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Sandesh Raykar
Junior resident, Department of General Medicine, Institute of Medical Sciences, Banaras Hindu University, Varanasi, Uttar Pradesh, India

Anju Bharti
Assistant professor, Department of Pathology, Institute of Medical Sciences, Banaras Hindu University, Varanasi, Uttar Pradesh, India

Lalit P Meena
Associate professor, Department of General Medicine, Institute of Medical Sciences, Banaras Hindu University, Varanasi, Uttar Pradesh, India

Madhukar Rai
Professor, Department of General Medicine, Institute of Medical Sciences, Banaras Hindu University, Varanasi, Uttar Pradesh, India

Correspondence
Lalit P Meena
Associate professor, Department of General Medicine, Institute of Medical Sciences, Banaras Hindu University, Varanasi, Uttar Pradesh, India

Study of coagulation and platelet dysfunction in chronic liver disease at a tertiary care hospital

Sandesh Raykar, Anju Bharti, Lalit P Meena and Madhukar Rai

Abstract
Introduction: The liver has a major role in hemostatic system. As most of the Indian studies concentrated mostly on the bleeding tendency in liver disease, in our study we included assessment of bleeding or thrombotic tendency by measuring, VWF, protein c and s, factor VIII level and platelet function.
Objectives: To determine the range of hemostatic defects in patients of chronic liver disease by measuring Coagulation profile, VWF, protein c and s, factor VIII level and platelet function.
Material and Method: Patients who were defined to have Chronic Liver Disease are included in study. The hemostatic tests which performed for procoagulant proteins were -Prothrombin time, Activated partial thromboplastin time, VWF, Factor VIII assay, Protein C, Protein S and assessment of Platelet Function.
Results: Present study included 75 patients of Chronic liver disease. The mean Age was 45.07±12.669 years with M: F ratio 5.8:1. 53% had thrombocytopenia. Mean value of Prothrombin in Class a was 15.340±1.4666, in Class B was 20.488±4.1447 and in Class C was 27.628±7.5530. Increased level (>150%) of VWF was seen in 34 cases (45%). The mean value of Factor VIII was115.66±38.08 %. The mean value of Protein C was 71.63±49.95%. Platelet aggregation test (PAT) was prolonged (>5seconds) with ADP as agonist in 33%.
Conclusion: it was observed in our study that laboratory evidences of both hypo and hypercoagulable state were present in patients of chronic liver disease We suggest that further studies with an increased number of samples and more tests of hypercoagulation like antithrombin III, α2-macroglobulin.

Keywords: coagulation, liver disease, platelets

Introduction
The liver has a major role in hemostatic system as it synthesizes the majority of coagulation factors, thrombopoeitin and proteins involves in coagulation pathway. Therefore, acute or chronic liver diseases frequently have a profound effect on the hemostatic system [1]. A low grade activation of endothelial cells result in continuous release of von Willebrand factor (VWF) whose level is therefore normally raised in patients with liver disease [2]. Liver synthesizes many coagulation proteins e.g. Factor I, II, V, VII, VIII, IX, X, XI, XII, XIII, anticoagulants protein C & S. Bleeding in liver disease could be due to portal hypertension, coagulopathies, or thrombocytopenia. Many studies have shown that patients with liver disease also develop deep venous thrombosis and pulmonary artery embolism [3-7]. Thrombotic disease has been attributed to decreased plasma levels of protein C, S and antithrombin. Prothrombin time which is a measure of factor I, II, V, VII and X is almost universally prolonged in patients with cirrhosis and other chronic liver diseases [8]. This test has also been employed as prognostic indicator. Therefore it is evident that patients with liver disease may experience both bleeding complications as well as thrombotic episodes. In patients with liver disease extremely complex alteration of hemostasis occur and therefore more complex tests and more comprehensive overview of hemostatic changes are required to appreciate haemostatic status. As most of the Indian studies concentrated mostly on the bleeding tendency in liver disease, in our study we included assessment of bleeding or thrombotic tendency by measuring, VWF, protein c and s, factor VIII level and platelet function.

Objectives
1. To determine the range of hemostatic defects in patients of chronic liver disease by measuring Coagulation profile, VWF, protein c and s, factor VIII level and platelet function.
2. To correlate the laboratory findings of hemostasis with clinical outcome of patients.

Material and Methods

The study was conducted in Department of Medicine/ Hematology section of Department of Pathology at a tertiary care centre between a periods from June 2016 to July 2017. The study was approved by institutional ethical committee. 75 patients of chronic liver disease who presented in General Medicine and Gastroenterology ward, were included in the study. Patients were defined to have Chronic Liver Disease based on following criteria: at least two signs of portal hypertension; platelet count <90,000/l, ascites, splenomegaly, or esophageal varices, and at least two signs of liver dysfunction; albumin level < 30g/l, INR > 1.5, or bilirubin level > 35 μmol/l, or solely by a liver biopsy showing evidence of liver cirrhosis. Patients included in the study did not have any thrombotic or bleeding complications except for variceal bleed. After taking detailed clinical history and performing clinical examination (as per format) and informed consent, blood sample was collected for investigations. Along with the all routine investigations test for hemostatic were done. The hemostatic tests which performed for procoagulant proteins were -Prothrombin time (PT), Activated partial thromboplastin time (aPTT) Von Will ebrand assay, Factor VIII assay. Tests for assessment of anticoagulant proteins were- Protein C, Protein S and assessment of Platelet Function was done by Platelet aggregation test. Liver biopsy was done wherever required.

Patients were divided into three groups based on Child Turcotte Pugh scoring system:

Score			
	1	**2**	**3**
Encephalopathy	None	Grade 1-2	Grade 3-4
Ascites	None	Mild/Moderate (diuretic responsive)	Severe (diuretic refractory)
Bilirubin (mg/dL)	<2	2-3	>3
Albumin (g/dL)	>3.5	2.8-3.5	<2.8
PT (sec prolonged) or INR	<4 <1.7	4-6 1.7-2.3	>6 >2.3

CTP **score** is obtained by adding the score for each parameter.
CTP **class**: A = 5-6 points, B = 7-9 points, C = 10-15 points

Blood for assessment of haemostasis was collected in 1 blue top (containing 3.2% sodium citrate) vacutainers in a ratio of 9 Vol blood to 1 Vol of anticoagulant. Test tubes were centrifuged at 1500-3000 rpm for 10 min for coagulation studies- Prothrombin time (PT), Activated partial thromboplastin time (aPTT). After separating, plasma will kept at -20 to -40ºC and may be tested in batches for- Factor VIII assay, Von Willebrand assay, Protein C, - Protein S. Tests were done on Diagnostica Stago semi-automated analyzer STARt^{R}4 as recommended by manufacturer. Platelet aggregation test was done by using platelet-aggregometer (Chronolog model 700) with platelet agonist: Adenosine diphosphate (ADP) and Adrenaline. Platelet aggregation time >5 seconds than control was considered Prolonged.

RESULTS- Present study included 75 patients of Chronic liver disease. The Age range of the patients were 18-70 years and the mean Age was 45.07±12.669 years. Male to female ratio was 5.8:1. 40 out of 75 cases (53%) had thrombocytopenia (<150x10^3/μl), 35 cases (47%) had platelet count in range (150-450) x10^3/μl. [Table 1) Mean value of Prothrombin in Class A was15.340±1.4666, in Class B was 20.488±4.1447 and in Class C was 27.628±7.5530. [Table 2] Mean value of INR in Class A was 1.168±0.1500, in Class B was 1.702±0.4443 and in Class C was 2.550±0.9616. Mean value of APTT in Class A was 31.016±4.7700, in Class B was 39.136±7.0614 and in Class C was 46.572±9.7093 [table 3]. Normal levels of VWF (50-150%) were seen in 41 cases (54.7%), with 25 cases (100%) in Class A, 12 cases (48%) in Class B and 4 cases (16%) in Class C. Increased level (>150%) of VWF was seen in 34 cases (45%), with none in Class A, 13cases (52%) in Class B and 21 cases (84%) in Class C. p value being <0.001 [Table4] the mean value of VWF was 104.77±27.163. In Class A the mean value of VWF was 81.24±21.90%, In Class B the mean value of VWF was147.40±18.53, %. In Class C the mean value of VWF was 168.24±15.96%. The mean value of Factor VIII was 115.66±38.08 %. In Class A the mean value of F VIII was 85.32±26.32 %, In Class B the mean value of F VIII was 127.42±36.047 %, In Class C the mean value of F VIII was 134.25±31.89 %. [Table 5] Decreased levels of Factor VIII (<60%) was seen in 2 cases (1.3%), with 2 cases (8%) in Class A, none of the cases in Class B and C. Normal levels (60-150%) were seen in 58 cases (77%), with 22 cases (88%) in Class A, 19 cases (76%) in Class B and 17 case (68%) in Class C. Increased levels (>150%) were seen in 15 cases (20%) with 1 case (4%) in Class A, 6 case (24%) in Class B and 8 cases (32%) in Class C. p value being <0.028

The mean value of Protein C was 71.63±49.95%. Mean value of protein C in Class A was 124.06±32.71 %. Mean value of protein C in Class B was 64.51±35.69%. Mean value of protein C in Class C was 26.33±17.23 %. [Table 6] Decreased levels of protein C (<70%) was seen in 39 cases (52%), with 2 cases (8%) in Class A, 13 cases (52%) in Class B and 24 cases (96%) in Class's C. Normal levels (70-130%) were seen in 18 cases (24%), with 6 cases (24%) in Class A, 11 cases (44%) in Class B and 1 case (4%) in Class C. Increased levels (>130%) were seen in 18 cases (24%) with 17 cases (68%) in Class A, 1 case (4%) in Class B and none in Class C. p value being <0.001. In present study, the mean value of Protein S was 61.41±23.09 %. Mean value of Protein S in Class A was 73.69±16.62 %. Mean value of protein S in Class B was 70.94±15.94%. Mean value of protein S in Class C was 39.61±19.14%. [Table 7] Decreased levels of Protein S (<90%) was seen in 69 cases (92%) with 23 cases (92%) in Class A, 22 cases (88%) in Class B and 24 cases (96%) in Class C. Normal levels (90-130%) were seen in 6 cases (8%), with 2 cases (8%) in Class A, 3 cases (12%) in Class B and 1 case (4%) in Class C. Platelet aggregation test (PAT) was normal (<5seconds) with ADP as agonist in 50 cases (66.6%), with 24 cases (96%) in Class A, 18 cases (72%) in Class B and 8 cases (32%) in Class C. Platelet aggregation test (PAT) was prolonged (>5seconds) with ADP as agonist in 25 cases (33%), with 1 case (4%) in Class A, 7 cases (28%) in Class B and 17 cases (68%) in Class C. p value being <0.001. Platelet aggregation test (PAT) was normal (<5seconds) with ADR as agonist in 48 cases (64%), with 23 cases (92%) in Class A, 18 cases (72%) in Class B and 7 cases (28%) in Class C. Platelet aggregation test (PAT) was prolonged (>5seconds) with ADR as agonist in 27 cases (36%), with 2 cases (8%) in Class A, 7 cases (28%) in Class B and 18 cases (72%) in Class C. p value being <0.001.

Discussion

The present study was conducted from June 2016 to July 2017 on patients with clinically suspected primary liver disease. 75 patients of chronic liver disease were included. Patient in our study did not have any thrombotic or bleeding manifestations except for variceal bleed. The age of patients at presentation ranged from 18-70 years. Mean age for chronic liver disease

patients (n=75) was 45.07± 12.669 years. Similar to our study Siddiqui *et al* (2011) found mean age of patients presenting with chronic liver disease to be 46±14 years.[9] The mean age for CTP (Child Turcotte Pugh) Class A (n=25) was 42.52±12.62 years, Class B (n=25) was 47.04±11.02 years and Class C (n=25) was 45.64±14.23 years. Male to female ratio was 5.8:1. Similar to present study Ahmadhameed *et al* (2006) found more male predominance with male to female ratio of 2:1.[10] 40 out of 75 cases (53%) had thrombocytopenia (platelet count <150x10^3/µl), 35 cases (47%) had platelet count in range (150-450) x10^3/µl. Mean platelet count in the study population was 152±66x10^3/µl. In Class A mean value of platelet count was 199±72x10^3/µl, with thrombocytopenia was seen in 4 cases (16%). In Class B mean value of platelet count was 154±50x10^3/µl, with thrombocytopenia was seen in 12 cases (48%). In Class C mean value of platelet count was 105±33x10^3/µl, with thrombocytopenia was seen in 24 cases (96%). N Afdhal *et al* 2008 found that thrombocytopenia (platelet counts <150,000/Dl) was a common complication in patients with chronic liver disease (CLD), reported in as many as 76% of cirrhotic patients [11]. Multiple factors can contribute to the development of thrombocytopenia, including splenic platelet sequestration, bone marrow suppression by chronic hepatitis C infection, and antiviral treatment with interferon-based therapy. Reductions in the level or activity of the hematopoietic growth factor, thrombopoietin (TPO) may also play a role. Possible reason behind this is 1) portal hypertension and hypersplenism in end stage liver disease (cirrhosis), 2) autoimmune reaction to platelets, 3) direct infection of platelets and megakaryocytes by HCV. Our study showed significant prolongation of prothrombin time (PT) and APTT in patients of liver cirrhosis which increased with increase in CTP class and MELD score. The mean PT in CTP Class A was 15.340±1.4666 seconds, Class B was 20.488±4.1447 and Class C was 27.628±7.5530 seconds. The mean APTT in Class A was 31.016±4.7700 seconds, Class B was 39.136±7.0614 and Class C was 46.572±9.7093. This is in concordance with study done by Saray *et al.* (2012) and Saja *et al* (2013) and confirmed that prolongation of conventional coagulation screening tests appear in advanced liver disease and are not sensitive markers of liver damage [12,13]. Furthermore, recent studies have shown that these global tests are not predictive of bleeding in patients with cirrhosis however PT has kept its place as one of the parameters of common prognostic indices in advanced liver disease. In present study, the mean value of Factor VIII was 115.66±38.08 %. Increased levels (>150%) of Factor VIII was seen in 15 cases (20%). In Class A the mean value of Factor VIII was 85.32±26.32 %, with increased levels of F VIII was seen in 1 case (4%). In Class B the mean value of F VIII was 127.42±36.047 %, with increased levels of F VIII was seen in 6 cases (24%). In Class C the mean value of F VIII was 134.25±31.89 %, with increased levels of F VIII was seen in 8 cases (32%). The elevated levels of factor VIII may be related to the elevated levels of its carrier protein VWF, or to the decreased expression of the low-density lipoprotein-related receptor, which is involved in factor VIII clearance. [14] In present study, the mean value of VWF was 104.77±27.163. Increased level (>150%) of VWF was seen in 34 cases (45%). In Class A the mean value of VWF was 81.24±21.90, with increased levels of VWF was seen in none of the cases. In Class B the mean value of VWF was 147.40±18.53, with increased levels of VWF was seen in 13 cases (52%). In Class C the mean value of VWF was 168.24±15.96, with increased levels of VWF was seen in 21 cases (84%), which shows an important compensatory mechanism for the defects in platelet number and function. It has been recently demonstrated that VWF plasma levels can be elevated more than 10-fold in severe cirrhosis. Moreover, despite subtle functional defects, these extremely high plasma levels of VWF are able to support platelet adhesion under flow conditions substantially better than plasma from healthy volunteers, and therefore to compensate, at least in part, for reduced platelet numbers with a possibly reduced function [15].

In present study, the mean value of Protein C was 71.63±49.95%. Decreased levels of protein C (<70%) was seen in 39 cases (52%). Mean value of protein C in Class A was 124.06±32.71 %, with 2 cases (8%) having decreased levels. Mean value of protein C in Class B was 64.51±35.69% with 13 cases (52%) having decreased levels. Mean value of protein C in Class C was 26.33±17.23 % with 24 cases (96%) having decreased levels. Study done by Saja *et al* 2013 and Sarey *et al* 2012 also found significantly low protein C value in cirrhosis group when compared with control group.[12,13] This was a sign of reduced hepatocyte synthetic capacity in chronic liver disease. Zocco *et al* 2009 showed that in chronic liver disease reduction in plasma levels of Protein C correlate with a higher model for end-stage liver disease (MELD) score. [16] Abdo *et al* 2010 documented Protein C as a potential predictor of hepatic fibrosis in chronic liver disease. [17] These findings, including the present one, confirm that levels of Protein C are sensitive markers. The mean value of Protein S in present study was 61.41±23.09 %. Decreased levels of Protein S (<90%) was seen in 69 cases (92%). Mean value of protein S in Class A was 73.69±16.62 % with 23 cases (92%) having decreased levels. Mean value of protein S in Class B was 70.94±15.94% with 22 cases (88%) having decreased levels. Mean value of protein S in Class C was 39.61±19.14% with 24 cases (96%) having decreased levels. In this study, Platelet aggregation test was prolonged (>5seconds) with ADP agonist in 25 cases (33%) and with ADR agonist in 27 cases (36%). In Class A, PAT was prolonged with ADP in 1 cases (4%) and with ADR in 2 cases (8%). In Class B PAT was prolonged with ADP in 7 cases (28%) and ADR in 7 cases (28%). In Class C PAT was prolonged with ADP in 17 cases (68%) and ADR in 18 cases (72%). Platelets from patients with liver disease have abnormal platelet aggregometry results, with the abnormalities proportional to the severity of liver disease. There are multiple causes for these abnormal results, including the presence of circulating inhibitors (d-dimers, DYSFIBRINOGENS), degradation of platelet receptors by plasmin, increased nitric oxide, defects in signal transduction pathways, and so forth [18, 19].

Table 1: Platelet distribution among Classes of CTP

PLT lakh	CTP					
	Class A		Class B		Class C	
	No.	%.	No.	%.	No.	%.
<1.5	4	16.0	12	48.0	24	96.0
>1.5	21	84.0	13	52.0	1	4.0
Total	25	100	25	100	25	100

$\chi 2$=32.571; p=<0.001

Table 2: Mean value of Prothrombin time among Classes of CTP

CTP	PT (Mean±SD)	Range	f-value	p-value
Classa	15.340±1.4666	13-19	37.393	.000
Class B	20.488±4.1447	16-29.9		
Class C	27.628±7.5530	15-44		
p-value				
Class A Vs Class B	Class A Vs Class C	Class B Vs Class C		
0.002	0.000	0.000		

Table 3: Mean value of APTT among Classes of CTP

CTP	APTT (Mean±SD)	Range	f-value	p-value
Class A	31.016±4.7700	24-41.7	27.205	.000
Class B	39.136±7.0614	26.9-49		
Class C	46.572±9.7093	31-67.3		
p-value				
Class A Vs Class B	Class A Vs Class C	Class B Vs Class C		
0.001	0.000	0.002		

Table 4: Distribution of Von Willebrand Factor among classes of CTP

Von willebrand factor	CTP					
	Class A		Class B		Class C	
	No.	%.	No.	%.	No.	%.
50-150	25	100	12	48.0	4	16.0
>150	0	0.0	13	52.0	21	84.0
Total	25	100	25	100	25	100

$\chi 2$=36.263**; p=<0.001**

Table 5: Mean value of FACTOR VIII among classes of CTP

CTP	Factor_VIII (Mean±SD)	f-value	p-value
Class A	85.320±26.3244	17.504	<0.001
Class B	127.428±36.0477		
Class C	134.256±31.8941		
p-value			
Class A Vs Class B	Class A Vs Class C	Class B Vs Class C	
<0.001	<0.001	1.000	

Table 6: Mean value of PROTEIN C among classes of CTP

CTP	Protein_C (Mean±SD)	f-value	p-value
Class A	124.064±32.7136	68.882	.000
Class B	64.516±35.6925		
Class C	26.336±17.2352		
p-value			
Class A Vs Class B	Class A Vs Class C	Class B Vs Class C	
<0.001	<0.001	1.000	

Table 7: Mean value of PROTEIN S among classes of CTP

CTP	Protein_S (Mean±SD)	f-value	p-value
Class A	73.696±16.6297	29.964	.000
Class B	70.944±15.9451		
Class C	39.612±19.1451		
p-value			
Class A Vs Class B	Class A Vs Class C	Class B Vs Class C	
1.000	<0.001	<0.001	

The following chemicals were purchased from their suppliers and used without further purification. Acetone (Merck

Conclusion

Thus it was observed in our study that laboratory evidences of both hypo and hypercoagulable state were present in patients of chronic liver disease. Since clinically there was neither any bleeding complication nor any evidence of thrombotic manifestations, our study therefore supported the concept of rebalanced hemostasis in liver diseases. As these balance appeared to be very precarious, patients should be kept under close watch for any of the two complications. Longstanding dogma that patients with liver disease have a hemostasis related bleeding tendency is not supported by data collected in our study. Although the prophylactic correction of abnormal hemostasis test results by blood product transfusion prior to invasive procedures to prevent bleeding is a commonly performed clinical practice, there is no scientific evidence for this, and this policy requires reconsideration [20]. Routine hemostasis tests such as the platelet count, PT and APTT did not reflect the increased tendency of bleeding complication in the patients of chronic liver disease. In our study it appeared that this hypocoaguable state was balanced by reduction in Protein C and Protein S level and by increase in Factor VIII and Von Willebrand level. The true picture of hemostasis in liver cannot be commented upon. Therefore we suggest that further studies with an increased number of samples and more tests of hypercoagulation like anti-thrombin III, α2-macroglobulin.

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