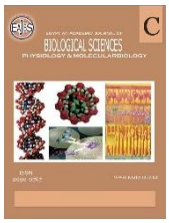
.

Citation: *Egypt.Acad.J.Biolog.Sci. (C.Physiology and Molecular biology) Vol. 17(1) pp69-80 (2025)*
DOI: 10.21608/EAJBSC.2025.418116

Egypt. Acad. J. Biolog. Sci., 17(1):69-80 (2025)

Egyptian Academic Journal of Biological Sciences
C. Physiology & Molecular Biology
ISSN 2090-0767
www.eajbsc.journals.ekb.eg



Assessment of Circulating Matrix Metalloproteinase 9 (MMP-9) Levels in Non-invasive Diagnosis and Predicting Prognosis of Hepatocellular Carcinoma After Successful DAA Therapy of HCV-Infected Egyptians

Shaimaa M. Abdelsamea[1], Hisham Ismail[1,*], Ahmed M. Sultan[2], Othman A. Othman[1], Mohamed Abdel-Wahab[2], Hoda M. El-Emshaty[2]

[1]Biochemistry Division, Chemistry Department., Faculty of Science, Minia University, Minia 61519, Egypt.

[2]Gastrointestinal Surgery Centre, Faculty of Medicine, Mansoura University, Mansoura 35516, Egypt.

***E-mail:** himosman@mu.edu.eg

ARTICLE INFO

Article History
Received:23/1/2025
Accepted:19/3/2025
Available:23/3/2025

Keywords:
HCV, DAA, Hepatocellular Carcinoma, Cirrhosis, MMP-9, Biomarker.
.

ABSTRACT

In spite of rapidly developing direct acting antiviral treatments of HCV, the incidence of hepatocellular carcinoma (HCC) in Egypt is still rising. Our objectives were to assess the diagnostic performance and the predictive value of serum MMP-9 in HCV-related chronic liver disease patients pre- and post DAAs therapy. The MMP-9 levels were measured by ELISA in sera of 84 patients with HCV-related chronic liver diseases and 20 healthy controls. The HCC patients presented the highest MMP-9 levels in comparison with patients with liver cirrhosis (LC) and healthy controls ($P < 0.0001$). For discriminating LC patients from healthy individuals, MMP-9 ROC curve showed AUC = 0.948 with $P < 0.0001$, using cutoff level 512.4 ng/L, for discriminating HCC patients from non-HCC individuals, it showed AUC = 0.830 with $P < 0.0001$ using cutoff value 730.9 ng/L, for discriminating LC patients from HCC patients, it showed AUC = 0.703 with $P < 0.0001$ using cutoff value 1348.4 ng/L and for discriminating HCV-treated HCC patients from HCV-untreated HCC patients, it showed AUC = 0.813 with $P < 0.001$ using cutoff value 785.2 ng/L. The MMP-9 levels were significantly reduced in HCC patients after DAA therapy ($P = 0.001$), while no significant decreased in LC patients after DAA therapy ($P > 0.05$). In conclusion, serum MMP-9 comprises a promising biomarker for assessment of LC and HCC before and after DAA therapy of HCV patients. Further research on larger sample size may validate these findings and support our suggestions.

INTRODUCTION

Hepatocellular carcinoma (HCC) is the 2nd main reason for fatality due to cancer and the 5th fastest-growing type of cancer overall. In cases of created liver cirrhosis brought on by alcohol, consumption of aflatoxin in the diet, non-alcoholic steatohepatitis (NASH), persistent infection through hepatitis C virus (CHC), acute hepatitis B virus infection (CHB), or non-alcoholic fatty liver disease (NAFLD), HCC is most commonly diagnosed (Liu *et al.*, 2019). In Egypt's general population, among cancers, HCC remains more frequent; yet, in females, it ranks second only to breast cancer. High HCV prevalence may have contributed to Egypt's high HCC rates (Chhatwal *et al.*, 2018; Ibrahim & Mikhail, 2015).

The utilization of direct-acting antivirals (DAAs) to treat HCV has led to higher sustained viral response (SVR) rates worldwide, reaching > 95% in the majority of genotypes and making them suitable for treating patients who were failed to respond to previous interferon-based treatments (Waked *et al.*, 2020). The occurrence of HCC continues to rise despite the fast evolving age of DAA strategies in the remedy of HCV (Pascut *et al.*, 2020). The illness's suffering can be lessened by an early identification of HCC, which can greatly improve the prognosis (Gao *et al.*, 2018). Late-stage HCC detection is possible with clinical techniques including imaging and histopathology (Benson 3ed *et al.*, 2009). Therefore, the development of novel alternative techniques to perform the initial identification of HCC is desperately needed (El-Emshaty *et al.*, 2022). The most widely used HCC biomarker worldwide is alpha-fetoprotein (AFP), which was initially identified as a blood diagnostic of HCC in 1960. AFP's specificity is still low, despite its 60% sensitivity in identifying HCC (Li *et al.*, 2024). Serum AFP is also normal in 15–30% of people with advanced HCC (Han *et al.*, 2014). Benign hepatic conditions including cirrhosis and hepatitis have also been linked to an increase in AFP. Accordingly, AFP is no longer advised by the American Association for the Study of Liver Diseases (AASLD) Practical Rules Commission for the early detection of HCC (Bruix & Sherman, 2011). Zinc-dependent proteases known as matrix metalloproteinase (MMPs) are categorized into four groups based on their activity: collagenases, matrilysins, stromelysins, and gelatinases (Abdel-Hamid & Abass, 2021). The primary MMP generated by human macrophages is MMP-9, a 92 kDa type IV collagenase or gelatinase B (Batroukha *et al.*, 2022). Type IV collagen, the basement membrane's main fundamental component and the extracellular matrix, is broken down by the proteolytic enzyme MMP-9, whose activity is frequently reported to be increased in tumor tissues and cancerous cells (Stetler-Stevenson, 2023). MMP-2 is primarily responsible for MMP-9 activation (Sienkiewicz *et al.*, 2021). In HCC, MMP-9 excessive production has been linked to intrahepatic metastases, tumor stage, vascular invasion, and capsular invasion. It is hypothesized that the two primary roles of MMP-9, the breakdown of the extracellular matrix and the restriction of angiogenesis, cause a complex shift in the equilibrium in HCV-related HCC since HCC is a highly vascular solid tumor in which angiogenesis plays a significant role (Bauer & Habior, 2022). Verifying the function of serum MMP-9 as indicators for the advancement of HCC in hepatic disorders associated with HCV following HCV RNA ablation was the goal of this investigation.

MATERIALS AND METHODS

1. Study Patients and Controls:

Between March 2022 and May 2023, 104 participants participated in a case-control study (40 HCV-related LC patients, 44 HCV-related LC evolved to HCC patients, and 20 healthy individuals served as control group). Abdominal ultrasonography, triphasic CT abdomen, serum AFP, and histological confirmation were used to diagnose the HCC. There was no indication of distant metastases or local invasion in the cirrhotic group. During the moment of recruitment, absolutely no medical or biochemical indication of liver disease or any known medical conditions in the control group. Patients with HCC who had undergone surgical, interventional, or medicinal treatment, patients with malignancies other than HCC, and patients with other viral infections (HBV infection or co-infection with HCV) were excluded from the study. Only 20 out of 40 LC patients and 24 out of 44 HCC patients underwent sofosobuvir-based DAA treatment in accordance with Egyptian national treatment recommendations for the care of genotype 4 CHC infection. For 12 weeks, the DAA treatment consisted of taking 60 mg of daclatasvir and 400 mg of sofosobuvir every day. At a 12-week follow-up, sustained virological response (SVR12) was described as imperceptible HCV-RNA using a

quantitative RT-PCR assay. Five milliliters of venous blood were drawn from each participant, allowed the coagulation process followed by centrifugation for ten minutes at 5000 rpm. Before being used, the serum was collected and kept at -20 ºC.

2. Laboratory Investigations:

All patients and controls underwent complete laboratory testing, including CBC, ELISA for HCV antibody, PCR for HCV-RNA viral load, serum ALT, AST, alkaline phosphatase, T. bilirubin, albumin, INR, creatinine, alpha-fetoprotein, CEA, and CA19-9. Fibrosis-4-score (FIB4), AAR, PLR, NLR, and APRI were computed by using hematological markers (platelets count, neutrophil count & lymphocyte count) and liver enzymes (ALT & AST), and each patient was categorized based on the Child-Pugh Score (Tarannum *et al.,* 2024) . All participants gave their written agreement, and the study was approved by the Mansoura University Faculty of Medicine's Ethics Committee in compliance with the Declaration of Helsinki, WHO. Universal Trial Number: MDP.23.10.135).

3. Measurement of Serum MMP-9:

A readily accessible ELISA kit was used to measure the serum amount of MMP-9 (Bldg., 501 Changsheng S Rd, Nanhu Dist., Jiaxing, Zhejiang, China) in accordance with the directions provided by the manufacturer. In brief, 50-µL/well of MMP-9 standards was add to standards wells, 40-µL/well of serum samples was add to sample wells and then 10-µL of anti-MMP-9 antibody was add to all wells, then 50-µL streptavidin-HRP was add to all wells and mixed well. Then, the plate was covered with a sealer and incubated for 60 minutes at 37 °C. Following the removal of the liquid and washing 5 times with wash buffer, 50-µL of substrate solution A was add to each well followed by 50-µL of substrate solution B. Then, the plate was incubated for 10 minutes at 37 °C in the dark and hence, 50-µL of stop solution was add to each well. The developed blue color changed into yellow immediately. Measurement of the optical density (OD) is conducted using a microplate reader (Biochrom EZ Read 400 Microplate Reader, Cambridge, UK) set to 450 nm within 10 minutes after the addition of stop solution. A standard curve representing the relationship between OD and the standard concentrations (ng/mL) of MMP-9 was established. By comparing the sample's OD to the standard curve, the concentration of circulating MMP-9 (ng/L) in the investigated samples was determined.

4. Statistical Analysis:

The SPSS software program, Version 24 was utilized. The interquartile range (IQR) and median were used to represent continuous variables. The Kruskal Wallis and Mann-Whitney tests were used to evaluate group comparisons. A P-value of less than 0.05 was deemed significant. Spearman's correlation coefficient was used to determine the correlation between the variables. The best cut-off values for the examined markers with sensitivity and specificity were found by plotting receiver operating characteristic (ROC) curves after a model for predictions was constructed using linear regression (Stochemer, 2019).

RESULTS

1. The Study's Subjects Clinical and Pathological Features:

A total of 84 older illnesses suffering from liver disorders related to HCV were split up into 44 HCC patients on top of LC (20 HCV-untreated HCC patients and 24 HCV-treated HCC patients) and 40 HCV-related LC patients (20 HCV-untreated LC patients and 20 HCV-treated LC patients). In addition to 20 healthy participants were assigned in the current study as controls. Among HCV- treated HCC patients, 12 (50%) patients were Child-Pugh A, 9 (37.4%) patients were Child-Pugh B and 3 (12.5 %) patients were Child-Pugh C, while in HCV-untreated HCC patients, 10 (50 %) patients were Child-Pugh B and 10 (50 %) patients were Child-Pugh C with significantly distinction between two groups (P value < 0.0001). However, no significant difference was detected in ALT, AST, AFP and Albumin, WBCs, Hb, neutrophil, lymphocyte and platelets count, between HCV-untreated HCC patients and HCV- treated HCC

patients, but significant difference was detected in Total Bilirubin (P = 0.002), liver cirrhosis activity (P < 0.0001) and Child Pugh score (P < 0.0001) between HCC groups (Tables 1 & 2).

Table 1. Demographic and clinical data of study patients with chronic liver disease (CLD) with or without HCC in comparison with healthy individuals.

Characteristics[#]		Healthy individuals (n = 20)	Group I (CLD without HCC) N = 40		Group II (CLD with HCC) N = 44		P value*
			Subgroup Ia HCV-untreated LC patients (n = 20)	*Subgroup Ib HCV treated -LC patients (n = 20)*	*Subgroup IIa HCV-untreated HCC patients (n = 20)*	*Subgroup IIb HCV- treated HCC patients (n = 24)*	
Biochemical markers:							
GGT (U/L)		19.5(15-34.8)	25 (14.3-86.5)	28 (22-51.8)	50 (24.3-70)	46.5 (27-68.3)	0.013
CRP (mg/L)		2.05 (1-3.8)	4 (3-10.5)	4 (3-7.3)	14.5 (5.8-18)	6.75 (3.63-14)	0.0001
AST (U/ml)		21 (21-25.5)	35 (23.8-52.5)	39 (29.3-63.8)	49 (29.5-57)	44 (33.3-106)	0.0001
ALT (U/ml)		20 (20-31.5)	22 (21-27.3)	24 (20.3-39.5)	34 (21.5-49.5)	37.5 (24.3-61)	0.007
Albumin (g/dL)		4.95 (4.3-5)	2.8 (2.4-3.6)	3.25 (2.9-3.5)	2.85 (2.6-3.1)	3.3 (2.8-3.8)	0.0001
T. BIL. (mg/dL)		0.55 (0.5-0.6)	1.85 (1.4-2.7)	1.4 (0.8-1.9)	3.1 (2.7-3.2)	1.3 (0.7-2.1)	0.0001
Alk. Phos. (U/L)		5 (5-5)	8 (5-9.75)	5 (5-7)	6 (5-7)	6 (5-8)	0.026
AFP (ng/mL)		1.35 (1.1-2.34)	3.1 (1.5-5.4)	4 (2.3-5.9)	45 (20.8-92)	15 (6.5-49.3)	0.0001
Hematological markers:							
WBCs		5.8 (5-7.4)	3.2 (2.2-4.05)	2.5 (1.6-5.04)	3.5 (2.6-5.2)	3.3 (2.6-5.5)	0.002
Lymphocytes		2.6 (1.95-3.14)	0.7 (0.41-0.98)	0.5 (0.38-0.68)	1.1 (0.62-1.5)	0.8 (0.57-1.36)	0.0001
Neutrophil		2.87 (2.2-3.59)	1.66 (1.1-2.5)	1.42 (0.93-3.1)	2.36 (1.3-3.2)	2.2 (1.13-3.73)	0.113
Hb (g/dL)		14.3 (13.7-15)	9.7 (7.9-11.2)	9.6 (8.2-10.9)	11.3 (10-13)	11.3 (8.2-12.5)	0.0001
Plt. Count (10^9/L)		246 (223.4-275.4)	51 (44.4-77.3)	59.4(34.5-86.4)	67 (47-80)	86.5(46.3-116.8)	0.0001
INR		1 (1-1.1)	1.4 (1.3-1.8)	1.5 (1.3-1.6)	1.45(1.15-1.7)	1.3 (1.2-1.5)	0.0001
Tumor markers:							
AFP (ng/mL)		1.35 (1.1-2.34)	3.1 (1.5-5.4)	4 (2.3-5.9)	45 (20.8-92)	15 (6.5-49.3)	0.0001
Demographic data:							
Age (yr)		29.5 (24-41)	53 (41-58)	53.5 (35.5-61)	55 (49.8-58)	57 (54-59)	0.0001
Sex	Male	13 (65%)	13 (65%)	14 (70%)	12 (60%)	16 (66.7%)	0.969
	Female	7 (35%)	7 (35%)	6 (30%)	8 (40%)	8 (33.3%)	
Clinical presentation:							
Ascites	Absent	0 (0%)	3 (15%)	7 (35%)	7 (35%)	12 (41.4%)	0.023
	Mild	0 (0%)	4 (20%)	8 (40%)	5 (25%)	5 (25%)	
	Moderate	0 (0%)	4 (20%)	2 (10%)	2 (10%)	3 (33.3%)	
	Marked	0 (0%)	9 (45%)	3 (15%)	6 (30%)	4 (20%)	
Activity of LC	Mild	0 (0%)	8 (40%)	12 (60%)	5 (25%)	19 (47.5%)	0.001
	Moderate	0 (0%)	12 (60%)	8 (40%)	15 (75%)	5 (13.9%)	
Child-Pugh Score	Class A	0 (0%)	4 (20%)	9 (45%)	0 (0%)	12 (50%)	0.001
	Class B	0 (0%)	11 (55%)	7 (35%)	10 (50%)	9 (37.5%)	
	Class C	0 (0%)	5 (25%)	4 (20%)	10 (50%)	3 (12.5%)	

Abbreviations: GGT, gama glutamyl transeferase; CRP, C creative protein; AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALB, Albumin; T.BIL, total bilirubin; Alk. Ph, Alkaine phosphatase; WBCs, white blood cells; Hb, hemoglobin; Plt; platelets, INR, international normalized ratio; AFP, alpha fetoprotein; CEA, carcinoembryonic antigen.

[#] Biochemical markers, Hematological markers, Tumor markers are represented as Median and interquartile range (25-75%); Kruskal Wallis tests were used for data analysis. Sex and clinical presentation are represented as frequency and percent.

* P > 0.05 is considered not significant; P < 0.05 considered significant; P < 0.001 considered very significant; P < 0.0001 is considered extremely significant.

Table 2. Fibrosis and inflammatory indexes of study patients with chronic liver disease (CLD) with or without HCC in comparison with healthy individuals.

Indexes[#]	Healthy individuals (n = 20)	Group I (CLD without HCC) (n = 40)		Group II (CLD with HCC) (n = 44)		P value*
		Subgroup Ia HCV-Untreated LC patients (n = 20)	Subgroup Ib HCV-Treated LC patients (n = 20)	Subgroup IIa HCV-Untreated HCC patients (n = 20)	Subgroup IIb HCV- Treated HCC patients (n = 24)	
PLR	93.9 (77-126)	82.2 (56.8-121)	115 (71-255)	61.6 (44-85)	82.3(59.4-179)	0.017
NLR	1 (0.79-1.5)	2.6 (1.2-3.9)	2.8 (2.1-5.6)	1.9 (0.85-3.2)	2.9 (1.5-4.1)	0.001
AAR	1.1 (0.78-1.1)	1.5 (1.1-1.77)	1.44 (1.1-1.6)	1.48 (1-2.25)	1.4 (0.87-1.8)	0.021
APRI	0.23 (0.19-0.26)	1.57 (1.1-2.24)	1.36 (0.97-2.8)	1.7 (0.77-2.7)	1.4 (1-2.4)	0.0001
FIB4	0.55 (0.41-0.74)	6.4 (4.29-10)	6.45 (3.37-8.79)	5.3 (3.65-11.3)	4.95 (3.59-11)	0.0001

Abbreviations: PLR, Platelet to Lymphocyte Ratio; NLR, Neutrophil to Lymphocyte ratio; AAR, AST/ALT ratio; APRI Index, AST to platelet ratio index; FIB4, Fibrosis index based-4 biomarkers. Data are represented as Median and interquartile range (25-75%); Kruskal Wallis tests were used for data analysis.

* P > 0.05 is considered not significant; P < 0.05 considered significant; P < 0.001 considered very significant; P < 0.0001 is considered extremely significant.

2. Diagnostic Performance of MMP-9 to Discriminate HCC Patients From LC Patients And Healthy Individuals:

The ROC curves were created for serum MMP-9 biomarker in order to distinguish between different study groups and to evaluate its diagnostic performances. To disseminate between HCV-treated HCC and nonmalignant individuals (HCV-treated LC patients + Healthy controls), MMP-9 biomarker showed sensitivity (62.5%), specificity (65.5%), NPV (70%), PPV (57.7%), and accuracy (64.3%) at a cut-off value of 0.573.32 with AUC = 0.655. To disseminate between HCV-untreated HCC and nonmalignant individuals (HCV-untreated LC patients + Healthy individuals), MMP-9 biomarker showed sensitivity (75%), specificity (60.7%), NPV (80.9%), PPV (52.2%), and accuracy (65.9%) at cut-off value of 0.730.9 with AUC = 0.830. To disseminate between HCV-untreated HCC & HCV-untreated LC patients, MMP-9 biomarker showed sensitivity (62.5%), specificity (93.8%), NPV (71.4%), PPV (90.9%), and accuracy (78%) at a cut-off value of 1348.4 with AUC = 0.730 and to disseminate between HCV-untreated HCC and HCV-treated HCC patients, MMP-9 biomarker showed sensitivity of 68.8%, specificity of 70.8%, NPV of 77.3%, PPV of 61.1%, and accuracy of 70% at a cut-off value of 785.2 ng/L with AUC 0.813 , as displayed in Table 3 & Figure 1

.

Table 3: The diagnostic value of serum MMP-9 distinguishing study groups.

Groups	AUC	*P*-Value	Cut off	Sens., %	Spec., %	NPV %	PPV %	Accuracy %
LC & HI	0.948	0.0001	512.4	93.8	100	92.3	100	96.4
LC(ttt) & HI	0.967	0.0001	459.3	85	100	80	100	84.4
LC & LC(ttt)	0.639	0.157	755.9	62.5	55	64.7	52.6	58.3
HCC & HI	1.000	0.0001	514.6	100	100	100	100	100
HCC(ttt) & HI	0.944	0.0001	434.8	87.5	91.7	78.6	95.5	88.9
HCC & HCC(ttt)	0.813	0.001	785.2	68.8	70.8	77.3	61.1	70
LC & HCC	0.703	0.049	1348.4	62.5	93.8	71.4	90.9	78
HCC & (HI + LC)	0.830	0.0001	730.9	75	60.7	80.9	52.2	65.9
HCC(ttt) & (HI + LC(ttt))	0.655	0.048	573.3	62.5	65.5	70	57.7	64.3

Abbreviations: HI; healthy individuals; LC, HCV-untreated-liver cirrhosis patients; LC(ttt), HCV-treated liver cirrhosis patients; HCC; HCV-untreated HCC patients, HCC(ttt); HCV-treated HCC patients.

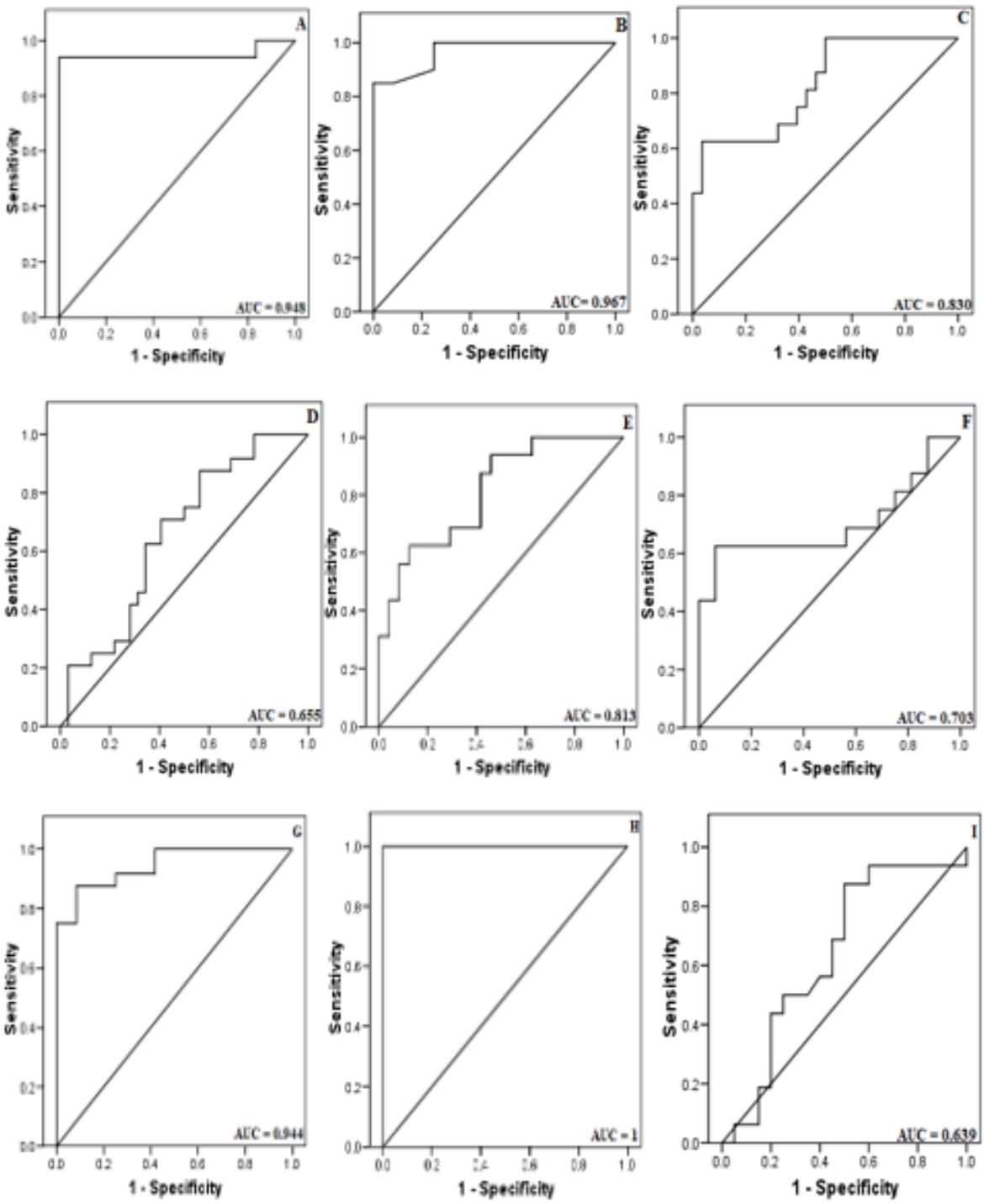


Fig. 1. The ROC curves and AUC for MMP-9 biomarker to differentiate between study groups. A. HCV untreated LC patients and healthy individuals (P value < 0.0001), **B.** HCV-treated LC patients and healthy controls (P < 0.0001), **C.** HCV-untreated HCC patients and Nonmalignant individuals (i.e. HCV- untreated LC patients + Healthy controls); (P < 0.0001). **D.** HCV-treated HCC patients & Nonmalignant individuals (i.e. HCV treated LC patients + Healthy individuals), (P = 0.048). **E.** HCV- untreated HCC patients and HCV-treated HCC patients (P < 0.001) & **F.** HCV- untreated HCC patients & HCV- untreated LC patients (P = 0.049). **G.** HCV-treated HCC patients & Healthy individuals (P < 0.0001). **H.** HCV untreated HCC patients & Healthy individuals (P < 0.0001). **I.** HCV-treated LC patients & HCV-untreated LC patients (P = 0.157).

3. Correlation of MMP9 and Laboratory Findings:

MMP-9 was substantially linked with ALT (r = - 0.593, P = 0.008), alkaline phosphatase level (r = -0.531, P = 0.017), NLR (r = 0.485, P = 0.028) & AAR (r = 0.511, P = 0.022) in patients with HCV-untreated HCC, and with CRP (r = - 0.349, P = 0.0047), total bilirubin (r = -0.425, P = 0.019), WBCs (r = - 0.346, P = 0.049), and INR (r = - 0.568, P = 0.002) in patients with HCV-treated HCC, as indicated in Table 4.

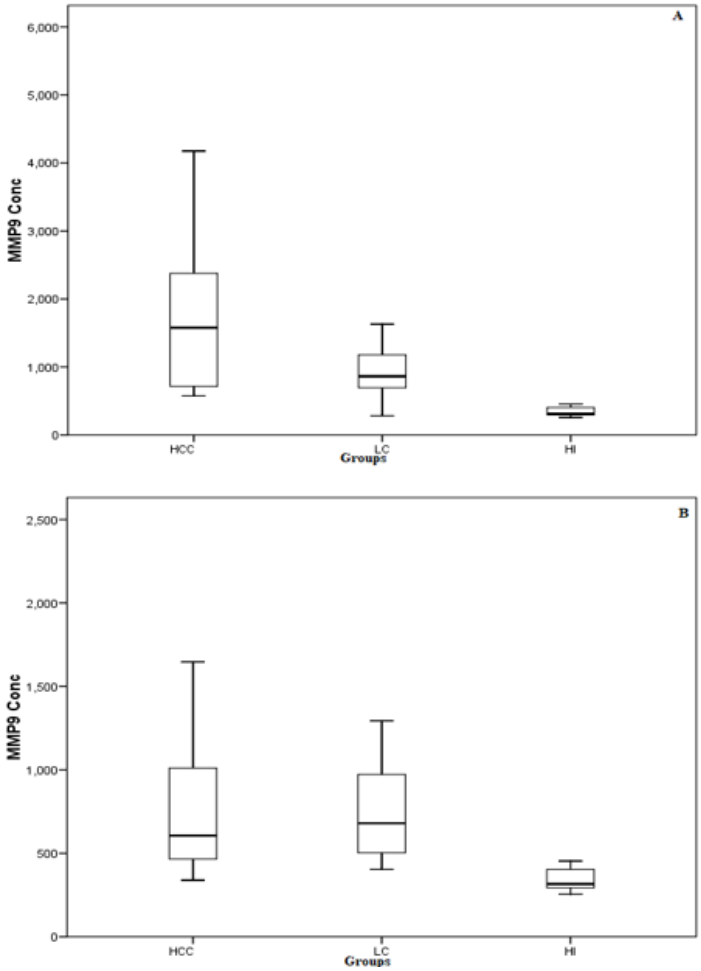
Table 4. Correlation between serum level of MMP-9 and laboratory markers and indexes.

Characteristics	Group II (CLD with HCC) (n = 44)		P value*	P value**
	Subgroup IIa HCV-Untreated HCC patients (n = 20)	Subgroup IIb HCV-Treated HCC patients (n = 24)		
Biochemical Markers:				
GGT (U/L)	r = -0.157	r = -0.064	0.281	0 .383
CRP (mg/L)	r = 0.137	r = -0.349	0.307	0 .047
AST (U/ml)	r = 0.043	r = -0.174	0.438	0.208
ALT (U/ml)	r = -0.593	r = -0.028	0.008	0.448
Albumin (g/dL)	r = 0.104	r = 0.191	0.351	0.186
T. BIL. (mg/dL)	r = 0.066	r = -0.425	0.404	0.019
Alk. Phos. (U/L)	r = -0.531	r = -0.081	0.017	0.354
AFP (ng/mL)	r = -0.105	r = -0.308	0.350	0.072
Hematological Markers:				
WBCs	r = 0.166	r = -0.346	0.269	0.049
Lymphocytes	r = -0.276	r = -0.241	0.151	0.129
Neutrophil	r = 0.329	r = -0.168	0.106	0.216
Hb (g/dL)	r = -0.167	r = -0.153	0.269	0.238
Plt Count (10^9/L)	r = -0.170	r = -0.140	0.265	0.258
INR	r = 0.405	r = -0.568	0.060	0.002
Inflammatory and Fibrotic Indexes:				
PLR	r = 0.409	r = 0.092	0.058	0.335
NLR	r = 0.485	r = 0.106	0.028	0.311
AAR	r = 0.511	r = -0.020	0.022	0.463
APRI	r = -0.001	r = 0.124	0.498	0.283
FIB4	r = 0.325	r = 0.050	0.109	0.407

* P > 0.05 is considered not significant and p value < 0.05 is considered significant for Subgroup IIa HCV-untreated HCC.
** P > 0.05 is considered not significant and p value < 0.05 is considered significant for Subgroup IIb HCV- treated HCC.

4. Assessment of MMP-9 Levels In Study Groups Before and after HCV Therapy with DAA:

The activity and blood level of MMP-9 in HCV-HCC patients were assessed in patients suffering from liver cirrhosis, HCC, in comparison with healthy controls (Fig. 2). MMP-9 activity varies significantly between groups HCC, LC & healthy individuals (Fig. 1). Compared to HCV-untreated LC patients & healthy individuals, MMP-9 activity was higher in HCV untreated HCC patients (1577 [702- 2407], 863.9 [680.9-1195], & 316.46 [294.42- 418.81], P < 0.0001). The activity of MMP-9 serum levels was assessed in individuals with HCV treated with DAA HCC (Figure 2). Compared to HCV-treated HCC, patients with LC showed increased MMP-9 level (606 [458.9-1105], 680.4 [497.9-1006.8] & 316.46 [294.42-418.81], P < 0.0001). MMP-9 serum level was much greater in HCV-untreated HCC patients, with significant difference between the two HCC groups (P value < 0.001). No discernible change of MMP-9 levels in HCV-HCC & HCV- LC (P = 0.832) & in HCV-treated groups (P value = 0.05), as indicated in Table 5.



Fig; 2. Box Plots Showing Distribution of Measured Serum MMP-9 Level with Median, Minimum and Maximum Values. **A:** HCC patients and LC patients before HCV treatment in comparison with healthy individuals, (*P* value < 0.0001), **B:** HCC patients and LC patients after HCV treatment in comparison with healthy individuals, (*P* value < 0.0001). The Box Defines the Boundaries of the First and third Quartiles of Data. Sample groups on X axis and marker concentrations on Y axis.

Table 5. Levels of MMP-9 in different study groups.

Pathological status		**No.**	**MMP-9 ng/L [Median and (IQR)]**	**P value***	**P value****
1. Healthy controls:		**20**	**316.46 [294.4-418.8]**	**-**	**< 0.0001**
2. Group I (CLD without HCC):	***Subgroup Ia (HCV-untreated LC patients)***	**20**	**863.9 [680.9-1195]**	**0.158**	
	Subgroup Ib (HCV-treated LC patients)	**20**	**680.4 [497.9-1006.8]**		
3. Group II (CLD with HCC):	***Subgroup IIa (HCV-untreated HCC patients)***	**20**	**1577[702- 2407]**	**0.001**	
	Subgroup IIb (HCV-treated HCC patients)	**24**	**606 [458.9-1105]**		

 # Data are presented as Median and Interquartile Range (IQR).

** P > 0.05 is considered not significant and p value < 0.05 is considered significant. The difference between HCV- treated LC & HCV-LC and HCV- treated HCC & HCV-HCC was calculated using Mann-Whitney Test.*

*** P > 0.05 is considered not significant and p value < 0.05 is considered significant. The difference between all study groups was calculated using Kruskal-Wallis Test.*

DISCUSSION

The poor prognosis and recurrence of HCC are caused by metastases and enhanced active angiogenesis in this vascularized tumor (Niu *et al.,* 2022). The prognosis of HCC is quite difficult, hence it is vital to find novel, appropriate individual or group of serum indicators that might be employed to diagnose HCC in high-risk people early. In addition to their involvement in angiogenesis, tumor growth, invasion, and metastasis, MMPs have the ability to breakdown the extracellular matrix and promote endothelial cell motility (Yan *et al.,* 2023). Those with HCV-infected HCC in the current study exhibited significantly higher serum AFP concentrations than those in other groups. These findings are consistent with a previous study that discovered that serum levels of AFP were significantly higher in HCC patients than in healthy controls and people with chronic hepatitis C (Zekri *et al.,* 2015). In line with a previous study that discovered liver function test values were significantly higher in HCC patients than in CLD patients, AST was also significantly higher in HCC patients than in LC patients (Zekri *et al.,* 2010). the ROC curve analysis of MMP-9 between HCC patients and nonmalignant (liver cirrhotic patients + healthy individuals) demonstrated high sensitivity of 75% and specificity of 60.7%, at a cutoff value of 730.94 ng/L, with an AUC of 0.830, in predicting the existence of HCC. The present findings aligned with earlier research that demonstrated MMP-9's superiority in predicting tumor survival and recurrence in HCC (Costa *et al.,* 2024). And they came to the conclusion that, at a cutoff value of 60 ng/mL, the ROC curve analysis of serum MMP-9 levels showed a sensitivity of 53% and a specificity of 89% for diagnosing HCC (Zhou & Qin, 2012). In comparison to liver cirrhosis and healthy individuals, as well as between HCV-treated and HCV-infected HCC patient groups, MMP-9 levels were markedly elevated in HCC patients. However, there was no discernible difference between LC and HCC patients. These findings are consistent with those of previous researchers, who found that patients with HCC had greater MMP-9/MMP-2 ratios than patients with chronic liver disorders and controls(El Tayebi *et al.,* 2011; Huang, 2018). In this investigation, the blood level of MMP-9 decreased following HCV clearance using DAA treatment in HCC and cirrhotic patients, although it was still high when compared to healthy individuals. Wnt/β-catenin is known to stimulate the development of MMPs, such as MMP2 and MMP9, which contribute to tumor metastasis Following HCV eradication by DAA, Wnt/β-catenin signaling remained active in chronic HCV-infected cells. Considering that Wnt/β-catenin signaling plays a significant role in carcinogenesis and that DAA was unable to restore it even after HCV was abolished(Chen *et al.,* 2021; Trujano-Camacho *et al.,* 2021). In this trial, in HCC patients treated with DAA from HCV There was highly substantial negative association between levels of blood MMP-9 and INR, CRP, Total Bilirubin, WBCs, while in HCC patients not treated with DAA from HCV Serum MMP-9 levels were correlated negatively with ALT and Alkaline Phosphatase and positively with AAR and NLR. disputes the notion that MMP-9 and HCC patients' levels of alkaline phosphatase, direct bilirubin, and total bilirubin are positively correlated. Consequently, when HCC advances and hepatic damage increases, MMP-9 rises (Kisseleva & Brenner, 2021). These findings supported earlier research that suggested a number of biochemical signs have been proposed as possible non-invasive serum indicators of fibro-proliferation. Because of an imbalance between increased matrix synthesis and decreased breakdown of connective tissue proteins, which leads to increased extracellular matrix deposition, it has been demonstrated that the MMPs and their TIMPs are associated with the development of cirrhosis induced by hepatitis C (Zhang *et al.,* 2003). The overexpression of their particular inhibitors (TIMPs) is the primary cause of the decreased activity of ECM-removing MMPs (Zu-hua *et al.,* 2006).

Maintaining the breakdown and synthesis of extracellular matrix depends on the balanced balance between MMP-9 and its TIMP-1 (Cui et *al.*, 2023). A loss of this equilibrium is linked to tumor invasion and metastasis and indicates a bad prognosis (Costa *et al.*, 2024). Tissue inhibitors of metalloproteinase gradually decreased in well-differentiated hepatocellular carcinoma and less differentiated tumors, whereas MMP levels increased. According to these findings, tissue expression of MMPs and their inhibitors may serve as a valuable indicator for predicting the development and spread of HCC (Costa *et al.*, 2024).

Conclusion:

Serum MMP-9 is a promising prognostic and predictive biomarker for assessment of liver cirrhosis and HCC in patients with chronic HCV patients pre- and post DAA therapy. Our recommendations may be supported by additional studies with a bigger sample size.

DECLERATIONS:

Ethical Approval: The present study approved by the Ethical Committee of Faculty of Medicine, Mansoura University with the assigned approval number MDP.23.10.135.

Conflicts of interest: The authors declare that there is no conflict of interest.

Formatting of funding sources: The present study received no fund from any funding authority.

Authors contributions: HME and HI. conception and design of the study. SMA and OAO. Laboratory investigations, collection of data and statistical analysis. MA and AMS: medical history, clinical investigations and endoscopy. HI, SMA and HME. confirm the accuracy and interpretation of the data and writhing the first draft of the manuscript. All authors have read and thoroughly reviewed the final version of the manuscript.

Availability of Data and Materials: All data supporting the described findings of the study can be obtained from the corresponding authors (HI) upon request.

Acknowledgments: The authors would like to thank all staff members of Gastrointestinal Surgery Center, Mansoura University for their kind help during the study.

REFERENCES

Abdel-Hamid, N. M., & Abass, S. A. (2021). Matrix metalloproteinase contribution in management of cancer proliferation, metastasis and drug targeting. *Molecular Biology Reports*, *48*(9), 6525-6538.

Batroukha, Y. A. F., Nour El Dein, M. M., Abou-Dobara, M. I., & El-Sayed, A. K. (2022). Production and Optimization of Gelatinase Producing Lentzea sp. Strain Isolated from the Soil Rhizosphere. *Scientific Journal for Damietta Faculty of Science*, *12*(1), 124-131.

Bauer, A., & Habior, A. (2022). Concentration of serum matrix metalloproteinase-3 in patients with primary biliary cholangitis. *Frontiers in Immunology*, *13*, 885229.

Benson 3rd, A. B., Abrams, T. A., Ben-Josef, E., Bloomston, P. M., Botha, J. F., Clary, B. M., . . . Davila, R. (2009). NCCN clinical practice guidelines in oncology: hepatobiliary cancers. *Journal of the National Comprehensive Cancer Network: JNCCN*, *7*(4), 350-391.

Bruix, J., & Sherman, M. (2011). Management of Hepatocellular Carcinoma: An Update. *Hepatology*, *53*(3), 1020-1022.

Chen, T., Chen, Z., Lian, X., Wu, W., Chu, L., Zhang, S., & Wang, L. (2021). MUC 15 promotes osteosarcoma cell proliferation, migration and invasion through livin, MMP-2/MMP-9 and wnt/β-catenin signal pathway. *Journal of Cancer*, *12*(2), 467.

Chhatwal, J., Chen, Q., Ayer, T., Bethea, E. D., Kanwal, F., Kowdley, K. V., . . . Gordon, S. C. (2018). Hepatitis C virus re-treatment in the era of direct-acting antivirals: projections in the USA. *Alimentary Pharmacology & Therapeutics*, *47*(7), 1023-1031.

Costa, D., Scalise, E., Ielapi, N., Bracale, U., Andreucci, M., & Serra, R. (2024).

Metalloproteinases as Biomarkers and Sociomarkers in Human Health and Disease. *Biomolecules* 2024, 14, 96.

Cui, Q., Wang, X., Zhang, Y., Shen, Y., & Qian, Y. (2023). Macrophage-derived MMP-9 and MMP-2 are closely related to the rupture of the fibrous capsule of hepatocellular carcinoma leading to tumor invasion. *Biological Procedures Online*, *25*(1), 8.

El Tayebi, H., Salah, W., El Sayed, I., Zekri, A., Zayed, N., Salem, E., . . . Abdelaziz, A. (2011). Expression of insulin-like growth factor-II, matrix metalloproteinases, and their tissue inhibitors as predictive markers in the peripheral blood of HCC patients. *Biomarkers, 16*(4), 346-354.

Gao, T., Zhi, J., Mu, C., Gu, S., Xiao, J., Yang, J., . . . Xiang, Y. (2018). One-step detection for two serological biomarker species to improve the diagnostic accuracy of hepatocellular carcinoma. *Talanta, 178*, 89-93.

Han, L.-L., Lv, Y., Guo, H., Ruan, Z.-P., & Nan, K.-J. (2014). Implications of biomarkers in human hepatocellular carcinoma pathogenesis and therapy. *World Journal of Gastroenterology, 20*(30), 10249.

Huang, H. (2018). Matrix metalloproteinase-9 (MMP-9) as a cancer biomarker and MMP-9 biosensors: recent advances. *Sensors, 18*(10), 3249.

Ibrahim, A. S., & Mikhail, N. N. (2015). The evolution of cancer registration in Egypt: from proportions to population-based incidence rates. *SECI Oncology, 4*, 1-21.

Kisseleva, T., & Brenner, D. (2021). Molecular and cellular mechanisms of liver fibrosis and its regression. *Nature Reviews Gastroenterology & Hepatology, 18*(3), 151-166.

Li, X., Li, Y., Yuan, J., Zhang, W., Xu, T., Jing, R., & Ju, S. (2024). Serum tRF-33-RZYQHQ9M739P0J as a novel biomarker for auxiliary diagnosis and disease course monitoring of hepatocellular carcinoma. *Heliyon, 10*(9), e30084.

Liu, Z., Jiang, Y., Yuan, H., Fang, Q., Cai, N., Suo, C., . . . Chen, X. (2019). The trends in incidence of primary liver cancer caused by specific etiologies: results from the Global Burden of Disease Study 2016 and implications for liver cancer prevention. *Journal of Hepatology, 70*(4), 674-683.

Niu, Z.-S., Wang, W.-H., & Niu, X.-J. (2022). Recent progress in molecular mechanisms of postoperative recurrence and metastasis of hepatocellular carcinoma. *World Journal of Gastroenterology, 28* (46), 6433.

Pascut, D., Pratama, M. Y., & Tiribelli, C. (2020). HCC occurrence after DAA treatments: molecular tools to assess the post-treatment risk and surveillance (Vol. 7, pp. HEP21): Taylor & Francis.

Stetler-Stevenson, W. G. (2023). The continuing saga of tissue inhibitor of metalloproteinase 2: emerging roles in tissue homeostasis and cancer progression. *The American Journal of Pathology*. 193(10), 1336-1352.

Stochemer, D. (2019). Quantitative Methods for the Social Sciences: A practical introduction with examples in SPSS and STATA. Springer Nature Publishing AG 2019, 1st eddition, (eBook). https://doi. org/10.1007/ 978-3-319-99118-4. 185PP.

Tarannum, S., Ilyas, T., Shaik, S. T., Sultana, N., Saniya, M. N., Mynampati, A. M., . . . Kumar, R. (2024). Assessment of the Child-Pugh Score, Model for End-Stage Liver Disease Score, Fibrosis-4 Index, and AST to Platelet Ratio Index as Non-endoscopic Predictors of the Presence of Esophageal Varices and Variceal Bleeding in Chronic Liver

Disease Patients. *Cureus, 16*(11), e73768.

Trujano-Camacho, S., Cantú-de León, D., Delgado-Waldo, I., Coronel-Hernández, J., Millan-Catalan, O., Hernández-Sotelo, D., . . . Campos-Parra, A. D. (2021). Inhibition of Wnt-β-catenin signaling by ICRT14 drug depends of post-transcriptional regulation by HOTAIR in human cervical cancer HeLa cells. *Frontiers in Oncology, 11*, 729228.

Waked, I., Esmat, G., Elsharkawy, A., El-Serafy, M., Abdel-Razek, W., Ghalab, R., . . . Kabil, K. (2020). Screening and treatment program to eliminate hepatitis C in Egypt. *New England Journal of Medicine, 382*(12), 1166-1174.

Yan, T., Cai, Y., Wei, Y., & Xie, Q. (2023). Expression of matrix metalloproteinases and their association with clinical characteristics of solid tumors. *Gene, 850*, 146927.

Zekri, A.-R., Youssef, A. S. E.-D., Bakr, Y. M., Gabr, R. M., El-Rouby, M. N. E.-D., Hammad, I., . . . Hamed, H. A. E.-H. (2015). Serum biomarkers for early detection of hepatocellular carcinoma associated with HCV infection in egyptian patients. *Asian Pacific Journal of Cancer Prevention, 16*(3), 1281-1287.

Zekri, A.-R. N., El-Din, H. M. A., Bahnassy, A. A., Zayed, N. A., Mohamed, W. S., El-Masry, S. H., . . . Esmat, G. (2010). Serum levels of soluble Fas, soluble tumor necrosis factor-receptor II, interleukin-2 receptor and interleukin-8 as early predictors of hepatocellular carcinoma in Egyptian patients with hepatitis C virus genotype-4. *Comparative Hepatology, 9*, 1-12.

Zhang, S., Li, L., Lin, J.-Y., & Lin, H. (2003). Imbalance between expression of matrix metalloproteinase-9 and tissue inhibitor of metalloproteinase-1 in invasiveness and metastasis of human gastric carcinoma. *World journal of gastroenterology: World Jornal of Gastroenterology, 9*(5), 899.

Zhou, T.-B., & Qin, Y.-H. (2012). The potential mechanism for the different expressions of gelatinases induced by all-trans retinoic acid in different cells. *Journal of Receptors and Signal Transduction, 32*(3), 129-133.

Zu-hua, G., Tretiakova, M. S., Liu, W.-h., Gong, C., Farris, P. D., & Hart, J. (2006). Association of E-cadherin, matrix metalloproteinases, and tissue inhibitors of metalloproteinases with the progression and metastasis of hepatocellular carcinoma. *Modern Pathology, 19*(4), 533.