Abdalla *et al. Egyptian Liver Journal* (2024) 14:11
https://doi.org/10.1186/s43066-024-00316-9

Egyptian Liver Journal

ORIGINAL RESEARCH ARTICLE

Open Access

Tissue inhibitor of matrix metalloprotinase-1 and collagen type IV in HCV-associated cirrhosis and grading of esophageal varices

Nasser Mohamed Abdalla[1], Fatma Mohamed Abd El Aziz[1], Akram Deghady[2], Mohamed Helmy Abaza[3*] and Walid Ismail Ellakany[1]

Abstract

Background Esophageal varices are abnormally dilated submucosal veins of the esophagus which develop as a result of portal hypertension due to cirrhosis. Collagen type IV is upregulated with a 14-fold increase in cirrhosis. Tissue inhibitor of metalloproteinases-1 (TIMP-1) is also upregulated during hepatic fibrogenesis and considered to promote fibrosis in the injured liver. The objective of this research was to study the serum levels of tissue inhibitor of matrix metalloprotinase-1 and serum collagen type IV in patients with post hepatitis C cirrhosis and their relation to the different grades of esophageal varices.

Patients and methods This study was carried out on one hundred and twenty individuals classified into three groups: Group I included thirty patients with liver cirrhosis without esophageal varices. Group II included sixty patients with liver cirrhosis with esophageal varices. Group III included thirty healthy volunteers as controls.

Results A significant positive correlation was found between collagen type IV and the presence of esophageal varices in esophageal varices group ($p=0001$*). Also, a significant positive correlation was found between TIMP-1 and the presence of esophageal varices in esophageal varices group ($p=0.033$*). After conducting multivariate logistic regression analysis, collagen type IV and INR were found to be independent risk factors for esophageal varices in patients with cirrhosis.

Conclusion The serum collagen type IV and TIMP-1 levels are useful markers for predicting of presence of esophageal varices.

Keywords Collagen type IV, Esophageal varices, Tissue inhibitor of matrix metalloprotinease-1

Introduction

Liver cirrhosis is characterized by formation of regenerative nodules and distortion of the hepatic architecture. Different liver diseases can progress to cirrhosis. Alcoholic liver disease, nonalcoholic liver disease, and hepatitis C are the most common causes of liver cirrhosis [1].

Hepatitis C virus (HCV) infection is an international problem. It is an important cause of acute hepatitis and chronic hepatitis. The World Health Organization (WHO) made an estimate of around 71 million people have chronic hepatitis C, with high mortality rate, predominantly due to cirrhosis and hepatocellular carcinoma (HCC) [2].

The prevalence of hepatitis C differs throughout the world. For example, Frank et al. documented in 2000 that Egypt had the greatest number of reported

*Correspondence:
Mohamed Helmy Abaza
drmohabaza1990@yahoo.com
[1] Tropical Medicine Department, Faculty of Medicine, Alexandria University, Alexandria, Egypt
[2] Clinical and Chemical Pathology Department, Faculty of Medicine, Alexandria University, Alexandria, Egypt
[3] Alexandria Fever Hospital, Ministry of Health, Alexandria, Egypt

Springer Open

© The Author(s) 2024. **Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit http://creativecommons.org/licenses/by/4.0/.

infections, largely due to the use of contaminated parenteral antischistosomal therapy [3].

Egypt achieved a successful HCV screening program that included more than 50 million Egyptians and treated more than 4 million patients [4].

Esophageal varices are dilated submucosal distal esophageal veins connecting the portal and systemic circulations. This occurs due to portal hypertension most commonly due to cirrhosis [5].

Esophageal varices are native veins that act as collaterals to the central venous circulation when flow through the portal vein is obstructed. These varices are liable to bleed causing life-threatening hemorrhage [6, 7].

To decompress the portal hypertension and restore blood flow to the systemic circulation, varices form. When the pressure gradient between the portal and hepatic veins increases over 12 mmHg, they become prominent on endoscopy [8].

For the majority of cirrhotic patients, endoscopic screening for esophageal varices is recommended in order to manage high risk varices which are liable to bleed [9]. A number of investigations have revealed a high correlation between the existence of esophageal varices (EV) in cirrhotic patients and non-invasive indicators such as platelet count, spleen diameter, and Child-Pugh score [10].

Although upper endoscopy is the preferred method for diagnosing esophageal varices in patients with cirrhosis, clinical, hematological, biochemical, and radiological markers can be used to detect patients who are at a high risk of developing EV [11].

Collagen IV is exclusively found in basement membranes [12]. In liver fibrosis, the expression of collagen IV and increased laminin deposition in the Disse space result in the formation of a perisinusoidal basement membrane [13].

Esophageal varices could be detected with high diagnostic accuracy after measuring type IV collagen, a non-invasive marker for hepatic fibrosis. Combining abdominal ultrasonography and type IV collagen correctly identified patients with esophageal varices [14].

Other potential noninvasive biomarker for the severity of cirrhosis is tissue inhibitor of metalloproteinases-1 (TIMP-1), the specific inhibitor of the matrix metalloproteinase. TIMP-1 is significantly increased in patients with cirrhosis and correlates with the severity of the disease and degree of portal hypertension. TIMP-1 is therefore a promising new noninvasive biomarker to predict hemodynamic-related complications in cirrhosis [15].

Aim of the work

The aim of this work was to study the serum levels of tissue inhibitor of matrix metalloprotinase-1 and serum collagen type IV in patients with post hepatitis C cirrhosis and their relation to the different grades of esophageal varices.

Patients and methods

This prospective controlled research was done on 120 participants visiting endoscopy unit at Alexandria fever hospital in Alexandria between August 2021 and April 2022.

Sample size was calculated using Power Analysis and Sample Size Software (PASS 2020) "NCSS, LLC. Kaysville, Utah, USA, ncss.com/software/pass ." A minimal total hypothesized sample size of 120 eligible is needed to study the serum levels of tissue inhibitor of matrix metalloprotinase-1 and serum collagen type IV in patients with post hepatitis C cirrhosis and their relation to the different grades of esophageal varices; taking into consideration 95% confidence level, effect size of 40%, and 80% power using Chi square-test.

Eligible patients were aged 37 years or older, HCV positive (took treatment for HCV), and cirrhotic by ultrasound.

Group I: 30 patients with post hepatitis C liver cirrhosis without esophageal varices.

Group II: 60 patients with post hepatitis C liver cirrhosis and esophageal varices. Esophageal varices were graded according to their sizes:

Group III: 30 healthy subjects of matched age and sex as control group.

Westaby classification of esophageal varices: [16]

Grade I: Varices extending just above the mucosal level

Grade II: Varices projecting by one-third of the luminal diameter that cannot be compressed with air insufflation.

Grade III: Varices projecting up to 50% of the luminal diameter and in contact with each other.

An informed consent was obtained from each person before any intervention.

Exclusion criteria

Patients with liver cirrhosis due to other causes than HCV such as viral or autoimmune hepatitis and non-alcoholic steatohepatitis were excluded from the study. Also, patients with focal hepatic lesions suspected for being hepatocellular carcinoma were excluded. Also,

other causes of portal hypertension as portal vein thrombosis and patients with past history of endoscopic treatment of esophageal varices were excluded.

All enrolled patients included in this study were subjected to complete history taking including demographic data and clinical data such as abdominal distension, jaundice, bleeding tendency, weight loss, hematemesis, and melena. They were clinically examined for hepatomegaly, splenomegaly, and the detection of ascites and manifestations of hepatocellular failure. They were subjected to laboratory investigations as complete blood picture (CBC), kidney function tests, liver enzymes, and liver function tests.

Serum samples from all subjects were assayed for our main study markers collagen type-4 and tissue inhibitor of matrix metalloprotinase type-1 by enzyme-linked immunosorbent assay (ELISA) technique. We calculated the Child-Pugh score and performed HCV antibodies (ELISA) and hepatitis B surface antigen (ELISA) for all participating patients.

Regarding ultrasonic parameters, we assessed all parameters; ultrasound evaluation of the liver and ascites was performed on all recruited patients to determine the existence of cirrhosis. They were evaluated with ultrasound measurements of splenic bipolar diameter and ultrasound Doppler measurements of portal vein diameter.

We calculated FIB-4 score for all cases. In addition, triphasic CT scans were performed on individuals with ultrasound-detected focal hepatic lesions, and UGIE was done for all patients.

Ethical approval

This research was approved by the ethics committee of the Faculty of Medicine at the University of Alexandria. All participants provided their written, informed consent.

Results

This study was conducted on 90 candidates in the Alexandria Fever Hospital and 30 healthy subjects as control group.

Concerning the demographic information of the analyzed groups, there were no significant variations in age and gender across all groups as shown in Table 1.

The most prevalent symptom in patients with EV was abdominal distension (83%) followed by lower limb swelling (53%) and jaundice (36%); hematemesis and melena were found in 23% and 18% of patients with EV respectively.

About 20% of patients without esophageal varices complained of weight loss, while it was reported only in 5% of patients with esophageal varices.

As shown in Fig. 1, ascites, lower limb swelling, and abdominal distension were present in almost all cases with grade III esophageal varices. Hematemesis and melena were present mainly in patients with grade III EV. Jaundice was present mainly in patients with grade II and grade III EV.

Regarding laboratory investigations, CBC findings showed a significant difference in white blood cell (WBC) count and platelets count between liver cirrhosis groups (I and II) and controls. Renal function tests were significantly higher in esophageal varices group than other groups. Total and direct bilirubin levels (mg/dl) were significantly different among the three studied groups. Serum albumin levels (g/dl) were considerably lower in groups II than other groups. Regarding liver enzymes levels, alanine aminotransferase (ALT) levels were significantly different among the three studied groups. Also, aspartate aminotransferase (AST) levels were significantly higher in cirrhotic groups than control group. Also, international normalized ratio (INR) level was significantly difference was found among the three groups, as shown in Table 2.

All cirrhotic patients without esophageal varices had splenomegaly. Most of patients with esophageal varices had splenomegaly. Splenectomy was present in minority of cases with esophageal varices.

The bipolar diameter of the spleen differed significantly among the three studied groups ($p < 0.001$).

Table 1 Comparison between the three studied groups according to demographic data

	Group 1 ($n = 30$)		Group 2 ($n = 60$)		Control ($n = 30$)		Test of Sig.	*P*
	No.	%	No.	%	No.	%		
Gender								
Male	14	46.7	29	48.3	16	53.3	$\chi^2 = 0.300$	0.861
Female	16	53.3	31	51.7	14	46.7		
Age (years)								
Min.–Max.	39.0–49.0		37.0–56.0		38.0–55.0		$F = 0.097$	0.907
Mean ± SD.	44.67 ± 3.10		45.05 ± 4.09		45.03 ± 4.78			
Median (IQR)	45.0 (43.0–47.0)		45.0 (42.0–48.0)		45.0 (42.0–49.0)			

IQR Interquartile range, *SD* Standard deviation, F F for one-way ANOVA test, χ2 Chi-square test

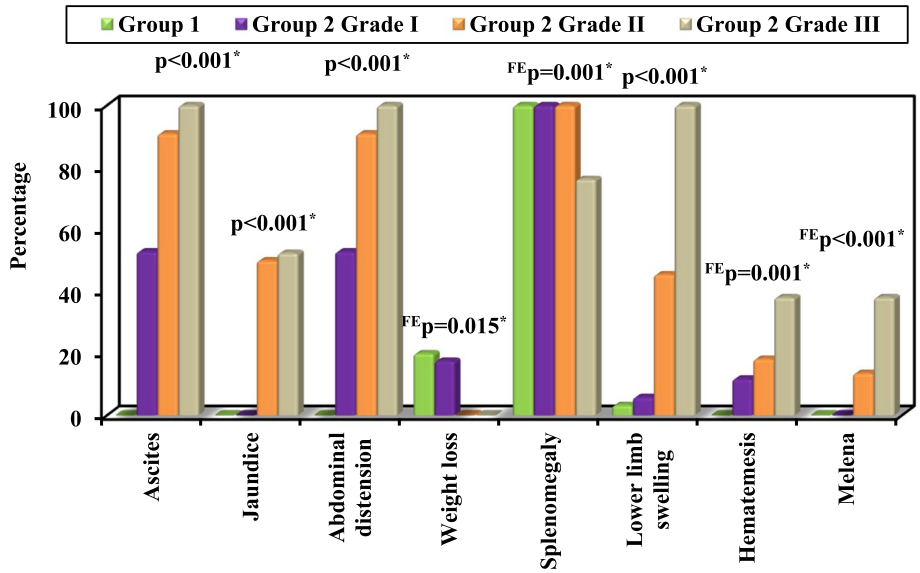

Fig. 1 Comparison between the different studied groups according to different parameters

PV diameter was statistically significantly different among the three groups, with a mean of 9.63 ± 1.22 mm in the control group, 14.77 ± 0.45 mm in group I, and 17.38 ± 1.10 mm in group II ($p < 0.001$).

None of the cirrhosis patients or participants of the control group had portal vein thrombosis (PVT).

Ascites was clinically identified in 83% of patients with EV, but not in patients without esophageal varices. Mild ascites was discovered in 18% of patients with esophageal varices, while moderate ascites was detected in 56% of patients with esophageal varices, and severe ascites was detected in 26% of patients with esophageal varices. Grade I hepatic encephalopathy was found in 10 % of group 1 and 95% of group 2 as shown in Table 3.

As shown in Table 4, all cases were classified according to Child score and FIB-4 score. For Child-Pugh classification, about 93% of patients without esophageal varices were Child-Pugh class A. For patients with esophageal varices, about 61.9% of patients with grade III EV were Child-Pugh class C.

Serum collagen type IV was significantly different between the control and liver cirrhosis groups, with a mean of 447.8 ± 283.2 pg/ml in the control group and 729.1 ± 80.29 pg/ml, 768.6 ± 137.9 pg/ml, 1018.8 ± 57.36 ng/ml, and 1770.7 ± 663.4 pg/ml in patients without esophageal varices, grade I EV, grade II EV, and grade III EV respectively. In addition, among patients with esophageal varices, statistically significant differences were among patients with grade I EV, grade II EV, and group III EV. However, there was no difference between the patients without EV and patients with grade I EV.

For collagen type IV, a positive correlation was found between the grade of esophageal varices and the level of collagen type IV in group II.

Serum TIMP-1 was significantly different between the control and liver cirrhosis groups, with a mean of 12.50 ± 11.59 µg/L in the control group and 24.87 ± 12.53 µg/L, 32.88 ± 25.97 µg/L, 41.86 ± 26.47 µg/L, and 55.14 ± 38.64 µg/L in patients without esophageal varices, grade I EV, grade II EV and grade III EV respectively. In addition, among patients with esophageal varices, statistically significant differences were among patients with grade I EV, grade II EV, and group III EV. However, there was no difference between the patients without EV and patients with grade I EV.

For TIMP-1, a positive correlation was found between the grade of esophageal varices and the level of TIMP-1 (Table 5).

The number of OV band ligation procedures necessary to eradicate the large OV in group II varied from 2 to 5 sessions over a period of 12 to 24 weeks.

ROC curve of serum collagen type IV level is used for diagnosis of esophageal varices at a cut off value of > 831 (pg/ml) with a sensitivity of 86.67%, specificity of 96.67%, a positive predictive value of 98.1%, and a negative predictive value of 78.4% (area under the curve = 0.883, 95% CI 0.806–0.960) (Fig. 2; Table 6).

ROC curve of serum TIMP-1 level is used for diagnosis of esophageal varices at a cut off value of > 23 (µg/L) with a sensitivity of 61.67%, specificity of 60%, a positive predictive value of 75.5, and a negative predictive value of 43.9 (area under the curve = 0.653, 95% CI 0.538–0.768).

Table 2 Comparison between the three studied groups according to laboratory investigations

	Group 1 ($n = 30$)	Group 2 ($n = 60$)	Control ($n = 30$)	Test of Sig. (p)	Sig. bet. grps.
Hemoglobin					
Min.–Max.	11.60–13.10	8.30–11.40	11.70–14.50	$F = 217.650^{*}$ $p < 0.001^{*}$	$p_1 < 0.001^{*}, p_2 = 0.002^{*}, p_3 < 0.001^{*}$
Mean ± SD.	12.45 ± 0.43	10.23 ± 0.73	13.07 ± 0.76		
Median (IQR)	12.45 (12.30–12.70)	10.40 (9.80–10.75)	13.20 (12.20–13.40)		
WBC					
Min.–Max.	4.0–9.0	2.70–9.0	6.0–9.0	$F = 20.804^{*} p < 0.001^{*}$	$p_1 = 0.183, p_2 < 0.001^{*}, p_3 < 0.001^{*}$
Mean ± SD.	5.75 ± 1.43	5.17 ± 1.66	7.26 ± 0.95		
Median (IQR)	5.50 (4.50–7.0)	5.10 (3.70–6.35)	7.10 (6.50–8.0)		
Platelets					
Min.–Max.	144.0–185.0	42.0–186.0	230.0–409.0	$F = 193.65^{*} p < 0.001^{*}$	$p_1 < 0.001^{*}, p_2 < 0.001^{*}, p_3 < 0.001^{*}$
Mean ± SD.	163.8 ± 12.77	116.3 ± 41.23	286.2 ± 49.11		
Median (IQR)	160.0 (156.0–172.0)	129.0 (75.50–147.5)	278.0 (242.0–319.0)		
Urea					
Min.–Max.	9.0–35.0	18.0–59.0	13.0–34.0	$F = 82.125^{*} p < 0.001^{*}$	$p_1 < 0.001^{*}, p_2 = 0.995, p_3 < 0.001^{*}$
Mean ± SD.	21.0 ± 8.11	42.45 ± 10.38	21.23 ± 7.08		
Median (IQR)	20.50 (14.0–28.0)	44.0 (35.0–51.0)	21.0 (15.0–27.0)		
Creatinine					
Min.–Max.	0.60–1.20	0.70–1.60	0.70–1.30	$F = 40.559^{*} p < 0.001^{*}$	$p_1 < 0.001^{*}, p_2 = 0.069, p_3 < 0.001^{*}$
Mean ± SD.	0.89 ± 0.17	1.27 ± 0.22	1.01 ± 0.19		
Median (IQR)	0.90 (0.80–1.0)	1.30 (1.15–1.40)	1.0 (0.90–1.20)		
Total bilirubin					
Min.–Max.	0.90–1.70	1.0–5.70	0.30–1.0	$H = 78.057^{*}$ $p < 0.001^{*}$	$p_1 < 0.001^{*}, p_2 < 0.001^{*}, p_3 < 0.001^{*}$
Mean ± SD.	1.35 ± 0.28	2.62 ± 1.54	0.68 ± 0.21		
Median (IQR)	1.40 (1.10–1.60)	1.70 (1.50–4.15)	0.70 (0.50–0.80)		
Direct bilirubin					
Min.–Max.	0.50–1.20	0.60–4.40	0.20–0.80	$H = 67.286^{*}$ $p < 0.001^{*}$	$p_1 < 0.001^{*}, p_2 < 0.001^{*}, p_3 < 0.001^{*}$
Mean ± SD.	0.86 ± 0.25	1.78 ± 1.13	0.48 ± 0.19		
Median (IQR)	0.90 (0.60–1.10)	1.20 (1.0–2.85)	0.40 (0.30–0.70)		
ALT					
Min.–Max.	9.0–98.0	12.0–124.0	16.0–32.0	$H = 32.049^{*}$ $p < 0.001^{*}$	$p_1 = 0.006^{*}, p_2 = 0.013^{*}, p_3 < 0.001^{*}$
Mean ± SD.	37.93 ± 25.74	51.28 ± 28.89	23.90 ± 4.45		
Median (IQR)	29.0 (23.0–54.0)	39.50 (29.0–73.5)	22.50 (21.0–27.0)		
AST					
Min.–Max.	41.0–131.0	32.0–124.0	12.0–29.0	$F = 90.793^{*} p < 0.001^{*}$	$p_1 = 1.000, p_2 < 0.001^{*}, p_3 < 0.001^{*}$
Mean ± SD.	74.13 ± 24.36	74.15 ± 21.57	18.27 ± 4.53		
Median (IQR)	73.50 (51.0–97.0)	72.0 (58.50–88.5)	17.0 (15.0–21.0)		
AST/ALT ratio					
Min.–Max.	1.27–4.89	0.77–4.08	0.48–1.0	$H = 71.431^{*}$ $p < 0.001^{*}$	$p_1 = 0.004^{*}, p_2 < 0.001^{*}, p_3 < 0.001^{*}$
Mean ± SD.	2.49 ± 0.99	1.74 ± 0.72	0.77 ± 0.12		
Median (IQR)	2.23 (1.80–3.07)	1.51 (1.18–2.25)	0.74 (0.68–0.86)		
Albumin					
Min.–Max.	3.20–4.60	2.10–4.20	3.90–4.50	$F = 93.250^{*} p < 0.001^{*}$	$p_1 < 0.001^{*}, p_2 = 0.138, p_3 < 0.001^{*}$
Mean ± SD.	3.96 ± 0.42	2.92 ± 0.58	4.20 ± 0.18		
Median (IQR)	4.0 (3.70–4.30)	2.85 (2.50–3.20)	4.20 (4.0–4.30)		
INR					
Min.–Max.	1.10–1.42	1.20–1.60	1.0–1.20	$F = 135.49^{*} p < 0.001^{*}$	$p_1 < 0.001^{*}, p_2 < 0.001^{*}, p_3 < 0.001^{*}$
Mean ± SD.	1.22 ± 0.11	1.40 ± 0.11	1.04 ± 0.07		
Median (IQR)	1.20 (1.10–1.31)	1.40 (1.30–1.45)	1.0 (1.0–1.10)		

IQR interquartile range, *SD* standard deviation, *F* F for one-way ANOVA test, pairwise comparison bet. each 2 groups was done using Post Hoc Test (Tukey)

H: H for Kruskal Wallis test, Pairwise comparison bet. each 2 groups was done using Post Hoc Test (Dunn's for multiple comparisons test)

p: *p* value for comparing between the three studied groups; p1: *p* value for comparing between group 1 and group 2

p2: *p* value for comparing between group 1 and control; p3: *p* value for comparing between group 2 and control

* Statistically significant at $p \leq 0.05$

Table 3 Comparison between the three studied groups according to physical examination

	Group 1 ($n=30$)		Group 2 ($n=60$)		Control ($n=30$)		Test of Sig.	P
	No.	%	No.	%	No.	%		
Ascites	0	0.0	50	83.3	0	0.0	$\chi^2 = 85.71^*$	$< 0.001^*$
Mild	–	–	9	18.0	–	–		
Moderate	–	–	28	56.0	–	–	–	–
Severe	–	–	13	26.0	–	–		
Splenomegaly	30	100.0	55	91.7	0	0.0	$\chi^2 = 2.647$	$^{FE}p = 0.165$
Spleen length								
Min. –Max.	14.0–17.0		15.0–20.0		10.0–12.0		$F = 402.74^*$	$< 0.001^*$
Mean ± SD.	15.60 ± 0.67		17.52 ± 1.19		11.43 ± 0.63			
Median (IQR)	16.0 (15.0–16.0)		17.0 (17.0–18.50)		11.50 (11.0–12.0)			
Sig. bet. grps.	$p_1 < 0.001^*, p_2 < 0.001^*, p_3 < 0.001^*$							
Portal vein diameter								
Min. –Max.	14.50–15.50		15.50–19.50		8.0–12.0		$F = 581.23^*$	$< 0.001^*$
Mean ± SD.	14.77 ± 0.45		17.38 ± 1.10		9.63 ± 1.22			
Median (IQR)	14.50(14.5–15.5)		17.50(16.5–18.5)		9.50 (9.0–11.0)			
Sig. bet. grps.	$p_1 < 0.001^*, p_2 < 0.001^*, p_3 < 0.001^*$							
Hepatic encephalopathy								
No	27	90.0	3	5.0	–	–	$\chi^2 = 65.025^*$	$< 0.001^*$
Grade 1	3	10.0	57	95.0	–	–		
Grade 2	0	0.0	0	0.0	–	–		
Grade 3	0	0.0	0	0.0	–	–		
Grade 4	0	0.0	0	0.0	–	–		

χ^2 Chi square test, *IQR* interquartile range, *SD* standard deviation, *F* F for one-way ANOVA test, pairwise comparison bet. each 2 groups was done using Post Hoc Test (Tukey)

p: *p* value for comparing between the three studied groups

p1: *p* value for comparing between group 1 and group 2

p2: *p* value for comparing between group 1 and control

p3: *p* value for comparing between group 2 and control

* Statistically significant at $p \leq 0.05$

Binary logistic regression analysis explored the risk factors associated with the presence of esophageal varices in patients with cirrhosis. In the univariate analysis, the patients with esophageal varices had lower PLT ($p < 0.001$), albumin (< 0.001), higher ALT ($p = 0.041$), alkaline phosphatase ($p = 0.001$), total bilirubin ($p = 0.004$), direct bilirubin ($p = 0.003$), collagen type IV ($p < 0.001$), TIMP-1 ($p = 0.008$), INR ($p < 0.001$), FIB-4 ($p = 0.005$), spleen length ($p < 0.001$), and Child-Pugh score (< 0.001). However, after the multivariate logistic regression analysis, it was found that Collagen type IV and INR were independent risk factors for esophageal varices in patients with cirrhosis, as shown in Table 7.

Discussion

One of the main effects of cirrhosis (CLD) is portal hypertension (PH), which can result in the development of collateral circulation [17]. Clinical significant portal hypertension (CSPH) is defined as hepatic venous pressure gradient (HVPG) ≥ 10 mmHg, which leads to clinical complications of PH such as esophageal varices (EV). Severe portal hypertension (SPH) defined as HVPG ≥ 12 mmHg is a risk factor of variceal hemorrhage [18]. In order to reduce the number of unnecessary endoscopies in patients with cirrhosis but without varices, thrombocytopenia, large spleen size, portal vein size, and platelet spleen diameter ratio strongly predict large number of esophageal varices [19].

Collagen is an important protein in mammals, accounting for 25–30% of the total protein [20], and the main structure of the extracellular matrix. The sinusoidal capillaries are injured in chronic hepatitis resulting in the disintegration of collagen type IV from the basement membrane and its presence in the blood. Detection of dynamic changes in the levels of collagen type IV in clinical practice is important for the diagnosis of chronic liver disease and monitoring disease progression [21, 22].

Table 4 Comparison between the three studied groups according to Child-Pugh classification and FIB-4 score

	Group 1 ($n=30$)		Group 2 ($n=60$)						Test of Sig.	P
			Grade I ($n=17$)		Grade II ($n=22$)		Grade III ($n=21$)			
	No.	%	No.	%	No.	%	No.	%		
Child-Pugh										
A	28	93.3	8	47.1	2	9.1	0	0.0	$\chi^2=69.285^*$	$<0.001^*$
B	2	6.7	9	52.9	10	45.5	8	38.1		
C	0	0.0	0	0.0	10	45.5	13	61.9		
FIB-4 score										
Min.–Max.	2.99–4.34		3.26–5.09		2.81–10.44		2.49–11.76		$H=18.652^*$	$<0.001^*$
Mean ± SD.	3.48 ± 0.24		3.75 ± 0.55		4.81 ± 1.87		5.50 ± 2.53			
Median	3.43		3.49		3.95		4.69			
IQR	3.37–3.62		3.39–3.67		3.49–5.83		3.87–6.74			
p_1			0.330		0.002^*		$<0.001^*$			
Sig. bet. grps			$p_2=0.076$, $p_3=0.014^*$, $p_4=0.453$							

IQR interquartile range, *SD* standard deviation, χ^2 Chi-square test

H: H for Kruskal Wallis test, Pairwise comparison bet. each 2 groups was done using Post Hoc Test (Dunn's for multiple comparisons test)

p: *p* value for comparing between the different studied groups

p_1: *p* value for comparing between Group 1 and each other grade

p_2: *p* value for comparing between Grade I and Grade II

p_3: *p* value for comparing between Grade I and Grade III

p_4: *p* value for comparing between Grade II and Grade III

* Statistically significant at $p \leq 0.05$

Table 5 Comparison between the different studied groups according to collagen type IV and TIMP

	Group 1 ($n=30$)	Group 2 ($n=60$)			Control ($n=30$)	H p
		Grade I ($n=17$)	Grade II ($n=22$)	Grade III ($n=21$)		
Collagen type IV (pg/ml)						
Min.–Max.	522.0–858.0	580.0–907.0	921.0–1107.0	1118.0–2901.0	200.0–1426.0	$H=93.318^*$ $p<0.001^*$
Mean ± SD.	729.1 ± 80.29	768.6 ± 137.9	1018.8 ± 57.36	1770.7 ± 663.4	447.8 ± 283.2	
Median	744.5	876.0	1023.5	1486.0	342.0	
IQR	694.0–772.0	627.0–896.0	963.0–1064.0	1251.0–2470.0	250.0–570.0	
p_0	0.004^*	0.005^*	$<0.001^*$	$<0.001^*$		
p_1		0.693	$<0.001^*$	$<0.001^*$		
Sig. bet. grps		$p_2=0.003^*$, $p_3<0.001^*$, $p_4=0.032^*$				
TIMP (µg/L)						
Min. –Max.	6.0–46.0	6.0–108.0	7.0–108.0	9.0–108.0	5.0–69.0	$H=44.366^*$ $p<0.001^*$
Mean ± SD.	24.87 ± 12.53	32.88 ± 25.97	41.86 ± 26.47	55.14 ± 38.64	12.50 ± 11.59	
Median	23.0	23.0	36.0	38.0	11.0	
IQR	16.0–36.0	20.0–32.0	22.0–47.0	22.0–90.0	5.0–13.0	
p_0	$<0.001^*$	$<0.001^*$	$<0.001^*$	$<0.001^*$		
p_1		0.584	0.044^*	0.025^*		
Sig. bet. grps		$p_2=0.216$, $p_3=0.150$, $p_4=0.819$				

IQR interquartile range, *SD* standard deviation, *H* H for Kruskal-Wallis test, pairwise comparison bet. each 2 groups was done using Post Hoc Test (Dunn's for multiple comparisons test)

p: *p* value for comparing between the different studied groups

p0: *p* value for comparing between Control and each other group

p1: *p* value for comparing between group 1 and each other group

p2: *p* value for comparing between group 2 (grade I) and group 2 (grade II)

p3: *p* value for comparing between group 2 (grade I) and group 2 (grade III)

p4: *p* value for comparing between group 2 (grade II) and group 2 (grade III)

* Statistically significant at $p \leq 0.05$

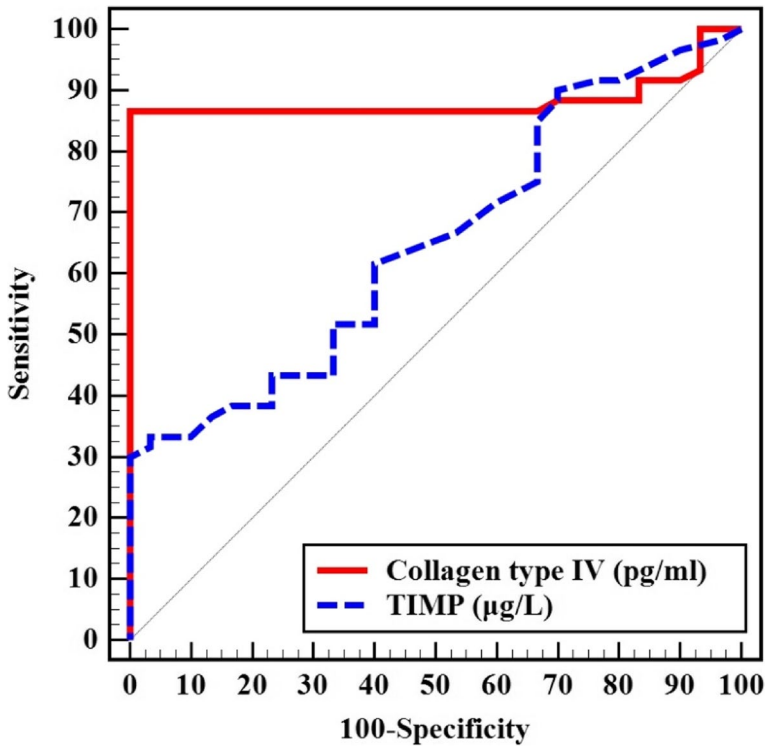

Fig. 2 ROC curve for collagen type IV and TIMP to discriminate patients with varices ($n=60$) (group 2) from patients without varices ($n=30$) (group 1)

Table 6 Diagnostic performance for collagen type IV and TIMP to discriminate patients with varices ($n=60$) (group 2) from patients without varices ($n=30$) (group 1)

	AUC	P	95% C.I	Cut off	Sensitivity	Specificity	PPV	NPV
Collagen type IV (pg/ml)	0.883	< 0.001*	0.806–0.960	> 831	86.67	96.67	98.1	78.4
TIMP (µg/L)	0.653	0.019*	0.538–0.768	> 23	61.67	60.0	75.5	43.9

AUC area under a curve, *p* value probability value, *CI* confidence intervals, *NPV* negative predictive value, *PPV* positive predictive value

* Statistically significant at $p \leq 0.05$

In this study, we found that collagen type IV and TIMP-1 were positively correlated with the presence of esophageal varices in a univariate analysis and that the increase in collagen type IV, not TIMP-1, was independently correlated with the presence of esophageal varices in multivariate analysis.

In our study, ROC curve of serum collagen type IV level is used for diagnosis of esophageal varices at a cut off value of > 831 (pg/ml) with a sensitivity of 86.67%, specificity of 96.67%, and area under the curve of 0.883.

The results of our study also matched with the result of Mamori S et al. who identified type IV collagen as the only independent variable predictive for esophageal varices in patients with alcoholic liver disease. Whenever the type IV collagen level raised every 150 ng/ml, the odds ratio of esophageal varices doubled. The positive predictive value of esophageal varices with type IV collagen value > 900 ng/ml was 100% [14].

In one study by Lehmann J, collagen type IV marker was significantly higher in patients with varices, suggesting an association with vascular formation and perisinusoidal fibrosis [23].

TIMP-1 is significantly increased in patients with cirrhosis and correlates with the severity of the disease, degree of portal hypertension, and vasodilatory state. TIMP-1 is therefore a promising new noninvasive marker to predict hemodynamic-related complications in cirrhosis [15].

Table 7 Univariate and multivariate logistic regression analysis for the parameters affecting varices (group 2) ($n=60$ vs. 30)

	Univariate		#Multivariate	
	P	OR (LL–UL 95%C.I)	P	OR (LL–UL 95%C.I)
Male	0.881	1.069 (0.444–2.572)		
Female	0.881	0.935 (0.389–2.250)		
Age (years)	0.649	1.028 (0.914–1.155)		
Collagen IV (pg/ml)	< 0.001*	1.010 (1.006–1.015)	0.007*	1.012 (1.003–1.021)
TIMP (µg/L)	0.008*	1.037 (1.010–1.065)	0.532	1.017 (0.964–1.074)
Platelets	< 0.001*	0.932 (0.900–0.966)		
ALP	0.001*	1.025 (1.010–1.041)		
ALT	0.041*	1.019 (1.001–1.038)	0.191	0.952 (0.885–1.025)
AST	0.997	1.000 (0.981–1.020)		
AST/ALT ratio	0.001*	0.350 (0.191–0.640)	0.829	0.786 (0.089–6.945)
Albumin	< 0.001*	0.047 (0.014–0.151)	0.289	6.141 (0.215–175.677)
Increasing in **Child-Pugh** classification	< 0.001*	39.752 (8.329–189.732)	0.080	23.327 (0.684–795.422)
Total bilirubin	0.004*	19.330 (2.588–144.367)	0.561	0.537 (0.066–4.366)
Direct bilirubin	0.003*	28.980 (3.237–259.450)		
INR$	< 0.001*	4.958 (2.538–9.685)	0.015*	5.307 (1.380–20.403)
Spleen length	< 0.001*	11.961 (4.122 – 34.712)		
FIB-4 score	0.005*	3.902 (1.521–10.008)	0.458	2.330 (0.249–21.778)

OR odd's ratio, *CI* confidence interval, *LL* lower limit, *UL* upper limit

$: for each 0.1 INR

#: All variables with $p < 0.05$ was included in the multivariate

* Statistically significant at $p \leq 0.05$

In our study, serum tissue inhibitor metalloproteinase type 1 was significantly different between the control and liver cirrhosis groups, In addition, between groups I and II, statistically significant differences was discovered

T. Medeiros et al. suggested that since circulating levels of MMP-9/TIMP-1 complex are significantly increased in chronic HCV patients, this molecule can be a promising biomarker of active fibrogenesis in HCV-induced liver fibrosis [24].

Busk TM et al. stated that TIMP-1 may represent a marker of portal hypertension and the level of circulating TIMP-1 appears to be significantly associated with disease severity and hemodynamic changes in cirrhotic patients [15].

This study matches with the findings of Metwally K et al. [25] which concluded that there was significant increase in TIMP-1 with the advancement of liver decompensation and its level could be used as an indirect measurement of the hepatic function state.

In this study, non-invasive markers for hepatic fibrosis, type IV collagen and TIMP-1, had a high diagnostic accuracy for the detection of esophageal varices in patients with chronic hepatitis C.

Conclusion

The serum Collagen type IV and TIMP-1 levels are useful markers for diagnosis of presence of esophageal varices in patients with cirrhosis related to HCV. Collagen type IV was independently associated with the presence of esophageal varices in patients with liver cirrhosis.

Abbreviations

TIMP-1	Tissue inhibitor of matrix metalloprotinease-1
WHO	World Health Organization
HCC	Hepatocellular carcinoma
HCV	Hepatitis C virus
SVC	Superior vena cava
kPa	Kilopascal
MDCT	Multidetector computed tomography
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
ELISA	Enzyme-linked immunosorbent assay
WBC	White blood cell
INR	International normalized ratio
PVT	Portal vein thrombosis
EV	Esophageal varices
PH	Portal hypertension
MMP	Matrix metalloprotinease
NAFLD	Non-alcoholic fatty liver disease
CLD	Chronic liver disease
CSPH	Clinically significant portal hypertension
HVPG	Hepatic venous pressure gradient

Acknowledgements
The authors are thankful to all the patients who took part in this study.

Authors' contributions
MA was in charge of the practical section, data analysis, and manuscript preparation. All authors provided critical feedback and helped shape and revise the research, the analysis, and the manuscript. All authors have read and approved the manuscript.

Funding
The authors declare that no funding, grants, or other forms of support were received.

Availability of data and materials
The article contains the information utilized to support the conclusions of this study.

Declarations

Ethics approval and consent to participate
This research was conducted in line with ethical principles. Before beginning the research, the Faculty of Medicine, Alexandria University Ethical Committee granted clearance on June 17, 2021, and the study protocol adheres to the ethical principles outlined in the 1975 Declaration of Helsinki. Each participant's consent was acquired in advance. The Committee's serial number is 0201524 and the reference number is FWA NO: 00018699.

Consent for publication
Both patients and the control group provided written informed consent. Patients participating in this research consent to data publication.

Competing interests
The authors declare no competing interests.

Received: 23 October 2023 Accepted: 8 February 2024
Published online: 19 February 2024

References

1. Wong RJ, Aguilar M, Cheung R, Perumpail RB, Harrison SA, Younossi ZM, Ahmed A (2015) Nonalcoholic steatohepatitis is the second leading etiology of liver disease among adults awaiting liver transplantation in the United States. Gastroenterology 148(3):547–555. https://doi.org/10.1053/j.gastro.2014.11.039
2. World Health Organization (WHO) (2018) Hepatitis C: fact sheet. WHO, Geneva, Switzerland
3. Frank C, Mohamed MK, Strickland GT, Lavanchy D, Arthur RR, Magder LS, El Khoby T, Abdel-Wahab Y, Aly Ohn ES, Anwar W, Sallam I (2000) The role of parenteral antischistosomal therapy in the spread of hepatitis C virus in Egypt. Lancet 355(9207):887–891. https://doi.org/10.1016/s0140-6736(99)06527-7
4. Hassanin A, Kamel S, Waked I, Fort M (2021) Egypt's ambitious strategy to eliminate hepatitis C virus: a case study. Glob Health Sci Pract 9(1):187–200. https://doi.org/10.9745/ghsp-d-20-00234
5. Shaheen AA, Nguyen HH, Congly SE, Kaplan GG, Swain MG (2019) Nationwide estimates and risk factors of hospital readmission in patients with cirrhosis in the United States. Liver Int 39(5):878–884. https://doi.org/10.1111/liv.14054
6. Cotran RS, Kumar V, Collins T (1999) Robbins pathologic basis of disease, 6th edn. WB Saunders Co, Philadelphia, Pa
7. Ellakany WI, Mahmoud MoheyEldin K, Invernizzi P, Mahmoud ElKady A, Eldin Fathy Abou Elkheir H, Abdel Haleem Abo Elwafa R, Ellakany A (2018) Study of the influence of heme oxygenase 1 gene single nucleotide polymorphism (rs2071746) on esophageal varices among patients with cirrhosis. Eur J Gastroenterol Hepatol 30(8):888–892
8. D'Amico G, Garcia-Pagan JC, Luca A, Bosch J (2006) Hepatic vein pressure gradient reduction and prevention of variceal bleeding in cirrhosis: a systematic review. Gastroenterology 131(5):1611–1624. https://doi.org/10.1053/j.gastro.2006.09.013
9. Garcia-Tsao G, Abraldes JG, Berzigotti A, Bosch J (2017) Portal hypertensive bleeding in cirrhosis: risk stratification, diagnosis, and management: 2016 practice guidance by the American Association for the study of liver diseases. Hepatology 65(1):310–335. https://doi.org/10.1002/hep.28906
10. Mahassadi AK, Bathaix FY, Assi C, Bangoura AD, Allah-Kouadio E, Kissi HY, Touré A, Doffou S, Konaté I, Attia AK, Camara MB, Ndri-Yoman TA (2012) Usefulness of noninvasive predictors of oesophageal varices in Black African cirrhotic patients in Côte d'Ivoire (West Africa). Gastroenterol Res Pract 2012:216390. https://doi.org/10.1155/2012/216390
11. Kumar P, Singh K, Joshi A, Thakur P, Mahto SK, Kumar B, Pasricha N, Patra BR, Lamba BMS (2020) Evaluation of non-invasive marker of esophageal varices in cirrhosis of liver. J Family Med Prim Care 9(2):992–996. https://doi.org/10.4103/jfmpc.jfmpc_854_19
12. Miner JH (2011) Basement membranes. In: Mecahm RP (ed) The extracellular matrix: an overview. Springer, New York, pp 117–145
13. Mak KM, Chen LL, Lee TF (2013) Codistribution of collagen type IV and laminin in liver fibrosis of elderly cadavers: immunohistochemical marker of perisinusoidal basement membrane formation. Anat Rec (Hoboken) 296(6):953–964. https://doi.org/10.1002/ar.22694
14. Mamori S, Searashi Y, Matsushima M, Hashimoto K, Uetake S, Matsudaira H, Ito S, Nakajima H, Tajiri H (2008) Serum type IV collagen level is predictive for esophageal varices in patients with severe alcoholic disease. World J Gastroenterol 14(13):2044–2048. https://doi.org/10.3748/wjg.14.2044
15. Busk TM, Bendtsen F, Nielsen HJ, Jensen V, Brünner N, Møller S (2014) TIMP-1 in patients with cirrhosis: relation to liver dysfunction, portal hypertension, and hemodynamic changes. Scand J Gastroenterol 49(9):1103–1110. https://doi.org/10.3109/00365521.2014.934910
16. Westaby D, Macdougall BR, Melia W, Theodossi A, Williams R (1983) A prospective randomized study of two sclerotherapy techniques for esophageal varices. Hepatology 3(5):681–684. https://doi.org/10.1002/hep.1840030509
17. Garcia-Tsao G, Bosch J, Groszmann RJ (2008) Portal hypertension and variceal bleeding—unresolved issues. Summary of an American Association for the study of liver diseases and European Association for the study of the liver single-topic conference. Hepatology 47(5):1764–1772. https://doi.org/10.1002/hep.22273
18. Bosch J, Abraldes JG, Berzigotti A, García-Pagan JC (2009) The clinical use of HVPG measurements in chronic liver disease. Nat Rev Gastroenterol Hepatol 6(10):573–582. https://doi.org/10.1038/nrgastro.2009.149
19. Sarangapani A, Shanmugam C, Kalyanasundaram M, Rangachari B, Thangavelu P, Subbarayan JK (2010) Noninvasive prediction of large esophageal varices in chronic liver disease patients. Saudi J Gastroenterol 16(1):38–42. https://doi.org/10.4103/1319-3767.58767
20. Meyer M (2019) Processing of collagen based biomaterials and the resulting materials properties. Biomed Eng Online 18(1):24. https://doi.org/10.1186/s12938-019-0647-0
21. Zhang HW, Wang ML, Zhang Z (2019) Effect of splenectomy combined with pericardial devascularization on liver function and liver fibrosis in patients with nonsclerotic portal hypertension. Chin J Hepatobiliary Surg 25:501–504
22. Chen GF, Ping J, Gu HT, Zhao ZM, Zhou Y, Xing F, Tao YY, Mu YP, Liu P, Liu CH (2017) Correlation of liver stiffness measured by FibroTouch and FibroScan with Ishak fibrosis score in patients with chronic hepatitis B. Zhonghua Gan Zang Bing Za Zhi 25(2):145–150. https://doi.org/10.3760/cma.j.issn.1007-3418.2017.02.013
23. Lehmann J, Praktiknjo M, Nielsen MJ, Schierwagen R, Meyer C, Thomas D, Violi F, Strassburg CP, Bendtsen F, Møller S, Krag A, Karsdal MA, Leeming DJ, Trebicka J (2019) Collagen type IV remodelling gender-specifically predicts mortality in decompensated cirrhosis. Liver Int 39(5):885–893. https://doi.org/10.1111/liv.14070

24. Medeiros T, Saraiva GN, Moraes LA, Gomes AC, Lacerda GS, Leite PE, Esberard EB, Andrade TG, Xavier AR, Quírico-Santos T, Rosário NF, Silva AA (2020) Liver fibrosis improvement in chronic hepatitis C after direct acting-antivirals is accompanied by reduced profibrogenic biomarkers-a role for MMP-9/TIMP-1. Dig Liver Dis 52(10):1170–1177. https://doi.org/10.1016/j.dld.2020.05.004
25. Metwally K, Fouad T, Shible N, Zaghla H, Sameea E (2017) Metalloproteinase inhibitor-1 closely correlates with the severity of liver disease in Egyptian patients. J Liver Dis Transplant 5:1–3

Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.