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Original Article

Assessment of Thrombomodulin Level in Plasma of Patients with Compensated and Decompensated Liver Cirrhosis

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ABSTRACT

Background: In liver cirrhosis (LC), broad hepatocyte necrosis, and diffuse hyperplastic connective tissue causing distortion, obliteration & narrowing of blood vessels by regenerating nodules, promoting damage of endothelial cell. Thrombomodulin (TM) expression in hepatic endothelial cells is highly affected in liver diseases like viral hepatitis and liver damage causing soluble thrombomodulin (sTM) release. This study aimed to determine sTM level in LC patients and to study the association between sTM levels and severity of hepatic dysfunction.

Methods: A case-control study was performed in GIT unit of Internal Medicine Department, Zagazig University Hospitals from October 2023 to March 2024. Ninety subjects were included, divided in 3 groups (A, B & C), 30 subjects in each group; Group A: Normal (as control). Group B: Compensated cirrhotic patients. Group C: Decompensated cirrhotic patients. All patients subjected to detailed history taking, clinical examination, radiological assessment, laboratory investigations and plasma sTM assessment.

Results: A statistically significant increment in plasma sTM level in decompensated cirrhotic group C (1231.4±513) compared to control group A and compensated group B (420.2±102) (561.1±198) respectively ($p<0.05$). A significant association of plasma sTM with (ALT, AST, ALB, BIL, INR, platelets, & Child Score) in group B & C patients ($p<0.05$). TM with Cut-off 500.11 pg/dl can discriminate patients of three groups with AUC 0.654, sensitivity 92.5%, specificity 95%, PPV 94.4% and NPV 92.6%.

Conclusions: The sTM level could have a role in early prediction of decompensated liver cirrhosis.

Keywords: liver Cirrhosis; soluble thrombomodulin; endothelial cells.

INTRODUCTION

Liver sinusoidal endothelial cells (LSECs), besides serving as a barrier between the bloodstream and hepatocytes, LSECs have several crucial functions, such as maintenance of vascular smooth myocyte contractile function, thrombosis, and inflammation, immunological response, involvement in liver fibrosis and regeneration [1]. Patients with compensated cirrhosis may be asymptomatic or they may report nonspecific symptoms, however, patients with decompensated cirrhosis may present with jaundice, pruritus, signs of upper GI bleeding, ascites, or confusion due to hepatic encephalopathy [2].

The hepatocytes acquire nodules and fibrosis & alteration in the normal histological hepatic lobular architecture because of chronic damage in liver cirrhosis (LC). Viruses, chemicals, autoimmune conditions, and hereditary problems can all cause hepatic injury. As endothelial damage plays a role in the advancement of LC and portal hypertension, various endothelial cell (VEC) damage markers were identified, including soluble thrombomodulin (sTM) and soluble VE-cadherin [3].

The most examined factor was vWF, which predicted the severity of portal hypertension and LC. However, vWF values vary greatly in normal individuals due to their genetic factors. As a result, highly elevated vWF readings are more useful in predicting LC prognosis than less vWF levels. sTM is a dependable indicator of endothelium damage in the early and later stages [4].

sTM is a trans membranous glycoprotein found in endothelial cells (EC) that functions as a thrombin receptor and is essential for anticoagulation. When an EC was activated by inflammation, TM was produced into the blood flow as sTM in four types with varying molecular weights. Earlier research has shown that sTM relates to elevated risk of coronary heart disease (CHD), renal impairment, and other conditions [5].

Several investigations have found that sTM values increased in chronic and acute liver failure, chronic hepatitis, and LC [6].

Recent researchers have investigated the possible correlation between sTM levels and liver dysfunction, so this study aims to determine plasma sTM level in LC patients either compensated cirrhotic or decompensated and to study the association between sTM levels and severity of liver dysfunction among Zagazig University Hospital liver cirrhosis patients.

METHODS

Patients

This case-control study was conducted in GIT unit of Internal Medicine Department, Zagazig University Hospitals. All cases met the inclusion and exclusion criteria, were included during the study period from October 2023 up to the end of March 2024, ninety cases were included and divided into 3 groups (A, B, & C), 30 subjects in each group. Group A: Normal (as control). Group B: cirrhotic compensated patients. Group C: cirrhotic decompensated patients. Written informed consent was obtained from all patients and the study protocol was approved by Zagazig University Institutional Review Board (IRB) no: ZU-IRB #11134/19-9-2023. The research was conducted under the World Medical Association's Code of Ethics (Helsinki Declaration) for human research. Cases with the following characteristics were included; Age ≥18 years, Samples of patients were collected either from outpatient clinic and/or hospitalized patients. However, cases with hepatocellular carcinoma or any other cancer, HIV infections, severe cardiac, pulmonary, renal impairment, and other serious disorders, patient 's age under 18 years, or the patient refused to participate in the research work were excluded.

Diagnosis of liver cirrhosis was confirmed based on liver biopsy (all patients with compensated cirrhosis) and/or who had clinical evidence of decompensation combined with endoscopic as well as radiological findings. Symptoms of hepatic decompensation like ascites, hepatic encephalopathy, internal bleeding from large blood vessels in the esophagus (varices) and yellowing of eyes and skin (jaundice). and signs, laboratory tests (e.g., liver function and coagulation tests), and abdominal images (small sized nodular liver ± caudate lobe hypertrophy, portal hypertension indicated by the presence of collateral vessels, varices, and/or splenomegaly), also FIB-4 score more than 3.25 was used to indicate liver cirrhosis more likely .Transient elastography was done in some patients with cutoff >11 to 14 kPa for cirrhosis. The classification as compensated cirrhosis precluded any history of the above criteria [2].

Severity of liver disease was assessed using length of stay in hospital. In addition, Child-Pugh and Model for End-stage Liver Disease (MELD) scores were calculated based on laboratory values obtained within 24 h of admission. Child-Pugh score: It included two continuous variables (bilirubin and albumin) and three discrete variables (ascites, encephalopathy and international normalized ratio [INR]). The score was divided into three classes: 5-6 points ⇒ Class A, 7-9 points ⇒ Class B, 10-15 points ⇒ Class C [2].

Methods

All study population underwent detailed history taking from the patients or their relatives. Clinical examination including general examination (skin, sclera, soft palate, temperature, flapping, and blood pressure) and local abdominal examination, abdominal Ultrasound assessment, laboratory and biochemical investigations.

Blood sampling: 10 ml of venous blood sample was obtained from all patients under complete aseptic condition by antecubital venipuncture and collected into 3.2% sodium citrate except for CBC sample which were drawn into EDTA tubes.

Serum preparation: The specimens were then processed using twofold centrifugation. All blood specimens were centrifuged for 15 minutes at 2500 g at room temperature, and 3 mL of platelet-poor plasma was obtained in the center of the tube, eliminating platelets on the top layer and buffy coat. The obtained plasma was centrifuged for 15 minutes

at 2500g at room temperature, and 2.6 mL of supernatant was recovered. The resultant PFP was kept in aliquots at -80°C for testing.

Complete blood count (CBC) was analyzed within 2 hours after venipuncture using an automated hematology analyzer. The parameters analyzed were hemoglobin level, RBCs, WBCs, and Platelet count.

Prothrombin Time (PT): Citrated plasma and thromboplastin were incubated at 37 degrees Celsius. The plasma was re-calcified, and time was recorded until fibrins were visible. Partial Thromboplastin Time (PTT): Citrated plasma, a stimulating agent, and phospholipid were mixed and incubated at 37°C. Calcium was added, and the time it took for the kaolin to clump was measured. The INR was computed as (Patient's PT / Normal PT).

Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT), Alkaline Phosphatase (ALP), were evaluated utilizing ELISA kits. Serum albumin was measured using BCG method.

Serum creatinine was determined by Jaffe's reaction. Albumin/ Creatinine Ratio was calculated. This ratio will be used to assess kidney function. Glomerular filtration rate was assessed. Alpha fetoprotein (AFP) and Virology (HBsAg, HCVAb, & HIVAb) tests were done using ELISA technique.

Thrombomodulin (TM) levels were determined by * ELISA Kit, (Catalog No: DL-TM-Hu, DLdebelop, Wuxi, Jiangsu, China), a "sandwich" ELISA employing a monoclonal antibody, which recognized the EGF, - EGF2 domains of TM. The normal lab reference of thrombomodulin levels ranged from 3 to 300 ng/ml.

STATISTICAL ANALYSIS

Data were analyzed employing SPSS version 20. The quantitative results were presented using means and standard deviations. Normally distributed data were expressed as the mean ± standard deviation (M ± SD), and comparisons were performed with independent samples t tests. Data with abnormal distributions are expressed as the median and the distributions between groups were compared using the nonparametric Mann-Whitney U test. Use the Z-test to compare the percentage of outcomes between the two groups. Pearson Correlation was utilized to investigate the link between quantitative variables. Multiple regression analysis was done, where Logistic regression coefficients are calculated and used to estimate Odds ratios for different independent factors that affect thrombomodulin level. $P < 0.05$ was considered significant.

RESULTS

A highly significant statistical difference was achieved regarding HCV patients in cirrhotic decompensated group C (15 patients) (50%). The Least significant difference (LSD) was in group C compared to group B ($P < 0.001$) (**Table 1 & 2**).

All control individuals got child A score, group B(compensated) patients; 21 patients got score A and 9 patients got score B, in group C(decompensated) patients; no patients got score A, 21 got score C and 9 got score B. The percentage of patients with score A child is significantly higher in groups A& B. While the percentage of patients with score B, C child is significantly higher in groups C. LSD was significantly high between groups A & B, A & C ($P < 0.001$) and B & C ($P < 0.05$) (**Tables 1 & 2**).

Table (2) shows a highly significant difference between the studied groups regarding ALT, AST, serum albumin, total bilirubin, INR, platelets count, serum creatinine, blood urea, serum sodium and potassium ($p< 0.001$) (Table 3). Serum Alpha fetoprotein (AFP) level in different studied groups was statistically insignificant ($p>0.05$) (**Table 1 & 2**).

There is increase in Thrombomodulin median level (pg/dl) in group C (1231.4± 513) compared to group A (420.2 ± 102) and group B (561.1 ± 198) which is statistically significant ($p< 0.05$) & LSD ($p< 0.001$) in (**Table 1 & 2**).

Thrombomodulin has significant correlation with laboratory parameters with liver functions assessment (ALT, AST, ALB, BIL INR, platelets & Child score) in group B & C patients ($p<0.05$) (Table 3).

The clinical performance of sTM in different groups revealed that, sTM with Cut-off 500.11 pg/dl can discriminate patients of groups A from B & C with area under curve of 0.654, sensitivity 92.5%, specificity 95%, positive predictive value (PPV) 94.4%, and negative predictive value (NPV) 92.6% (**Table 4 & Figure S1**).

Thrombomodulin with Cut-off 500.11 pg/dl can discriminate the diagnosis of compensated cirrhotic patients with area under curve of 0.701, sensitivity 77.5%, specificity 61.5%, PPV 76.5%, NPV 64% and accuracy 71.2% ($p<0.05$) (**Table 5 & Figure S 2**).

Thrombomodulin with Cut-off 500.11 pg/dl can discriminate the diagnosis of decompensated cirrhotic patients without complications among the studied patients with area under curve of 0.651, sensitivity 67.5%, specificity 50%, PPV 67.5%,

NPV 50% and accuracy 60.7% (p<0.05) (**Table 6 & Figure S 3**).

Albumin, total bilirubin, INR, Platelet and Creatinine are independent factors that affect thrombomodulin level. Their (95% CI) were (5.22–6.26), (7.31–8.47), (0.81–1.33), (3.47–4.53) and (4.01–8.17) respectively, OR were 1.16, 1.01, 1.13, 1.42 and 0.16 for each respectively (**Table 7**).

Table (1): Demographic and clinical presentation parameters in studied groups.

parameter	Group A n=30	Group B n=30	Group C n=30	Test	LSD	P value
Sex [n (%)] **Male:** **Female:**	 10 (33%) 20(67%)	 15(50%) 15(50%)	 20(67%) 10(33%)	X2=2.96	0.09 [1] 0.64 [2] 0.22 [3]	>0.05
Smoking	1 (3.3%)	3(10%)	1 (3.3%)	X2=2.78	0.09 [1] 0.29 [2] 0.5 [3]	>0.05
HCVAb positive cases	0.00	9(30%)	15(50%)	54.8	0.02* [1] 0.64* [2] 0.02* [3]	**H. S**
HBsAg positive cases	0.00	6(20%)	3(10%)	7.78	0.06 [1] 0.35 [2] 0.33 [3]	>0.05 **N. S**
Mixed HBV and HCV cases	0.00	3(10%)	9(30%)	8.4	0.09 [1] 0.64 [2] 0.22 [3]	>0.05 **N. S**
Child score (A)	30(100%)	21(70%)	0.00	60.4	0.01* [1] 0.٠5* [2] 0.٠3* [3]	<0.001
Child score (B)	0.00	9(30%)	9(30%)	44.4	0.04 [1] 0.٠3* [2] 0.٠5* [3]	<0.001
Child score (C)	0.00	0.00	21(70%)	11	0.٠2* [2] 0.٠3* [3]	**<0.05**
Fever	0(0%)	0(0%)	9 (30%)	12.6	0.٠4* [2] 0.٠2* [3]	0.013
Abdominal pain	0(0%)	0(0%)	3(10%)	33.4	0.٠1* [2] 0.٠1* [3]	0. 001
Hematemesis/Melena	0(0%)	0(0%)	2(6.6%)	10.7	0.٠5* [2] 0.٠3* [3]	0.001
Jaundice	0(0%)	3(10%)	9(30%)	75.5	0.02 [1] 0.٠1* [2] 0.٠4* [3]	<0. 001
Ascites	0(0%)	0 (0%)	6(20%)	68.8	0.٠5* [2] 0.٠3* [3]	<0. 001
Splenomegaly	0(0%)	5(16.5%)	10(33.4%)	49.6	0.01 [1] 0.٠2* [2] 0.٠4* [3]	<0. 001
Hepatomegaly	0(0%)	5(16.5%)	10(33.4%)	65.5	0.04 [1] 0.٠1* [2] 0.٠3* [3]	<0. 001
Lower limb edema	0(0%)	0(0%)	2(6.6%)	43.4	0.٠2* [2] 0.٠1* [3]	<0. 001
Abdominal tenderness	0(0%)	0(0%)	1(3.3%)	30.7	0.٠1* [2] 0.٠1* [3]	<0. 001
Encephalopathy	0(0%)	0(0%)	1(3.3%)	33.4	0.٠1* [2] 0.٠1* [3]	<0. 001

P1: Group A versus Group B

P2: Group A versus Group C

P3: Group B versus Group C
*: Significant

Table (2): Descriptive and comparative data of laboratory parameters in the different studied groups.

Lab.parameter **[No. (%)]**	**Group A** n=30	**Group B** n=30	**Group C** n=30	Test	LSD	P value
ALT (n=7-31 IU/L)	21.34±6.89	37.75 ±7.19	89.85±32.58	32.3*	0.01* 1 0.٠3* 2 0.٠1* 3	<0. 001
AST (n=7-40 IU/L)	19.1±9.5	61.5±14.02	136.05±46.07	46.2*	0.01* 1 0.٠2* 2 0.٠4* 3	<0. 001
Albumin (n=3.5-5 gm)	4.3±0.41	3.34±0.32	2.86±0.193	106.9*	0.03* 1 0.٠5* 2 0.٠1* 3	<0. 001
T.bil. (n=0.3-1.2 mg/dl)	0.892±0.16	1.01±0.14	1.85±0.369	76.5*	0.01* 1 0.٠5* 2 0.٠3* 3	<0. 001
INR (n=0.8-1.1)	1.01±0.07	1.07±0.12	1.09±0.12	32.4*	0.01* 1 0.٠4* 2 0.٠5* 3	<0. 001
Platelet count (n=150-400 X10 /L)	335.18±38	267.75±89.49	118±43.65	64.4*	0.02* 1 0.٠2* 2 0.٠5* 3	<0. 001
Creatinine (mg/dl) (n=0.7-1.3mg/dl)	0.8 ± 0.1	0.9 ± 0.2	0.9 ± 0.2	32.4*	0.03* 1 0.٠5* 2 0.٠4* 3	<0. 001
Urea (U/L) (n=20-40mg/dl)	19.6 ± 1.8	20.4 ± 1.9	19.8 ± 1.6	64.4*	0.04* 1 0.٠5* 2 0.٠3* 3	<0. 001
Sodium (mmol/L) (n=135-145)	130.5(118-190)	129.5(118-41)	133.5(122-141)	106.9*	0.01* 1 0.٠5* 2 0.٠3* 3	<0. 001
Potassium (n=-3.5-5.5meq/l)	4.5(2.4-7.1)	2.8(1.5-3.5)	3.5(3.1-4.1)	76.5*	0.01* 1 0.٠5* 2 0.٠3* 3	<0. 001
Thrombomodulin (pg/dl)	420.2 ± 102	561.1 ± 198	1231.4± 513	36#	0.01* 1 0.٠5* 2 0.٠3* 3	<0. 001
AFP (ng/ml)	6 (3-11)	10.5 (5 -25)	11.4 (4 -27)	26#	0.01* 1 0.٠5* 2 0.٠3* 3	<0. 001

*ANOVA test, #Kruskal Wallis test
P1: Group A versus Group B
P2: Group A versus Group C
P3: Group B versus Group C
*: Significant

Table (3): Correlation between the plasma level of thrombomodulin and other parameters of groups B & C.			
Variables	**Thrombomodulin**		
	R	**P**	**Sig.**
ALT (n=7-31 IU/L)	0.193	0.009	**S**
AST (n=7-40 IU/L)	0.207	0.048	**S**
Albumin (n=3.5-5 gm)	-0.489	0.030	**S**
T.bil. (n=0.3-1.2 mg/dl)	0.287	0.05	**S**
INR	0.292	0.038	**S**
Child Score	0.225	0.042	**S**
Platelets count	-0.195	0.010	**S**

Ranked Spearman Correlation Test.

Table (4): Performance characteristics of thrombomodulin for discriminating patients of groups A from B & C.						
	Cut-off	**AUC**	**SN%**	**SP%**	**NPV%**	**PPV%**
Thrombomodulin	500.11pg/dl	0.654	92.5%	95%	92.6%	94.4%

SP: specificity, SN: sensitivity, NPV: negative predictive value, PPV: positive predictive value

Table (5): Performance of thrombomodulin in diagnosis of compensated cirrhotic patients among the studied patients.							
Cutoff	**AUC**	**Sensitivity**	**Specificity**	**PPV**	**NPV**	**Accuracy**	**P**
500.11 pg/dl	0.701	77.5	61.5	76.5	64	71.2	0.006*

*p<0.05 is statistically significant

Table (6): Performance of thrombomodulin in diagnosis of decompensated cirrhotic patients among the studied patients.							
Cutoff	**AUC**	**Sensitivity**	**Specificity**	**PPV**	**NPV**	**Accuracy**	**P**
500.11 pg/dl	0.651	67.5	50	67.5	50	60.6	0.039*

*p<0.05 is statistically significant

Table (7): Logistic regression analysis to evaluate factors that affect thrombomodulin level**.**

	Univariate			**Multivariate**		
	OR	**(95% CI)**	**P value**	**OR**	**(95% CI)**	**P value**
Age	1.54	(2.47–3.43)	**0.5***	1	(0.93–2.38)	0.678
ALT	8.45	(0.01–٠.60)	0.280	5.93	(0.26–1.36)	0.24
AST	0.37	(0.30–3.67)	0.168	0.15	(0.09–1.12)	0.52
Albumin	1	(1.80–2.52)	**0.04***	1.16	(5.22–6.26)	0.04*
T.bil.	1.01	(١.58–٢.42)	**0.07***	1.01	(7.31–8.47)	0.06*
INR	1.11	(0.31–0.47)	**0.91**	1.13	(0.81–1.33)	**0.03**
Platelet	3.46	(1.81–4.33)	0.05	1.42	(3.47–4.53)	0.04*
Creatinine	1.12	(0.47–0.53)	0.178	0.16	(4.01–8.17)	0.03*
Urea (U/L)	0.97	(0.09–0.70)	0.161	0.97	(0.30–1.63)	0.12

DISCUSSION

LC is extensively prominent globally and can be the result of various etiology including obesity, heavy alcohol intake, non-alcoholic fatty liver disorder, HBV or HCV infection, autoimmune disorders, and copper or iron accumulation [7].

In LC, broad hepatocytic necrosis, the development of regenerative clusters, architectural alterations of liver lobules, and diffuse hyperplastic connective tissue causing distortion and obliteration, as well as the narrowing of blood vessels by regenerating nodules, promoting damage of endothelial cell. As

endothelial damage has a potential effect in the advancement of LC and portal hypertension, various endothelial damage markers were identified, including soluble P-selectin vWF, sTM, and soluble VE-cadherin [3].

This study is case control study conducted on LC cases. The objectives of of the study were to determine sTM level in LC patients and to study the association between sTM levels and severity of hepatic dysfunction. The study was conducted on 90 cases, the cases divided into three groups (A, B & C). Group A: Normal (as control), Group B: Cirrhotic compensated patients & Group C: Cirrhotic decompensated patients (Thirty subjects in each group).

Regarding the demographic data of included populations, there was non-significant statistical difference concerning age, sex (male and female) distribution, the mean age in groups A, B & C was (55.1±13.87 years, 60.9±8.18 years, 59.3±8.6 years respectively), and smoking as a special habit among studied groups was insignificant (P>0.05).

The current study findings were supported by Jeon et al. [8], who revealed that the mean age in compensated liver disease versus decompensated was (59.8 years vs. 59.9 years), and males (60.1%), females (39.9%) vs. males (59.7%), females (40.3%) respectively. On the other hand, Garrido et al. [9] reported that, non-chronic liver disease group; the mean age was 70.5 years, Smokers 6.93%. Whereas, chronic liver disease group, the mean age was 68 years, smokers 21.4% smokers. There was significant difference between groups concerning gender and smoking. In addition to, Moreau et al. [10] found statistical significance concerning age. This difference might be attributed to the fact that the study of Moreau et al. was carried on many patients.

In the present study, 24 HCV patients were included, 9 patients (30%) in compensated group B and 15 patients (50%) in the decompensated group C. Meanwhile, 9 HBV patients, 6 patients (20%) in compensated group B and 3 patients (10%) in decompensated group. Lastly, 12 patients with mixed (HBV&HCV), 3 (10%) in compensated group B and 9 (30%) in decompensated group C.

We have 15 cirrhotic patients free of viral infection, 12 patients within group B, 3 patients within group C. A highly significant statistical difference were achieved regarding HCV patients in decompensated group C (15 patients) (50%) While, insignificant difference regarding HBV patients in compensated group B (6 patients) (20%) (>0.05) & mixed group (HCV & HBV) in decompensated group C (9 patients) (30%) (>0.05). The Least significant difference (LSD) was higher in group C compared to group B (P < 0.001) (Table 1 & 3).

The present study findings were like some extent with study by Sartorius et al., [11] who had found that HCV was found in 52% of total cases presented with cirrhosis in their study. In addition, HBV was in 16% of cases. Also, Francoz et al. [12] had reported that HCV was found in 59%, HBV in 32% and both together were found in 3% of the cases.

Meanwhile, Butterworth et al., [13] had reported that HBV was found in 28% of the studied population and mixed HCV + HBV was found in 7% of the cases.

Concomitant HCV and S. mansoni infection contributes to a higher incidence of hepatic cirrhosis, hepatocellular carcinoma, and a much higher liver related mortality rate [14]. In developed countries, common causes of cirrhosis include Chronic viral hepatitis (hepatitis B, C), Alcoholic liver disease, Hemochromatosis, Nonalcoholic fatty liver disease [15] & [16] .

Thus, HCV infection was considered the most common cause of cirrhosis in our included patients coming with the explanation of being endemic in our locality. Additionally, the distribution of viral hepatitis whether HCV or HBV or even mixed infection has greatly related to geographic distribution of infected patients with these virus strains.

In the present study, it was noticed that all control individuals got child A score, in group B patients (cirrhotic compensated group); 21 patients got score A and 9 patients got score B, in group C patients (cirrhotic decompensated group); no patients got score A , 21 got score C and 9 got score B. The percentage of patients with score A child is significantly higher in control and compensated groups. While the percentage of patients with score B, C child is significantly higher in decompensated groups. The LSD was significantly high between groups A&B (p< 0.001), A&C (p< 0.001) and B&C (p< 0.05) (Table 1 & 3).

The present study findings agreed with Naga et al., [17] study who reported that cirrhotic compensated patients were maximum in child class B (55.4%) followed by (36%) patients in child class C and, (8.6%) patients in child class A. Cirrhotic decompensated patients were maximum in child class C (60%) followed by (35%) patients in child class C. In contrary to a study done by Goel et al. [18] 48.4% of patients has child score B.

This difference confirms the fact that the incidence of chronicity and severity of liver diseases with varying etiologies might be higher than India and Europe, in addition to large numbers of patients infected with HCV or/and HBV in Egypt [19].

In the present study, the rate of fever, abdominal pain, upper GIT bleeding was in decompensated group C (30 %, 10% and 6.6% respectively)., and significantly higher rate of clinical signs in group C with arrangement of splenomegaly & hepatomegaly (33.4%), jaundice (30%), ascites (20%), lower limb edema (6.6%), abdominal tenderness (3.3%) and lastly encephalopathy (3.3%) (table 1) So, splenomegaly, hepatomegaly, jaundice & ascites were the most common signs in decompensated cirrhotic group C. However, Aberg et al., [20] who reported that, in total of 171 patient jaundice was the most common sign (n=118, (69%)) followed by ascites (n=108, (63.15%)), altered conscious level (n=56,(32.74%)) and melena (n=51,(29.82%)). Moreover, Wei et al., [21] showed that the most prevalent clinical sign was icterus in 88 patients (29%), followed by lower limb edema in 61 patients (20%).

In contrast to Moreau et al. [10] who reported that ascites was the most clinical finding (63%), followed by splenomegaly (25.14%) and hepatomegaly (14.6%). Again, the differences may be due to factors concerning etiology, ethnicity, geographical and social factors.

A highly significant difference between the three studied groups (A, B & C) regarding laboratory parameters such as, ALT (21.34±6.89, 37.75 ±7.19 vs. 89.85±32.58 respectively), AST (19.1±9.5, 61.5±14.02 vs. 136.05±46.07 respectively), serum bilirubin (0.892±0.16,1.01±0.14 vs.1.85±0.369 respectively), serum albumin (4.3±0.41, 3.34±0.32 vs.2.86±0.193 respectively), INR (1.01±0.07, 1.07±0.12 vs. 1.09±0.12 respectively) and platelets count (335.18±38, 267.75±89.49 vs. 118±43.65). High significant differences between groups A & B, A & C, and B & C ($p< 0.001$) (Table 3).

In the present study, it was noticed that higher levels of ALT, AST, serum bilirubin, INR in decompensated cirrhotic than compensated group, in contrast to, serum albumin and platelets count that had lower levels in the same decompensated group than compensated group.

In accordance with Aberg et al. [20] who assumed that decompensated group was found to have higher levels of WBC, total serum bilirubin, ALT, AST, creatinine and INR. Also had lower levels of platelets, serum albumin and sodium levels than the compensated group.

The plasma level of Thrombomodulin (sTM) (pg/dl) was measured in different groups in our study. A statistically significant increment in sTM levels in decompensated group C (1231.4±513) compared to control group A and compensated group B (420.2±102) (561.1±198) respectively ($p<0.05$) & LSD ($p< 0.001$) in (Table 2 & 3).

Wei et al. [21] found that all fragments of sTM, increased in acute liver failure patients. sTM, on the other hand, remained stable in cirrhosis patients, whereas the ratio of smaller sTM to larger sTM decreased. In chronic hepatitis and early cirrhosis, the plasma sTM maintained similar to healthy controls. This explains why TM levels in cirrhotic compensated group B came near to that obtained from control group A

In agreement with the result of Wei et al. [21] in their prospective single-center study, patients had followed up every 6 months until transplantation or death. They discovered that, among 219 cases with decompensated LC, after controlling for sex, age, bilirubin, and other possible variables, plasma sTM rise causes an 8% rise in death. They noticed that plasma sTM levels in LC elevated dramatically in proportion to liver impairment severity. sTM levels greater than 18 TU/ml predicted a bad prognosis for decompensated LC.

Several studies verified that sTM was a sensitive marker of endothelial cell injury. Boehme et al., [22] reported that sTM rapidly elevated with induction of endothelial injury. So, the researchers believed that wounded endothelium cells released sTM rather than produced it, and that it may be employed as both an early and advanced stage marker.

sTM is also a marker of LSECs injury. In a previous study, sTM and the expression of sinusoidal TM increased during acute liver injury induced by D-galactosamine, especially in the necrotic area and around the central vein, suggesting that sTM was related to endothelial injury and parenchymal necrosis [23].

Performance characteristics of thrombomodulin for discriminating patients of groups A from B & C in the present study: thrombomodulin with Cut-off 500.11 pg/dl can discriminate patients of groups A from B & C with AUC 0.654, sensitivity 92.5%, specificity 95%, PPV 94.4% and NPV 92.6% The power of receiver operating characteristic (ROC) test was statistically higher in diagnosis of compensated cirrhotic patient (p=0.006) than

decompensated group (p=0.039). So, it is useful test in predicting cirrhosis higher than the severity of liver dysfunction.

sTM ought to function as a marker for portal hypertension. But ascites volume was the only parameter that sTM was shown to be associated with; while hepatic encephalopathy, platelet count, and esophageal gastric variceal hemorrhages were not. Since hepatocytes eliminate sTM, the current study anticipated liver function would also impact sTM concentrations. The connection between sTM and creatinine was another finding from the study. Additionally, the kidney eliminated sTM, supposing that the clearance rates by the kidney and liver significantly influenced the levels of sTM. They concluded that sTM, rather than portal hypertension, was a predictor of survival mainly because of its relationship to liver function [21].

La Mura et al., [24] also found that TM immune reactivity was almost totally lost in endothelial cells 24 h after LPS injection, thus further confirming the involvement of the endothelium in the pathogenesis of liver damage induced by endotoxemia.

Other research also demonstrated that tumor necrosis factor and other cytokines also aggravate vascular endothelial injury in liver cirrhosis, which can lead to the continuous increase of sTM level. Thus, inflammation may damage endothelial cells, thus resulting in an increase in sTM levels [25].

TM expressions in hepatic endothelial cells are highly affected in liver diseases like viral hepatitis and liver damage which also cause sTM release. Overall, liver enzymes could be modulators of sTM and sTM levels as well. The increase in plasma sTM levels in liver disease may be due to defective hepatic degradation of the circulating sTM. Elevated sTM levels reflect endothelial injury and can contribute to the overall understanding of the severity of liver cirrhosis. Several studies had previously determined sTM in various liver diseases, but the results were inconsistent. In a case–control study, sTM was elevated in hepatocellular carcinoma patients (42.1 ± 2.0 ng/ml) than in cirrhosis patients (28.3 ± 2.1 ng/ml; P = 0.039), and sTM level did not relate to the outcome of cirrhosis individuals [21].

The study's strengths include being one of the updated studies to investigate thrombomodulin level in plasma of patients with compensated and decompensated liver cirrhosis at Zagazig University Hospitals. The investigations inside the lab were carried out by one person. There was a high selectivity of cases, and their samples were collected and stored very carefully.

Limitations of this study include a small sample size (total of 90 subjects); and its limited follow-up duration, which limited the study's ability to generalize to longer postoperative outcomes.

Author contribution: AAHE for collected patients' samples and clinical data from outpatient clinic and hospitalized patients of Internal Medicine Department, Zagazig University Hospitals, Zagazig, Egypt, and prepared sample for laboratory investigations. All laboratory investigations were supervised by SEI in Clinical Pathology Department. Statistical analysis, interpretation of data, and writing the manuscript were done by AAHE. Critical revision of the manuscript was performed by SAA & MAMA. All authors have read and approved the final manuscript.

CONCLUSIONS

Early cirrhosis has the potential to regress as liver fibrosis is a dynamic condition. With the advent of effective non-invasive tools for detecting hepatic fibrosis, more and more patients with CLC are currently being recognized. Plasma sTM level in LC cases was significantly elevated in parallel with the severity of hepatic impairment. The sTM level could have a role in early prediction of decompensated liver cirrhosis. Thus, further studies are needed on large scale and large numbers of patients in the future to make a focus on predicting early cirrhosis that could be reversible and treated.

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List of abbreviations

ALP : Alpha fetoprotein, ALP : alkaline phosphatase, ALT : Alanine Aminotransferase, AST : Aspartate aminotransferase, AUC : Area under the curve, CBC : Complete blood count, CHD : Coronary heart disease,CLC : Compensated liver cirrhosis, EGF : Epidermal growth factor, GIT : Gastro-intestinal tract, INR : International normalized ratio, IRB : Institutional review board, LC : Liver cirrhosis, LPS : Lipopolysaccharide, LSD : Least significant difference, LSECs : Liver sinusoidal endothelial cells, NPV : Negative predictive value, OR : Odds ratio, PPV : Positive predictive value, PT : Prothrombin time, PTT : Partial thromboplastin time, ROC : Receiver operating characteristic, SN : Sensitivity, SP : Specificity, sTM : Soluble thrombomodulin, VEC :

Various endothelial cells, vWF : Von Willebrand factor, X2 : Chi-square.

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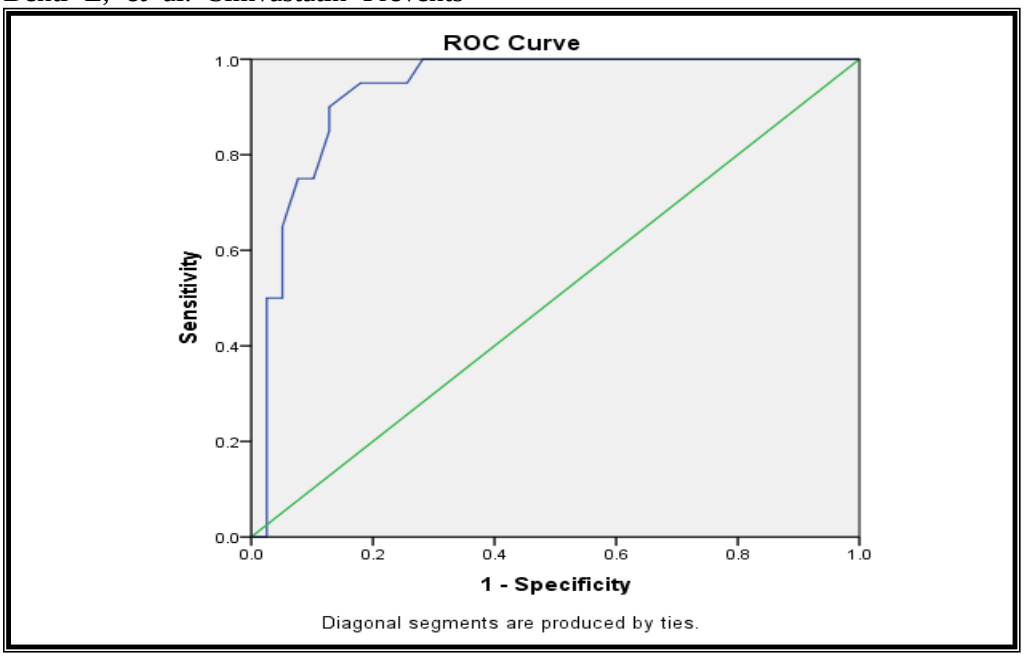

Figure (S 1): Receiver operating characteristic (ROC) curve of thrombomodulin with cut of (<500.11ng/ml) in discriminating patients of groups A from B & C.

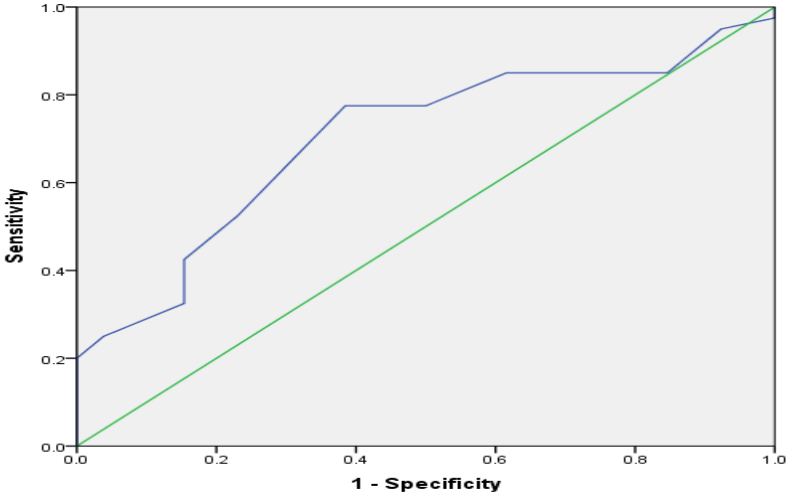

Figure (S 2): ROC curve showing performance of thrombomodulin in diagnosis of compensated cirrhotic patients among the studied patients

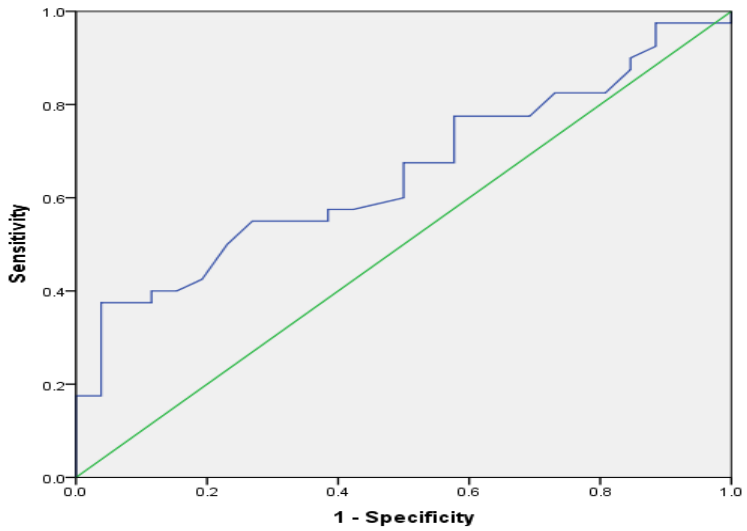

Figure (S 3): ROC curve showing performance of thrombomodulin in diagnosis of decompensated cirrhotic patients among the studied patients

Citation

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