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Platelet Function Abnormalities in Patients with Liver Cirrhosis and Esophageal Varices

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ABSTRACT

Background: Severe thrombocytopenia and platelet dysfunctions are common amongst patients with cirrhosis and can contribute to increase risk of bleeding in patient with cirrhosis and portal hypertension (PH). Many studies confirmed the close association between thrombocytopenia and accordance of variceal bleeding. But the relationship of platelet function disorder and risk of variceal bleeding is still a matter of debate.

Objectives: This study aimed to evaluate effect of platelet count, platelets indices and platelet functions in cirrhotic patients with different grade of oesophageal varices (OV) and identifying potential risk factors associated with variceal bleeding. **Patients and methods:** 60 cirrhotic patients with different grades of OV were included in the study, 30 of them had a history of variceal bleeding and 30 patients never bled as well as 30 apparently healthy control subjects. They were subjected to laboratory investigations including: liver function tests, coagulation tests, platelet count, platelet indices and platelet function assessment using light transmission aggregometry.

Results: The collagen corrected area at cut off point ≥ 0.76 (AUC=0.832), with a sensitivity of 70% and a specificity of 96.7%. Ristocetin corrected area at cut off point ≥ 0.76 (AUC=0.832) with a sensitivity of 66.7% and a specificity of 93.3% but ADP had a poor diagnostic performance as ADP corrected area at cut off point ≥ 0.51 (AUC=0.646) sensitivity is 53.3% and specificity 90%. **Conclusion**: The corrected areas of ADP, collagen, and ristocetin were significantly associated with risk of OV bleeding, in which the corrected area of collagen and ristocetin were good diagnostic markers and superior to ADP for prediction of bleeders OV group.

Keywords: Oesophageal varices, platelet function tests, ADP, collagen, ristocetin, light transmission aggregometry.

INTRODUCTION

The last stage of any chronic liver damage, liver cirrhosis, is characterised by vascular fibrosis and distortion, which increases hepatic resistance and may result in portal hypertension [1]. Oesophageal varices (OV) are a frequent and dangerous cirrhosis consequence that are linked to higher mortality and morbidity rates [2]. In Egypt, the incidence of large varices is 47% and the incidence of OV in hepatitis C virus (HCV) patients with liver cirrhosis is around 62% [3]. Low platelet counts might increase bleeding risk and make it more severe. However, there is proof that people with thrombocytopenia and cirrhosis still have relatively high levels of functional platelet capacity [4]. The most important portosystemic collaterals are the OV because variceal haemorrhage, the most common and deadly cirrhosis consequence, is caused when they rupture [5]. Seventy percent of all instances of upper gastrointestinal haemorrhage in people with PH are due to variceal bleeding, a serious consequence of liver cirrhosis [6]. Platelet dysfunctions are present in patients with cirrhosis, so the laboratory investigations for platelet disorders may include: platelet count, mean platelet volume (MPV), platelet morphology in the blood smear, and platelet function tests, such as activated clotting time (ACT), bleeding time (BT), platelet aggregation, flowcytometry-measuring precense of platelet membrane glycoproteins, and the platelet aggregation metabolites may be detected by ELISA technique[7]. It might be challenging to choose the best platelet function testing technique for patients with liver cirrhosis. For evaluating platelet function, impedance aggregometry (IE) is now regarded as the "gold standard." Thrombin, adenosine diphosphate (ADP), collagen, and ristocetin are used as platelet receptor agonists to assess the capacity of platelets to aggregate [8]. This study aimed to evaluate effect of platelet count, platelets indices and platelet functions in cirrhotic patients with different grades of oesophageal varices (OV) and identifying potential risk factors associated with variceal bleeding.

PATIENTS AND METHODS

This case control study, was conducted on 90 subjects, 60 of them were collected from inpatient, outpatient clinic and endoscopic unit of Gastroenterology and Hepatology department of National Liver Institute of Menoufia University from August 2020 to September 2021, as well as 30 age and sex-matched apparently healthy subjects were enrolled as controls. After a complete clinical history, clinical examination, abdominal ultra-sonography and upper endoscopy. The cirrhotic patients were divided into 2 groups: Group I, 30 patients with cirrhosis and oesophageal varices, who had never bled and Group II, 30 patients with cirrhosis with a history of variceal bleeding. They were 42 male and 18 female patients and their ages ranged from 40 to 72 years with mean of age 51.97 ± 7.46 and 54.40 ± 8.24 years for GI and GII, respectively.

Sampling: Nine mL of venous blood sample were taken

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by sterile venipuncture and then divided into four tubes; 2ml was withdrawn in K2 EDTA for complete blood count and platelet indices (MPV and PDW) using Sysmex XS- 1800i hematology analyzer (Sysmex , Kobe, Japan). Three ml plain tube with clot activator for serum preparation for liver and kidney function tests using Cobas 6000 analyser (c 501 module-Diagnostics , Germany) and FDPS by latex agglutination kit. Other two tubes containing 3.2% sodium citrate to prepare platelet poor plasma for PT, INR, PTT, fibrinogen using Sysmex CS-1600 automated hemostasis analyser (Sysmex, Kobe- Japan), and D-Dimer using Cobas 6000 analyser. The last tube containing 3.2% sodium citrate was used for separation of platelet rich plasma (PRP) for assessment of platelet aggregation using a lumi-aggregometer (Apact 4004, Labitec, Ahrenssburg-Germany).

Precaution and procedure and of Light transmission aggregometry (LTA):

To reduce platelet activation during the operation, the following measures should be taken while collecting blood samples for LTA studies:

- Venostasis should be avoided or kept to minimum while drawing blood samples for LTA.
- A needle with at least a gauge of 21 should be used to collect blood samples for LTA.
- The initial 3–4 mL of obtained blood should be discarded or utilized for testing other than LTA [9].

Assessment of PRP quality [9]:

No lipemic samples are allowed, platelet count must be performed on the PRP sample being evaluated, and LTA study findings may be incorrect if the PRP sample's platelet count is less than 150×10^9 L^{-1}.

Pre-analytical variables [9]:

1. To reduce the effect of the release of adrenaline during exercise on platelet aggregation, blood samples for LTA should be taken after a brief time of rest for the participant.
2. At least 3 days should pass before sample if you're using medications (like NSAIDs) that are known to reversibly impair platelet activity.
3. It is recommended to cease using any medications known to permanently impair platelet function, such as aspirin and thienopyridines, at least 10 days before to sampling.
4. To prevent the creation of chylomicrons in plasma, which would obstruct light transmission, patients should not be analysed after consuming meals with a high fat content.

Procedure [9]:

LTA was done using Apact 4004 (labitec, Ahrensburg, Germany) as the following procedure: Platelet rich plasma (PRP) was prepared by centrifugation at 20°C for 10-15 minutes at 150-200g. and platelet count was adjusted to 300000- 600000/ul by diluting PRP with platelet poor plasma then it was taken out and put in a plastic container using a plastic transfer pipette (with cap) labelled 'PRP'. The container was capped, kept at room temperature and allowed to rest for 15 minutes while platelet poor plasma (PPP) was prepared by centrifuging the sample at 2000 x g for 20 minutes Then PPP with a plastic transfer pipette was taken out and put in a plastic container (with cap) labelled 'PPP'. The container was capped and kept at room temperature.

200 ul of PRP was pipetted in to an aggregation cuvette and a stir bar was added and re-warmed to 37° C for 120 seconds then, according to agonist used, 20 ul of ADP, 20 ul of collagen and 35 ul of ristocitin were added directly in to the cuvettes. The reagent wasn't allowed to run down the wall of the cuvette. The pattern of aggregation was allowed to form for a minimum of 5 minutes after setting 0% and 100% aggregation levels on the aggregometer.

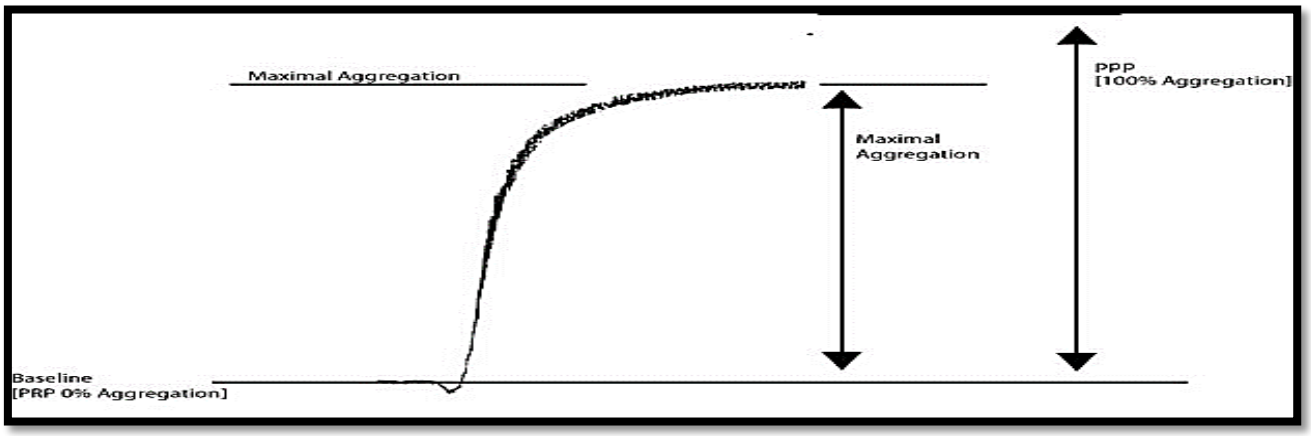

Figure (1): Analyzing aggregation curves. When examining aggregation curves in the past, maximal aggregation as a percentage [%] has been reported. The maximal aggregation [X] is divided by the distance [Y] between the baseline [0% aggregation - platelet rich plasma] and platelet poor plasma [100% aggregation]. Therefore, in the aforementioned example, if Y = 100mm and X = 87mm, then X/Y = 87% is the percentage of maximal aggregation **[10].** It was proposed that the findings be expressed as the ratio between AUC and the sample platelet count (AUC/platelet count ratio) in order to eliminate the confusing platelet count implications on the results of the aggregometer **[11].**

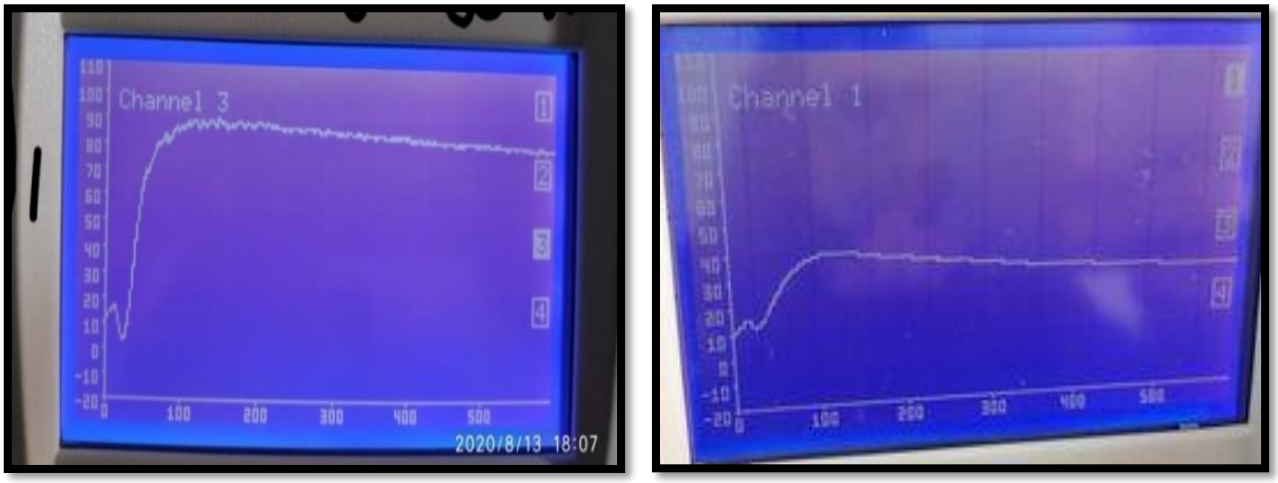

Figure (2): Example of collagen aggregation response in healthy subjects (left one) and hypofunction response in patient group (right one).

Ethical approval:

A written consent was taken and agreed by ethical committee of National Liver Institute of Menoufia University with IRB number 00353/2022. All study participants provided written informed permission after being informed of our research's goals. The Declaration of Helsinki for human beings, which is the international medical association's code of ethics, was followed during the conduct of this study.

Statistical analysis

The current study's statistical analysis was done with SPSS version 27.0. (SPSS Inc., Chicago, IL, USA). The mean, standard deviation, and range of quantitative values were displayed. Frequency and percentage were used to represent qualitative data. To assess the relationship between the qualitative variables, the chi-square test was performed.

When comparing the mean±SD of two sets of normally distributed data, the Student t-test was employed, while the Mann Whitney test was used when the quantitative data was not normally distribute (results were presented as Median) Estimates of the test's sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and accuracy were also computed at the ideal cutoff in order to evaluate the test's efficacy.

Analysis of receiver operating characteristics (ROC). It is a visual representation of the sensitivity measured against one minus the specificity (rate of false positives) for various cutoffs.

The Youden index J, which is the point on the ROC curve that is furthest away from the diagonal line of equality [maximum (sensitivity + specificity)-1], was used to establish the ideal cutoff value. The ability of a test to accurately distinguish between people with and without illness depends on how great the AUC is. When the P-value is less than 0.05, it was deemed statistically significant.

RESULTS

Table (1) shows that there were significant lower levels of hemoglobin, platelet count and fibrinogen were detected in non- bleeder (G I) ($p < 0.001$ for each) and in bleeder (G II) ($p<0.001$ for each) compared to healthy control group. Also, a significant lower level of hemoglobin, platelet count and fibrinogen level were found among G II compared to G I ($p < 0.001$ for each).

Table (1): Comparison between Laboratory data of the studied patients.

Hematological and coagulation parameters	Healthy controls (n= 30)	Non bleeders G I (n= 30)	Bleeders G II (n= 30)	Kruskal-Wallis test	Pairwise comparisons*
Hemoglobin (g/dL)					**p1<0.001** HS
Median (IQR)	13.00 (1.65)	10.45 (3.48)	8.50 (1.30)	χ^2= 55.24	**p2<0.001** HS
Range (min-max)	11.30 - 15.40	7.90 - 14.30	6.70 - 10.00	P-value **<0.001**HS	**p3<0.001** HS
Platelets count (10^3 cells/µL)					**p1<0.001** HS
Median (IQR)	252.00 (67.75)	93.50 (34.75)	50.00 (21.25)	χ^2= 71.86	**p2<0.001** HS
Range (min-max)	161.00 - 400.00	51.00 - 134.00	26.00 - 93.00	P-value **<0.001**HS	**p3<0.001** HS
MPV(fL)					**p**1=0.805 NS
Median (IQR)	10.55 (1.35)	10.30 (1.20)	12.25 (1.82)	χ^2= 26.22	**p2<0.001** HS
Range (min-max)	9.00 - 12.00	8.70 - 11.80	9.40 - 14.00	P-value **<0.001**HS	**p3<0.001** HS
PDW (fL)					**p1<0.001** HS
Median (IQR)	12.00 (3.65)	14.50 (2.24)	15.00 (3.25)	χ^2= 24.09	**p2<0.001** HS
Range (min-max)	9.50 - 18.40	10.30 - 17.60	9.90 - 18.30	P-value **<0.001**HS	p3=0.335 NS
INR					**p1<0.001** HS
Median (IQR)	1.02 (0.04)	1.40 (0.30)	1.84 (0.43)	χ^2= 67.50	**p2<0.001** HS
Range (min-max)	1.00 - 1.05	1.10 - 1.87	1.30 - 2.40	P-value **<0.001**HS	**p3<0.001** HS
aPTT(seconds)					p1=0.648 NS
Median (IQR)	33.00 (5.55)	35.65 (12.00)	45.80 (11.48)	χ^2= 26.34	**p2<0.001** HS
Range (min-max)	24.00 - 45.00	23.40 - 53.00	27.00 - 76.00	P-value **<0.001**HS	**p3<0.001** HS
Fibrinogen (µg/mL)					**p1<0.001** HS
Median (IQR)	256.00 (72.45)	223.15 (50.10)	186.20 (21.30)	χ^2= 51.97	**p2<0.001** HS
Range (min-max)	210.00 - 366.00	122.70 -299.00	110.80 -201.00	P-value **<0.001**HS	**p3<0.001** HS
D-Dimer (ng/mL)					
Median (IQR)	0.30 (0.20)	0.30 (0.10)	0.30 (0.23)	χ^2= 4.67	-
Range (min-max)	0.10 - 0.40	0.10 - 0.70	0.02 - 0.50	P-value =0.097NS	
FDPs (mg/dL)					
Median (IQR)	0.25 (0.10)	0.25 (0.20)	0.30 (0.13)	χ^2= 1.85	-
Range (min-max)	0.10 - 0.40	0.10 - 0.50	0.10 - 0.40	P-value =0.397 NS	
Biochemical parameters	**Healthy controls (n= 30)**	**Non bleeders G I (n= 30)**	**Bleeders G II (n= 30)**	**Kruskal-Wallis test**	**Pairwise comparisons***
AST (U/L)				χ^2= 42.51	**p1<0.001**HS
Median (IQR)	18.00 (9.25)	33.00 (17.50)	45.50 (23.50)	P-value	**p2<0.001** HS
Range (min-max)	11.00 - 35.00	12.00 - 98.00	23.00 - 120.00	**<0.001**HS	**p3=0.003** HS
ALT (U/L)				χ^2= 37.98	**p1=0.021** S
Median (IQR)	23.00 (9.75)	37.50 (29.50)	50.50 (27.00)	P-value	**p2<0.001** HS
Range (min-max)	13.00 - 38.00	11.00 - 87.00	27.00 - 138.00	**<0.001**HS	**p3=0.003** HS
Albumin (g/dL)				χ^2= 66.09	**p1<0.001** HS
Median (IQR)	4.55 (1.05)	3.10 (0.30)	2.75 (0.32)	P-value	**p2<0.001** HS
Range (min-max)	3.60 - 5.35	2.10 - 3.80	2.32 - 3.20	**<0.001**HS	**p3<0.001** HS
AST/ALT ratio				χ^2= 24.09	**p1=0.012** S
Median (IQR)	0.82 (0.08)	0.88 (0.10)	0.89 (0.06)	P-value	**p2<0.001** HS
Range (min-max)	0.71 - 0.92	0.65 - 2.91	0.82 - 1.05	**<0.001**HS	p3=0.459 NS

Range (min-max), median, IQR: non- parametric test, IQR: Interquartile range (difference between 1st and 3rd quartiles)

*: **After significant Kruskal-Wallis test, multiple pairwise comparisons was adjusted by Dunn-Sidak post hoc test, p1: Difference between healthy controls and non-bleeders groups, p2: Difference between healthy controls and bleeder groups, p3: Difference between non-bleeders and bleeders groups, NS: Non-significant at P ≥0.05, S: Significant at P < 0.05, HS: Highly significant at P <0.01.**

There were significant high levels of PDW and INR in G I (p <0.001 for each) and a significant high level of MPV, PDW, INR and aPTT were noticed in G II (p <0.001 for each) compared to healthy group. Also, the MPV, INR and aPTT were significantly higher in G II compared to G I (p <0.001 for each).

There was no significant difference between healthy group and G I regarding MPV, aPTT (p= 0.805, 0.648 respectively). Also, no significant difference was found between G I compared G II regarding PDW (p= 0.335) and among studied groups regarding D-Dimer and FDPs (p= 0.097& 0.397 respectively).

There was a significant decreased serum levels of albumin among G I and G II compared to the healthy group (p-value= <0.001) for each, G I and G II had significant elevated levels of ALT, AST, AST/ALT ratio compared to healthy control group (p <0.001, 0.021, 0.012 respectively) among G I, among G II (p <0.001 for each) and among G II compared to G I (p= 0.003, 0.003, <0.001 respectively).

But, there was no significant difference between G I and G II regarding AST/ALT ratio (p= 0.459).

Table (2): Comparison between platelet function tests in studied groups

Aggregometry measurements	Healthy controls (n= 30)	Non bleeders G I (n= 30)	Bleeders G II (n= 30)	Kruskal-Wallis test	Pairwise comparisons*
ADP corrected area (%)					
Median (IQR)	0.32 (0.10)	0.27 (0.22)	0.52 (0.81)	χ^2= 5.93	-
Range (min-max)	0.20 - 0.49	0.01 - 0.98	0.08 - 1.19	P-value =0.051 NS	
ADP area (%)					**p1<0.001 HS**
Median (IQR)	77.96 (1.80)	25.74 (16.44)	28.25 (38.53)	χ^2= 59.40	**p2<0.001 HS**
Range (min-max)	77.01 - 80.70	0.51 - 67.80	5.10 - 57.20	P-value **<0.001 HS**	p3=0.997 NS
ADP max aggregation (%)					**p1<0.001 HS**
Median (IQR)	85.41 (8.80)	32.03 (14.47)	33.72 (35.24)	χ^2= 59.62	**p2<0.001 HS**
Range (min-max)	77.67 - 91.49	3.12 - 76.56	10.27 - 66.36	P-value **<0.001 HS**	p3=0.846 NS
ADP lag phase (°)					**p1<0.001 HS**
Median (IQR)	36.50 (2.32)	16.11 (13.01)	24.65 (11.73)	χ^2= 60.43	**p2<0.001 HS**
Range (min-max)	35.50 - 38.88	5.59 - 36.33	9.16 - 35.49	P-value **<0.001 HS**	p3=0.030 S
Collagen corrected area (%)					**p1<0.001 HS**
Median (IQR)	0.30 (0.11)	0.61 (0.21)	0.96 (0.55)	χ^2= 55.01	**p2<0.001 HS**
Range (min-max)	0.20 - 0.49	0.18 - 0.91	0.26 - 2.01	P-value **<0.001 HS**	**p3<0.001 HS**
Collagen area (%)					**p1<0.001 HS**
Median (IQR)	78.60 (9.43)	50.00 (24.78)	55.33 (20.40)	χ^2= 47.47	**p2<0.001 HS**
Range (min-max)	61.40 - 82.01	14.00 - 73.40	24.30 - 78.40	P-value **<0.001 HS**	p3=0.981 NS
Collagen max aggregation (%)					**p1<0.001 HS**
Median (IQR)	87.54 (10.34)	55.73 (25.80)	62.83 (22.73)	χ^2= 52.24	**p2<0.001 HS**
Range (min-max)	75.68 - 89.67	17.89 - 83.21	27.51 - 86.10	P-value **<0.001 HS**	p3=0.963 NS
Collagen lag phase (°)					**p1<0.001 HS**
Median (IQR)	38.67 (2.28)	29.58 (9.96)	30.96 (8.91)	χ^2= 55.95	**p2<0.001 HS**
Range (min-max)	36.36 - 40.57	18.05 - 35.37	15.45 - 36.98	P-value **<0.001 HS**	p3=0.295 NS
Ristocetin corrected area (%)					**p1=0.003 HS**
Median (IQR)	0.29 (0.08)	0.51 (0.36)	0.94 (0.60)	χ^2= 51.38	**p2<0.001 HS**
Range (min-max)	0.18 - 0.47	0.09 - 1.13	0.37 - 2.61	P-value **<0.001 HS**	**p3<0.001 HS**
Ristocetin area (%)					**p1<0.001 HS**
Median (IQR)	75.40 (8.13)	45.15 (35.37)	55.04 (20.57)	χ^2= 58.59	**p2<0.001 HS**
Range (min-max)	69.10 - 79.40	7.30 - 70.00	21.20 - 70.30	P-value **<0.001 HS**	p3=0.169 NS
Ristocetin max aggregation (%)					**p1<0.001 HS**
Median (IQR)	84.01 (7.77)	54.91 (37.70)	62.27 (25.49)	χ^2= 51.61	**p2<0.001 HS**
Range (min-max)	77.84 - 87.67	13.20 - 80.07	6.19 - 81.49	P-value **<0.001 HS**	p3=0.707 NS
Ristocetin lag phase (°)					**p1<0.001 HS**
Median (IQR)	36.07 (4.45)	25.33 (16.25)	22.81 (7.63)	χ^2= 56.84	**p2<0.001 HS**
Range (min-max)	32.02 - 38.67	5.36 - 37.34	7.08 - 31.48	P-value **<0.001 HS**	p3=0.979 NS

Range (min-max), median, IQR: non- parametric test, IQR: Interquartile range (difference between 1st and 3rd quartiles)

*: **After significant Kruskal-Wallis test, multiple pairwise comparisons was adjusted by Dunn-Sidak post hoc test**

p1: Difference between healthy controls and non-bleeders groups, p2: Difference between healthy controls and bleeder groups, p3: Difference between non-bleeders and bleeders groups, NS: Non-significant at P ≥0.05, S: Significant at P < 0.05, HS: Highly significant at P <0.01

Table (2) shows that there was a significant lower levels of ADP area, ADP max aggregation, ADP lag phase, collagen area, collagen max aggregation, collagen lag phase, ristocetin area, ristocetin max aggregation and ristocetin lag phase were found in G I and GII patients compared to healthy control group (p <0.001 for each). There was significant higher levels of collagen corrected area and ristocetin corrected area was detected among G I (p <0.001, 0.003, respectively) and G II (p <0.001 for each) compared to healthy control group. There was a significantly higher levels of collagen corrected area and ristocetin corrected area among G II (bleeder) compared to (p <0.001 for each).

There was no significant difference between GI compared to G II regarding ADP area, ADP max aggregation, ADP lag phase, collagen area, collagen max aggregation, collagen lag phase, ristocetin area, ristocetin max aggregation and ristocetin lag phase (p=0.997, 0.846, 0.03, 0.981, 0.963, 0.295, 0.169, 0.707 & 0.979, respectively).

Table (3): Test characteristics of corrected areas of ADP, collagen, and ristocetin as diagnostic markers for bleeders versus non-bleeders

Test characteristics	**Bleeders versus non-bleeders**		
	ADP corrected area (%)	Collagen corrected area (%)	Ristocetin corrected area (%)
Best cutoff value	≥ 0.51	≥ 0.76	≤ 0.76
AUC	0.646	0.832	0.858
P-value	0.053 NS	**<0.001 HS**	**<0.001 HS**
Sensitivity %	53.3	70.0	66.7
Specificity %	90.0	96.7	93.3
PPV %	84.2	95.5	90.9
NPV %	65.8	76.3	73.7
Accuracy %	71.7	83.4	80.0

PPV: Positive predictive value; NPV: Negative predictive value, NS: Non-significant at P-value ≥0.05, HS: Highly significant at P-value <0.01

Table (3) assess the ability of ADP corrected area, collagen corrected area, ristocetin corrected area in discrimination between Bleeders versus non-bleeders and it showed that; ADP corrected area at cut off point ≥ 0.51 (AUC=0.646) sensitivity is 53.3% and specificity 90% and P= 0.53. Collagen corrected area at cut off point ≥ 0.76 (AUC=0.832) sensitivity is 70% and specificity 96.7% and P <0.001. Ristocetin corrected area at cut off point ≥ 0.76 (AUC=0.832) sensitivity is 66.7% and specificity 93.3% and P <0.001.

Table (4): Potential risk factors associated with bleeding in patients with OV

Variables	**Univariable analysis**		
	B	**OR (95% CI)**	**P-value [a]**
Age	0.04	1.04 (0.97 - 1.11)	0.233 NS
Gender (female)	0.32	1.38 (0.45 - 4.17)	0.574 NS
ADP corrected area (10x scaled)	0.26	**1.29 (1.07 - 1.56)**	**0.008 HS**
Collagen corrected area (10x scaled)	0.55	**1.74 (1.27 - 2.37)**	**<0.001 HS**
Ristocetin corrected area (10x scaled)	0.56	**1.74 (1.30 - 2.34)**	**<0.001 HS**
MPV	1.45	**4.25 (2.06 - 8.77)**	**<0.001 HS**
Oesophgeal varices (OV):	-	-	0.079 NS
OV (grade I)		Ref	-
OV (grade II)	-0.82	0.44 (0.14 - 1.38)	0.160 NS
OV (grade III)	1.67	5.29 (0.57 - 49.13)	0.143 NS
Spleen size (cm)	0.25	1.29 (0.98 - 1.70)	0.071 NS

Abbreviations: **MPV**, mean platelets volume; **OR (95%CI)**, odd ratio with 95% confidence interval; **[a]**: Wald test
NS: Non significant at P ≥ 0.05, S: Significant at P-value < 0.05; HS : Highly significant at P < 0.01

Table (4) shows the univariable analysis used to identify the potential risk factors associated with bleeding among esophageal varices patients. Aggregometry measurements including corrected areas of ADP, collagen, and ristocetin (all 10x scaled) were significantly associated with risk of bleeding (OR=1.29, 95% CI: 1.07-1.56, P=0.008; OR=1.74,95% CI: 1.27-2.37, P<0.001; OR=1.74 95% CI: 1.30-2.34, P<0.001, respectively). Additionally, MPV was significantly associated with occurrence of bleeding (OR=4.25, 95% CI: 2.06-8.77, P<0.001).Other risk factors and confounders did not associate significantly (P > 0.05) with bleeding status, those included age, gender, grades of varices, and spleen size.

Table (5): Multivariable Logistic regression models of aggregometry results:

Model Variables	Coefficient	SE	Wald Square	P-value [a]	OR (95% CI)
Model 1					
ADP corrected area (10x scaled)	0.21	0.16	1.69	0.193[NS]	1.24 (0.90 - 1.70)
Collagen corrected area (10x scaled)	0.43	0.20	4.55	**0.033[S]**	1.54 (1.04 - 2.29)
Restocetin corrected area (10x scaled)	0.52	0.18	8.90	**0.003 [HS]**	1.68 (1.20 - 2.37)
Constant	-7.20	1.94	13.78	**<0.001 [HS]**	
Equation	Logit(p)*= 0.21 × (ADP corrected area 10x scaled) + 0.43 × (Collagen corrected area 10x scaled) +0.52 × (Restocetin corrected area 10x scaled) -7.2				
χ^2- value (P-value [b])	**41.38 (< 0.001 [HS])**				
Model 2					
Collagen corrected area (10x scaled)	0.55	0.26	4.40	**0.036 [S]**	1.73 (1.04 - 2.89)
Restocetin corrected area (10x scaled)	0.58	0.23	6.59	**0.010 [HS]**	1.79 (1.15 - 2.79)
MPV	1.66	0.55	9.23	**0.002 [HS]**	5.27 (1.80 - 15.39)
Constant	-25.96	7.38	12.38	**<0.001 [HS]**	
Equation	Logit(p)*=0.55× (Collagen corrected area 10x scaled) +0.58 ×(Restocetin corrected area 10x scaled) +1.66 ×(MPV)–25.96				
χ^2- value (P-value [b])	**55.14 (< 0.001 [HS])**				

- Predicted probability: $p = \frac{1}{1+e^{-logit(p)}}$ *$logit(p) = \ln\left(\frac{p}{1-p}\right)$

- χ^2 value: model chi square, -a : P-value of Wald test squared.

-b :P-value corresponds to overall chi square of the model, NS : Non significant at P-value ≥ 0.05 S : Significant at P-value < 0.05 HS : Highly significant at P-value < 0.01, OR (95% CI): Odds ratio with 95 % confidence interval.

Table (5) for multivariable logistic regression model, the P-value at 0.1 was set for variable inclusion in the shown two models: model 1 included aggregometry measurements of corrected areas of ADP, collagen, and ristocetin (all 10x scaled). However, ADP corrected area did not contribute significantly in the model fit (P=0.193). In model 2, the ADP measurement was replaced with MPV.

The conducted multivariable logistic regression analysis was used to evaluate the contribution of combined measurement of corrected areas of ADP, collagen, and ristocetin (all 10x scaled) in model 1 and collagen, ristocetin (both 10x scaled), and MPV in model 2 to assign patients to the disease outcome whether bleeders or non-bleeders.

A patient would be expected to have bleeding OV if he has a covariate pattern values (measurement combinations) that corresponds to a predicted probability greater than a usual cutoff value of 0.5. Alternatively, if the output for the applied model equation is a positive value then bleeding is predicted and if it is a negative value, no bleeding is predicted.

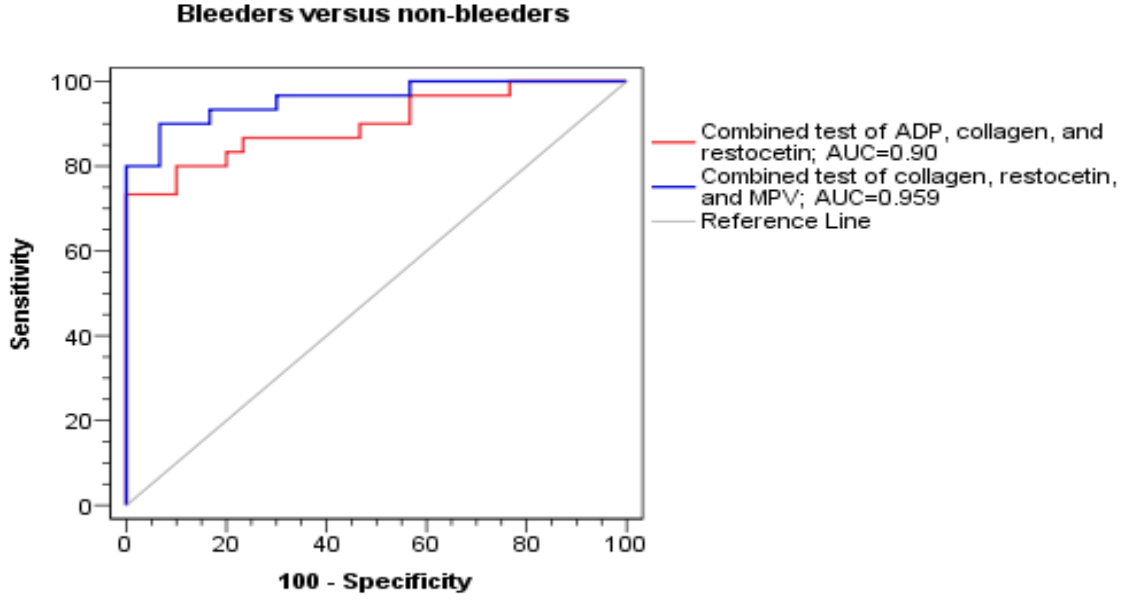

Figure (3): ROC curves of combined measuremnts of ADP, collagen, and ristocetin corrected areas and combined measuremnts with replacing ADP with MPV for discrimination between bleeders and non-bleeders.

In model 1 (combined testing of ADP, collagen and ristocetin), to discriminate between bleeders and non-bleeders groups, the ROC analysis estimated 73.3% sensitivity 100.0% specificity, 100.0% PPV, 78.9% NPV, and 86.7% accuracy at the best cutoff value of 0.69 (predicted probability). The AUC was 0.90 with a highly significant P <0.001

In model 2 (combined testing of collagen , ristocetin and MPV) , we replaced ADP with MPV, the estimates were 90.0% sensitivity 9 3.3% specificity, 93.1% PPV, 93.3% NPV, and 91.7% accuracy at the best cutoff value of 0.69 (predicted probability). The AUC was 0.96 with a highly significant P <0.001.

DISCUSSION

The most prevalent portosystemic collaterals are gastric varices, which rupture and produce significant variceal bleeding, which is regarded to be the most common serious and fatal PH complication. In cirrhosis, 70% of gastrointestinal tract bleeding is due to the rupture of oesophageal varices [12]. Severe thrombocytopenia and platelet dysfunctions associated cirrhosis can contribute to increased risk of bleeding[13].

This study aimed to evaluate effect of platelet count, platelets indices and platelet functions tests in cirrhotic patients with oesophageal varices (OV) and identifying potential risk factors associated with bleeding tendency. The study included 60 cirrhotic patients with different grades of OV, 30 of them had a history of variceal bleeding and 30 patients never bled. They were subjected to liver function tests, CBC, platelet count and indices and platelet aggregation tests with different agonist.

In the current study, there was a significant decrease in the mean hemoglobin concentration in cirrhotic patients with bleeding OV than without bleeding varices which was in accordance with **Liu *et al.*** study [14], who suggested a decreased hemoglobin concentration is due to splenomegaly, congestive gastropathy, in cirrhotic patients, bleeding risk may be exacerbated by recurrent bleeding and decreased hematocrit as a result of the rheological influence of erythrocytes on platelet adhesion. Additionally, the platelet count was significantly decreased in patients with bleeding OV and patients without bleeding varices compared to healthy control group. The lower platelet counts were noticed in bleeding varices group.

The high frequency of cirrhotic chronic HCV patients in the research groups may help to explain the incidence of thrombocytopenia. In addition to poor thrombopoietin production and platelet sequestration and death in the spleen, megakaryocytes directly infected by viruses and auto-immune processes have also been hypothesized as contributing factors to the pathogenesis of thrombocytopenia [15, 16].

There was a significant increase in INR among patients with bleeding OV and patients without bleeding varices compared to the healthy control group. Similar results obtained by **Zermatten *et al.*** [17] who reported that, INR was significantly increased among patients with liver cirrhosis than healthy controls. Because the primary prothrombotic modifications include increased factor VIII and reduced natural anticoagulants, such as proteins C, protein S, and antithrombin III, this prothrombotic condition appears to worsen with the severity of liver disease.

The fibrinogen was significantly lower in patients with bleeding OV and patients without bleeding varices compared to healthy control group and the lower level was detected in patients with bleeding OV. Similarly **Hessien *et al.*** [18] revealed a significant reduction in fibrinogen level in comparison to healthy persons in cirrhotic patients. This is because the liver's hepatocytes have been severely damaged, which causes inadequate generation of plasma proteins and fibrinogen, which lowers their levels.

In the present study, there was increased fibrinogen degradation products in patients with bleeding OV than patients without bleeding varices although this difference still non-significant. In accordance to our findings a previous study by **Surawong *et al.*** [19] showed that FDP was significantly increased with bleeding in cirrhotic patients. The observations imply that hyperfibrinolysis occurs often, even in cirrhosis that is not yet progressed. Although earlier studies suggested that ascites could be the cause of plasma hyperfibrinolysis [20].

Regarding platelet indices, there was a significant increase in the MPV among patients with bleeding OV in compared to healthy control group. Similar to our finding **Xianghong *et al.*** [21] who showed that MPV value was 13.26fl in cirrhosis patients versus 9.73fl in healthy control group. This may be understood by the fact that, as compared to healthy controls, patients with non-alcoholic fatty liver disease and chronic viral hepatitis had higher MPV levels and a poorer histological fibrosis stage. This was due to the probable involvement of activated big platelets in the development of microthrombi that destroyed the intrahepatic arteries and the portal vascular bed, progressing liver disease by eradicating parenchymal tissue [22]. Furthermore, a significant decrease in platelet function was described as lower levels of ADP area, ADP max aggregation, ADP lag phase, collagen area, collagen max aggregation, collagen lag phase, ristocetin area, ristocetin max aggregation and ristocetin lag phase among patients with bleeding OV and patients without bleeding varices compared to healthy group. Similar to our findings, **Vinholt *et al.*** [23] demonstrated that patients with hepatic cirrhosis had lower platelet activation and aggregation capability. Low platelet counts and severe liver illness were both related with impaired platelet activation and aggregation.

Also, **Jüttner *et al.*** [24] similarly showed lower platelet aggregation in cirrhotic patients with diverse aetiologies, after adjusting for platelet count. **Wosiewicz *et al.*** [25] cirrhotic patients with or without PVT awaiting liver transplantation were found to have

reduced platelet aggregation. Strangely, platelet aggregation was lower in PVT patients than in those without PVT (platelet count was similar between groups).

When we correct platelet functions to platelet counts, we revealed significant higher levels of collagen corrected area and ristocetin corrected area among patients with bleeding OV and patients without bleeding compared to healthy group.

Moreover, platelet aggregation showed a significantly higher level of collagen corrected area and ristocetin corrected area among patients with bleeding OV (in spite of low platelet count) compared to patients without bleeding varices. In agreement to our finding **Rogalski *et al.*** [26] who found that when compared patients who had never bled variceally, those who had previously experienced variceal haemorrhage had significantly lower platelet counts, which are crucial for platelet aggregation. Additionally, patients who had previously experienced haemorrhages displayed enhanced platelet activity; the fraction of platelets in blood clots was considerably higher in the bleeding group compared to the non-bleeding group. These results imply that despite more severe thrombocytopenia, platelets in patients with a history of variceal bleeding play a compensating role, supporting successful hemostasis. Similarly, **Raparelli *et al.*** [27] looked into the possibility that bacterial lipopolysaccharide (LPS) has a role in platelet activation. The study demonstrated that cirrhotic patients' platelets are more responsive to common agonists than normal platelets are, and it showed that LPS may play a role in initiating platelet aggregation.

The corrected areas of ADP, collagen, and ristocetin were significantly associated with risk of bleeding (OR=1.29, 95% CI: 1.07-1.56, P=0.008; OR=1.74, 95% CI: 1.27-2.37, P<0.001; OR=1.74 95% CI: 1.30-2.34, P<0.001, respectively), while the corrected areas of collagen, and ristocetin were good diagnostic markers to discriminate between bleeders versus non-bleeders. Collagen corrected area at cut off point ≥0.76 (AUC=0.832) with a sensitivity of 70% and specificity of 96.7%. Ristocetin corrected area at cut off point ≥ 0.76 (AUC=0.832) and a sensitivity of 66.7% and specificity of 93.3%, but ADP had a poor diagnostic performance as ADP corrected area at cut off point ≥0.51 (AUC=0.646) with a sensitivity of 53.3% and specificity of 90%. **Rogalski *et al.*** [26] reported no statistically significant differences between patients with bleeding OV and patients without bleeding varices after platelet activation with the ADP**.**

In this study, the collagen corrected area and ristocetin corrected area is superior to ADP corrected area to discriminate patients with OV (either with bleeding tendency or without bleeding) in comparison to healthy controls

Finally, univariable and multivariate analysis were done to identify the potential risk factors associated with occurrence of bleeding in patients with OV. Aggregometry measurements including corrected areas of ADP, collagen, and ristocetin were significantly associated with risk of bleeding followed by MPV value. Other risk factors and confounders did not associate significantly with bleeding status, those included age, gender, grades of varices, and spleen size.

Similar to this, **Rogalski *et al.*** [26] described how platelet aggregation is heavily reliant on the platelet numbers that serve as a compensating function, maintaining good hemostasis despite more severe thrombocytopenia. According to **Seeff *et al.*** [28]**,** who showed that severe thrombocytopenia (<50,000/μL) could predict major bleeding and re-bleeding in the peri-interventional setting**.** According to our findings, a research by **Erdogan *et al.*** [29] demonstrated that MPV is a potentially useful measure for cirrhosis-related variceal haemorrhage that is easily accessible. This can be explained as platelet shape and hemostatic activity are linked [30].

CONCLUSION

Corrected areas of collagen, ristocetin and MPV are potential risk factors associated with bleeding in patients with oesophageal varices and had better diagnostic performance. Further large scale studies on non-bleeders group and these corrected areas in addition to MPV are recommended. Also evaluation the effect of other platelet functions on bleeding varices to ensure using of platelet agonist as a therapy to reduce risk of bleeding.

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