Plasma Von Willebrand Factor Level and Hepatic Microvascular Thrombosis in Acute-on-Chronic Liver Failure Patients

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ABSTRACT

Background: Acute-on-chronic liver failure (ACLF) is associated with features of systemic inflammation. Plasma VWF levels are increased 5:7 fold in patients with ACLF. The imbalance between VWF and ADAMTS13 in sepsis/inflammatory conditions can be a predisposition to platelet microthrombi and an impedance to vital organ microcirculation. **Objective:** to assess the possibility of hepatic microvascular thrombosis in patients with acute on chronic liver failure and its relationship with plasma Von Willebrand Factor level in such cases. **Patients and methods:** This study was carried out on patients in the Gatroenterology and Hepatology Unit of Internal medicine department in Zagazig University Hospitals. A total number of 90 individuals they were divided equally into group (I) ACLF patients; group (II) chronic compensated hepatic patients; and group (III) healthy control. All patients were submitted to full history taking and physical examination, specific investigations included plasma Von Willebrand Factor and D-dimer. **Results:** There was a significant difference between the studied groups regarding hemoglobin, albumin, platelet count, and D dimer. A significant difference between the studied groups regarding WBCs, direct bilirubin, total bilirubin, total protein, ALT, AST, INR. on comparing each two individual groups, the difference is significant between patients with ACLF and each other group. Regarding VWF, the difference is significant between ACLF patients with each two individual groups. There was a significant difference between the studied groups regarding RI, flow volume, and portal vein diameter, the difference is significant between patients within each two individual groups. Among factors significantly correlated to VWF, only D dimer and flow volume significantly independently associated with it in patients with ACLF. There was a significant correlation between degree of ascites, direction of blood flow and VWF among patients of ACLF. Positive correlation between VWF and all of ALT, AST, INR, D dimer, and portal vein diameter among patients with compensated CLD. A significant relation between VWF and both presence of splenomegaly and cirrhosis among patients with compensated CLD. A negative correlation between VWF and both hemoglobin and platelet count among patients with compensated CLD. **Conclusion** Von Willebrand Factor (VWF) is increased in patients with chronic liver diseases and correlates positively with severity of liver impairment.

Keywords: Liver Failure; Microvascular Thrombosis; Von Willebrand Factor Level

INTRODUCTION

Acute decompensation of chronic liver disease associated with organ failures and high short-term mortality is known as acute-on-chronic liver failure (ACLF) **(1)**. The initial consensus on ACLF was supplied by the Asian Pacific Association for the Study of

the Liver (APASL), which described it as an acute hepatic insult manifesting as jaundice and coagulopathy, complicated within 4 weeks by ascites and/or encephalopathy. 'High 28-day mortality' was added to the definition as it was subsequently enlarged **(2)**.

According to researchers with the EASL-CLIF consortium, acute decompensation is the sudden onset of hepatic encephalopathy, massive ascites, bacterial infections, gastrointestinal haemorrhage, or any combination of these. They also identified diagnostic thresholds for extrahepatic organ failure and divided individuals with ACLF into 4 subgroups **(3).**

The World Gastroenterology Organization's updated working definition of ACLF refers to it as "a syndrome in patients with CLD, with or without previously diagnosed cirrhosis, which is characterised by acute hepatic decompensation leading to liver failure, jaundice, prolongation of the INR, and one or more extrahepatic organ failures, which is associated with increased mortality within a period of 28 days and up to 3 months from onset." Thus, individuals who have chronic hepatitis, compensated cirrhosis, or cirrhosis with a history of decompensation are included in this group of individuals **(4).**

In the remaining patients, sepsis, active drinking, and return of chronic viral hepatitis are the most frequently reported precipitating events. Up to 40% to 50% of cases of ACLF have no known trigger. Overactive systemic inflammation appears to be a major factor in the emergence of ACLF **(1)**.

Changes in hemostasis in both procoagulant and anticoagulant chemicals are significantly more obvious in severe chronic liver disease. Processes related to coagulation, fibrinolysis, and platelet function change **(5).** Not all haemostatic changes in hepatopathy patients favour bleeding. According to several studies, the plasma of cirrhotic patients is unbalanced in favour of procoagulant chemical compoundS **(6).**

As liver disease progresses more severely, portal vein thrombosis becomes increasingly common.Up to 25% of liver transplant candidates and 1% of patients with compensated cirrhosis experience this. The only indicator of portal venous thrombosis is a decrease in the speed and velocity of the portal flow **(7).**

One of the most effective catalysts for the production of thrombin is factor VIII. High amounts of VWF, which make factor VIII interact with plasma and prevent it from being degraded by proteases, mediate reduction in the purification of factor VIII **(5)**.

Platelets adhere to Von Willebrand factor (VWF), a protein that is produced in very high molecular weight forms from active endothelium **(8).** The imbalance between VWF and ADAMTS13 (a VWF-cleaving protease), which occurs in patients with cirrhosis (of various aetiologies, including viral and alcohol), acute liver failure, and systemic inflammation, may be the pathogenic mechanism of hepatic microvascular thrombosis **(9)**. VWF levels are correlated with the degree of hepatic impairment, the gradient of the hepatic vein pressure, and patient survival **(10).** This study aims to evaluate the possibility of developing hepatic microcirculatory thrombosis in patients with ACLF and its correlation with VWF levels in these cases.

PATIENTS AND METHODS

This case control study was carried out on patients in the Gatroenterology and Hepatology Unit of Internal medicine department in Zagazig University Hospitals during

2019-2020. The study included a total number of 90 individuals they were divided into 3 groups (30 individuals in each group):

Group I: Acute on chronic liver failure (ACLF) patients group.

Group II: Chronic compensated hepatic group.

Group III: Healthy control group.

Ethical Consideration:

The scientific and ethical committee at Zagazig University approved the project. All of the subjects' written informed permission was acquired. The Declaration of Helsinki, the World Medical Association's code of ethics for studies involving humans, guided the conduct of this work.

Inclusion criteria:

Subjects included in this study were within age group >18 years old, of both sex, they were divided into 3 groups:

Group (1) (ACLF patients): patients diagnosed with chronic hepatitis, based on laboratory, ultrasonographic,or histological criteria (if available) who developed new-onset jaundice, encephalopathy, and/or ascites with elevated international normalisation ratio (INR) within few days before enrolling in the study. All had regular follow-up in the Hepatology Clinic in Zagazig University Hospital with compensated clinical course in the past 3 months.

Group (2): patients with compensated chronic hepatitis.

Group (3): normal individuals that act as control group.

Exclusion criteria:

Patients with hepatocellular carcinoma, portal vein thrombosis, deep venous thrombosis, recent esophageal variceal bleeding or injected varices, previously treated by anticoagulants, and evidence of disseminated intravascular coagulation were excluded.

Steps of performance and techniques used:

All patients were submitted to full history taking and physical examination.

Laboratory and radiological investigations included in this study were subjected to:

(A) Routine investigations in the form

Complete blood count, liver and kidney function tests, coagulation profile (PT, PTT, INR), and viral markers (HBsAg, HBcAb, HCV ab ,HAVIgM).

(B) Special investigations in the form of:

1- Plasma von willebrand factor level

2- D-dimer

(C) Radiological investigations:

1- Doppler sonography of portal vein for detection of hepatic microvascular thrombosis.

2- Pelvi-abdominal ultrasound.

3- Triphasic CT abdomen for group (1) to diagnose and exclude hepatocellular carcinoma.

Estimation of Von Willebrand Factor:

Measuring the concentration of human von Willebrand factor (vWF) in samples using an enzyme-linked immunosorbent test (ELISA) with a double-antibody sandwich. Von Willebrand Factor (vWF) was added to a monoclonal antibody-coated enzyme well after it had been pre-coated with a human von Willebrand Factor (vWF) monoclonal antibody. Next, von Willebrand Factor (vWF) antibodies were labelled with biotin and combined with streptavidin-HRP to form an immune complex. Finally, incubation and washing were repeated to remove the uncombined enzyme. After adding Chromogen Solutions A and B, the liquid's colour changed to blue. Under the influence of acid, the colour eventually turned yellow. The concentration of the human substance von Willebrand Factor (vWF) in the sample and the colour chroma had a positive correlation.

Statistical analysis:

SPSS (Statistical Package for the Social Sciences) version 26 was used for data analysis. The chi square test, fisher exact test, monte carlo tests, Shapiro-Wilk, Spearman rank correlation coefficients, Kruskal-Wallis, one-way ANOVA, and independents sample t test were used. The means, standard deviations, or median and range of quantitative variables were used. Pairwise comparison and LSD comparison were used. The associated independent factors for the dependent factor were measured using a ROC curve and a linear regression analysis. The level statistical significance was set at $P<0.05$. Highly significant difference was present if $p\leq0.001$.

RESULTS

There is statistically significant difference between the studied groups regarding age. On LSD comparison, the difference is significant between patients with ACLF and control groups. There is statistically non-significant difference between the studied groups regarding gender **(Table 1)**.

There is statistically significant difference between the studied groups regarding liver parenchyma, splenomegaly, ascites and flow direction. Four patients within group II had mild ascites. Within group I, 60% had moderate ascites while 40% had tense ascites. All patients within group II had hepatopetal flow versus 20% within group I. All patients within group I were cirrhotic and had splenomegaly versus 80% and 63.3% within group II respectively.On the other hand, there is non-significant relation between groups regarding virology **(Table 2)**.

Within patients with ACLF, 6.7%, 36.7%, 36.7% and 20% had grades 1,2,3 and 4 respectively **(Figure 1)**.

There is statistically significant difference between the studied groups regarding hemoglobin, albumin, platelet count, and D dimer. On comparing each two individual groups, the difference is significant between patients with ACLF and each other group. There is statistically significant difference between the studied groups regarding serum creatinine. On LSD comparison, the difference is significant between control group and each other group. There is statistically significant difference between the studied groups regarding WBCs, direct bilirubin, total bilirubin, total protein, ALT, AST, INR. on comparing each two individual groups, the difference is significant between patients with ACLF and each other group **(Table 3)**.

There is statistically significant difference between the studied groups regarding VWF. On pairwise comparison, the difference is significant between ACLF patients with each two individual groups **(Figure 2)**.

There is statistically significant difference between the studied groups regarding RI, flow volume, and diameter. On pairwise comparison, the difference is significant between patients within each two individual groups.There is statistically significant difference between the studied groups regarding PI. On pairwise comparison, the difference is significant between patients with ACLF and each other group **(Table 4)**.

There is statistically significant positive correlation between VWF and serum albumin, total protein, ALT, AST, INR, D dimer, RI, PI, diameter, and grades of hepatic encephalopathy.There is statistically significant negative correlation between VWF and platelet count and flow volume.There is statistically non-significant correlation between VWF and either hemoglobin, age, total protein, creatinine or white blood cells **(Table 5, Figure 3)**.

Among factors significantly correlated to VWF, only D dimer (unstandardized β=0.077) and flow volume (unstandardized β=-0.001) significantly independently associated with it **(Table 6)**. There is statistically significant correlation between degree of ascites, direction of blood flow and VWF among patients of ACLF **(Figure 4)**.

The best cutoff of VWF in diagnosis of reverse direction (hepatofugal flow) is ≥0.5525, with area under curve 0.992, sensitivity 95.8%, specificity 95.5%, positive predictive value 88.5%, negative predictive value 98.4%, and overall accuracy 95.6% **(Figure 5)**.

Table (1) Comparison between studied groups regarding demographic data:

	Group I	**Group II**	**Group III**	**F**	**p**
	Mean ± SD	**Mean ± SD**	**Mean ± SD**		
Age (year)	**60.87 ± 5.3**	**56.77 ± 7.33**	**43.47 ± 13.49**	**28.236**	**<0.001****
LSD	**P_1 0.094**	**P_2 0.077**	**P_3 <0.001****		
Sex:					
Female	**11 (36.7%)**	**10 (33.3%)**	**11 (36.7%)**	**0.097**	**0.953**
Male	**19 (63.3%)**	**20 (66.7%)**	**19 (63.3%)**		

*F One way ANOVA test χ^2 Chi square test **p≤0.001 is statistically highly significant p1 difference between group I and group II p2 difference between group II and III p3 difference between group I and III LSD Least significant difference*

Table (2) Comparison between studied groups regarding disease-specific data:

	Group I	**Group II**	**χ^2**	**p**
	N=30(%)	**N=30(%)**		
Virology:				
HBV	6 (20%)	4 (13.3%)	0.48	0.488
HCV	24 (80%)	26 (86.7%)		
Cirrhotic	30 (100%)	26 (80%)	Fisher	0.024*
Splenomegaly	30 (100%)	19 (63.3%)	13.469	<0.001**
Ascites:				
No	0 (0%)	26 (86.7%)		
Mild	0 (0%)	4 (13.3%)	51.827	<0.001**
Moderate	18 (60%)	0 (0%)		
Tense	12 (40%)	0 (0%)		
Flow direction:				
Hepatofugal	24 (80%)	0 (0%)	40	<0.001**

Hepatopetal	6 (20%)	30 (100%)		

*χ^2 Chi square test **p≤0.001 is statistically highly significant *p<0.05 is statistically significant*

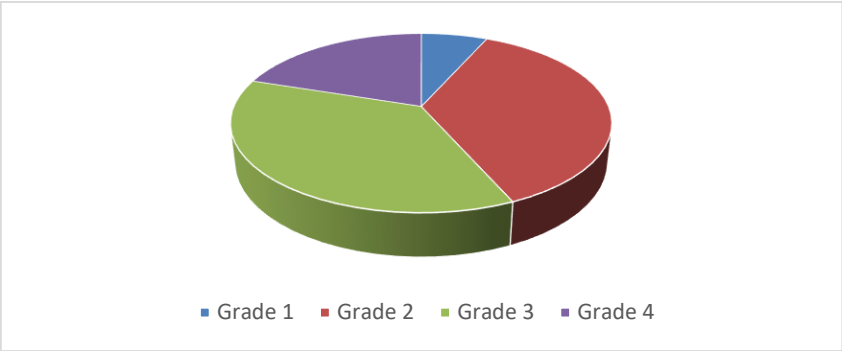

Figure (1) Pie chart showing distribution of patients with ACLF

Table (3) Comparison between studied groups regarding laboratory data:

	Group I	**Group II**	**Group III**	**F**	**p**
	Mean ± SD	**Mean ± SD**	**Mean ± SD**		
Hemoglobin	9.54 ± 1.6	10.99 ± 2.3	13.09 ± 0.94	32.851	<0.001**
LSD	P_1 0.001**	P_2 <0.001**	P_3 <0.001**		
Albumin	2.23 ± 0.3	3.38 ± 0.38	3.79 ± 0.23	205.876	<0.001**
LSD	P_1 <0.001**	P_2 <0.001**	P_3 <0.001**		
T protein	5.76 ± 0.7	6.29 ± 0.55	6.46 ± 0.5	11.475	<0.001**
LSD	P_1 <0.001**	P_2 0.268	P_3 <0.001**		
Creatinine	1.0 ± 0.29	1.09 ± 0.38	0.74 ± 0.19	11.37	<0.001**
LSD	P_1 0.206	P_2 0.001**	P_3 <0.001**		
INR	3.09 ± 1.02	1.09 ± 0.15	0.98 ± 0.08	119.651	<0.001**
LSD	P_1 <0.001**	P_2 0.478	P_3 <0.001**		
	Median (IQR)	**Median (IQR)**	**Median (IQR)**	**KW**	**p**
WBCs	10.5(8.28 – 12.23)	8(6.08 – 10)	7.8(6.48 – 9.13)	12.189	0.002*
Pairwise	P_1 0.007*	P_2 0.578	P_3 <0.001**		
Platelet	83.5(60.5 – 97.75)	193.5(144.25–255)	279.5(241. –318.75)	64.479	<0.001**
Pairwise	P_1 <0.001**	P_2 0.01*	P_3 <0.001**		
Total bilirubin	8.4(5.23 – 13.73)	1(0.54 – 1.23)	0.73(0.65 – 0.84)	62.527	<0.001**
Pairwise	P_1 <0.001**	P_2 0.077	P_3 <0.001**		
D bilirubin	6.6(4.15 – 10.23)	0.4(0.2 – 0.8)	0.5(0.4 – 0.62)	59.73	<0.001**
Pairwise	P_1 <0.001**	P_2 0.553	P_3 <0.001**		
ALT	105.5(87.5 – 166)	39(18.75–46.25)	33(29.75 – 38.25)	56.617	<0.001**
Pairwise	P_1 <0.001**	P_2 0.336	P_3 <0.001**		
AST	176(129.25 –225)	32(24.25 – 45)	29.5(27 – 34)	59.371	<0.001**
Pairwise	P_1 <0.001**	P_2 0.556	P_3 <0.001**		
D dimer	4.25(3.58 – 6.43)	0.61(0.26 – 0.35)	0.3(0.26 – 0.35)	76.398	<0.001**
Pairwise	P_1 <0.001**	P_2 <0.001**	P_3 <0.001**		

*F One way ANOVA KW Kruskal Wallis test **p≤0.001 is statistically highly significant *p<0.05 is statistically significant p1 difference between group I and group II p2 difference between group II and III p3 difference between group I and III LSD Least significant difference*

There is statistically significant positive correlation between VWF and all of ALT, AST, INR, D dimer, and diameter.There is statistically significant negative correlation between VWF and both hemoglobin and platelet count.There is statistically non-significant correlation between VWF and either hemoglobin, age, albumin, INR, total, direct bilirubin, total protein, creatinine, PI, RI or white blood cells **(Table 7)**.

There is statistically significant relation between VWF and both presence of splenomegaly and cirrhosis (VWF was significantly higher with cirrhosis and splenomegaly). There is statistically non-significant relation between VWF and either virology or presence of ascites **(Table 8)**.

A case of female patient 50 years old, known chronic compensated liver disease, admitted to ICU by deterioration of conscious level, yellowish discoloration of sclera. The radiological examination was illustrated in **Figure (6)**.

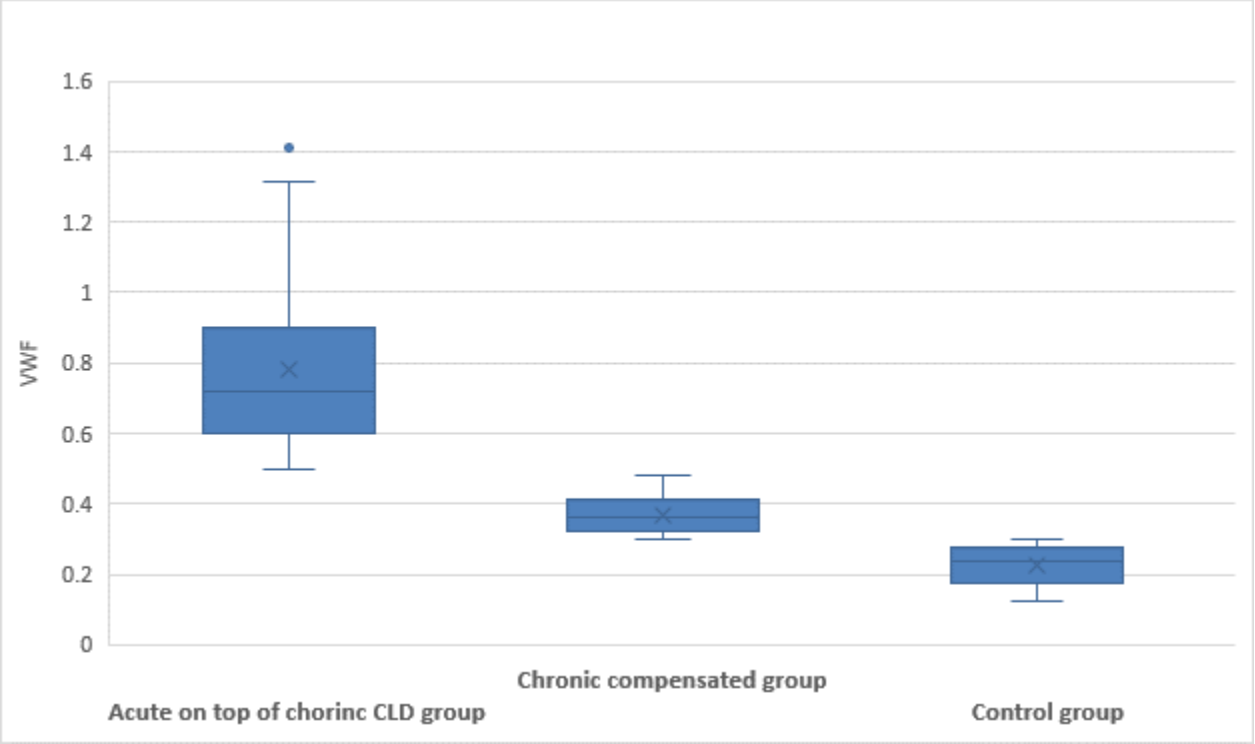

Figure (2) Boxplot showing comparison between the studied groups regarding VWF

Table (4) Comparison between studied groups regarding Doppler data:

	Group I	Group II	Group III	KW	p
	Median (IQR)	Median (IQR)	Median (IQR)		
RI	0.93(0.87 – 0.97)	0.36(0.33 – 0.37)	0	82.238	<0.001**
Pairwise	P_1 <0.001**	P_2 <0.001**	P_3 <0.001**		
PI	5.36(5.08 – 5.74)	3.81(3.35 – 4.24)	3.5(3.26 – 3.79)	43.426	<0.001**
Pairwise	P_1 <0.001**	P_2 0.261	P_3 <0.001**		
Diameter	11.15(10.77–11.33)	8.15(7.68 – 8.6)	7.4(7.2 – 7.8)	70.705	<0.001**
Pairwise	P_1 <0.001**	P_2 <0.001**	P_3 <0.001**		
Flow volume (ml/min)	419.76(343.1–487.2)	809.91(788.9–850.1)	947.78(892.1–984.7)	73.372	<0.001**
Pairwise	P_1 <0.001**	P_2 <0.001**	P_3 <0.001**		

*KW Kruskal Wallis test **p≤0.001 is statistically highly significant p1 difference between group I and group II p2 difference* between group II and III p3 difference between group I and III

Table (5) Correlation between VWF and the studied laboratory data of patients with ACLF:

Age (year)	-0.105	0.582
Albumin (g/dl)	0.413	**0.023***
Protein (g/dl)	0.11	0.561
T bilirubin (mg/dl)	0.776	**<0.001****

D bilirubin (mg/dl)	0.714	**<0.001****
ALT (U/L)	0.73	**<0.001****
AST (U/L)	0.821	**<0.001****
Creatinine (mg/dl)	0.163	0.389
INR	0.594	**<0.001****
WBCs	0.28	0.133
Hemoglobin (g/dl)	0.215	0.255
Platelet count	-0.409	**0.025***
D dimer	0.988	**<0.001****
Flow volume (ml/minute)	-1	**<0.001****
Diameter	0.731	**<0.001****
Grades of HE	0.591	**<0.001****

*r Spearman rank correlation coefficient *p<0.05 is statistically significant **p≤0.001 is statistically highly significant*

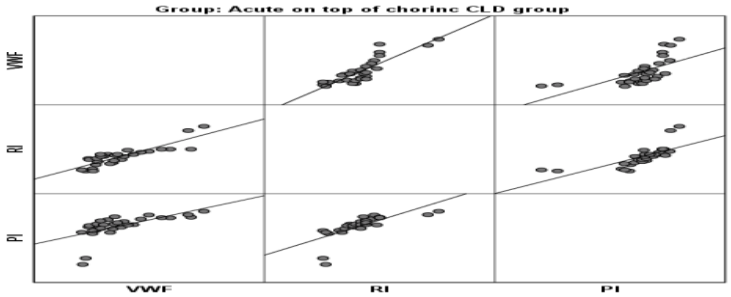

Figure (3) Scatter matrix showing significant positive correlation between VWF and both PI and RI among patients with ACLF

Table (6) Linear stepwise regression analysis of factors significantly associated with VWF among patients with ACLF:

	Unstandardized Coefficients		Standardized Coefficients			95.0% Confidence Interval	
	β	Std. Error	Beta	t	P	Lower	Upper
(Constant)	0.673	0.114		5.903	<0.001**	0.439	0.907
D dimer	0.077	0.006	0.778	12.099	<0.001**	0.064	0.090
Flow volume (ml/min)	-0.001	0.000	-0.230	-3.580	0.001**	-0.001	0.000

***p≤0.001 is statistically highly significant*

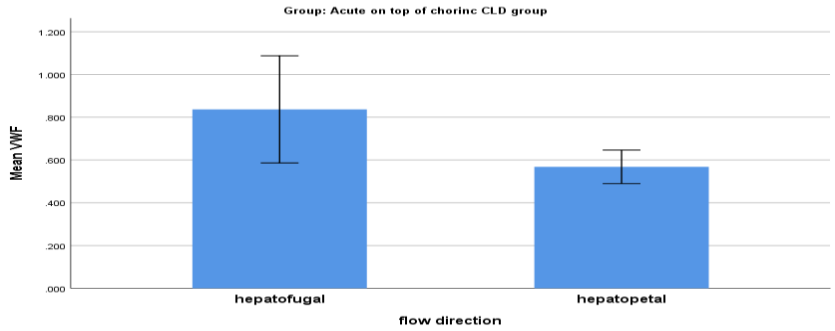

Figure (4) Simple bar chart showing relation between VWF and direction of flow in patients with ACLF

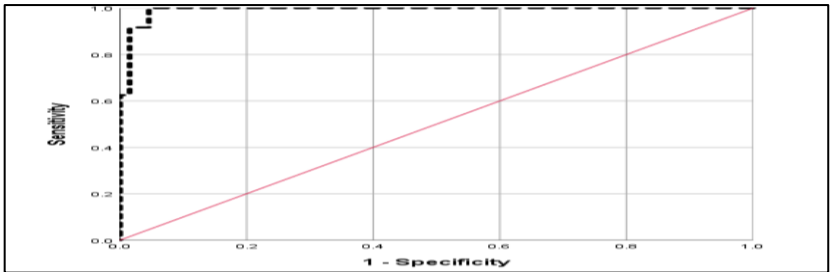

Figure (5) ROC curve showing performance of VWF in prediction of hepatofugal flow

Table (7) Correlation between VWF and the studied laboratory data of patients with compensated CLD:

Age (year)	0.202	0.284
Albumin (g/dl)	-0.102	0.59
Protein (g/dl)	-0.188	0.319
T bilirubin (mg/dl)	0.223	0.216
D bilirubin (mg/dl)	0.222	0.238
ALT (U/L)	**0.385**	**0.036***
AST (U/L)	**0.409**	**0.025***
Creatinine (mg/dl)	0.327	0.078
INR	**0.379**	**0.039***
WBCs	-0.243	0.197
Hemoglobin (g/dl)	**-0.409**	**0.025***
Platelet count	**-0.445**	**0.015***
D dimer	**0.993**	**<0.001****
RI	0.113	0.552
PI	0.139	0.463
Flow volume (ml/minute)	-0.136	0.472
Diameter	**0.486**	**0.007***

*r Spearman rank correlation coefficient *p<0.05 is statistically significant **p≤0.001 is statistically highly significant*

Table (17) Relation between VWF and disease-specific data among patients with compensated CLD:

	Mean ± SD	**t/F**	**p**
Virology:			
B	**0.39 ± 0.05**	**1.125**	**0.27**
C	**0.36 ± 0.05**		
Ascites:			
Absent	**0.36 ± 0.05**	**-0.999**	**0.326**
Mild	**0.39 ± 0.05**		
Spleen			
Normal	**0.34 ± 0.03**	**-2.303**	**0.029***
Splenomegaly	**0.38 ± 0.06**		
Liver:			
Normal	**0.33 ± 0.03**	**-2.702**	**0.015***
Cirrhotic	**0.38 ± 0.06**		

*t independent sample t test *p<0.05 is statistically significant **p≤0.001 is statistically highly significant*

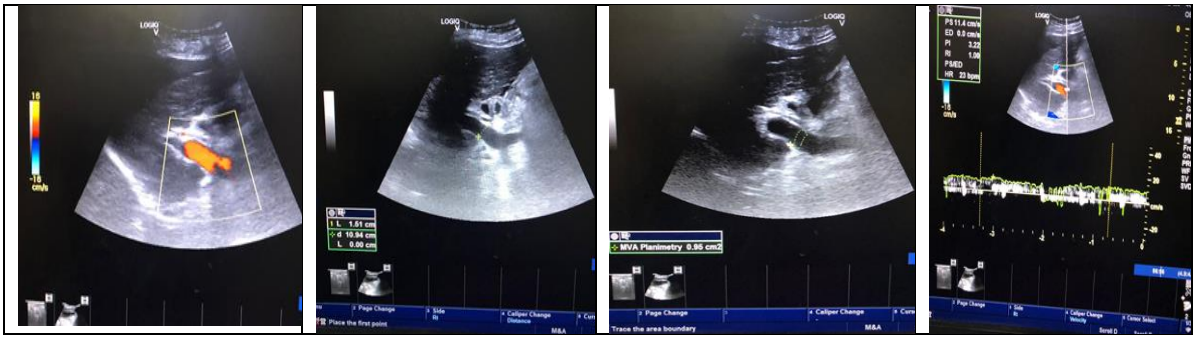

Figure (6): Radiological finding of female patient 50 years old, known chronic compensated liver disease, admitted to ICU by deterioration of conscious level, yellowish discoloration of sclera.

DISCUSSION:

Acute-on-chronic liver failure (ACLF) is a terms which is becoming more widely accepted to describe an acute decline in liver function in cirrhotic patients, either as a result of a superimposed liver injury or as a result of extrahepatic precipitating factors like infection, which results in end-organ dysfunction. Uncontrolled inflammation is believed to be a major contributing factor to the development of ACLF, despite the fact that the specific pathophysiology of this condition is yet unknown **(11).**

According to a study utilising NACELD criteria, the 30-day death rate for infected decompensated cirrhosis without advanced cardiac life failure (ACLF) was 8%, and it rose to 77% in patients with ACLF with four organ failure **(12).**

In our study, there is statistically significant difference between the studied groups regarding direct bilirubin, total bilirubin, total protein, ALT, AST, INR. On comparing each two individual groups, the difference is significant between patients with ACLF and each other group, this reflects the severe deterioration of liver functions in ACLF patients.

These results are in agreement with a study carried out by **Edoardo et al.** who reported that biochemical changes in one of the two different hepatic systems or in liver function are frequently indicative of hepatic disease. Although liver function tests are frequently used to measure the amount of serum liver enzymes, these tests actually represent more hepatocyte integrity or cholestasis than hepatic activity. Prothrombin time or serum albumin changes are frequently linked to a decrease in hepatic mass, which is supported by an elevated INR level.

Auto anticoagulation has always been linked to chronic liver disease, but the coagulation changes associated with this illness appear to be more nuanced. According to recent statistics, persons with this condition have a 0.5 to 1.9% rate of venous thromboembolism **(13).**

Coagulation may be impacted by any disorder that compromises liver health. Alterations in hemostasis in both pro-coagulant and anticoagulant chemicals are significantly more noticeable in advanced chronic liver disease. Processes related to coagulation, fibrinolysis, and platelet function change **(5).**

Prolonged partial thromboplastin time (PTT), extended prothrombin time (PT), thrombocytopenia, and reduced fibrinogen are the primary changes in patients with chronic hepatopathy that are discovered in laboratories **(14).**

According to some researches, the plasma of cirrhotic patients has an unfavourable balance in favour of procoagulant chemicals. Patients with cirrhosis produce more thrombin than healthy people do, and thrombomodulin, a potent anticoagulant, is resistant in these patients. **(6).** This explains frequent thrombosis in the portal venous system in acute on chronic liver failure patients.

In general, procoagulant factors decline in individuals with chronic liver disorders, with the exception of factor VIII and von Willebrand factor (VWF). Natural anticoagulants such ATIII and protein C are decreasing at the same time. Factor VII declines first due to its short lifespan. Its blood levels are inversely related to the severity of cirrhosis. Factors II, V, and X are likewise decreased in acute hepatopathy, while factors IX and XI are also lost in chronic hepatopathy. Although fibrinogen levels are normal in stable liver disease, they fall as the severity of the hepatopathy increases **(15).**

It is not surprising to suppose that isolated PT measurement is inadequate for measuring the coagulation state of patients with chronic liver illness given these changes in the hemostasis of these individuals. Although thrombomodulin and protein C activation are also altered in these patients, thrombomodulin's role and its relationship to procoagulant factors are not taken into account by PT, which only assesses the time needed for thrombin generation. As a result, even in the presence of thrombomodulin, thrombin can still develop **(5).**

Plasminogen is transformed into plasmin by a process called fibrinolysis. This procedure results in bleeding and the dissolution of clots. According to certain reports, individuals with chronic hepatopathy experience hyperfibrinolysis **(16)**.

The procedure that turns plasminogen into plasmin is known as fibrinolysis. Blood is lost during this procedure, and clots are eliminated. Patients with chronic hepatopathy reportedly experience hyperfibrinolysis **(17).**

The tendency to bleed in liver disease patients cannot be primarily attributed to changes in hemostasis. Haemodynamic changes such as portal hypertension, endothelial dysfunction, renal failure, and synthesis of chemicals comparable to heparin released by bacterial infections provide a partial explanation of hemorrhagic episodes in individuals with decompensated liver disease **(18).**

In cirrhotic patients, decreases in anticoagulant factors balance out decreases in coagulation-favoring factors. These results contribute to the understanding of the pathophysiology of hemostasis in cirrhosis and indicate that the coagulation status of these patients' bleeding is not fully reflected by traditional lab testing **(19)**.

In our study, 30 ACLF patients were studied (group 1), 24 (80%) of them showed hepatofugal blood flow, also there is a significant increase in RI, PI, diameter of portal vein and a significant reduction in flow volume among this group than the other 2 groups.

A study carried out on 50 ACLF patients by **Mostafa et al. (20)** reported at the time of admission, these patients' decreased mean portal flow velocity and preponderance of the no forward portal flow worsened, reaching a peak after 2 weeks, and then beginning to improve in both flow direction and mean portal flow velocity along with clinical and laboratory improvements after 2 months.

Patients with ACLF have higher levels of factor VIII, lower levels of the VWF cleaving enzyme ADAMS 13, and lower levels of natural anticoagulants like protein C

and antithrombin III (ATIII). As the liver condition worsens, these changes become more obvious. These changes may help to explain why people with ACLF are more likely to develop venous thromboembolism **(6).**

Blood glycoprotein known as Von Willebrand Factor (VWF) is made by megakaryocytes, subendothelial connective tissue, and endothelium. There is a close relationship between the amounts of FVIII and VWF, which serves as a carrier protein for FVIII **(21)**

This hypothesis is supported by genetic studies (the VWF rs1063856 single-nucleotide polymorphism in exon 8 is associated with an elevated venous thrombotic risk). High levels of VWF cause high levels of FVIII, which in turn raise the risk of venous thrombotic problems. Additionally, immunohistochemistry testing found VWF in the thrombi of individuals who passed away from venous thrombotic problems **(22).**

The enzyme ADAMTS13 breaks down VWF into smaller pieces that are then broken down by other peptidases. The amount of VWF is higher in conditions like thrombotic thrombocytopenic purpura (TTP) and hemolytic uremic syndrome (HUS), which increases the risk of microvascular thrombosis **(23).**

Patients with chronic liver diseases (cirrhosis patients in the outpatient setting) have plasma VWF levels that are 2-3 times higher than in healthy individuals. Patients with acute hepatic dysfunction (acute liver injury and acute liver failure) have plasma VWF levels that are 4.0–4.5 times higher than in healthy individuals, and patients with ACLF have plasma VWF levels that are 5-7 times higher **(24).**

Increased endotoxin and cytokine release is frequently linked to acute liver failure, which may result in a decline in ADAMTS13 activity and a parallel increase in VWF levels **(25).**

In sepsis/inflammatory situations, the imbalance of high VWF and low ADAMTS13 can be a risk factor for platelet microthrombi and a hindrance to important organ microcirculation, which can result in multi-organ failure and death in critically ill individuals. Raised plasma VWF levels may have a role in disease aetiology in critically ill patients with severe ACLF or acute liver failure, as these individuals frequently experience multi-organ failure **(26).**

In our study, VWF levels were higher in ACLF patients than chronic compensated hepatic group and healthy control group, median was 0.72(0.6 – 0.9), 0.36(0.32 – 0.41) and 0.24(0.18 – 0.28) respectively, the difference was statistically significant between the studied groups, also there was significant difference between patients with each two individual groups. We found that VWF levels correlated positively with severity of liver impairement among ACLF group, There was statistically significant positive correlation between VWF and ALT, AST, INR, RI, PI, portal vein diameter, and grades of HE ,There was statistically significant negative correlation between VWF and platelet count and portal flow volume, also there was a statistically significant difference between degree of ascites, direction of blood flow and VWF among these patients. This is in agreement with **Lisman et al. (17)** found when compared to the control group, VWF:Ag levels in the plasma of patients with Child C cirrhosis were significantly higher. VWF:Ag levels were higher in five patients with acute liver failure compared to controls. There was shown to be a significant association between VWF:Ag levels and disease severity as determined by the MELD score when patients were categorised based on the model for end-stage

liver disease (MELD) score. In another study carried out by **Prasanna et al. (27)** found that patients with ACLF and patients who had a composite bad outcome had day 1, 3, and plasma VWF antigen and activity that were increased. There was a moderate but significant positive correlation of day 1 MELD score with day 1 VWF antigen and activity. With increasing grade of ACLF on day 1, there was a trend to increase in day 1 VWF antigen and significant increase in day 1 VWF activity.

Our study showed that D dimer is elevated in ACLF patients when compared to chronic compensated hepatic group and control group with significant difference between studied groups: 4.25 (3.58: 6.43),0.61(0.26: 0.35) and 0.3(0.26 – 0.35) respectively, this indicates that D dimer correlates positively with degree of liver injury.

We found that among factors significantly correlated to VWF, D dimer (unstandardized β=0.077) and flow volume (unstandardized β=-0.001) are significantly independently associated with it in ACLF patients. We also found that There is statistically significant positive correlation between VWF and all of ALT, AST, INR, D dimer, and portal vein diameter among patients with compensated CLD.

In the study carried out by **Mostafa et al. (20)** revealed that when compared to the control group, ACLF patients had significantly greater fibrin monomer levels, a no significant difference in D-dimer, a reduced mean portal flow velocity, and a higher proportion of patients with no forward portal flow.

Follow-up for 3 months determining 2 weeks as the peak of deterioration and 2 months for improvement, significant differences in FM, ALT, total bilirubin, INR and portal flow mean velocity, and direction with no significant difference in platelet count in ACLF patients were observed.

From the previous results, we can conclude that proofing and acceptance of the hypothesis of hepatic microcirculatory thrombosis and its role in the deterioration of liver function and progression of cirrhosis, an emerging query is whether this microthrombosis is a sequel with presence of a precipitating factor or a cause of developing acute deterioration in the absence of precipitating reason and may give us the value of using different therapeutic options like low molecular weight heparin or antithrombin 3 and studying its effect on protecting the liver from the hypercoagulable state in patients with acute-on-chronic and chronic hepatic disease.

These new findings clearly established VWF as a crucial factor in venous thrombosis and demonstrate its involvement in processes other than atherothrombosis. Therefore, targeting VWF may be an effective strategy for the management and/or avoidance of these vaso-occlusive complications **(28).**

According to preliminary reports in and acute liver failure, therapeutic plasma exchange is the most effective method of lowering VWF. A small number of patients experienced increased survival without liver transplantation in patients with acute liver injury and acute liver failure after the implementation of VWF-reducing medication in accordance with a management protocol suited to the degree of liver dysfunction **(29).**

Fresh frozen plasma contains VWF, but it is unclear if transfusing or exchanging plasma with patients who have acute liver failure or injury and already have elevated plasma VWF levels is safe. In patients with acute liver failure, it's likely that high VWF content plasma is removed during plasma exchange and replaced with plasma from

healthy donors (with normal VWF concentration). Patients with ADAMTS13 deficiency and portopulmonary hypertension as well as those with acute liver injury appeared to benefit from the transfusion of fresh frozen plasma to replenish ADAMTS13 and lower VWF. Further studies are needed to explore the risks and benefits of plasma exchange and VWF-lowering strategy in patients with liver failure **(30).**

Our study is limited because ADAMTS13, the VWF cleavage protease whose levels become variable in hepatic patients was not included in this study, also, only few studies have described the role of VWF in cases of ACLF with the emergence of microvascular thrombosis, further studies are needed to confirm this possibility.

CONCLUSION:

Uncontrolled inflammation is believed to be a primary contributing cause to acute-on-chronic liver failure (ACLF), which is distinguished by organ failures, abrupt deterioration of chronic liver disease, and increased short-term mortality.

Not all haemostatic alterations in patients with hepatopathy favor bleeding. Some evidences showed that there is an imbalance in favor of procoagulant substances in the plasma of cirrhotic patients.

Hypercoaguability and hepatic microvascular thrombosis in ACLF patients are due to resistance to thrombomodulin (which is explained by increased factor VIII levels).

Von Willebrand Factor (VWF), a glycoprotein produced in endothelium, functioning as a carrier protein for FVIII, its level is increased in patients with chronic liver diseases and correlates positively with severity of liver impairement.

D dimer is elevated in ACLF patients when compared to compensated chronic hepatic patients and correlates positively with degree of liver injury.

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