statistically significant difference between the groups under investigation. When pairwise comparisons are made, each of the two separate groups shows a substantial difference. greatest in the liver cirrhosis group, followed by the HCC group and the control group.) $p<0.001$ (Table 3).

With an area under the curve of 0.945, the sensitivity of 93.3%, specificity of 76.7%, positive predictive value of 80%, negative predictive value of 92%, and overall accuracy of 85%, the optimal cutoff of alpha-fetoprotein for the diagnosis of liver cirrhosis is ≥19.5. A serum Glypican-3 cutoff of ≥2.25, with an area under the curve of 0.859, sensitivity of 83.3%, specificity of 70%, positive predictive value of 73.5%, negative predictive value of 80.8%, and overall accuracy of 76.7%, is optimal for the diagnosis of liver cirrhosis ($p\leq0.001$) (Table 4) & Figure (IS).

With an area under the curve of 0.858, the sensitivity of 80%, specificity of 75%, positive predictive value of 61.5%, negative predictive value of 88.2%, and overall accuracy of 76.7%, the optimal cutoff of alpha-fetoprotein for the diagnosis of hepatocellular carcinoma is ≥32.5. A serum Glypican-3 cutoff of ≥3.65, with an area under the curve of 0.873, sensitivity of 90%, specificity of 76.7%, positive predictive value of 65.9%, negative predictive value of 93.9%, and overall accuracy, is suitable for the diagnosis of hepatocellular carcinoma of 81.1% ($p\leq0.001$) (Table 4) & Figure (2S).

There is a statistically significant inverse connection between serum Glypican-3 and serum albumin. Serum Glypican-3 exhibits a statistically significant positive connection with age, total bilirubin, AST, ALT, alpha-fetoprotein, and INR (Table 5).

A statistically significant inverse relationship has been observed between serum albumin and alpha-feto protein. The alpha-feto protein exhibits a statistically significant positive connection with age, total bilirubin, AST, ALT, and INR. Nevertheless, the relationship between age and alpha-feto protein is not statistically significant (Table 6).

Serum albumin (unstandardized β=67.467, p<0.001) and INR (unstandardized β=203.278, p<0.001) were two parameters that substantially connected with alpha-fetoprotein in the individuals under study. Among factors significantly correlated to serum glypican-3 among studied patients, serum albumin (unstandardized β=-1.159, p<0.001), INR (unstandardized β=0.641, p=0.009), AST (unstandardized β=-0.04, p=0.012) and age (unstandardized β=0.043, p=0.026) significantly independently associated with it. (Table 7).

About two thirds of HCC group had tumor size less than 3 cm. Single lesion was the most frequent (56.7%) among studied cases, 23.3 % of the studied cases had portal vein thrombosis, 10% extra-hepatic spread and 13.3% had lymph node metastasis. Stage D & A BCLC were least frequent (16.7% each). While, Stage B & C represent 33.3% each (Table 1S).

Table 1: Comparison between the studied groups regarding demographic data

	HCC group N=30 (%)	**Liver cirrhosis group N=30 (%)**	**Control group N=30 (%)**	**χ^2**	**p**
Sex: **Female** **Male**	 9 (30%) 21 (70%)	 5 (16.7%) 25 (55.6%)	 12 (40%) 18 (60%)	4.002	0.135
Special habits: **NAD** **Smoking**	 28 (93.3%) 2 (6.7%)	 29 (96.7% 1 (3.3%)	 26 (86.7%) 4 (13.3%)	MC	0.567
Comorbidities: **NAD** **CAD** **Diabetes** **Hypertension** **Diabetes, hypertension**	 17 (56.7%) 0 (0%) 10 (33.3%) 2 (6.7%) 1 (3.3%)	 22 (73.3%) 0 (0%) 5 (16.7%) 3 (10%) 0 (0%)	 24 (80%) 1 (3.3%) 3 (10%) 2 (6.7%) 0 (0%)	MC	0.244
	Mean ± SD	**Mean ± SD**	**Mean ± SD**	**F**	**p**
Age (year)	62.03 ± 6.58	62.2 ± 6.58	59.97 ± 5.37	1.28	0.283

-NAD (No abnormality detected), CAD (coronary artery disease), χ^2Chi square test F One-way ANOVA test

Table 2: Comparison between the studied groups regarding symptoms and ultrasonography

	HCC group N=30 (%)	**Liver cirrhosis group N=30 (%)**	**χ^2**	**p**
Symptoms				
Jaundice	24 (80%)	13 (43.3%)	8.531	**0.003***
Lower limb edema	26 (86.7%)	17 (56.7%)	Fisher	**0.02***
Ascites	28 (93.3%)	15 (50%)	Fisher	**<0.001****
Ecchymosis	25 (83.3%)	10 (33.3%)	15.429	**<0.001****
Pallor	28 (93.3%)	20 (66.7%)	Fisher	**0.021***
Flapping tremor	28 (93.3%)	17 (56.7%)	Fisher	**0.002***
Nausea	26 (86.7%)	3 (10%)	Fisher	**<0.001****
Vomiting	26 (86.7%)	5 (16.7%)	Fisher	**<0.001****
Weight loss	28 (93.3%)	7 (23.3%)	Fisher	**<0.001****
Fatigue	30 (100%)	10 (33.3%)	30	**<0.001****
Constipation	26 (86.7%)	10 (33.3%)	Fisher	**<0.001****
Hematemesis&melena	27 (90%)	13 (43.3%)	Fisher	**<0.001****
Abdominal pain	30 (100%)	10 (33.3%)	30	**<0.001****

Ultrasonography				
Liver size: Shrunken	30 (100%)	14 (46.7%)		**<0.001****
Liver texture: Bright Coarse	0 (0%) 30 (100%)	10 (33.3%) 20 (66.7%)	12	**<0.001****
Spleen size: Average Enlarged	0 (0%) 30 (100%)	13 (43.3%) 17 (56.7%)	16.596	**<0.001****
Focal lesion	30 (100%)	3 (10%)	Fisher	**<0.001****
PV thrombosis	26 (86.7%)	0 (0%)	Fisher	**<0.001****
Ascites: Absent Mild Severe	0 (0%) 4 (13.3%) 26 (86.7%)	12 (40%) 10 (33.3%) 8 (26.7%)	23.33¥	**<0.001****

**p≤0.001 is statistically highly significant χ^2Chi square test MC Monte Carlo test

Table 3: Comparison between the studied groups regarding laboratory data

	HCC group N=30 (%)	**Liver cirrhosis group N=30 (%)**	**Control group N=30 (%)**	**F**	**p**
	Mean ± SD	**Mean ± SD**	**Mean ± SD**		
Albumin (g/dl)	2.07 ± 0.29	2.59 ± 0.6	4.37 ± 0.4	217.36	**<0.001****
LSD	**P_1 <0.001****	**P_2 <0.001****	**P_3 <0.001****		
	Median (IQR)	**Median (IQR)**	**Median (IQR)**	**KW**	**p**
AST	44(40 – 50)	46(43 – 50.75)	33(30.75 – 34.25)	54.765	**<0.001****
ALT	47(45 – 55.25)	46(43.75 – 53)	29.5(28 – 31.25)	55.755	**<0.001****
Pairwise	P_1 0.566	**P_2 0.001****	**P_3 <0.001****		
Total bilirubin (mg/dl)	3(2 – 5)	1.5(1.1 – 3)	1(0.9 – 1.2)	51.968	**<0.001****
Pairwise	**P_1 <0.001****	**P_2 0.001****	**P_3 <0.001****		
INR	2.1(1.9 – 3)	1.65(1.5 – 2)	1(0.8 – 1.2)	65.993	**<0.001****
Pairwise	**P_1 0.01***	**P_2 0.001****	**P_3 <0.001****		
α feto-protein	65(35 – 130)	35(20 – 50)	7 (5 – 10)	56.656	<0.001**
Pairwise	P_1 0.025*	P_2 0.001**	P_3 <0.001**		
GPC-3	6(5.43 – 6.2)	3.45(2.4 – 6.1)	2(1.58 – 2.63)	50.412	<0.001**
Pairwise	P_1 0.004*	P_2 0.001**	P_3 <0.001**		

F One-way ANOVA test LSD Fisher least significant difference, C KW Kruskal Wallis test, **p≤0.001 is statistically highly significant *p<0.05 is statistically significant p1 difference between groups A and B p2 difference between groups B and C p3 difference between groups A and C.

Table 4: Performance of alpha-fetoprotein and Serum Glypican-3 in the diagnosis of liver cirrhosis and hepatocellular carcinoma

	Cutoff	**AUC**	**Sensitivity**	**Specificity**	**PPV**	**NPV**	**Accuracy**	**p**
In diagnosis of liver cirrhosis								
A-fetoprotein	≥19.5	0.945	93.3%	76.7%	80%	92%	85%	<0.001**
GPC3	≥2.25	0.859	83.3%	70%	73.5%	80.8%	76.7%	<0.001**
In diagnosis of hepatocellular carcinoma								
A-fetoprotein	≥32.5	0.858	80%	75%	61.5%	88.2%	76.6%	<0.001**
GPC3	≥3.65	0.873	90%	76.7%	65.9%	93.9%	81.1%	<0.001**

**p≤0.001 is statistically highly significant PPV positive predictive value NPV negative predictive value AUC area under the curve

Table 5: Correlation between Serum Glypican-3 and the studied parameters

	r	p
Age (year)	0.267	0.011*
Albumin (g/dl)	-0.794	<0.001**
Total bilirubin (mg/dl)	0.710	<0.001**
AST (U/L)	0.41	<0.001**
ALT (U/L)	0.528	<0.001**
INR	0.786	<0.001**
Alpha-fetoprotein	0.717	<0.001**

r Spearman rank correlation coefficient *p<0.05 is statistically significant **p≤0.001 is statistically highly significant

Table 6: Correlation between alpha-fetoprotein and the studied parameters

	r	**p**
Age (year)	0.184	0.083
Albumin (g/dl)	-0.769	**<0.001****
Total bilirubin (mg/dl)	0.664	**<0.001****
AST (U/L)	0.569	**<0.001****
ALT (U/L)	0.664	**<0.001****
INR	0.827	**<0.001****

r Spearman rank correlation coefficient *p<0.05 is statistically significant **p≤0.001 is statistically highly significant

Table 7: Linear regression analysis of factors associated with alpha-fetoprotein and serum Glypican-3

	Unstandardized Coefficients		**Standardized Coefficients**			**95% Confidence Interval**	
	β	**Std. Error**	**Beta**	**t**	**p**	**Lower**	**Upper**
Alpha-fetoprotein							
(Constant)	-483.812	90.809		-5.328	<0.001**	-664.31	-303.319
INR	203.278	24.256	1.025	8.381	<0.001**	155.067	251.489
Serum albumin (g/dl)	67.465	17.487	0.472	3.858	<0.001**	32.708	102.223
Serum Glypican-3							
(Constant)	5.237	1.785		2.934	**0.004***	1.689	8.786
S. Albumin(g/dl)	-1.159	0.198	-0.666	-5.855	**0.001****	-1.553	-0.765
INR	0.641	0.238	0.266	2.693	**0.009***	0.168	1.115
AST (U/L)	-0.040	0.016	-0.193	-2.557	**0.012***	-0.072	-0.009
Age (year)	0.043	0.019	0.138	2.262	**0.026***	0.005	0.081

*p<0.05 is statistically significant **p≤0.001 is statistically highly significant

DISCUSSION

In addition to liver cirrhosis associated to HCV, our study evaluated blood Glypican-3 versus alpha-fetoprotein in patients with hepatocellular carcinoma. Ninety participants participated in our study; they were divided into three groups: thirty patients with hepatocellular carcinoma, thirty patients with liver cirrhosis linked to HCV, and thirty healthy people who served as the control group.

The demographic data of the analyzed groups revealed that the median age of cirrhotic non-HCC patients versus HCC patients was (62.2 ± 6.58 Vs. 62.03 ± 6.58) years respectively. This was consistent with research conducted by **Waziry et al. [10]** and **Ziada et al. [11].** Patients older than fifty

had both cirrhosis and HCC. According to a previous study by **Kew [12],** HCC is uncommon in individuals under 40 and gradually becomes more common in those in their ages 60s and 70s.

Patients with cirrhosis and HCC had M/F ratios of 2:1. The variations in how risk factors are exposed could be the cause, such as HCV-related viral hepatitis, which affects men more often than women. Males are also more likely to be exposed to environmental carcinogens. Furthermore, other x-linked genetic variables and sex hormones might also be significant. The idea that estrogens play a protective function in hepatocellular carcinoma incidence is supported by the sex disparity in incidence's age-dependent patterns **[13]**.

According to the current data, there was no significant difference in the smoking status of the three study groups (cirrhotic, HCC, and healthy control). The cirrhotic group smoked more frequently than the healthy control group, while the HCC group smoked less frequently. Most of them had never smoked or had quit ($p>0.05$). This study has similarities to that of **Ezzat et al. [14],** who showed no significant difference in the smoking status of HCC and non-HCC groups. However, **Chen et al. [15]** identified a considerably elevated risk of HCC among cigarette smokers, contradicting the findings of our study. Furthermore, a noteworthy dose-response correlation was seen between the number of substance use behaviors and the risk of HCC.

The study's findings demonstrated that there is no statistically significant difference in the levels of DM or hypertension in the groups under investigation ($p>0.05$). Study groups with diabetes and hypertension in HCC, Cirrhotic, and control groups were (33.3% and 6.7%) vs. (16.7% and 10.0%) vs. (10% and 6.7%) respectively. Our findings concurred with those of **Ziada et al. [11]**, who found that patients with HCC had a considerably greater incidence of diabetes than individuals with HCV-related cirrhosis. On the other hand, **Li et al. [16]** discovered that a small percentage of both groups had hypertension and DM.

Pertaining to the patients under study's laboratory results, between the non-HCC (cirrhotic) and HCC groups, The levels of total and direct bilirubin, AST, and ALT in the serum were statistically significantly different ($p<0.001$).

Hepatic enzymes that represent the necro-inflammatory process, including AST and ALT, were found in our investigation on liver function tests to be considerably higher in HCC patients. This aligns with the conclusions of **Elgamal et al. [17],** who discovered a statistically significant difference in serum bilirubin, albumen level, AST, ALT, and INR, separating the other groups and the HCC group.

On the other hand, **Li et al. [16]** discovered no appreciable variations between the respectable HCC group and the non-HCC group to the previously tracked metrics.

According to the results, over half of the HCC patients (17 patients (56.7%)) had a single focal lesion, and two-thirds of the cases had tumors less than three centimeters (20 patients (66.7%)). This result is consistent with that of El-**Azab et al. [18],** who found that 68.9% of HCC cases had a single focal lesion that measured ≤ 5 cm. Furthermore, the majority of HCC patients typically appeared with modest single lesions and had Child-Pugh grades of A or B, according to **Abdelaziz et al [19].**

However, **Sakr et al. [20]** revealed that 40% of HCC patients had a single hepatic focal lesion, while 60% of patients had numerous hepatic focal lesions. Their average size was 4.16±1.36 cm, with a range of 2 to 6.3 cm.

The current study's findings on BCLC staging of HCC revealed that, of the entire HCC group, stages B and C accounted for 2/3 (33.3% each), while stages A and D accounted for 16.7% each.

Similar results were observed in a recent study by **Elgamal et al. [17],** which discovered that in accordance with BCLC staging, Stages C and D diagnoses accounted for 40.5% and 17.9% of HCC patients, respectively. **Raphe et al. [21]** carried out a cross-sectional study to examine epidemiological features of first-line treatment, staging, diagnosis, and risk factors in a closed community. Stage A was found to be 32.71%, B to be 21.96%, C to be 30.37%, and D to be 14.95%. In contrast, 705 instances of HCC were categorized by the BCLC into four categories in multicenter research conducted in Spain: early-stage A (49.8%), intermediate-stage B (19.8%), advanced stage C (18.8%), and terminal-stage D (11.6%) **[22].** This might be because doctors are becoming more aware of the unique characteristics of HCC patients and are working to provide a more precise surveillance schedule for high-risk patients in industrialized nations.

The three groups' GLP-3 values for our novel biomarker, Glypican-3, differ statistically significantly, with the HCC group having the highest value (5.43-6.2), followed by the cirrhotic

group (2.4–6.1) and the last healthy control group (1.58-2.63) ($p < 0.001$). This is consistent with the results of **Rojas et al. [23]**; they found that, although remaining undetectable in healthy liver tissue, patients with HCC had significantly increased GLP-3 levels, suggesting that GLP-3 is a useful tumor marker for HCC diagnosis.

This is consistent with the results of **Rojas et al. [23],** who discovered that patients with HCC have considerably higher GLP-3 levels. Additionally, we noted that Glypican-3 had higher overall accuracy (81.1%), positive predictive value (65.9%), negative predictive value (93.9%), specificity (76.7%), and sensitivity (90%) than the other two. The ideal cutoff of alpha-fetoprotein for the diagnosis of hepatocellular carcinoma is ≥32.5, with an area under the curve of 0.858, a sensitivity of 80%, specificity of 75%, positive predictive value of 61.5%, negative predictive value of 88.2%, and overall accuracy of 76.7%.

These findings were similar to those of **Hashem et al. [24]**; they discovered that the HCC group's serum GLP-3 sensitivity was 84%, and the sensitivity of the serum GLP-3 and AFP combination was 81.9%.

GLP-3 was shown to be significantly higher in HCC patients than in persons who were in good condition. by **El-Saadany et al. [25].** Additionally, **Badr et al. [26]** and **Yu et al. [27]** demonstrated increased sensitivity and specificity of HCC diagnosis by a combination of both AFP and GLP-3, as well as greater sensitivity of combined GLP-3 and serum AFP levels in the diagnosis of HCC at all stages. Furthermore, they discovered that serum Glypican-3 expression was higher in HCC patients. Additionally, they discovered that the accurate and efficient diagnosis of HCC can be enhanced by the simultaneous detection of serum AFP and GLP-3.

Ultimately, based on our findings, we can say that compared to AFP, serum GLP-3 is a more reliable and sensitive diagnostic tool for HCC (the sensitivity of AFP vs. GLP-3 in the diagnosis of HCC was 80% vs. 90%, respectively). Combining GLP-3 and AFP increases the sensitivity of HCC diagnosis, suggesting that GLP-3 may be a helpful marker for HCC identification even in patients without elevated AFP.

CONCLUSION

In many regions of the world, particularly in Egypt, HCC is a common and deadly cancer. The most crucial element in an HCC patient's effective treatment is an early diagnosis. In our location, HCC has been reported to be more common in older male patients as well as those who live in rural areas. Serum GLP-3 has great sensitivity and specificity in the detection of HCC. Potential diagnostic biomarkers for HCC include AFP at a cutoff value ≥32.5 (ng/ml) while GLP-3 at a cutoff value ≥ 3.5 (ng/ml). Combining GLP-3 and AFP increases the sensitivity of HCC diagnosis, screening, and follow-up treatment of HCC. GLP-3 may be a potential marker in the diagnosis of HCC, especially in patients without AFP elevation.

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Consent for publication

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Competing interests

The authors declare that they have no competing interest.s

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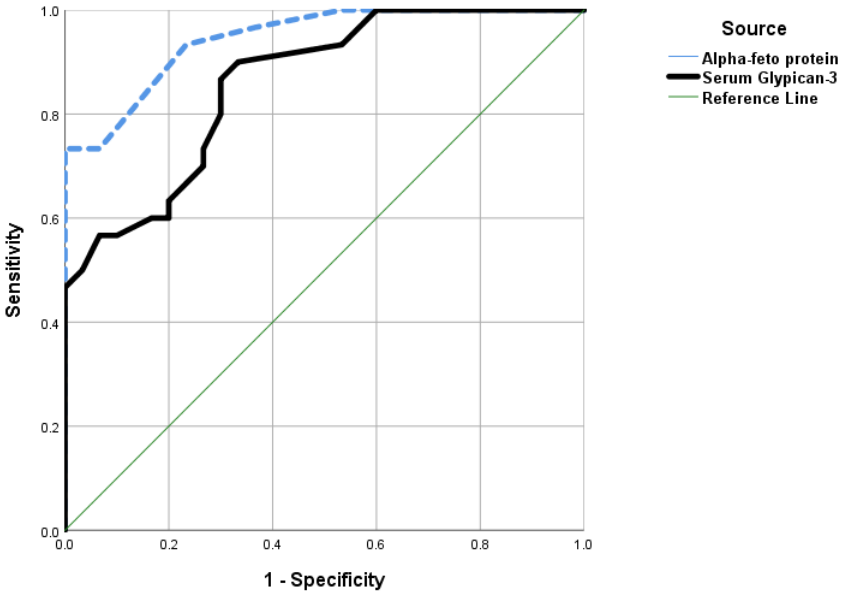

Figure 1S: ROC curve showing performance of alpha-feto protein and Serum Glypican-3 in the diagnosis of liver cirrhosis.

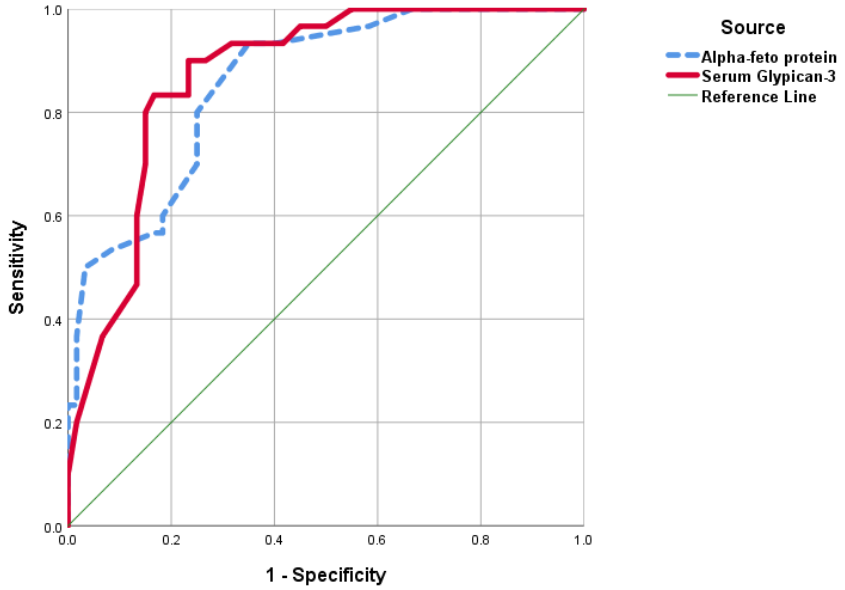

Figure 2S: ROC curve showing performance of alpha-fetoprotein and Serum Glypican-3 in the diagnosis of hepatocellular carcinoma

Table 1S: Tumor characteristics of the HCC group (n=30)

Tumor characteristic	Frequency	Percentage
Tumor size: < 3 cm: > 3 cm:	 20 10	 66.7% 33.3%
Number of focal lesions: Single Multiple <3cm: Multiple > 3 cm:	 17 6 7	 56.7% 20% 23.3%
Presence of portal vein thrombosis	7	23.3%
Presence of extra-hepatic spread	3	10.0%
Presence of lymph node metastases	4	13.3%
BCLC Stage A **Stage B** **Stage C** **Stage D**	 5 10 10 5	 16.7% 33.3% 33.3% 16.7%

Citation

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