Original Article

DOI: 10.7860/JCDR/2017/24975.10451

Pathology Section

Haemostatic Profile of Patients with Chronic Liver Disease- its Correlation with Severity and Outcome

VARNIKA RAI[1], NEERAJ DHAMEJA[2], SANDIP KUMAR[3], JYOTI SHUKLA[4], RAJEEV SINGH[5], VINOD KUMAR DIXIT[6]

ABSTRACT

Introduction: The liver plays an important role in the haemostatic system as it synthesizes the majority of coagulation factors and fibrinolytic proteins.

Aim: The present study was planned to determine the range of haemostatic defects in patients of chronic liver diseases.

Materials and Methods: Test performed included Prothrombin Time (PT), activated Partial Thromboplastin Time (aPTT), Thrombin Time (TT), Fibrinogen, Protein C, D Dimer and platelet count. Comparisons between groups frequencies and groups means were made using Chi-square test and Student's t-test, respectively.

Results: In cirrhosis group PT, aPTT, TT and D Dimer level were significantly increased compared to Chronic Hepatitis (CH) and control group ($p<0.001$ for all comparisons). Serum fibrinogen, Protein C and platelet count were significantly reduced in cirrhosis patients compared to CH and control group. ($p<0.001$ for all comparisons). All studied coagulation parameters were within normal limit in CH group. However, statistically significant difference was found in protein C and mean platelet count in CH group compared to control ($p=0.03$ and $p<0.001$ respectively). No evidence of bleeding or thrombosis was present in study group.

Conclusion: In cirrhosis patients severe derangement in both anti and procoagulant factors occurs. Haemostatic profile in chronic hepatitis patient remains within normal limits.

Keywords: Chronic hepatitis, Cirrhosis, D Dimer, Fibrinogen, Protein C

INTRODUCTION

The liver has a cardinal role in the haemostatic system. Liver synthesizes plasma proteins including many coagulation proteins e.g., Factor I, II, V, VII, VIII, IX, X, XI, XII, XIII, many natural anticoagulants like protein C & S [1,2]. Chronic or acute liver diseases frequently have an intense impact on the haemostatic system [3].

Bleeding in liver disease could be due to decreased plasma levels of haemostatic proteins synthesized by the liver [3]. It could also be due to thrombocytopenia, coagulopathy, enhanced fibrinolysis or portal hypertension [4-6]. Recent studies have shown that patients with liver disease also develop deep venous thrombosis and pulmonary artery embolism at rates between 0.5%-1.9% [6-9].

A large population based study done by Sogaard KK et al., also showed an increased risk for development of venous thrombosis in patients with liver disease as compared with healthy persons [10]. This thrombotic tendency has been attributed to decreased plasma levels of the natural anticoagulants, protein C, S and antithrombin. Therefore, it is evident that patients with liver disease may experience both bleeding complications as well as thrombotic episodes.

The present study was planned to determine the range of haemostatic defects in patients of chronic liver diseases. In contrast to previous studies done in India focussing mainly on the bleeding tendency in chronic liver disease, our study also included assessment of thrombotic tendency by measuring protein C levels in these patients.

MATERIALS AND METHODS

Our study was retrospectively done on 60 patients of chronic liver disease who presented in Department of Gastroenterology of SS hospital, Benaras Hindu University. Patients who were referred from outside over a period of one and half years (Jan 2014 to Jun 2015) were also included. Patients were divided into two groups. First group (n=30) included serologically diagnosed cases of chronic hepatitis. All the patients in this group had undergone liver biopsy and showed mild to moderate fibrosis (Ishak fibrosis score 0-3). Second group (n=30) consisted of histologically (Ishak fibrosis score 4 and 5), radiologically or clinically established cases of liver cirrhosis with or without oesophageal varices or ascites. Informed consent was taken from every patient included in the study.

Control group comprised of 30 age and sex matched healthy subjects recruited randomly from blood donors, academic staff and volunteers from the general public. General exclusion criteria included history of bleeding or thrombotic disorder, history of renal disease, diabetes mellitus, ongoing or recent pregnancy, recent history of transfusion of blood products, current anticoagulation therapy. After getting informed consent detailed history was taken.

For coagulation studies blood was collected in blue top (containing 3.2% sodium citrate) vacutainers in a ratio of 9 volume blood to 1 volume of anticoagulant. Platelet Poor Plasma (PPP) was made by centrifugation at 2000 g for 10 minutes. Coagulation tests were done immediately or PPP were stored in cuvettes at temperature of -20 to -40 degree celcius for later dates. Almost 2 ml blood was collected in EDTA vial for complete blood count by Beckman Coulter Haematology Fully Automated Autoanalyser LH750. The coagulation screening tests; PT (STA-Neoplastine RCl), activated partial thromboplastin time (CK PRESTR) and thrombin time (STA-THROMBIN) were performed by the conventional methods and the clotting times were registered by an optical coagulation system (STartR4; Diagnostica Stago). Plasma fibrinogen was measured by the turbidometric method of Clauss (FIBRI-PRESTR). Assessment of Protein C levels was done by kit provided by Diagnostic Stago (Staclot Protein C). D-Dimer test was performed using D-DI latex kit (Diagnostica Stago, France).

STATISTICAL ANALYSIS

Statistical analysis was done using SPSS 16.0 Comparisons between two group frequencies were made using Chi-square test. For the comparison of group means, Student's t-test was applied.

RESULTS

The age of chronic liver disease patients (n=60) ranged between 26-67 years and mean age was 46.62 years. There were 47 male and 13 female patients with M:F ratio of 3.6:1. Mean age for CH, cirrhosis and control group were 42.13, 51.10 and 40.8 years respectively. Fatigue was most common clinical symptom present in all 60 cases (100%) of patient group. The aetiology of chronic liver disease of the overall study group is summarized in [Table/Fig-1].

In our study mean platelet count was 197000±44277/mm^3 and 91400±41692/mm^3 in chronic hepatitis and cirrhosis group respectively and significantly lower than that of control group (292130+72824/mm^3) (p-value<0.001). Platelet count was significantly lower in cirrhosis group in comparison to chronic hepatitis group. In our study 33 (55%) out 60 cases of Chronic Liver Disease (CLD) had thrombocytopenia (platelet count <150x10^3/µl). Thrombocytopenia were found in five cases (16.7%), 25 cases (93.3%) and 0% cases of chronic hepatitis, cirrhosis and control respectively.

PT, aPTT and TT in cirrhosis group were significantly higher when compared to CH and control group (p-value <0.001). There were no significant difference in PT, aPTT and TT of CH group compared to control [Table/Fig-2].

Fibrinogen level in cirrhosis group was significantly lower than that of control and CH group (p-value <0.001). There was no significant difference in CH group compared to control.

Cirrhosis group had significantly lower protein C value compared to CH and control group (p<0.001). Though chronic hepatitis group had mean protein C level within normal limit, compared to control group it was significantly reduced (p=0.030) [Table/Fig-2].

In the present study, cirrhosis group had 21 cases out of 30 (70%) with increased D-Dimer level (>0.5ug/ml-FEU). When compared with chronic hepatitis and control group, cirrhosis group had significant number of cases with increased D Dimer level. Chronic hepatitis had two cases (6.67%) with increased D-Dimer level which was not significant when compared with control group [Table/Fig-3].

Aetiology	Chronic hepatitis group	Cirrhosis group	Total
Hepatitis B	23	15	38
Hepatitis C	5	4	9
Alcohol	-	11	11
Cryptogenic	2	-	2

[Table/Fig-1]: Aetiology of chronic liver disease.

Parameters (Mean ±SD)	Chronic Hepatitis (n=30)	Cirrhosis Group (n=30)	Control group (n=30)
PT(sec)	14.32±0.89	21.98±5.05 p*<0.001, p**<0.001	13.24±0.53
aPTT(sec)	29.64±3.37	45.65±16.64 p*<0.001, p**<0.001	27.31±2.10
TT(sec)	19.77±1.92	35.16±14.80 p*<0.001, p**<0.001	19.30±1.57
Fibrinogen (mg/dL)	307.80±78.76	198.50±104.05 p*<0.001, p**<0.001	303.43±41.01
Protien-C (%)	98.72±22.26 p*0.03	46.48±29.10 p*<0.001, p**<0.001	108.85±11.57

[Table/Fig-2]: Results and comparison of haemostatic assays in chronic liver disease patients.
Quantitative values are expressed as mean ± standard deviation, PT: Prothrombin Time, aPTT: activated partial thromboplastin time. *significant difference in comparison with controls **significant difference in comparison with group 1

D-dimer (0.5ug/ml-FEU)	Chronic hepatitis (n=30)		Cirrhosis(n=30)		Control	
	No.	%	No.	%	No.	%
<0.5	28	93.34	9	30	30	100
≥0.5	2	6.66	21	70	0	0
Total	30	100	30	100	30	100

[Table/Fig-3]: D-dimer distribution in chronic hepatitis and cirrhosis group vs control group.
Chronic hepatitis Vs cirrhosis – p-value = <0.001; χ^2=25.45
Chronic hepatitis Vs control p-value = 0.4915;χ^2=2.069
Cirrhosis Vs control p-value = <0.001;χ^2=32.31

DISCUSSION

In present study, 33 out of 60 cases (55%) had thrombocytopenia (platelet count <150x103/µl). Afdhal N et al., found that thrombocytopenia (platelet counts <150,000/dL) was a common complication in patients with CLD, reported in as many as 76% of cirrhotic patients [11]. Various factors can lead to thrombocytopenia like splenic platelet sequestration, bone marrow suppression by chronic hepatitis C infection, and antiviral treatment with interferon-based therapy [11]. Reduced level or activity of the Thrombopoietin (TPO) may also play a role [11]. Similar to our study, Papatheodoridis GV et al., found mean platelet count in chronic hepatitis to be within normal limit (208X 103/mm^3) and Zocco MA et al., found mean platelet count in cirrhosis to be reduced (96 X 103/ mm^3) [12,13].

Findings of PT and aPTT in our study are in concordance with study done by Saray A et al., and Saja MF et al., and confirmed that prolongation of conventional coagulation screening tests appears in advanced liver disease and are not sensitive markers of liver damage [14,15]. 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 [16].

Al-Ghumlas AK et al., documented TT as a measurement of the final step of the coagulation cascade, viz., the conversion of fibrinogen to fibrin. Prolongation of the TT reflects quantitative as well as qualitative fibrinogen abnormalities (Dysfibrinogenemia). Our findings of TT were in agreement with Saja et al., [15,17].

Our findings of serum fibrinogen level were in line with similar study done by Saray A et al., [14]. Al-Ghumlas et al., found that plasma fibrinogen as an acute-phase reactant remains normal or increases in chronic liver disease [17]. Fibrinogen is synthesized almost exclusively in the liver and low levels in cirrhotics are generally attributed to decreased liver synthetic capacity, consumption during Disseminating Intravascular Coagulation (DIC), destruction by abnormal plasma fibrinolytic activity or accelerated catabolism [18].

Our finding of protein C was in concordance with Saja MF et al., and Saray A et al., who also found significantly low protein C value in both chronic hepatitis and cirrhosis group when compared with control group [14,15]. This was a sign of reduced hepatocyte synthetic capacity in chronic hepatitis. Zocco MA et al., showed that in CLD reduction in plasma levels of PC correlate with a higher Model For End-Stage Liver Disease (MELD) score [13]. Abdo AA et al., documented PC as a potential predictor of hepatic fibrosis in chronic liver disease [19].

These findings, including the present one, confirm that levels of PC are sensitive markers [19]. Study done by Ahmadhameed SN et al., also found 49 patients out of 50 of chronic liver disease had FDP (D-dimer) levels above normal (> 250 ng/ml) [20]. Our findings of fibrinolytic activity in CLD were compatible with other studies done by Cioni G et al., and Takahashi H et al., [21,22]. The underlying mechanism for increased fibrinolysis is increased conversion of plasminogen to plasmin, increased tissue plasminogen activators and impaired clearance of circulating plasminogen activators. It may also be due to defect of the antiplasmin and other inhibitory factors. The major inhibitor of plasmin i.e., α-2 antiplasmin is reduced in cirrhotic patients with resultant increased fibrinolysis. Moronglu F et al., stated that fibrinolysis may also be secondary to disseminated intravascular coagulation [23].

LIMITATION

Due to some technical issue we could not include other markers of thrombosis and bleeding tendencies like protein S, Von Willebrand factor, thromboelastography and platelet function tests which would have helped in better understanding as well as enhancing the quality of our study.

CONCLUSION

Thus it was observed in our study that in cirrhosis patients severe derangement in both anti and procoagulant factors occurs. Haemostatic profile in chronic hepatitis patient remains within normal limits.

Since clinically there was neither any bleeding complication nor any evidence of thrombotic manifestations, our study therefore supported the concept of rebalanced haemostasis 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 haemostasis related bleeding tendency is not supported by data collected in our study. Routine haemostasis tests such as the platelet count, PT and aPTT did not reflect the increased bleeding complication in the patients of chronic liver disease.

We also found that patients with chronic hepatitis exhibited significant reduction in protein C levels in comparison with healthy controls. All other haemostatic assays did not show any significant fluctuation in this patient group. Thus it supported use of protein C as one of the sensitive markers for hepatic dysfunction.

REFERENCES

[1] Mammen EF. Coagulation abnormalities in liver disease. Haematol Oncol Clin North Am. 1992;6:1247-57.
[2] McCormick PA, Murphy KM. Spleno-megaly, hypersplenism and coagulation abnormalities in liver disease. Bailliere's Clin Gastroentrol. 2000;14:1009-31.
[3] Lisman T, Leebeek FWG, de Groot PG. Haemostatic abnormalities in patients with liver disease. J Hepatol. 2002;37(2):280-87.
[4] Witters P, Freson K, Verslype C, Peerlinck K, Hoylaerts M, Nevens F, et al. Review article: blood platelet number and function in chronic liver disease and cirrhosis. Aliment Pharmacol Ther. 2008;27(11):1017-29.
[5] Bakker CM, Knot EA, Stibbe J, Wilson JH. Disseminated intravascular coagulation in liver cirrhosis. J Hepatol. 1992;15(3):330-35.
[6] Northup PG, McMahon MM, Ruhl AP, Altschuler SE, Volk-Bednarz A, Caldwell SH, et al. Coagulopathy does not fully protect hospitalized cirrhosis patients from peripheral venous thromboembolism. Am Gastroenterol. 2006;101(7):1524-28.
[7] Senzolo M, Sartori MT, Lisman T. Should we give thromboprophylaxis to patients with liver cirrhosis and coagulopathy? HPB (Oxford). 2009;11(6):459-64.
[8] Gulley D, Teal E, Suvannasankha A, Chalasani N, Liangpunsakul S. Deep vein thrombosis and pulmonaryembolism in cirrhosis patients. Dig Dis Sci. 2008;53(11):3012-17.
[9] Garcia-Fuster MJ, Abdilla N, Fabia MJ, Fernandez C, Oliver V, Forner MJ. Venous thromboembolism and liver cirrhosis. Rev Esp Enferm Dig. 2008;100(5):259-62.
[10] Sogaard KK, Horvath-Puho E, Gronbaek H, Jepsen P, Vilstrup H, Sorensen HT. Risk of venous thromboembolism in patients with liver disease: a nationwide population- based case control study. Am J Gastroenterol. 2009;104(1):96-101.
[11] Afdhal N, McHutchison J, Brown R, Jacobson I, Manns M, Poordad F, Thrombocytopenia associated with chronic liver disease. Journal of Hepatology. 2008;48(6):1000-07.
[12] Papatheodoridis GV, Papakonstantinou E, Andrioti E, Cholongitas E, Petraki K, Kontopoulou I, et al. Thrombotic risk factors and extent of liver fibrosis in chronic viral hepatitis. Gut. 2003;52(3):404-09.
[13] Zocco MA, Di Stasio E, De Cristofaro R, Novi M, Ainora ME, Ponziani F, et al. Thrombotic risk factors in patients with liver cirrhosis: correlation with MELD scoring system and portal vein throm-bosis development. J Hepatol. 2009;51(4):682-89.
[14] Saray A, Mesihovic R, Vanis N, Gornjakovic S, Prohic D. Clinical significance of haemostatic tests in chronic liver disease. Med Arh. 2012;66(4):231-35.
[15] Saja MF, Abdo AA, Sanai FM, Shaikh SA, Gader AG. The coagulopathy of liver disease: does vitamin K help? Blood Coagul Fibrinolysis, 2013;24:10–17.
[16] Tripodi A, Caldwell SH, Hoffman M, Trotter JF, Sanyal AJ. The prothrom-bin time test as a measure of bleeding risk and prognosis in liver disease. Ali-ment Pharmacol Ther. 2007;26(2):141-48.
[17] Al Ghumlas AK, Abdel Gader AG, Al Faleh FZ. Haemostatic abnormalities in liver disease: could some haemostatic tests be useful as liver function tests? Blood Coagulation and Fibrinolysis. 2005;16:329-35.
[18] Amitrano L, Guardascione MA, Brancac-cio V, Balzano A. Coagulation disorders in liver disease. Semin Liver Dis. 2002;22(1):83-96.
[19] Abdo AA, Sanai FM, Azzam N, Al Sawat K, Al Dukhayil M, Al Ghumlas A, et al. Natural anticoagulants can be useful predictors of severity in chronic liver disease. Blood Coagul Fibrinolysis. 2010;21(2):122-27.
[20] Ahmadhameed SN, Khursheed ASSI, Hamid A, Naveed I. An assessment of coagulation parameters in liver cirrhosis. Biomedica. 2006;22:74-77.
[21] Cioni G, Cristani A, Vignoli A, Ventura E. High Ddimer level. A possible index of risk of onset DIC and / or digestive bleeding in advanced liver cirrhosis. Recenti Prog Med. 1994;85(4):230-34.
[22] Takahashi H, Tatewaki W, Wada K, Niwano H, Shibata A. Fibrinolysis and fibrinogenolysis in liver disease. Am J Hematol. 1990;34(4):241-45.
[23] Moronglu F, Mamusa AM, Mameli G, Mulas G, Solinas A, Demelia L, et al. alpha 2 Antiplasmin and disseminated intravascular coagulation in liver cirrhosis. Thrombosis Research. 1985;37:287-94.

PARTICULARS OF CONTRIBUTORS:
1. Resident, 24 Hour Laboratory, SGPGI, Lucknow, Uttar Pradesh, India.
2. Assistant Professor, Department of Pathology, IMS BHU, Varanasi, Uttar Pradesh, India.
3. Assistant Professor, Department of Pathology, IMS BHU, Varanasi, Uttar Pradesh, India.
4. Professor, Department of Pathology, IMS BHU, Varanasi, Uttar Pradesh, India.
5. Senior Resident, Department of Radiodiagnosis, SGPGI, Lucknow, Uttar Pradesh, India.
6. Professor, Department of Gastroenterology, IMS BHU, Varanasi, Uttar Pradesh, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:
Dr. Varnika Rai,
Resident, 24 Hour Laboratory, SGPGI, Lucknow-226014, Uttar Pradesh, India.
E-mail: vrachievers@gmail.com

FINANCIAL OR OTHER COMPETING INTERESTS: None.

Date of Submission: **Nov 05, 2016**
Date of Peer Review: **Dec 11, 2016**
Date of Acceptance: **Feb 05, 2017**
Date of Publishing: **Aug 01, 2017**