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Evaluation the Role of Lipocalin 2 as a New Non-Invasive Diagnostic Marker for Hepatocellular Carcinoma in Egyptian Cirrhotic Patients

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ABSTRACT

Background: Hepatocellular carcinoma (HCC) is the sixth most common cancer globally and a major health challenge in Egypt, where it ranks as fourth most prevalent cancer. Current diagnostic approaches, including serum alpha-fetoprotein (AFP) and imaging, have limitations, particularly in early detection. Lipocalin-2 (LCN2), a protein secreted during hepatic damage, shows promise as a novel non-invasive biomarker for HCC diagnosis.

Objective: This study aimed to evaluate diagnostic potential of serum LCN2 in distinguishing HCC from liver cirrhosis and healthy controls in an Egyptian cohort.

Patients and methods: A case-control study was conducted on 96 participants: 32 HCC patients, 32 cirrhotic patients without HCC, and 32 healthy controls. Serum LCN2 levels were measured using enzyme-linked immunosorbent assay (ELISA). Comparative and diagnostic performance analyses were performed using ANOVA and ROC curve analysis.

Results: LCN2 levels were significantly higher in HCC patients 293 (140–445 ng/L) compared to cirrhotic patients 129 (20–239 ng/L, $P < 0.001$) and healthy controls 53 (38–68 ng/L, $P < 0.001$). ROC curve analysis demonstrated a sensitivity and specificity of 96.87% each and an accuracy of 98.4% at a cutoff of >160 ng/L. Serum AFP was also elevated in HCC patients 2737 (0–22303 ng/mL, $P < 0.05$), but LCN2 exhibited superior diagnostic performance.

Conclusion: Serum LCN2 is a highly sensitive and specific biomarker for HCC diagnosis. Combined with AFP, it could enhance early detection in surveillance programs. Further studies are recommended to validate its role in diverse populations and clinical settings.

Keywords: Hepatocellular carcinoma, Lipocalin-2, Biomarker, Liver cirrhosis, Early detection.

INTRODUCTION

Hepatocellular carcinoma (HCC) is the sixth most common cancer globally and fourth most prevalent in Egypt. It predominantly arises in patients with cirrhosis, occurring in 80%-90% of cases, with an annual incidence of 2-4% [1]. Major risk factors for HCC include hepatitis B and C infections, alcoholic liver disease, and non-alcoholic fatty liver disease (NAFLD). The disease presents variably depending on tumor stage and underlying cirrhosis, often leading to complications such as hepatic encephalopathy, portal vein thrombosis, worsening ascites, and variceal bleeding. Despite advancements in screening and diagnostic tools, late diagnosis remains common, reducing chances of curative treatment [2].

Surveillance for HCC typically involves ultrasound and alpha-fetoprotein (AFP) measurement in high-risk populations. Suspicious lesions detected during surveillance are confirmed using contrast-enhanced computed tomography (CT) or dynamic magnetic resonance imaging (MRI). However, these methods are insufficient for early detection in some cases, emphasizing need for novel diagnostic biomarkers [3].

Lipocalin-2 (LCN2), a protein overexpressed in HCC tissues, has shown potential as a non-invasive biomarker. Elevated LCN2 levels correlate with hepatic damage, disease progression, and shorter survival in HCC patients. While, LCN2 effectively discriminates HCC from other NAFLD stages, its ability to distinguish between early and late HCC remains limited [4, 5].

This study aimed to evaluate diagnostic potential of LCN2 as a novel biomarker for HCC in Egyptian patients.

PATIENTS AND METHODS

This case-control study was conducted at Ain Shams University Hospital over a 6-month period. A total of 96 Egyptian participants were recruited and divided into three groups: 32 patients with HCC, 32 patients with liver cirrhosis without HCC, and 32 healthy individuals serving as controls.

Inclusion criteria: Participants aged 18 years or older who were diagnosed with liver cirrhosis, with or without HCC.

Exclusion criteria: Participants under 18 years of age, pregnant or nursing females, individuals diagnosed with malignancies other than HCC, and those with other organ dysfunctions such as chronic kidney disease (CKD) or ischemic heart disease (IHD).

Study procedures: All subjects had a thorough assessment, including an extensive medical history and clinical examination. Laboratory investigations encompassed a CBC, coagulation profile (PT, PTT, and INR), liver function tests (AST, ALT, serum albumin, total and direct bilirubin, ALP, and GGT), kidney function tests (BUN and serum creatinine), electrolytes (sodium and potassium), and viral markers (HBsAg and HCV Ab). Tumor markers, including AFP and LCN2 were quantified. Imaging investigations, such as pelvi-abdominal ultrasonography and contrast-enhanced triphasic CT or dynamic MRI, were conducted to verify existence of hepatic focal lesions. A statistical study

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was performed to assess sensitivity and specificity of LCN2 in HCC diagnosis.

Principle of the ELISA assay: Serum LCN2 concentrations were quantified via a sandwich ELISA technique. Kit included a microplate that was pre-coated with antibodies against human CACNA2D1. CACNA2D1 was present in samples and bound to these antibodies, after which biotinylated antibodies were introduced to create complexes. Streptavidin-HRP was then added to bind to biotinylated antibodies. Following washing process, a substrate solution was introduced to generate color in accordance to concentration of CACNA2D1. The reaction was terminated using an acidic solution, and absorbance was quantified at 450 nm.

Sample preparation and handling: Blood samples were allowed to clot at room temperature for 10–20 minutes. Clot was then removed by centrifugation at 2000–3000 rpm at 2–8°C for 20 minutes. If precipitation occurred during storage, samples were re-centrifuged before analysis to ensure accurate measurements.

Assay procedure: The test process was conducted in accordance with established protocols. All reagents were equilibrated to ambient temperature prior to use. The standard was created by combining 120 µl of standard solution (3200 ng/L) with 120 µl of standard diluent to produce a 1600 ng/L standard stock solution. Standard was let to rest for 15 minutes with moderate agitation prior to performing dilutions. Duplicate standard points were generated by serially diluting standard stock solution (1600 ng/L) in a 1:2 ratio with a standard diluent, resulting in concentrations of 800 ng/L, 400 ng/L, 200 ng/L, and 100 ng/L. Standard diluent functioned as zero standard (0 ng/L). Any residual stock solution was frozen at -20 °C and used within one month. Wash buffer was formulated by diluting 20 ml of 30x wash buffer concentrate in deionized or distilled water to produce 500 ml of 1x wash buffer. If crystals had developed in the concentrate, solution was gently agitated until crystals were entirely dissolved.

ELISA plate assay procedure: Reagents, standard solutions, and samples were prepared according to instructions. Experiment was performed at ambient temperature. The necessary quantity of strips was determined, and surplus strips were preserved at 2–8 °C. Fifty microliters of standard solution was added to each standard well. Antibody was omitted from standard wells since standard solution already included biotinylated antibodies. In the sample wells, 40 µl of sample and 10 µl of anti-CACNA2D1 antibody were introduced, followed by addition of 50 µl of streptavidin-HRP to both sample and standard wells, omitting blank controls. The plate was meticulously mixed, sealed, and incubated for 60 minutes at 37°C.

Following incubation, sealer was detached, and plate underwent five washes with wash buffer. Each well was saturated with a minimum of 0.35 ml of wash buffer for a duration of 30 seconds to 1 minute every wash. The automated washing process included aspirating all wells and overfilling with wash buffer five times. Subsequent to cleaning, plate was patted dry using absorbent material. Thereafter, 50 µl of substrate solution A and 50 µl of substrate solution B were introduced into each well. The plate was sealed and incubated for 10 minutes at 37 °C in darkness. Fifty microliters of stop solution were applied to each well, resulting in a color change from blue to yellow. Optical density (OD) values were assessed at 450 nm within 10 minutes using a microplate reader.

Calculation of results: A standard curve was generated by graphing mean OD for each standard on vertical (Y) axis vs concentration on horizontal (X) axis. A best-fit curve was constructed via data points in graph. The computations were performed via computer-based curve-fitting software, with optimal fit line established by regression analysis.

Ethical considerations: The study was done after being accepted by Research Ethics Committee, Ain Shams University. All patients provided written informed consents prior to their enrolment. The consent form explicitly outlined their agreement to participate in the study and for the publication of data, ensuring protection of their confidentiality and privacy. This work has been carried out in accordance with Code of Ethics of World Medical Association (Declaration of Helsinki) for studies involving humans.

Statistical methods

Data management and statistical analysis were conducted using SPSS version 26 (IBM, Armonk, New York, United States). Quantitative data were expressed as mean ± standard deviation (SD). The Student's t-test was used to compare means of two groups, whilst one-way analysis of variance (ANOVA), accompanied by a post hoc test, was utilized for comparing means of three groups. Nonparametric quantitative data were presented as median (range), and Tukey's test was used for mean comparisons. Qualitative data were presented as frequency and percentage. Relationships among qualitative variables were examined via chi-square test. A P value of ≤ 0.05 was deemed statistically significant, and $P < 0.01$ was regarded as extremely significant. A P value beyond 0.05 was considered nonsignificant.

RESULTS

Demographic and clinical, and laboratory characteristics among HCC, cirrhotic, and control groups were shown in tables (1) and (2).

Table (1): Comparison of demographic and clinical characteristics among HCC, cirrhotic, and control groups

Variable	HCC (n [%])	Cirrhotic (n [%])	Control (n [%])	Chi-Square (X^2)	P-value
Gender: Male	24 (75.00)	22 (68.75)	22 (68.75)	0.403	0.817
Gender: Female	8 (25.00)	10 (31.25)	10 (31.25)	0.403	0.817
Smoking: No	21 (65.63)	20 (62.50)	32 (100.00)	15.232	0.004*
Smoking: Current	8 (25.00)	9 (28.13)	0 (0.00)	15.232	0.004*
Smoking: Ex-smoker	3 (9.38)	3 (9.38)	0 (0.00)	15.232	0.004*
DM: No	17 (53.13)	21 (65.63)	32 (100.00)	19.095	<0.001*
DM: Yes	15 (46.88)	11 (34.38)	0 (0.00)	19.095	<0.001*
HTN: No	14 (43.75)	24 (75.00)	32 (100.00)	25.741	<0.001*
HTN: Yes	18 (56.25)	8 (25.00)	0 (0.00)	25.741	<0.001*
HBsAg: No	27 (84.38)	27 (84.38)	32 (100.00)	5.581	0.061
HBsAg: Yes	5 (15.63)	5 (15.63)	0 (0.00)	5.581	0.061
HCV Ab: No	14 (43.75)	15 (46.88)	32 (100.00)	27.608	<0.001*
HCV Ab: Yes	18 (56.25)	17 (53.13)	0 (0.00)	27.608	<0.001*
PVT: No	10 (31.25)	27 (84.38)	32 (100.00)	41.121	<0.001*
PVT: Yes	22 (68.75)	5 (15.63)	0 (0.00)	41.121	<0.001*
Child Grade: A	0 (0.00)	7 (21.88)		8.048	0.018*
Child Grade: B	11 (34.38)	10 (31.25)		8.048	0.018*
Child Grade: C	21 (65.63)	15 (46.88)		8.048	0.018*

HCC: Hepatocellular Carcinoma, DM: Diabetes Mellitus, HTN: Hypertension, HBsAg: Hepatitis B Surface Antigen, HCV Ab: Hepatitis C Virus Antibody, PVT: Portal Vein Thrombosis, X^2: Chi-Square.

Table 2: Clinical and laboratory characteristics of the HCC, cirrhotic, and control groups

Variable	HCC	Cirrhotic	Control	F	P-value	H&CI	H&CO	CI&CO
Age (Years	58.719 ± 5.670	53.031 ± 8.034	38.469 ± 6.686	74.057	<0.001*	0.004*	<0.001*	<0.001*
BMI (kg/m^2)	21.506 ± 2.158	22.281 ± 1.920	22.231 ± 1.477	1.715	0.186			
WBCs ($10^3/mm^3$)	6.366 ± 1.013	6.450 ± 1.267	6.966 ± 1.680	0.843	0.434			
Hb (gm/dl)	9.884 ± 0.932	9.909 ± 1.072	12.597 ± 1.108	71.862	<0.001*	0.995	<0.001*	<0.001*
PLTs ($10^3/mm^3$)	94.313 ± 15.931	102.250 ± 24.185	265.188 ± 85.099	105.064	<0.001*	0.822	<0.001*	<0.001*
BUN (mg/dL)	19.563 ± 4.256	18.844 ± 1.659	12.875 ± 1.287	4.954	0.009*	0.949	0.014*	0.032*
Creat. (mg/dL)	0.838 ± 0.156	0.794 ± 0.161	0.825 ± 0.208	0.162	0.850			
Na (mEq/L)	138.406 ± 4.918	138.000 ± 5.565	139.469 ± 4.096	0.768	0.467			
K (mEq/L)	4.166 ± 0.607	4.050 ± 0.590	4.231 ± 0.451	0.879	0.419			
Bilirubin T (mg/dL)	3.316 ± 0.966	1.603 ± 0.138	0.909 ± 0.268	15.386	<0.001*	0.001*	<0.001*	0.271
Bilirubin D (mg/dL)	1.994 ± 0.206	0.794 ± 0.121	0.375 ± 0.016	13.072	<0.001*	0.001*	<0.001*	0.413
AST (U/L)	49.875 ± 8.522	34.219 ± 7.487	19.813 ± 4.817	17.686	<0.001*	0.007*	<0.001*	0.015*
ALT (U/L)	38.750 ± 5.415	32.844 ± 3.969	13.688 ± 3.188	13.092	<0.001*	0.484	<0.001*	0.001*
ALP (U/L)	166.625 ± 2.633	115.688 ± 37.761	81.469 ± 11.648	25.782	<0.001*	<0.001*	<0.001*	0.014*
GGT (U/L)	75.500 ± 5.176	40.344 ± 2.700	23.000 ± 5.263	60.047	<0.001*	<0.001*	<0.001*	0.002*
Albumin (g/dL)	2.597 ± 0.478	2.722 ± 0.728	4.291 ± 0.523	82.875	<0.001*	0.671	<0.001*	<0.001*
INR	1.488 ± 0.311	1.416 ± 0.364	1.072 ± 0.130	19.267	<0.001*	0.576	<0.001*	<0.001*

HCC: Hepatocellular Carcinoma, BMI: Body Mass Index, WBCs: White Blood Cells, Hb: Hemoglobin, PLTs: Platelets, BUN: Blood Urea Nitrogen, Creat: Creatinine, Na: Sodium, K: Potassium, AST: Aspartate Aminotransferase, ALT: Alanine Aminotransferase, ALP: Alkaline Phosphatase, GGT: Gamma-Glutamyl Transferase, INR: International Normalized Ratio, H &CI: Comparison between HCC and Cirrhotic groups, H & CO: Comparison between HCC and Control groups, CI &CO: Comparison between Cirrhotic and Control groups, F: ANOVA test statistic, P-value: Probability value.

For AFP, the mean levels were 2737.278 ± 7455.428 in HCC group, 4.103 ± 3.116 in the cirrhotic group, and 2.644 ± 1.510 in the controls. The ANOVA test yielded an F-statistic of 4.303 with a P-value of 0.016, showing significant differences. Post-hoc analysis indicated a significant difference between HCC and cirrhotic groups (P = 0.034) and between HCC and control groups (P = 0.034), but no significant difference between cirrhotic and control groups (P = 1.000). For LCN2, the mean levels were 292.750 ± 50.940 in HCC group, 129.313 ± 36.605 in cirrhotic group, and 53.250 ± 4.958 in controls. ANOVA test yielded an F-statistic of 363.117 with a P-value of < 0.001. Post-hoc analysis showed significant differences between all groups (P < 0.001 for all comparisons) (Table 3).

Table (3): Comparison of alpha-fetoprotein (AFP) and lipocalin-2 (LCN2) levels among the hepatocellular carcinoma (HCC), cirrhotic, and control groups

Variable	Group	Median (Range)	Test Value (Kruskal-Wallis)	P-value	H&CI (Post hoc)	H&CO (Post hoc)	CI&CO (Post hoc)
AFP	HCC	2737 (0–22303)	10.5	0.016*	0.034*	0.034*	1.000
	Cirrhotic	4 (0–12)					
	Control	3 (0–9)					
LCN2	HCC	293 (140–445)	40.2	<0.001*	<0.001*	<0.001*	<0.001*
	Cirrhotic	129 (20–239)					
	Control	53 (38–68)					

H&CI: Comparison between HCC and cirrhotic groups; H&CO: Comparison between HCC and control groups; CI&CO: Comparison between cirrhotic and control groups; P-value: Significance level ($P < 0.05$ is significant).

For Child score, mean ± SD was 10.281 ± 1.938 in HCC group and 8.906 ± 2.190 in cirrhotic group. T-test yielded a t-value of 2.659 with a P-value of 0.010, indicating a significant difference between groups. For MELD score, mean ± SD was 16.000 ± 4.303 in HCC group and 13.594 ± 4.500 in cirrhotic group. T-test yielded a t-value of 2.186 with a P-value of 0.033, also indicating a significant difference between groups (Table 4).

Table (4): Child score and MELD score among HCC and cirrhotic groups

		Groups						T-Test	
		HCC			Cirrhotic			t	P-value
Child Score	**Range**	7	-	14	5	-	12	2.659	0.010*
	Mean ±SD	10.281	±	1.938	8.906	±	2.190		
MELD Score	**Range**	9	-	27	7	-	24	2.186	0.033*
	Mean ±SD	16.000	±	4.303	13.594	±	4.500		

MELD Score: Model for End-Stage Liver Disease, t: T-test statistic; P-value: Significance level ($P < 0.05$ is significant).

In HCC group, number of focal lesions was 2.281 ± 0.851. The size of focal lesions (in cm) was 2.656 ± 1.254. In HCC group, LCN2 levels were significantly higher in patients without DM (311.059 ± 38.359) compared to those with DM (272.000 ± 56.532) (*t = 2.311, P = 0.028*). Additionally, LCN2 levels were significantly higher in patients with Child C classification (318.810 ± 33.791) compared to those with Child B (243.000 ± 40.147) (*t = -5.652, P < 0.001*) (Table 5).

Table (5): LCN2 levels in patients with HCC in relation to gender, DM, HTN, HBsAg, HCV Ab, PVT, Child score, and smoking

HCC		LCN2				T-Test	
		N	Mean	±	SD	t	P-value
Gender	Male	24	286.833	±	55.052	-1.144	0.262
	Female	8	310.500	±	32.457		
DM	No	17	311.059	±	38.359	2.311	0.028*
	Yes	15	272.000	±	56.532		
HTN	No	14	285.571	±	58.742	-0.697	0.491
	Yes	18	298.333	±	44.920		
HBsAg	No	27	291.370	±	52.414	-0.351	0.728
	Yes	5	300.200	±	46.596		
HCV Ab	No	14	279.500	±	38.702	-1.313	0.199
	Yes	18	303.056	±	57.702		
PVT	No	10	277.800	±	64.112	-1.124	0.270
	Yes	22	299.545	±	43.751		
Child Grade	Child B	11	243.000	±	40.147	-5.652	<0.001*
	Child C	21	318.810	±	33.791		
ANOVA						**F**	**P-value**
Smoking	No	21	295.952	±	54.914	0.190	0.828
	Current	8	282.875	±	47.544		
	Ex-smoker	3	296.667	±	40.415		

HCC: Hepatocellular Carcinoma, LCN2: Lipocalin-2, DM: Diabetes Mellitus, HTN: Hypertension, HBsAg: Hepatitis B Surface Antigen, HCV Ab: Hepatitis C Virus Antibody, PVT: Portal Vein Thrombosis, SD: Standard Deviation, ANOVA: Analysis of Variance, F: F-statistic, P-value: Probability Value.

In cirrhotic group, LCN2 levels varied significantly across Child grades. Patients with Child C had highest levels (143.267 ± 11.653), followed by Child A (140.857 ± 66.934) and Child B (100.300 ± 7.334) (*F = 6.080, P = 0.006*) (Table 6).

Table (6): LCN2 levels in the cirrhotic group in relation to gender, DM, HTN, HBsAg, HCV Ab, PVT, Child score, and smoking

Cirrhotic		LCN2				T-Test	
		N	Mean	±	SD	t	P-value
Gender	**Male**	22	132.136	±	41.061	0.641	0.526
	Female	10	123.100	±	24.875		
DM	**No**	21	131.476	±	42.134	0.456	0.652
	Yes	11	125.182	±	23.970		
HTN	**No**	24	130.875	±	39.489	0.413	0.683
	Yes	8	124.625	±	27.867		
HBsAg	**No**	27	134.370	±	37.141	1.890	0.068
	Yes	5	102.000	±	17.678		
HCV Ab	**No**	15	136.867	±	45.028	1.100	0.280
	Yes	17	122.647	±	26.856		
PVT	**No**	27	131.000	±	38.582	0.600	0.553
	Yes	5	120.200	±	24.201		
		ANOVA				**F**	**P-value**
Child Grade	**Child A**	7	140.857	±	66.934		
	Child B	10	100.300	±	7.334	6.080	0.006*
	Child C	15	143.267	±	11.653		
Smoking	**No**	20	128.950	±	43.685		
	Current	9	125.000	±	22.377	0.313	0.734
	Ex-smoker	3	144.667	±	14.048		

HCC: Hepatocellular Carcinoma, LCN2: Lipocalin-2, DM: Diabetes Mellitus, HTN: Hypertension, HBsAg: Hepatitis B Surface Antigen, HCV Ab: Hepatitis C Virus Antibody, PVT: Portal Vein Thrombosis, SD: Standard Deviation, ANOVA: Analysis of Variance, F: F-statistic.

In HCC group, there were significant positive correlations between LCN2 levels and age (*r = 0.370, P = 0.037*) and between LCN2 levels and size of focal lesions (*r = 0.384, P = 0.030*). Additionally, a significant negative correlation was observed between LCN2 levels and albumin levels (*r = -0.358, P = 0.044*). In cirrhotic group, there was a significant negative correlation between LCN2 levels and age (*r = -0.370, P = 0.037*) (Table 7).

Table (7): LCN2 levels among the study population in relation to age, laboratory findings, imaging data, Child score, and other markers of HCC

Correlations	LCN2			
	HCC		Cirrhotic	
	r	P-value	r	P-value
Age	0.370	0.037*	-0.370	0.037*
WBCs	0.156	0.393	0.009	0.963
Hb	-0.105	0.566	-0.303	0.092
PLTs	0.016	0.932	0.053	0.775
BUN	-0.068	0.711	-0.184	0.313
Creat.	0.155	0.396	-0.198	0.277
Na	0.059	0.747	0.041	0.824
K	-0.081	0.661	0.028	0.880
Bilirubin T	0.249	0.169	0.170	0.352
Bilirubin D	0.166	0.363	0.167	0.360
AST	-0.104	0.572	-0.322	0.073
ALT	0.011	0.954	-0.291	0.106
ALP	0.276	0.126	0.069	0.707
GGT	0.347	0.052*	0.095	0.606
Albumin	-0.358	0.044*	0.274	0.129
INR	0.178	0.329	0.027	0.884
AFP	0.040	0.828	-0.085	0.643
BMI	-0.097	0.597	0.047	0.800
Child Score	0.555	0.001*	0.154	0.401
MELD Score	0.267	0.140	-0.130	0.477
No. of Focal Lesion	0.275	0.128	.	.
Size of Focal Lesion (cm)	0.384	0.030*	.	.

HCC: Hepatocellular Carcinoma, LCN2: Lipocalin-2, WBCs: White Blood Cells, Hb: Hemoglobin, PLTs: Platelets, BUN: Blood Urea Nitrogen, Creat.: Creatinine, Na: Sodium, K: Potassium, Bilirubin T: Total Bilirubin, Bilirubin D: Direct Bilirubin, AST: Aspartate Aminotransferase, ALT: Alanine Aminotransferase, ALP: Alkaline Phosphatase, GGT: Gamma-Glutamyl Transferase, Albumin: Serum Albumin, INR: International Normalized Ratio, AFP: Alpha-Fetoprotein, BMI: Body Mass Index, MELD Score: Model for End-Stage Liver Disease Score.

At a cutoff value of >160, LCN2 showed a sensitivity of 96.87% and a specificity of 96.87% for distinguishing HCC from cirrhotic cases. The positive predictive value (PPV) was 96.9%, negative predictive value (NPV) was 96.9%, and overall accuracy was 98.4%.

DISCUSSION

HCC is the sixth most common cancer globally and fourth in Egypt [6]. Its rising incidence in Egypt is linked to improved screening, better diagnostics, increased cirrhotic survival, and a high prevalence of HCV, a major risk factor [7]. Surveillance involves ultrasound and AFP testing, with diagnostic confirmation via CT or MRI [8, 9]. Late diagnoses often limit cure rates, underscoring need for new markers like LCN2, a protein highly expressed in liver cancer tissue [10]. This study evaluated LCN2 as a diagnostic marker for HCC in Egyptian patients.

In the present study, age of cirrhotic and HCC groups was significantly higher than that of healthy controls. This difference is attributed to relatively younger age of healthy controls, while the age of HCC patients (58.719 ± 5.670 years) was within sixth decade, on top of liver cirrhosis, in Egyptian cohort. This finding aligns with previous reports indicating that HCC typically affects individuals in fifth to sixth decades [11].

Males were more affected than females in this study, with more male cirrhotic patients than females. However, this difference did not reach statistical significance (P-value = 0.817), likely due to relatively small sample size. These findings are consistent with previous studies reporting a higher prevalence of HCC in males, attributed to greater prevalence of risk factors, particularly chronic viral hepatitis, among males [12, 13].

The study also found that smoking was not a significant risk factor for HCC. In HCC group, 21 subjects (65.63%) were non-smokers, 8 (25%) were smokers, and 3 (9.38%) were ex-smokers. This result is likely influenced by small sample size and contrasts with previous studies suggesting that smoking may contribute to HCC risk [14].

In studied HCC patients, 17 subjects (53.13%) did not have DM, while 15 subjects (46.88%) had DM. Additionally, 18 subjects (56.25%) had HTN, and 14 subjects (43.75%) did not. These findings reflect role of DM and HTN in development of liver cirrhosis and their predisposition to HCC, which is aligning with findings of **Li *et al.*** [15].

The study demonstrated that chronic viral infection was a significant risk factor for HCC. HCV infection was present in 18 patients (56.25%), and hepatitis B surface antigen (HBsAg) positivity was found in 5 patients (15.63%), while 14 patients (43.75%) were negative for HCV. This highlights burden of HCV infection in Egypt and its contribution to HCC development, even in era of effective antiviral treatments [1].

Serum AFP levels were significantly elevated in HCC patients compared to cirrhotic and healthy controls. This finding agrees with reports that high AFP levels, combined with triphasic CT or dynamic MRI, are diagnostic for HCC. Current guidelines recommend using serum AFP with abdominal ultrasound for early detection of HCC in cirrhotic patients [16].

Regarding serum LCN2, this study found significantly higher levels in HCC patients compared to cirrhotic patients and healthy controls. This result is consistent with the findings of **Barsoum *et al.*** [10]. High serum LCN2 levels in HCC patients compared to cirrhotic patients, irrespective of comorbidities such as DM, HTN, or viral markers, demonstrate its potential as a promising marker for early detection of HCC in cirrhotic patients, regardless of etiology. The study revealed a sensitivity of 96.87% and specificity of 96.87% for LCN2 as a marker for HCC, with an accuracy of 98.4% at a cutoff value of >160. These findings align with previous reports by **Barsoum *et al.*** [10].

Limitations: However, the study highlights the need for a larger sample size and prospective studies to evaluate serum LCN2 levels after different HCC treatments.

CONCLUSION

Serum LCN2 levels were significantly higher in HCC patients and mildly elevated in cirrhotic patients compared to controls, supporting its use as a tumor marker for HCC diagnosis. Combining LCN2 with AFP or other markers may enhance diagnostic accuracy and facilitate early HCC detection in surveillance programs. Future studies on larger HCC cohorts and metastatic liver tumors are needed to further establish diagnostic utility of LCN2.

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Conflict of Interest: Nil.

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