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Original paper

CD62P (P-selectin) expression as a platelet activation marker in patients with liver cirrhosis with and without cholestasis

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Abstract

Aim of the study: P-selectin (CD62P) is a platelet activation marker that was claimed to mediate the accumulation of platelets induced by cholestasis. The nature of platelet dysfunction and hemostasis abnormalities in cholestatic liver disease needs to be more explored. The aim of this study was to assess platelet CD62P expression in cirrhotic patients with and without cholestasis, and to evaluate its relationship with a bleeding tendency.

Material and methods: 150 patients were included in this case-control study. Participants were divided into 84 patients with liver cirrhosis (group I), 44 of whom had cholestasis (Group Ia) and 40 patients were without cholestasis (group Ib); 36 patients who were cholestatic without liver cirrhosis (group II); and 30 healthy subjects who formed the control group (group III). Platelet CD62P expression was assessed by a flow cytometer.

Results: Platelets expressing CD62P were significantly increased in all patient groups compared to controls ($p < 0.001$). Platelets expressing CD62P were significantly increased in gastrointestinal (GIT) bleeders compared to non-bleeders in cirrhotic and cholestatic groups ($p < 0.001$ each). Among group I patients at cut-off > 12.4, up-regulation of platelet CD62P yielded 72% sensitivity and 44.1% specificity to discriminate bleeders from non-bleeders ($p = 0.01$), while among group II at cut-off > 12.9, it yielded 90% sensitivity and 80.8% specificity ($p < 0.001$). In cirrhotic patients, platelet CD62P expression was significantly increased in patients with an advanced Child-Pugh class ($p < 0.001$). Platelet expressing CD62P was shown as an independent risk factor for bleeding among cirrhotic cases with an odds ratio of 1.07 and CI 0.99-1.15.

Conclusions: Up-regulation of platelet CD62P expression can serve as a GIT bleeding predictor in liver cirrhosis.

Key words: CD62P, platelet activation, liver, cirrhosis, cholestasis, selectin, gastrointestinal, bleeding, marker, overexpression.

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Introduction

Liver cirrhosis is the result of liver injury by different mechanisms that lead to necroinflammation and fibrogenesis [1]. Cholestasis is stagnation, or marked reduction, in bile secretion and flow that results from either functional impairment of hepatocytes and/or obstruction at any level of the excretory bile pathway [2].

Thrombocytopenia is the commonest hematological disorder in patients with chronic liver disease, affecting 64-84% of patients with cirrhosis or fibrosis [3]. Thrombocytopenia is associated with poor prognosis since it frequently prevents crucial interventions such as medications and diagnostic or therapeutic procedures [4].

CD41 is used as a platelet identifier, as it is only present on platelets and no other circulating blood cells [5]. P-selectin (CD62P) is a special index of platelet activation that is usually stored in platelet granules and Weibel-Palade bodies of endothelial cells. Upon appropriate activation; by histamine, oxygen radicals, interleukin 1 (IL-1), and tumor necrosis factor (TNF), it is rapidly mobilized to the cell surface [6].

The platelet is known for its important role in bile duct ligation-induced liver injury since it promotes leukocyte recruitment and deteriorates microvascular perfusion. P-selectin is expressed by activated platelets and mediates the accumulation of platelets induced by cholestasis. Moreover, inhibition of P-selectin prevents cholestasis-induced platelet and leukocyte recruitment, as well as the associated hepatocellular damage. Thus, targeting platelet activation and accumulation is suggested to be helpful against liver damage induced by obstructive jaundice [7].

Some clinical studies have examined the platelet activation markers in patients with chronic liver disease as one of these studies [8, 9] reported the expression of P-selectin on the endothelium of hepatic artery branches, platelets, and few mononuclear inflammatory cells and delineated the aberrant expression of P-selectin on blood endothelial cells for the first time. These studies did not explain the relationship between the platelet activation markers, jaundice, and bleeding tendency among patients with cholestasis. Therefore, the present study was designed to evaluate the changes in CD62P expression as a platelet activation marker in patients with liver cirrhosis with and without cholestasis and to evaluate its relationship with other platelet functional parameters, clinical status, and bleeding tendency.

Material and methods

This case-control study was conducted on 150 subjects and carried out at outpatient clinics of hepatology, clinical pathology departments, National Liver Institute, Menoufia University in the period from April 2018 to December 2019. All participates were divided into 84 patients with liver cirrhosis (group I), 44 of whom had liver cirrhosis with cholestasis (group Ia) and 40 patients had liver cirrhosis without cholestasis (group Ib); 36 patients with cholestasis without liver cirrhosis (group II); and 30 apparently healthy subjects who were enrolled in the study as a control group (group III).

All patients were subjected to full history taking and clinical and radiological examination. The following laboratory investigations were conducted: liver and kidney function tests [total bilirubin, direct bilirubin, total protein, albumin, alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase, γ-glutamyl transferase (GGT), urea, and creatinine) using a Cobas 6000 auto-analyzer (c501 module) (Roche-Germany). Hepatitis C virus antibody (HCV-Ab) was done by electrochemiluminescence immunoassay "ECLIA" using a Cobas 6000 (e 601 modules) (Roche-Germany). Complete blood counts (CBC) with complete platelet parameters were done using the Sysmex XT1800i (Sysmex Corporation, Kobe, Japan). Prothrombin concentration in percent (%) and international normalized ratio (INR) were determined by Sysmex CS-1600 Automated hemostasis testing (Sysmex Corporation, Kobe, Japan).

CD62P expression on platelets was assessed by a FACSCalibur (BD, Biosciences, San Jose, USA) at the Flow cytometry Lab, Alzahraa Hospital, Al-Azhar University. Cell Quest Pro software (BD Biosciences, San Jose, USA) was used for data analysis. A compensation setting was established before acquiring the samples using color-calibrated beads (BD, Biosciences, San Jose, USA). Unstained samples were acquired to detect the sample auto-fluorescence.

Briefly, immediately after citrated sample withdrawal, samples were centrifuged for 10 min at 800 ×g at room temperature to obtain the platelet-rich plasma. Fifty μl of platelet-rich plasma (PRP) was obtained for final concentration of cells = 1-5 × 10^6 ml and incubated with: 5 μl of monoclonal Ab of lineage-specific CD41a-pythoerythrine (PE) conjugated (BD, Biosciences, San Jose, USA, Cat. No. 555467, Lot No. 5245924) and 5 μl of monoclonal Ab of activation marker CD62p-fluorescein isothiocyanate conjugated (FITC) (BD, Biosciences, San Jose, USA, Cat. No. 561922, Lot No. 8270765), for 20 min on ice in the dark at room temperature before washing the sample once, resuspend in 400 μl of buffer.

A total of 500,000 cells were counted, setting forward scatter/side scatter on logarithmic amplification. Only events in the size range of platelets [larger than debris and smaller than red blood cells (RBCs)] were gated, then the CD41a population was assessed as the platelet identifier in another graph, and finally, a single histogram was created to measure the mean fluorescence intensity (MFI) of the anti-CD 62P-FITC positive population (area under curve) and their percentage of positivity taken only from the CD41a positive population. Also, co-expression of anti-CD41a-PE and anti-CD62P-FITC was detected on a double parameter quadrant plot histogram (Fig. 1).

Statistical methods

Results were statistically analyzed using SPSS 22.0 (IBM Corp., Armonk, NY). Two types of statistical analysis were conducted. Student's *t*-test and the Mann-Whitney test were used to compare the quantitative variables. Chi-square (χ^2) test, Kruskal-Wallis test, and Fisher's exact test were used to compare the qualitative variables. A logistic stepwise regression

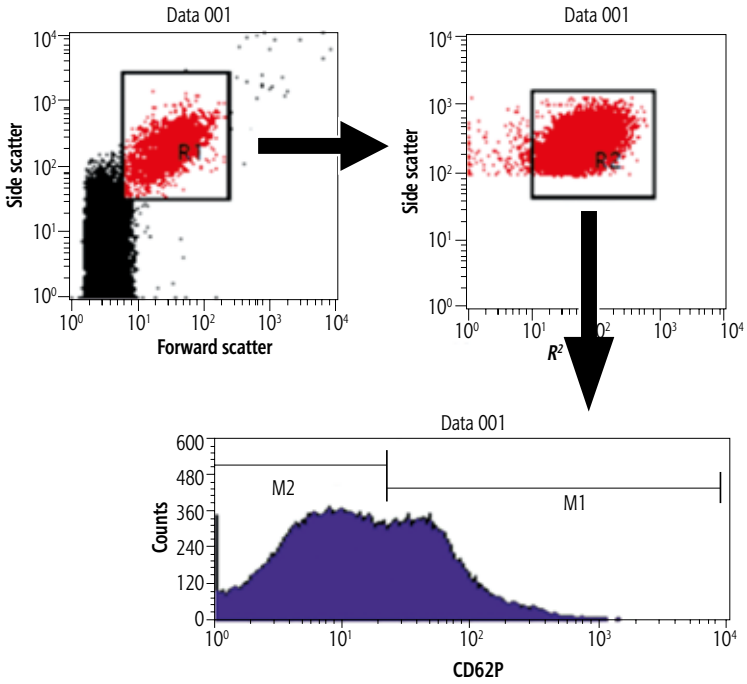

Fig. 1. Gating strategy to detect percentage of positivity of CD62P expressed on platelets using a single histogram gated on CD 41a +ve cells and CD62P +ve cells MFI which was measured in area under M1 marker

Table 1. Socio-demographic and clinical data among studied groups

Parameters	Cirrhosis and cholestasis (group Ia) *n* = 44		Cirrhosis (group Ib) *n* = 40		Cholestasis (group II) *n* = 36	Control (group III) *n* = 30	Test	*P* value
Age (years)								
Mean ±SD	58.3 ±7.8		56.7 ±7.6		54.2 ±12.3	48.2 ±4.3	*F* test 1.95	0.12
Range	44-75		33-70		31-77	40-56		
Gender								
Male	37 (84.1%)		27 (67.5%)		27 (75.0%)	18 (60%)	χ^2 5.95	0.11
Female	7 (15.9%)		13 (32.5%)		9 (25.0%)	12 (40%)		
	n	%	*n*	%	–	–		
Virology								
HBs Ag	2	4.5	2	5.0			Fe 0.01	1.0
HCV Ab	42	95.5	38	95.0				
PV								
Patent	25	56.8	40	100			χ^2 22.3	< 0.001
Thrombosed	19	43.2	0	0.0	–	–		
Encephalopathy								
Positive	38	86.4	7	17.5			χ^2 40	< 0.001
Negative	6	13.6	33	82.5	–	–		
Child-Pugh classification								
A	0	0.0	26	65.0			χ^2 84.0	< 0.001
B	0	0.0	14	35.0				
C	44	100	0	0.0	–	–		

F – ANOVA test, χ^2 – chi-square, Fe – Fisher's exact test, p > 0.05 is statistically non-significant, p < 0.05 is statistically significant

Table 2. Comparison between patient groups vs. control group regarding CD62P and CD41 expression

Parameter	Cirrhosis with cholestasis (group Ia) *n* = 44	Cirrhosis (group Ib) *n* = 40	Cholestasis (group II) *n* = 36	Control (group III) *n* = 30	Test of signif.	*P* value
Platelet CD62P					$t = 24.05$	< 0.001[a]
Mean ±SD	22.2 ±4.2	9.5 ±3.4	12.2 ±1.5	6.3 ±1.01	$U = 3.69$	< 0.001[b]
Range	15-30	2.5-14.7	10-16	4.4-7.4	$t = 19.07$	< 0.001[c]
CD62P MFI					$t = 11.7$	< 0.001[a]
Mean ±SD	19.3 ±4.4	16.1 ±3.4	15.71 ±2.83	9.64 ±2.6	$t = 9.03$	< 0.001[b]
Range	14-33	11-23	11-22	6-21.7	$t = 8.91$	< 0.001[c]
CD41%					$t = 3.5$	0.001[a]
Mean ±SD	60.61 ±9.95	62.7 ±10.60	64.14 ±10.95	51.3 ±12.9	$t = 4.04$	< 0.001[b]
Range	48-80	43-81	36-82	29-75	$t = 4.36$	< 0.001[c]

MFI – mean fluorescence intensity, t – Student t test, U – Mann-Whitney U test, p > 0.05 is statistically non-significant, p < 0.05 is statistically significant
[a] comparing cirrhosis and cholestasis group with control group, [b] comparing cirrhosis group with control group, [c] comparing cholestasis group with control group

Table 3. Comparison between patient groups regarding CD62P and CD41 expression

Parameter	Cirrhosis with cholestasis (group Ia) *n* = 44	Cirrhosis (group Ib) *n*= 40	Cholestasis (group II) *n* = 36	Test of signif.	*P* value
Platelet				$U = 7.88$	< 0.001[1]
CD62P					
Mean ±SD	22.2 ±4.0	9.0 ±3.42	12.2 ±1.5	$*t = 14.63$	< 0.001[2]
Range	15-30	2.5-14.7	10-16	$U = 3.26$	0.001[3]
CD62P MFI				$*t = 3.62$	0.001[1]
Mean ±SD	19.3 ±4.4	16.19 ±3.4	15.7 ±2.8	$*t = 4.24$	< 0.001[2]
Range	14-33	11-23	11-22	$T* = 0.67$	0.51[3]
CD41%				$*t = 0.93$	0.35[1]
Mean ±SD	60.6 ±9.9	62.7 ±10.6	64.1 ±10.9	$*t = 1.51$	0.14[2]
Range	48-80	43-81	36-82	$*t = 0.58$	0.56[3]

MFI – mean fluorescence intensity, t – Student t test, U – Mann-Whitney U test, p > 0.05 is statistically non-significant, p < 0.05 is statistically significant
[1] comparing cirrhosis and cholestasis group with cirrhosis group, [2] comparing cirrhosis and cholestasis group with cholestasis group, [3] comparing cirrhosis group with cholestasis group

model was used to give an adjusted odds ratio and 95% confidence interval of the effect of the different independent and dependent factors for detecting the association with the CD62P expression as a platelet activation marker in patients with liver cirrhosis. All these tests were used as tests of significance at $p < 0.05$.

Results

All studied groups were matched for age and gender ($p > 0.05$). Portal vein thrombosis, hepatic encephalopathy, and Child-Pugh class C were significantly prevalent ($p < 0.001$ for each) among patients with cirrhosis and cholestasis group (Ia) when compared to the cirrhosis (Ib) group. No significant difference ($p > 0.05$) was found between the two groups regards the virology screen (HCV Ab and HBs Ag) (Table 1).

Statistical analysis revealed that the percentage of platelet expressing CD62P, CD62P MFI were significantly higher in all patient groups compared to group III ($p < 0.001$ for each) (Table 2).

The percentage of platelets expressing CD62P was significantly higher in group Ia and group II compared to group Ib ($p < 0.001$), and CD62P MFI was significantly higher in group Ia compared to group Ib and group II ($p = 0.001$ and $p < 0.001$ respectively) (Table 3).

There was a positive correlation between percentage of platelets expressing CD62P and each of serum bilirubin (total and direct) ($r = 0.67$, $p < 0.001$ and $r = 0.65$, $p < 0.001$) (Fig. 2), ALT ($r = 0.47$, $p < 0.001$),

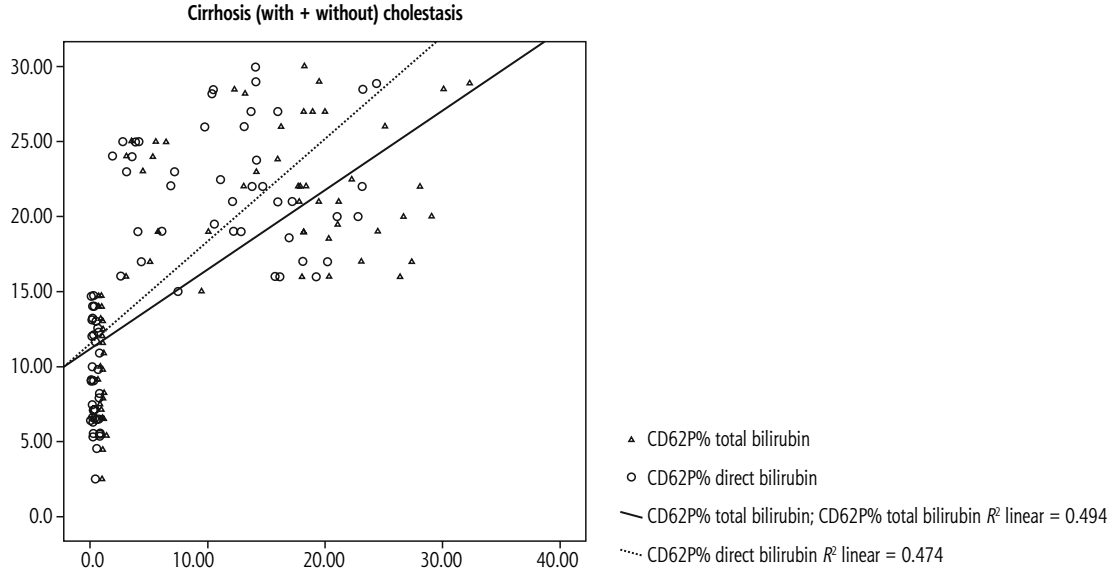

Fig. 2. Correlation between percentage of platelets expressing CD62P with total bilirubin ($r = 0.67$, $p < 0.001$) and direct bilirubin $r = 0.65$, $p < 0.001$)

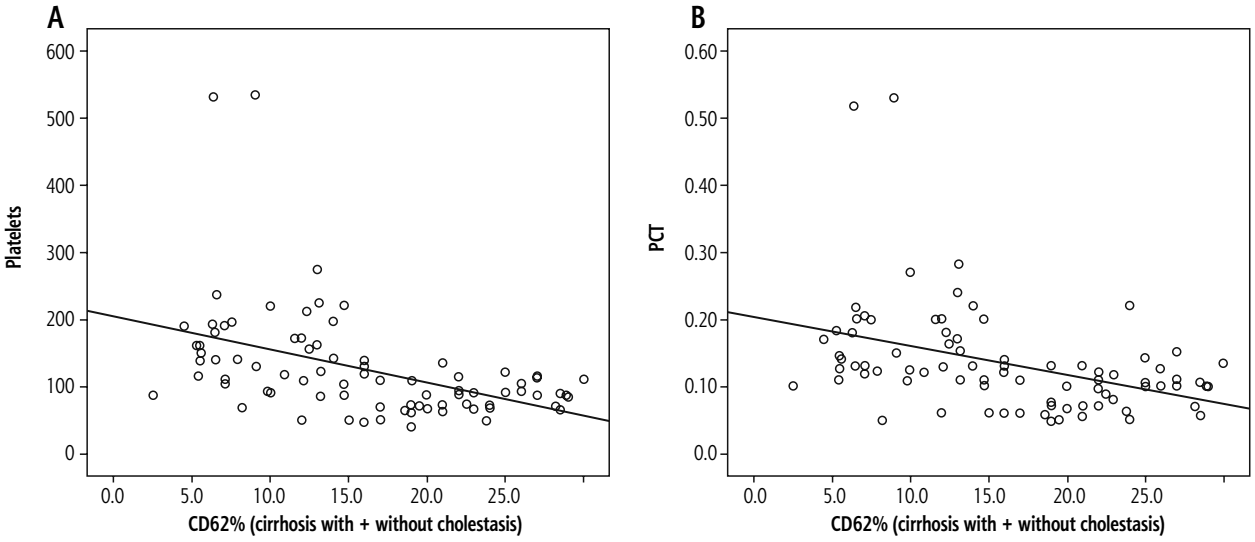

Fig. 3. Scatter dot figure of correlation between CD62P% and platelet count and PCT in cirrhosis groups (with and without cholestasis). **A**) Percentage of platelets expressing CD62P was negatively correlated with platelet count ($r = -0.39$, $p < 0.001$). **B**) Percentage of platelets expressing CD62P was negatively correlated with plateletcrit (PCT) ($r = -0.35$, $p < 0.001$)

AST ($r = 0.43$, $p < 0.001$), alkaline phosphatase ($r = 0.50$, $p < 0.001$), GGT ($r = 0.64$, $p < 0.001$) and INR ($r = 0.43$, $p < 0.001$) in the cirrhotic group with and without cholestasis (group Ia and Ib). Serum albumin and prothrombin concentration showed an inverse relationship in the cirrhotic group I (Ia and Ib) ($r = -0.50$, $p = 0.006$ and $r = -0.40$, $p = 0.006$, respectively). No significant correlation was observed between percentage of platelets expressing CD62P and both of urea and creatinine. In group II (group with cholestasis), CD62P% was positively correlated with INR ($r = 0.43$, $p = 0.006$), while it inversely correlated with prothrombin concentration ($r = 0.45$, $p = 0.006$). No significant correlation was observed between percentage of platelets expressing CD62P and other studied parameters (Table 3).

Percentage of platelets expressing CD62P was positively correlated with CD62P (MFI) ($r = 0.37$, $p = 0.006$) in the cirrhotic group (Ia and Ib), while it was negatively correlated with platelet count and plateletcrit (PCT) ($r = -0.39$, $p < 0.001$ and $r = -0.35$, $p < 0.001$, respectively) (Fig. 3). No significant correlation was observed between CD62P% and platelet count or platelet indices in group II or group III.

In cirrhotic patients (group Ia and Ib), we found that the percentage of platelets expressing CD62P was

Table 4. Comparison between percentage of platelets expressing CD62P and clinical data among all cirrhotic group (with and without cholestasis) and cholestatic group

Parameter	Cirrhosis with and without cholestasis (group Ia + Ib) *N* = 84			Cholestasis (group II) *N* = 36		
	Percentage of platelets expressing CD62P			Percentage of platelets expressing CD62P		
	(X ±SD)	Test of signif.	*P*-value	(X ±SD)	Test of signif.	*P*- value
Bleeding						
Positive	17.8 ±7.11	*U* = 2.47	0.01	13.9 ±0.9	U = 4.30	< 0.001
Negative	13.7 ±7.3			11.62 ±1.1		
Portal vein						
Patent	15.04 ±7.9	*U* = 2.75	0.006	–	–	–
Thrombosed	20.08 ±2.9					
Encephalopathy						
Positive	20.8 ±3.9	*U* = 3.04	0.002	–	–	–
Negative	7					
Ascites						
Positive	20.5 ±5.9	*U* = 5.76	< 0.001	–	–	–
Negative	11.2 ±5.7					
Child-Pugh class						
A	9.5 ±3.1	K test 62.23	< 0.001	–	–	–
B	9.4 ±4.0					
C	21.7 ±5.3					

U – Mann-Whitney U test, K – Kruskal-Wallis test, p > 0.05 is statistically non-significant, p < 0.05 is statistically significant

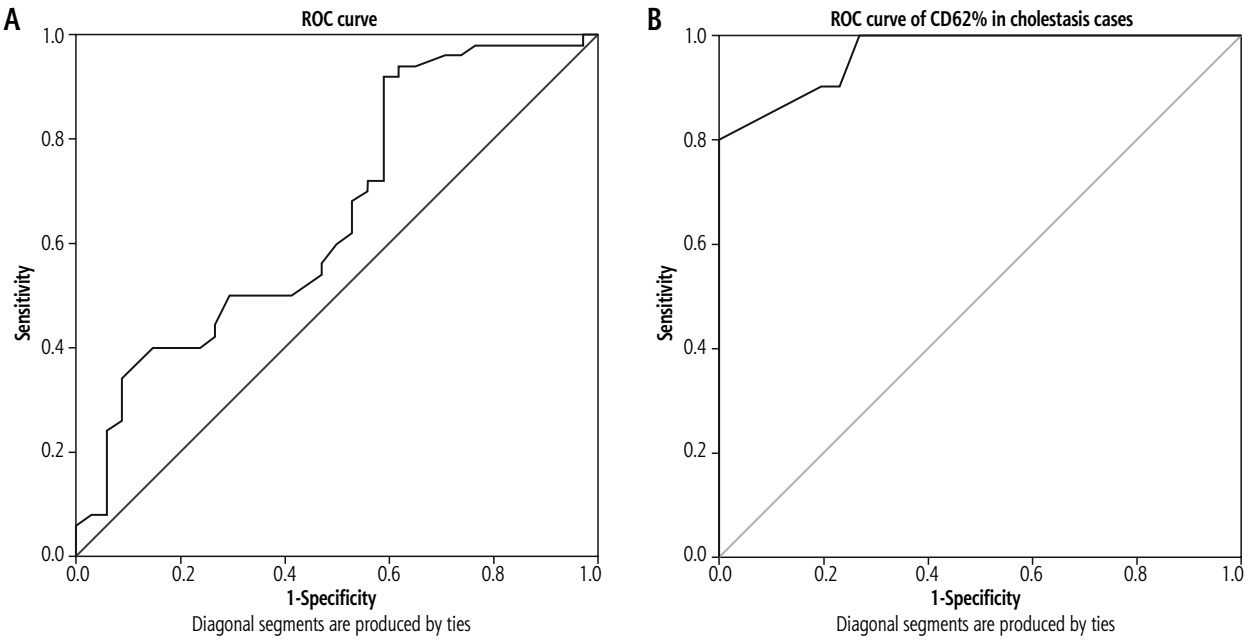

Fig. 4. ROC curve analysis of CD62P% to predict bleeding among cases of cirrhosis with and without cholestasis (Group I) (**A**) and among cases of cholestasis (Group II) (**B**)

significantly higher in patients with gastrointestinal (GIT) bleeding than non-bleeders, in patients with portal vein thrombosis than a patent one, in presence of encephalopathy, ascites, and an advanced Child-Pugh class C than Child A and B ($p = 0.01$, $p = 0.006$, $p = 0.002$, $p < 0.001$, $p < 0.001$ respectively). In group II,

Table 5. Comparison between bleeders and non-bleeders in cirrhotic group and cholestatic group regarding platelet count, indices, CD62P and CD41 expression

Parameters	Cirrhotic group (Group Ia + Ib) *N* = 84		Test	*P* value	Group with cholestasis (Group II) *N* = 36		*U* test	*P* value
	Bleeders *n* = 50	Non-bleeders *n* = 34			Bleeders *n* = 10	Non-bleeders *n* = 26		
Platelets								
Mean ±SD	115.9 ±73.6	138.3 ±91.7	*U* = 1.02	0.31	274.5 ±100.6	265.5 ±82.5	0.19	0.85
Range	40-535	50-533			134-427	150-416		
MPV (fl)								
Mean ±SD	11.46 ±1.36	9.85 ±0.98	*t* = 5.95	< 0.001	9.85±1.38	10.36 ±1.25	1.29	0.20
Range	7.5-14	8.3-11.9			8.3-12.7	7.8-12.2		
PCT%								
Mean ±SD	0.13 ±0.08	0.13 ±0.09	*U* = 0.32	0.75	0.27 ±0.12	0.27 ±0.09	0.25	0.78
Range	0.05-0.53	0.05-0.52			0.12-46	0.16-0.44		
PDW								
Mean ±SD	15.81 ±4.70	13.6 ±2.63	*t* = 2.48	0.002	13.13 ±2.60	14.49 ±3.4	1.33	0.19
Range	10.1-41.9	10.9-20.1			11-19.5	10.9-24.3		
HCV-PCR								
Mean ±SD	32.6 ±8.1	26.9 ±6.8	*t* = 3.32	0.001	25.9 ±9.4	29.2 ±9.1	0.94	0.35
Range	9-46.3	15.9-39.5			16.3-4	14.1-44.6		
Platelet CD62P								
Mean ±SD	17.82 ±7.11	13.76 ±7.39	*U* = 2.47	0.01	13.96 ±0.98	11.62 ±1.1	4.30	< 0.001
Range	4.5-30	2.5-28.5			12.7-16	10.13		
CD62P MFI								
Mean ±SD	18.23 ±4.08	17.3 ±4.6	*t* = 0.99	0.33	16.8 ±3.15	15.29 ±2.6	1.22	0.22
Range	11-32	11-33			12-22	11-21		
CD41%								
Mean ±SD	60.5 ±10.22	63.1 ±10.2	*t* = 1.14	0.26	61.3 ±8.8	65.2 ±11.6	1.11	0.27
Range	43-78	45-81			52-78	36-82		

MPV – mean platelet volume, PCT – plateletcrit, PDW – platelet distribution width, PLCR – platelet large cell ratio, MFI – mean fluorescence intensity, U – Mann-Whitney U test, t – Student t test, p > 0.05 is statistically non-significant, p < 0.05 is statistically significant

higher CD62P% expression was significantly detected in patients with GIT bleeding than in non-bleeders ($p < 0.001$) (Table 4).

Furthermore, in the bleeder cirrhotic group, platelet distribution width (PDW), mean platelet volume (MPV), platelet large cell ratio (PLCR), and percentage of platelets expressing CD62P were significantly higher ($p = 0.002$, $p < 0.001$, $p = 0.001$, $p = 0.01$ respectively) compared to the non-bleeder group. However, platelet count, PCT, and MFI of CD62P showed no significant difference between groups ($p > 0.05$), whereas in the bleeder cholestatic group percentage of platelets expressing CD62P was significantly higher ($p < 0.001$) compared to the nonbreeder group, while no significant difference was detected regarding all studied parameters between bleeders and non-bleeders ($p > 0.05$) (Table 5).

At cut-off > 12.4 the percentage of platelets expressing CD62P yielded AUC = 0.66, $p = 0.01$, with a sensitivity of 72% and specificity of 44.1%, which can discriminate between bleeders and non-bleeders among the group I patients. Moreover, at cutoff > 12.9 the CD62P expression (AUC = 0.96, $p < 0.001$), with sensitivity 90% and specificity 80.8%, can discriminate between bleeders and non-bleeders among group II patients (Fig. 4).

Mean platelet volume was an independent risk factor for bleeding among cirrhotic cases with an odds ratio of 2.96 and CI 1.47-5.94 followed by percentage of platelets expressing CD62P with an odds ratio of 1.07 and CI 0.99-1.15 (Table 6).

Table 6. Binary logistic regression analysis (inter method) for independent risk factors for bleeding among cirrhosis (with and without cholestasis) cases

	SE	Wald χ^2	*P* value	Odds ratio	95% CI
MPV	0.36	9.30	0.002	2.96	(1.47-5.94)
PDW	0.11	0.09	0.767	0.97	(0.78-1.20)
PLCR	0.06	0.06	0.80	0.99	(0.88-1.10)
CD62P%	0.04	2.56	0.11	1.07	(0.99-1.15)

MPV – mean platelet volume, PDW – platelet distribution width, PLCR – platelet large cell ratio, SE – standard error, CI – confidence interval

Discussion

P-selectin, also known as CD62P, is a cell-surface glycoprotein adhesion molecule that is mobilized to the cell surface after activation by different inflammatory or thrombogenic agents [10]. Studies have examined the platelet activation markers in patients with chronic liver disease, e.g. the study of Martí-Carvaja *et al.* [8], but most of these studies did not explain the relationship between the platelet activation markers and jaundice or the bleeding tendency among those patients. Therefore, the present study was designed to evaluate the changes in CD62P expression as a platelet activation marker in patients with liver cirrhosis with and without cholestasis and to evaluate its relation to other platelet functional parameters, clinical status, and bleeding tendency.

All our studied groups were matched for age and gender ($p > 0.05$). Another study conducted by Ghoneim *et al.* included 70 individuals divided according to the diagnosis into biliary atresia ($n = 30$) and cholestasis groups ($n = 20$). Both were age and sex-matched with a third healthy control group ($n = 20$). Baseline demographic characteristics were comparable in both biliary atresia and cholestasis groups.

The present study found that portal vein thrombosis, hepatic encephalopathy, and Child-Pugh class C were significantly prevalent ($p < 0.001$) among patients with cirrhosis and cholestasis group (Ia) when compared to the cirrhosis (Ib) group. Also, no significant difference ($p > 0.05$) was found between the two groups regards the virology screen (HCV Ab and HBs Ag). Kalaitzakis *et al.* found that 61% had cirrhosis (37% Child-Pugh class A, 23% Child-Pugh class B, and 2% Child-Pugh class C).

Regarding CD62P as a platelet activation marker, this study showed that the percentages of platelets expressing CD62P and CD62 MFI were significantly higher in the cirrhotic patients (group I) and cholestatic groups (group II) compared to healthy controls (group III). This finding was in agreement with Xianghong *et al.* [11], who found that CD62P was significantly higher in hepatic cirrhosis patients than in healthy controls. In addition, Tacke *et al.* [12] reported that P-selectin was generally higher in liver disease patients compared to healthy controls.

Interestingly, Sira *et al.* [9] reported that P-selectin expression (CD62P) was significantly higher in liver tissue of patients with biliary atresia (BA) patients compared with non-biliary atresia patients. It has been hypothesized that primary vascular abnormalities participate in the pathogenesis of BA since the biliary tree receives its blood supply from the arterial system exclusively and impaired arterial flow results in necrosis and fibrous obliteration of extrahepatic bile ducts.

Moreover, the percentage of platelets expressing CD62P was significantly higher in group Ia and group II compared to group Ib, and its MFI was significantly higher in group Ia compared to group Ib and group II. This is consistent with the study of Tacke *et al.* [12], who reported elevated CD62P levels in chronic hepatitis and liver cirrhosis patients and claimed that the elevation of P-selectin is not related to the stage of liver cirrhosis or the underlying cause of liver disease.

Panasiuk *et al.* [13] also found that platelet P-selectin expression, serum platelet factor 4, and plasma β-thromboglobulin activities were increased in patients with liver disease. Pihusch *et al.* [14] found that the platelet granule marker CD62P was identical before and after platelet agonists stimulation in patients with PBC/PSC (primary biliary cirrhosis/primary sclerosing cholangitis) and HCV.

There was a positive correlation between the percentage of platelets expressing CD62P and each of serum bilirubin, ALT, AST, alkaline phosphatase, GGT, and INR in the cirrhotic group with and without cholestasis (group Ia and Ib), while serum albumin showed an inverse relationship in group I (Ia and Ib). On the other hand, the percentage of platelets expressing CD62P was positively correlated with INR and inversely correlated with prothrombin concentration in group II (cholestasis group). This agreed with the study carried out by Tacke *et al.* [12]; they found that P-selectin plasma levels correlated with albumin concentration. However, serum albumin and prothrombin concentrations are the most important synthetic function of the liver; this may ex-

plain the increased CD62P expression with the deterioration of the liver function tests (decreased serum albumin and decreased prothrombin concentration). Ali *et al.* [15] found that P-selectin was correlated with higher fibrosis score, bilirubin, alkaline phosphatase, prothrombin time (PT), and MELD score, with a negative correlation with albumin and platelet numbers. P-selectin also was associated with reduced ALT and PT, with a positive association with platelet counts.

The current study revealed an inverse correlation between CD62P expression and Hb level in group I (Ia and Ib). No significant correlation was observed between it and all other CBC parameters among studied groups. Tacke *et al.* [12] noted a correlation between P-selectin levels and white blood cells (WBC), monocyte, neutrophil, and lymphocyte counts. They evaluated also concomitant liver biopsies and observed higher P-selectin levels in patients with severe leukocyte infiltration, indicating the association between P-selectin and leukocyte recruitment in chronic liver disease.

Furthermore, the percentage of platelets expressing CD62P was positively correlated with CD62P (MFI) in the cirrhotic group (Ia and Ib), while it was negatively correlated with platelet count and PCT. This agreed with Kamel *et al.* [16] as they demonstrated that higher P-selectin levels can be proposed as a marker of *in vivo* platelet activation since MFI of CD62P was significantly higher in all HCV infected groups than that of controls.

Kamel *et al.* [17] also reported that plasma soluble P-selectin level was markedly elevated in chronic HCV especially in patients with low platelet counts with a direct correlation with serum HCV-RNA. Guzmán-Fulgencio *et al.* [18] noted significantly increased serum P-selectin during anti-HCV therapy.

Our study showed that, in cirrhotic patients (group Ia and Ib), the percentage of platelets expressing CD62P was significantly increased in patients with GIT bleeding, portal vein thrombosis, encephalopathy, presence of ascites, and advanced Child-Pugh class. In group II, higher CD62P% expression was significantly detected in patients with GIT bleeding. This agreed with the study of Tacke *et al.* [12] as they found a negative correlation between P-selectin and spleen size as a major site of platelet clearance with no correlation with ChildPugh class of cirrhosis as the cause of liver disease, which disagreed with our finding.

Additionally, MPV, PDW, PLCR, and CD62P% were significantly higher in the bleeder cirrhotic group than in the non-bleeder group. Also, in the bleeder cholestatic group, the percentage of platelets expressing CD62P was significantly higher compared to the non-bleeder group, while no significant difference was detected regarding all studied parameters between bleeders and non-bleeders. This agreed with Xianghong *et al.* [11] as they found that CD62P expression was higher in patients with liver cirrhosis combined with upper gastrointestinal (GI) bleeding than in the non-bleeding group.

At a cutoff > 12.4, the percentage of platelets expressing CD62P yielded AUC = 0.66, $p = 0.01$, with sensitivity 72% and specificity 44.1%, which was able to discriminate bleeders and non-bleeders among the group I patients. Moreover, at cutoff > 12.9 the percentage of platelets expressing CD62P yielded AUC = 0.96, $p < 0.001$, with a sensitivity of 90% and specificity of 80.8%, which was able to discriminate bleeders and non-bleeders among group II patients. The study which was conducted by Wang *et al.* [19] on 191 patients diagnosed with HCC, revealed that the AUC for CD62P% was 0.697 (95% CI: 0.641-0.750) and at cut-off value 18.4%, the sensitivity and specificity were 39.27% and 95.96%, respectively.

Finally, our study revealed that MPV was an independent risk factor for bleeding among cirrhotic cases with an odds ratio of 2.96 and CI 1.47-5.94, followed by the percentage of platelets expressing CD62P, with an odds ratio 1.07 and CI 0.99-1.15. Kalafateli *et al.* [20] found a larger MPV, a marker of platelet activation, in chronic hepatitis B (CHB) patients with the inactive disease than in controls.

Conclusions

Mean platelet volume and percentage of platelets expressing CD62P are independent risk factors for the bleeding tendency among cirrhotic cases, which can provide guidance for clinical treatments and prognostics. Future large-scale studies are recommended to evaluate the role of platelet activation markers in patients with liver disease.

Ethics approval

All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (Ethical Committee of National Liver Institute, Menoufia University No. NLI:018/33) and with the Helsinki Declaration of 1975, as revised in 2008.

Informed consent was obtained from all patients for being included in the study.

Disclosure

The authors declare no conflict of interest.

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