Serum MFAP4 as a Non Invasive Diagnostic Marker of Oesophageal Varices in Cirrhotic Hepatitis C Virus Patients

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Background and study aim: HCV induced liver cirrhosis is the primary cause of liver-related mortality, with liver disease ranking as the world's tenth greatest cause of death. The portal pressure remains below the threshold at which varices develop during the compensated phase. Conversely, those who are decompensated get clinically evident portal hypertension. MFAP4 was first proposed as a new prospective parameter for fibrotic liver disease after recent research showed that it is one of the substantially elevated proteins in fibrotic liver. The purpose of this investigation was to assess the efficacy of serum MFAP4 as a diagnostic biomarker for identifying OV in HCV induced liver cirrhosis patients. It also compared the effectiveness of MFAP4 with other non-invasive markers, as well as upper gastrointestinal endoscopic findings across different grades of OV

Patients and Methods: Our study was carried out on ninety participants which were divided into four groups after doing upper GIT endoscopy: group I (n = 20) consisted of patients without oesophageal varices; group II (n = 25) had small varices; group IIIa (n = 25) consisted of large size varices; group IIIb consisted of the same patients as group IIIa but after eradicated varices; group IV (n=20) as healthy control group. Routine laboratory investigations (CBC, liver and renal functions), non-invasive marker of liver fibrosis were done and our main study marker serum level of MFAP4 was assessed via ELISA.

Results: All cirrhotic patients had serum MFAP4 levels that were statistically considerably greater than those of control group (p = <0.001). Additionally, its level was higher in patients with large varices as opposed to patients without varices or small varices (p < 0.001) and in patients with small varices as opposed to those without varices (p = <0.001). Nevertheless, p = 0.082 indicates that groups IIIa and IIIb were not statistically different. Serum MFAP4 did not correlate with the APRI or FIB-4 in any of the studied groups, with the exception of patients with large varices, who showed a negative correlation with the APRI score.

Conclusion: Serum MFAP4 may be used as a useful non-invasive biomarker of oesophageal varices in cirrhotic patients and its grading, but not after esophageal varices eradication.

INTRODUCTION

Liver disease ranks tenth in global mortality, [1] with cirrhosis being the most prevalent killer [2]. ongoing non-resolved wound healing from chronic liver disease will finally end by cirrhosis, the final stage of hepatic fibrosis. This damage is usually caused by hepatitis B and C viruses, persistent alcohol abuse, and NAFLDs [3]. Portal hypertension (PH) is the principal mechanism for oesophageal varices (OV), and a significant contributor to the manifestation of the disease's clinical symptoms. The presence of PH is denoted by an HVPG (hepatic venous pressure gradient) that exceeds 5 mmHg. PH is classified as clinically severe when the HVPG is 10 mm Hg or higher. Bleeding from oesophageal varices happens when the HVPG exceeds 12 mmHg [4]. Hepatic fibrosis and regenerating nodules lead to a rise in intrahepatic resistance, thereby facilitating the onset of OV. Esophageal variceal bleeding (EVB) leads to liver cirrhosis deaths and morbidity.

EVB causes 11%–40% of deaths [5]. Varices form at 3%-12% every year and mature into massive varices at 8%-12% [6]. The stage of chronic liver disease and grades of endoscopic varices can indicate variceal hemorrhage [7]. The current Baveno VI agreement requires surveillance endoscopies for all liver cirrhosis patients at diagnosis and every one to three years afterward, depending on screening results and liver condition [8].

In cirrhotic patients with OV with risk signs of bleeding, nonselective beta blockers (NSBBs) must be given as the main preventive medication against (EVB). Additionally, they can be combined with endoscopic band ligation (EBL) to prevent EVB later on. Both NSBB and EBL worked incredibly well at stopping bleeding at an early stage [9].

A more serious and perhaps fatal outcome is associated with EBL causing bleeding ulcers, requiring surveillance endoscopies to check for recurrent varices [9]. This indicates that NSBBs are the most effective treatment overall.

For the diagnosis and grading of OV, esophagogastroduodenoscopy (EGD) is considered the gold standard due to its excellent sensitivity and specificity. The drawbacks of EGD include its invasiveness, need for conscious sedation [10] and relatively high cost. Furthermore, EGD is not often accessible in nations with little resources. A large number of people with chronic liver illness will not present with OV on an EGD. Thus, many non-invasive techniques have been developed as a simple marker for OV detection in order to get over these challenges [11].

The gene responsible for encoding the 36 KDa extracellular matrix Microfibrillar-associated protein 4 (MFAP4) is found on chromosome 17 inside the deleted region linked with Smith-Magenis syndrome [12]. MFAP4 exists in its active state as a homodimer, which is connected by disulfide bonds and has the ability to form oligomeric structures through cross-linking [13].

MFAP4 is heavily expressed in elastic tissues like the skin, heart, and lungs due to its ability to bind to elastin and other extracellular matrix fibers [14]. MFAP4 has been associated with various disorders that involve tissue remodeling, like fibrotic diseases [15], asthma, and cardiovascular conditions, particularly atherosclerosis [16].

A new possible marker for cirrhotic liver disease, MFAP4, has been proposed for the first time [15]. Subsequent investigations have confirmed the significance and high precision of blood MFAP4 in detecting liver fibrosis related to alcoholic abuse and HCV [17].

In this study, we are going to investigate possibility of using serum MFAP4 levels as a non-invasive marker for OV prediction in patients with HCV-induced liver cirrhosis.

This study assessed the efficacy of serum MFAP4 as a diagnostic biomarker for identifying OV in HCV induced liver cirrhosis patients. It also compared the effectiveness of MFAP4 with other non-invasive markers, as well as upper gastrointestinal endoscopic findings across different grades of OV.

PATIENTS/MATERIALS AND METHODS

There were many studies estimating prevalence of HCV in Egyptian patients which ranged from 3%-13%, so we choose a study with prevalence of 7.3% with precision of 5 and α of 5% [18]. The minimum number needed for our study was calculated to be 86 patients [19].

This prospective controlled research was done on ninety people who were attending the Tropical Medicine Department at the Main University Hospital in Alexandria and GIT endoscopy unit at Medical Research Institute participated in this prospective controlled study.

Individuals were classified into four groups after doing upper GIT endoscopy. Twenty patients with liver cirrhosis without OV are in Group I. Twenty-five individuals with small size OV grades I and II due to hepatic cirrhosis comprise Group II. There are twenty-five patients in Group IIIa who have large size OV grades (III, IV) due to hepatic cirrhosis. Group IIIb is made of the same 25 patients as Group IIIa but with OV eradicated by both NSBBs and band ligation in 3-6 months. Group IV has 20 healthy persons as control group. The age range of the patients was 40 to 71 years old.

Every patient who was enrolled in the research had a thorough history taking, clinical examinations and the following laboratory tests were carried out on them: CBC, serum Alpha Fetoprotein (AFP), liver and renal function tests, HCV antibodies (ELISA), and hepatitis B

surface antigen (ELISA).

All subjects were tested for the marker (MFAP4) using serum samples. Additionally, patients in group IIIb had their serum samples measured using an enzyme-linked immunosorbent assay (ELISA) method employing the Human Microfibrillar-associated protein 4 (Cloud-Clone Corp., China) at the time when varices eradicated. For each patient who took part, we determined the severity of liver disease using Child Pugh score.

In terms of abdominal ultrasound parameters, evaluations to ascertain whether they had cirrhosis or bilharzial hepatic fibrosis. The right hepatic lobe diameter, splenic bipolar diameter, and portal vein diameter were measured by Doppler ultrasonography.

The AST to platelet ratio index (APRI) [20], the Index for Liver Fibrosis FIB4 [20], platelet count to spleen diameter [21], and the AST/ALT ratio were measured for all cirrhotic patients [22]. Furthermore, all patients underwent UGIE with grading of oesophageal varices according to Paquet classification [23].

Exclusion Criteria:

The study excluded patients with sepsis, non-HCV-related liver cirrhosis, portal vein thrombosis, diabetes mellitus, malignancies, acute liver failure, and rheumatoid arthritis.

Statistical analysis

Computer data was entered and analyzed using IBM SPSS 20.0. Qualitative data were numbers and percentages. Quantitative data was characterized by range, mean, standard deviation, median, and IQR.

RESULTS

Ninety candidates participated in this study, which was done in the Department of Tropical Medicine at Alexandria Main University Hospital and GIT endoscopy unit at medical research institute. There were four grouping of subjects.

With respect to the demographic data of the studied groups, no statistically significant difference was observed with respect to age or gender across any of the categories. Females predominated males in groups I (60 percent), II (56 percent), and the control group (55 percent) while males predominated in group IIIa (56 percent) as indicated in Table 1. The mean age of the participants in these groups were 47.4 ± 4.72 years, 49.6 ± 7.64 years, 47.5 ± 5.99 years, and 43.3 ± 4.61 years, respectively.

In groups I and II, dyspepsia was the frequent symptom reported by patients at the time of admission. Table 1 indicated that all patients in group IIIa experienced abdominal distension and swelling of the lower limbs.

Table 1 illustrated that pallor was the most prevalent finding in 25% of the individuals in group I based on the general examination of groups I. Hematemesis was found in 36% of patients in group II, whereas palmer erythema, hepatic encephalopathy, and jaundice were the most frequent signs in patients with large varices.

As seen in Table 2, ascites was in 30%, 48%, and 100% of cirrhotic groups (I.II and IIIa), respectively.

According to ultrasonography, all patients in groups I, II, and IIIa had cirrhosis, whereas group IV participants' livers were normal. Furthermore, as Table 2 shows, patients with significant varices had spleens that were statistically substantially larger than those without or small varices.

Additionally, Table 2 demonstrates that 10% of individuals in Group I, 12% of individuals in Group II, and 36% of individuals in Group IIIa had combined cirrhosis with periportal hepatic fibrosis as a result of Schistsomiasis.

Table 2 displays statistically significant differences in diameter of portal vein ($p1<0.001$), ($p2<0.001$) and ($p3<0.001$) among all cirrhotic groups.

All CBC measures differed significantly between groups of liver cirrhosis and control group. Table 3 shows a difference with statistically significant in platelet count between groups II and IIIa, I and III, yet not between I and II.

There were no significant discrepancies between the serum AFP, FBG, and kidney function test results for any of the groups. Additionally, a normal ESR level and a negative CRP was noted in all cases.

The liver profile showed substantial differences ($p < 0.001$) between the cirrhotic and control groups for all metrics. Table 3 shows that serum levels of total bilirubin and ALP were considerably higher in cirrhotic patients

compared to controls. Also, patients with small and large varices had lower serum albumin levels than those without varices. Furthermore, all cirrhotic groups showed considerably higher serum levels of liver enzymes (AST and ALT) than the healthy group. Furthermore, Table 3 revealed that INR was significantly higher in groups I, II, and IIIa compared to healthy group, also, PA was significantly lower in all three cirrhotic groups compared to healthy subjects.

Groups I, II, and IIIa all included individuals with post-viral liver cirrhosis. All of them obtained a negative PCR for HCV and underwent treatment for their chronic HCV infection. They also tested negative for the autoimmune hepatitis marker and the Hepatitis B Virus (HBV). The Child-Pugh classification and score are shown in Table 4.

Table 4 indicates that a total of two to five OV band ligation sessions were required over a period of three to six months in order to completely eradicate the large oesophageal varices in group IIIa.

AST/ALT, platelet count-to-spleen diameter, APRI, FIB4, and conventional prognostic scores differed considerably between cirrhotic and control groups. Table 5 reveals a statistically significant difference in all predicted scores between cirrhotic groups I and IIIa, II and IIIa, yet not between I and II.

Serum MFAP4, our main research marker, varies significantly across the liver cirrhosis groups and controls. The mean concentration in the control group was 613.3 ± 243.5 ng/ml, while in groups I, II, and IIIa, it was 1455.0 ± 428.3 ng/ml, 2341.6 ± 406.6 ng/ml, and 3842.4 ±807.6 ng/ml, respectively. In addition, there were statistically significant differences between groups I and II, I and IIIa, and II and IIIa. Nonetheless, as Table 5 and Figure 1 demonstrate, there was no significant difference between the IIIa and IIIb groups.

In addition, Figure 2 demonstrates that a cutoff value of >1900 (ng/ml) for serum MFAP4 is a highly accurate diagnostic of the presence of OV, with a sensitivity of 80% and specificity of 85%. Furthermore, Figure 3 demonstrates that serum MFAP-4 has the ability to differentiate between small and large varices using a threshold value of >2952.6 (ng/ml), with a sensitivity of 80% and specificity of 96%.

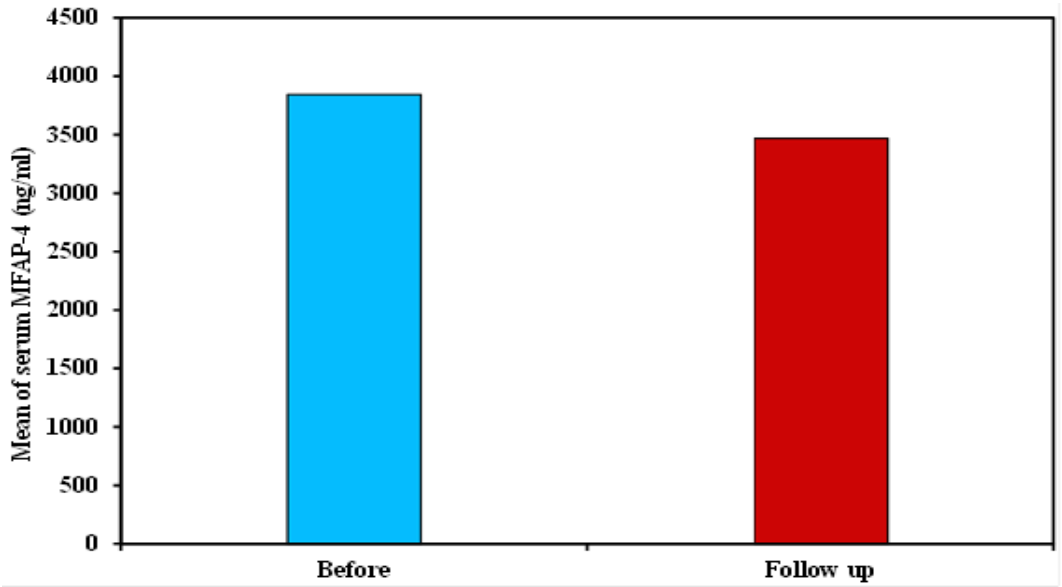

Figure 1. Comparison between before and after according to Serum MFAP-4 in Group III (n = 25)

Table 1. Comparison between the four studied groups according to different parameters

	GroupI (n = 20)	GroupII (n = 25)	GroupIIIa (n = 25)	GroupIV (n = 20)	Test of Sig.	p
Age (years)						
Mean ± SD.	47.4 ± 4.72	49.6 ± 7.64	47.5 ± 5.99	43.3 ± 4.61	F= 1.285	0.285
Median (Min. – Max.)	47 (40 – 55)	47 (40 – 71)	47 (38 – 61)	43.5 (36 – 53)		
Sex						
Male	8 (40.0%)	11 (44.0%)	14 (56.0%)	9 (45.0%)	χ^2= 1.326	0.723
Female	12 (60.0%)	14 (56.0%)	11 (44.0%)	11 (55.0%)		
Lower limb swelling	2 (10.0%)	2 (8.0%)	25 (100.0%)	0 (0.0%)	χ^2=73.333*	<0.001*
Abdominal distension	7 (35.0%)	10 (40.0%)	25 (100.0%)	0 (0.0%)	χ^2=47.612*	<0.001*
Dyspepsia	11 (55.0%)	17 (68.0%)	19 (76.0%)	0 (0.0%)	χ^2=30.082*	<0.001*
Weight loss	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	–	–
Hematemesis	5 (25.0%)	9 (36.0%)	8 (32.0%)	0 (0.0%)	χ^2=10.929*	^{MC}p=0.012*
Melena	3 (15.0%)	4 (16.0%)	12 (48.0%)	0 (0.0%)	χ^2=16.260*	^{MC}p=0.001*
Pallor	5 (25.0%)	7 (28.0%)	11 (44.0%)	0 (0.0%)	χ^2=11.418*	0.010*
Palmer erythema	0 (0.0%)	3 (12.0%)	15 (60.0%)	0 (0.0%)	χ^2=32.131*	^{MC}p<0.001*
Jaundice	0 (0.0%)	1 (4.0%)	12 (48.0%)	0 (0.0%)	χ^2=25.534*	^{MC}p<0.001*
Hepatic encephalopathy	2 (10.0%)	3 (12.0%)	12 (48.0%)	0 (0.0%)	χ^2=18.038*	^{MC}p<0.001*

SD: **Standard deviation** χ^2: **Chi square test** MC: **Monte Carlo**
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 four studied groups
p_0: p value for comparing between **Group IV** and **each other group**
p_1: p value for comparing between **Group I** and **Group II**
p_2: p value for comparing between **Group I** and **Group III**
p_3: p value for comparing between **Group II** and **Group III**
*: Statistically significant at $p \leq 0.05$
a: Significant with **Group I** b: Significant with **Group II** c: Significant with **Group III**

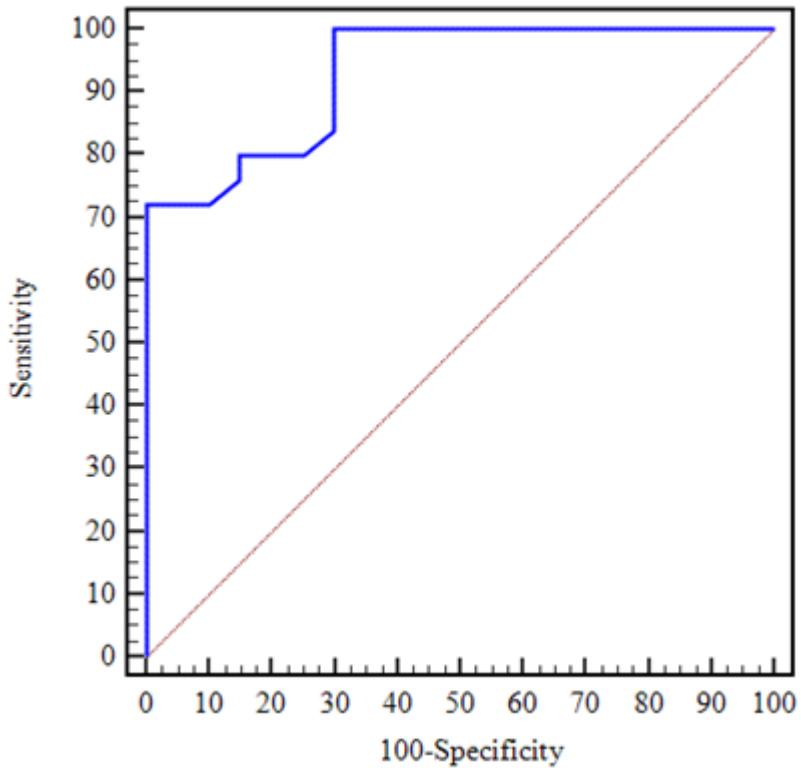

Figure 2. ROC curve for Serum MFAP-4 to discriminate group II (n = 20) from group I (n = 20)

Table 2. Comparison between the four studied groups according to radiological findings

Radiological findings	GroupI (n = 20)	GroupII (n = 25)	GroupIIIa (n = 25)	GroupIV (n = 20)	Test of Sig.	p
Ascites						
No	14 (70.0%)	13 (52.0%)	0 (0.0%)	20 (100.0%)		
Mild	6 (30.0%)	10 (40.0%)	0 (0.0%)	0 (0.0%)	χ^2=	^{MC}p
Moderate	0 (0.0%)	2 (8.0%)	16 (64.0%)	0 (0.0%)	92.001*	<0.001*
Massive	0 (0.0%)	0 (0.0%)	9 (36.0%)	0 (0.0%)		
Hepatomegaly	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	–	–
Splenomegaly	20 (100.0%)	25 (100.0%)	25 (100.0%)	0 (0.0%)	χ^2=82.579*	^{MC}p<0.001*
Spleen size(cm)						
Mean ± SD.	14.3 ± 0.95	15.3 ± 0.90	18.1^{ab} ± 2.18	11.4^{abc} ± 0.68	F=	<0.001*
Median (Min. – Max.)	14 (12.3 – 16)	16 (12.8 – 16)	18 (16 – 23)	11.5 (10 – 12)	91.584*	
p_0	<0.001*	<0.001*	<0.001*			
Sig. bet. Grps.	p_1=0.064,p_2<0.001*,p_3<0.001*					
Bilharzia hepatic fibrosis	2 (10.0%)	3 (12.0%)	9 (36.0%)	0 (0.0%)	χ^2=11.092*	^{MC}p=0.006*
Liver cirrhosis	20 (100.0%)	25 (100.0%)	25 (100.0%)	0 (0.0%)	χ^2=82.579*	^{MC}p<0.001*
Liver right lobe size(cm)						
Mean ± SD.	13.5 ± 1.33	13.3 ± 1.42	11.4^{ab} ± 1.67	13.9^{c} ± 0.94	F=	<0.001*
Median (Min. – Max.)	13.8(10.5 – 15)	13.5 (11 – 15)	12 (8.50 – 13)	14 (12 – 15)	15.797*	
p_0	0.805	0.537	<0.001*			
Sig. bet. Grps.	p_1=0.979,p_2<0.001*,p_3<0.001*					
Portal vein diameter(mm)						
Mean ± SD.	14.3 ± 0.47	15.6^{a} ± 0.65	17.7^{ab} ± 0.66	9.75^{abc} ± 1.21	F=	<0.001*
Median (Min. – Max.)	14 (14 – 15)	16 (15 – 17)	18 (17 – 19)	10 (8 – 12)	400.772*	
p_0	<0.001*	<0.001*	<0.001*			
Sig. bet. Grps.	p_1<0.001*,p_2<0.001*,p_3<0.001*					

SD: Standard deviation χ^2: Chi square test MC: Monte Carlo
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 four studied groups
p_0: p value for comparing between Group IV and each other group
p_1: p value for comparing between Group I and Group II
p_2: p value for comparing between Group I and Group III
p_3: p value for comparing between Group II and Group III
*: Statistically significant at $p \leq 0.05$
a: Significant with Group I b: Significant with Group II c: Significant with Group III

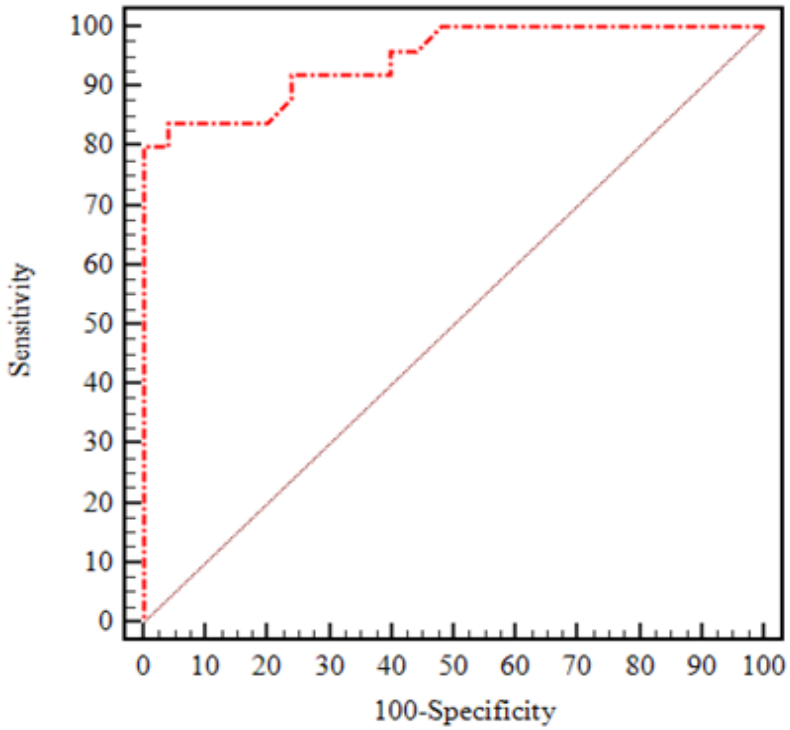

Figure 3: ROC curve for Serum MFAP-4 to discriminate group III (n = 20) from group II (n = 20)

Table 3: Comparison between the four studied groups according to different lab tests

CBC	Group I (n = 20)	Group II (n = 25)	Group IIIa (n = 25)	Group IV (n = 20)	F	p
Hemoglobin (g/dl)						
Mean ± SD.	10.8 ± 0.40	10.7 ± 0.38	10.5 ± 0.59	12.8^{abc} ± 0.83	74.681*	<0.001*
Median (Min. – Max.)	11 (9.9 – 11.3)	10.8(10.1–11.3)	10.6 (9.1–11.5)	13 (11.3 – 14.3)		
p_0	<0.001*	<0.001*	<0.001*			
Sig. bet. Grps.	p_1=0.936,p_2=0.175,p_3=0.405					
WBC (×10³/µl)						
Mean ± SD.	5.95 ± 1.46	5.19 ± 1.25	4.90 ± 1.81	6.39^{bc} ± 1.11	4.874*	0.004*
Median (Min. – Max.)	6 (4 – 9)	5 (3.4 – 8.3)	5 (2.7 – 8.9)	6.25 (5 – 8.10)		
p_0	0.772	0.036*	0.005*			
Sig. bet. Grps.	p_1=0.314,p_2=0.086,p_3=0.896					
Platelets (×10³/µl)						
Mean ± SD.	175.7 ± 29.1	158.8 ± 19.9	99.6^{ab} ± 37.8	272.8^{abc} ± 53.2	85.602*	<0.001*
Median (Min. – Max.)	169.5(149–279)	152 (130–191)	90 (51–168)	268.5(210–389)		
p_0	<0.001*	<0.001*	<0.001*			
Sig. bet. Grps.	p_1=0.415,p_2<0.001*,p_3<0.001*					
Liver function test	**Group I (n = 20)**	**Group II (n = 25)**	**Group IIIa (n = 25)**	**Group IV (n = 20)**	**Test of Sig.**	**p**
ALT(IU/L)						
Mean ± SD.	48.0 ± 27.33	41.32 ± 21.36	52.48 ± 26.62	19.90^{abc} ± 4.62	F= 8.878*	<0.001*
Median (Min. – Max.)	47 (11 – 99)	35 (13 – 81)	44 (20 – 120)	19.5 (12 – 28)		
p_0	<0.001*	<0.001*	<0.001*			
Sig. bet. Grps.	p_1=0.942,p_2=0.999,p_3=0.875					
AST(IU/L)						
Mean ± SD.	70.15 ± 29.60	74.84 ± 34.86	68.96 ± 28.38	13.10^{abc} ± 4.45	F= 23.342*	<0.001*
Median (Min. – Max.)	64.5 (31 – 132)	69 (39 – 203)	65 (25 – 152)	12 (7 – 24)		
p_0	<0.001*	<0.001*	<0.001*			
Sig. bet. Grps.	p_1=0.942,p_2=0.999,p_3=0.875					
Albumin (g/dl)						
Mean ± SD.	3.91 ± 0.47	3.66 ± 0.44	2.92^{ab} ± 0.26	4.21^{bc} ± 0.22	F= 51.987*	<0.001*
Median (Min. – Max.)	4.05 (3.2 – 4.6)	3.8 (2.9 – 4.3)	3 (2.4 – 3.3)	4.2 (3.8 – 4.7)		
p_0	0.054	<0.001*	<0.001*			
Sig. bet. Grps.	p_1=0.112,p_2<0.001*,p_3<0.001*					
Total bilirubin (mg/dL)						
Mean ± SD.	0.67 ± 0.26	0.82 ± 0.22	2.68 ± 1.95	0.78 ± 0.20	H= 28.520*	<0.001*
Median (Min. – Max.)	0.73 (0.20 – 1)	0.90 (0.30 – 1)	1^{ab}(0.70 – 6.50)	0.80^{c} (0.30 – 1)		
p_0	0.333	0.411	<0.001*			
Sig. bet. Grps.	p_1=0.065,p_2<0.001*,p_3=0.001*					
Direct bilirubin (mg/dL)						
Mean ± SD.	0.46 ± 0.23	0.59 ± 0.18	2.12 ± 1.62	0.53 ± 0.17	H= 30.171*	<0.001*
Median (Min. – Max.)	0.5 (0.1 – 0.8)	0.7 (0.2 – 0.8)	0.9^{ab} (0.4 – 5.3)	0.6^{c} (0.2 – 0.8)		
p_0	0.510	0.300	<0.001*			

Sig. bet. Grps.	p_1=0.084,p_2<0.001*,p_3=0.001*					
ALP						
Mean ± SD.	94.40 ± 27.57	101.9 ± 27.05	153.4^{ab} ± 64.63	78.45^{c} ± 19.28	F= 15.117*	<0.001*
Median (Min. – Max.)	91 (50 – 135)	92 (67 – 170)	129 (59 – 251)	77.5 (50 – 111)		
p_0	0.595	0.219	<0.001*			
Sig. bet. Grps.	p_1=0.926,p_2<0.001*,p_3<0.001*					
INR						
Mean ± SD.	1.12 ± 0.11	1.29^{a} ± 0.11	1.70^{ab} ± 0.20	1.05^{bc} ± 0.08	F= 106.867*	<0.001*
Median (Min. – Max.)	1.1 (1 – 1.32)	1.3 (1.1 – 1.5)	1.62 (1.4 – 2.1)	1 (0.9 – 1.2)		
p_0	0.334	<0.001*	<0.001*			
Sig. bet. Grps.	p_1<0.001*,p_2<0.001*,p_3<0.001*					
Prothrombin activity (%)						
Mean ± SD.	84.35 ± 14.67	65.4 ± 10.5	43.2 ± 8.27	94.3 ± 8.41	F= 100.878*	<0.001*
Median (Min. – Max.)	90 (60 – 100)	63 (50 – 92)	45 (27 – 56)	98.5 (79 – 110)		
p_0	0.021*	<0.001*	<0.001*			
Sig. bet. Grps.	p_1<0.001*,p_2<0.001*,p_3<0.001*					

SD: **STANDARD DEVIATION** AST: ASPARTATE TRANSFERASE, ALT: ALANINE TRANSFERASE, ALP: ALKALINE PHOSPHATASE, PA: PROTHROMBIN ACTIVITY, INR: INTERNATIONAL NORMALIZED RATIO, WBC: WHITE BLOOD CELLS
F: F FOR ONE-WAY ANOVA TEST, PAIRWISE COMPARISON BET. EACH 2 GROUPS WERE DONE USING **POST HOC TEST (TUKEY)**
P: P VALUE FOR COMPARING BETWEEN THE FOUR STUDIED GROUPS
P_0: P VALUE FOR COMPARING BETWEEN **GROUP IV** AND **EACH OTHER GROUP**
P_1: P VALUE FOR COMPARING BETWEEN **GROUP I** AND **GROUP II**
P_2: P VALUE FOR COMPARING BETWEEN **GROUP I** AND **GROUP III**
P_3: P VALUE FOR COMPARING BETWEEN **GROUP II** AND **GROUP III**
*: STATISTICALLY SIGNIFICANT AT $P \leq 0.05$
A: SIGNIFICANT WITH **GROUP I** B: SIGNIFICANT WITH **GROUP II** C: SIGNIFICANT WITH **GROUP III**

Table 4: Comparison between the three studied groups according to different parameters

	GroupI (n = 20)	**GroupII (n = 25)**	**GroupIIIa (n = 25)**	**Test of Sig.**	**p**
Child PUGH					
A	14 (70.0%)	13 (52.0%)	0 (0.0%)	χ^2= 41.957*	^{MC}p <0.001*
B	6 (30.0%)	12 (48.0%)	13 (52.0%)		
C	0 (0.0%)	0 (0.0%)	12 (48.0%)		
Child score					
Mean ± SD.	5.70 ± 0.92	6.08 ± 1.19	9.52^{ab} ± 2.43	F= 36.748*	<0.001*
Median (Min. – Max.)	5 (5 – 7)	5 (5 – 8)	9 (7 – 13)		
Sig. bet. Grps.	p_1=0.736,p_2<0.001*,p_3<0.001*				
Esophageal varices					
No	20 (100.0%)	0 (0.0%)	0 (0.0%)	χ^2 =125.148*	$=^{MC}p$ <0.001*
Grade I	0 (0.0%)	10 (40.0%)	0 (0.0%)		
Grade II	0 (0.0%)	15 (60.0%)	0 (0.0%)		
Grade III	0 (0.0%)	0 (0.0%)	11 (44.0%)		
Grade IV	0 (0.0%)	0 (0.0%)	14 (56.0%)		
Number of esophageal band sessions					
Mean ± SD.	–	–	3.60 ± 0.96	–	–
Median (Min. – Max.)	–	–	4 (2 – 5)		
No weeks eradication					
Mean ± SD.	–	–	16.2 ± 4.04	–	–
Median (Min. – Max.)	–	–	16 (12 – 24)		

SD: Standard deviation χ^2: Chi square test MC: Monte Carlo
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
p_1: p value for comparing between Group I and Group II
p_2: p value for comparing between Group I and Group III
p_3: p value for comparing between Group II and Group III
*: Statistically significant at $p \leq 0.05$
a: Significant with Group I b: Significant with Group II

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Table 5: Comparison between the four studied groups according to non –invasive to assess liver fibrosis

	GroupI (n = 20)	GroupII (n = 25)	GroupIIIa (n = 25)	GroupIV (n = 20)	Test of sig.	p
AST/ALT ratio						
Mean ± SD.	1.87 ± 0.96	2.05 ± 0.83	1.47[b] ± 0.64	0.65[abc] ± 0.14	F= 15.750*	<0.001*
Median (Min. – Max.)	1.9 (0.56 – 3.8)	2.03 (0.7 – 3.8)	1.32 (0.6 – 3.3)	0.6 (0.4 – 0.95)		
p_0	<0.001*	<0.001*	0.002*			
Sig. bet. Grps.	p_1=0.842,p_2=0.255,p_3=0.028*					
FIB 4						
Mean ± SD.	2.93 ± 0.87	3.74 ± 1.71	5.73[ab] ± 2.09	0.42[abc] ± 0.15	F= 48.440*	<0.001*
Median (Min. – Max.)	3.2 (1.14 – 4.2)	3.4 (1.8 – 11.4)	5.8 (2 – 11.5)	0.42 (0.2 – 0.8)		
p_0	<0.001*	<0.001*	<0.001*			
Sig. bet. Grps.	p_1=0.269,p_2<0.001*,p_3<0.001*					
PLT spleen diameter						
Mean ± SD.	1241.4 ± 269.2	1048.2 ± 187.1	570.8[ab] ± 254.3	2404.4[abc]±508.9	F= 129.323*	<0.001*
Median (Min. – Max.)	1171.4 (931.2 – 2113)	950 (812.5 – 1364)	515.7 (243 – 1050)	2350 (1758 – 3536)		
p_0	<0.001*	<0.001*	<0.001*			
Sig. bet. Grps.	p_1= 0.187,p_2<0.001*,p_3<0.001*					
APRI						
Mean ± SD.	1.33 ± 0.79	1.29 ± 0.59	2.21 ± 1.28	0.15 ± 0.06	H= 55.285*	<0.001*
Median (Min. – Max.)	1.21 (0.4 – 3.9)	1.1 (0.58 – 3.6)	1.8 (0.54 – 6.8)	0.14 (0.1 – 0.3)		
p_0	<0.001*	<0.001*	<0.001*			
Sig. bet. Grps.	p_1=0.905,p_2=0.015*,p_3=0.007*					
Serum MFAP-4 (ng/ml)						
Mean ± SD.	1455.0 ± 428.3	2341.6[a] ± 406.6	3842.4[ab]±807.6	613.3[abc] ± 243.5	F= 152.992*	<0.001*
Median (Min. – Max.)	1495.3 (718.2–2063.4)	2416.8 (1740.4–2990.6)	3822.8 (2481.4–4970.4)	579.5 (304 – 1113.4)		
p_0	<0.001*	<0.001*	<0.001*			
Sig. bet. Grps.	p_1<0.001*,p_2<0.001*,p_3<0.001*					

SD: Standard deviation, AST: Aspartate Transferase, ALT: Alanine Transferase, MFAP4: Microfibrillar-associated protein 4, APRI: AST to platelet ratio index
F: F for One-way ANOVA test, Pairwise comparison bet. each 2 groups were done using **Post Hoc Test (Tukey)**
H: H for **Kruskal Wallis test,** Pairwise comparison bet. each 2 groups were done using **Post Hoc Test (Dunn's for multiple comparisons test)**
p: p value for comparing between the four studied groups
p_0: p value for comparing between **Group IV** and **each other group**
p_1: p value for comparing between **Group I** and **Group II**

Table 6: Correlation between serum MFAP-4 and different parameters in each group

	Group I		Group II		Group IIIa	
	r	**p**	**r**	**p**	**r**	**p**
Child PUGH	-0.110	0.645	0.068	0.745	-0.238	0.251
Child score	-0.302	0.196	0.008	0.971	-0.042	0.842
FIB-4	0.367	0.111	0.013	0.952	-0.292	0.157
APRI	0.275	0.241	0.080	0.703	-0.446*	0.026*
PLT/ spleen diameter	-0.189	0.425	-0.026	0.901	0.185	0.376
AST/ALT ratio	0.298	0.202	0.280	0.176	0.076	0.717

r: Pearson coefficient, AST: Aspartate Transferase, ALT: Alanine Transferase, MFAP4: Microfibrillar-associated protein 4, APRI: AST to platelet ratio index
*: Statistically significant at $p \leq 0.05$

DISCUSSION

Recent research has indicated that MFAP4, a protein that is significantly increased in fibrotic liver, could potentially serve as a new biomarker for fibrotic liver disease. [15] Nevertheless, there is a lack of previous studies investigating the utilization of serum MFAP4 as a means for detecting OV in cirrhotic patients.

The importance of various clinical, laboratory and ultrasonographic parameters that are associated with portal hypertension can be evaluated by using non-invasive parameters like thrombocytopenia, splenomegaly, APRI [20], platelet count to spleen diameter ratio [21] and AST/ALT ratio. [22]

One of MFAP4's advantages is that it may be added to the routine liver function test that is

frequently conducted during a primary care visit and doesn't require the installation of costly equipment like elastography. The evaluation of serological fibrosis using patented markers is still quite expensive; while, novel biomarkers, such as MFAP4 or others, may offer a more appealing and affordable option.

This study aimed to assess the potential of serum MFAP4 as a noninvasive diagnostic biomarker for OV. We observed statistically significant differences between the groups with liver cirrhosis and the control group. These findings are consistent with the study conducted by Bracht T et al., [24], which determined that MFAP4 can serve as a beneficial blood biomarker for identifying individuals at a greater risk of severe fibrosis stages in HCV patients and for assessing hepatic fibrosis.

This was similar with Kanaan R et al. [25] finding that patients with advanced stage cirrhosis and fibrosis (stage F4) had greater serum MFAP4 levels than those with F1–F2–F3. He found no significant difference between F3/F2/F1 and healthy controls, F3 against F2, or F2 versus F1. This may discuss absence of a significant difference in blood MFAP-4 levels between NAFLD patients and healthy controls. Transient elastography (TE) also correlated positively with serum MFAP4. TE measurement strongly correlates with NAFLD patients' advanced fibrotic stages.

Furthermore, we observed in our study that, when OV was eradicated by simultaneous band ligation and beta blockers, serum MFAP4 did not differ significantly between IIIa and IIIb., which reduces its prognostic value. Nevertheless, more extensive randomised investigations are necessary to validate this result.

The results of Madsen BS et al. [26] were in agreement with our findings. They observed the presence of MFAP4 in fibrotic liver tissue and noted that serum levels of MFAP4 increased with stage of fibrosis. Additionally, their analysis showed that MFAP4 had similar diagnostic accuracy as the enhanced liver fibrosis test or transient elastography (TE) in the studied subjects. In addition, he mentioned that the optimal threshold for diagnosis advanced fibrosis and cirrhosis was 88.7 U/L for blood MFAP4 in his study while in our study it was 1900 ng/ml which equal 50 U/L. The elevated threshold value seen in his study may be ascribed to a range of factors, including different etiologies of cirrhosis (Alcholic liver disease in his study), diverse ethnicities, and varying sample sizes.

In this study, we also correlated serum MFAP4 with other non-invasive cirrhosis scores in each group. As indicated in Table 6, we discovered that serum MFAP4 did not correlate with the FIB-4 or APRI in any of the cirrhotic patients, with the exception of a negative correlation with the APRI score in patients with large varcies.

Similarly, Kanaan R et al. [25] found no correlation between serum MFAP4 levels and liver function parameters, including ALT, AST, ALP, GGT, and bilirubin, in their study.

CONCLUSION

For the diagnosis and grading of OV, serum MFAP4 may be a sensitive non-invasive predictor; however, it is not recommended for use in treatment follow-up.

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Conflict of Interest: According to the authors, they have no conflicting interests.

Ethical approval: The study was conducted in accordance with ethical principles. The research was granted clearance by the Ethical Committee of the Faculty of Medicine, Alexandria University on December 3, 2023. procedure was adherent to the ethical principles outlined in the 1975 Declaration of Helsinki. Prior consent was acquired from each individual. FWA NO: 00018699 is the reference number. the Committee's serial number is 0306411, IRB NO:00012098.

List of abbreviations:

AFP: Alfa Feto-Protein, AST: Aspartate Transferase, ALT: Alanine Transferase, ALP: Alkaline Phosphatase, APRI: AST to platelet ratio index, CBC: Complete Blood Count, CRP: C Reactive Protein, ESR: Erythrocyte Sedimentation Rate, EVB: Esophageal variceal bleeding, EGD: Esophagogastroduodenoscopy, EBL: Endoscopic band ligation, , ELISA: Enzyme-linked immunosorbent assays, FBG: Fasting blood sugar, HCV: Hepatitis C Virus, HBV: Hepatitis B Virus , HVPG: hepatic venous pressure gradient, INR: International normalized ratio, MFAP4: Microfibrillar-associated protein 4, NAFLDs: non-alcoholic fatty liver diseases,

NSBBs: Non selective beta blockers, OV: Oesophageal varices, PA: Prothrombin activity, PH: Portal hypertension, ULN: Upper limit of normal, VH: Variceal hemorrhage.

Availability of data and materials:

The data used to back up the study's conclusions is included in the paper.

HIGHLIGHTS

- Esophagogastroduodenoscopy (EGD) is considered the gold standard for OV detection due to its excellent sensitivity and specificity.
- The drawbacks of EGD include its invasiveness, need for conscious sedation and relatively high cost. Furthermore, EGD is not often accessible in nations with little resources.
- Thus, many non-invasive techniques have been developed as a simple marker for OV detection in order to get over these challenges like MFAP4.

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