International Journal of Biomedical And Advance Research

ISSN: 2229-3809 (Online)
Journal DOI:10.7439/ijbar
CODEN:IJBABN

Research Article

Haemostatic alterations in liver diseases

Sheetal Waghmare*, Narayan Ingole and Nitin Gangane

Department of Pathology, Mahatma Gandhi Institute of Medical Sciences Sewagram, Wardha, Maharashtra, India

***Correspondence Info:**
Dr. Sheetal Waghmare,
Department of Pathology,
Mahatma Gandhi Institute of Medical Sciences, Sewagram, Wardha, Maharashtra, India
E-mail: sheetalwaghmare12@gmail.com

Abstract

Background - The liver is the major site of synthesis for all the clotting factors. The concentrations of these factors, especially factor V has been demonstrated to fall proportionately to the increasing severity of the disease. Liver disease also leads to vitamin K deficiency, mainly by the decreased absorption from the gut due to the reduced bile salts secretion from the liver. The physiology of the hemostatic system is closely linked to liver function because the liver parenchymal cells produce most of the factors of clotting and fibrinolytic systems and because the liver regulates the activation or inhibition of both systems.

Objectives - The present study is designed to assess the values of various haemostatic parameters in liver disease. The parameters studied are Platelet counts, Prothrombin time (PT), Activated partial thromboplastin time (APTT), Thrombin time (TT), and fibrinogen level.

Results and Conclusions - On comparing the mean prothrombin time, activated partial thromboplastin time (APTT), thrombin time, and mean fibrinogen level in cases of liver disease with that in the controls, it was found to be significantly ($p<0.001$) increased in liver disease. The mean platelet count observed in liver diseases was significantly decreased than in controls.

Keywords: Liver diseases, Platelet count, Prothrombin time, Activated Partial Thromboplastin time, Thrombin time, Fibrinogen level

1. Introduction

The physiology of the hemostatic system is closely linked to liver function because the liver parenchymal cells produce most of the factors of clotting and fibrinolytic systems and because the liver regulates the activation or inhibition of both systems. Professor Eberhard F. Mammen greatly contributed to the understanding of the relationship between hemostatic abnormalities and liver diseases.[1] A disturbed liver parenchymal cell function adversely impacts the hemostatic system, the extent of which correlates with the degree of disease.

The liver is the major site of synthesis for all the clotting factors. The concentrations of these factors, especially factor V, has been demonstrated to fall proportionately to the increasing severity of the disease.[2] Liver disease also leads to vitamin K deficiency, mainly by the decreased absorption from the gut due to the reduced bile salts secretion from the liver.[3] Prothrombin time (PT), which measures these vitamin K dependent factors, has been well recognised as an accurate predictor of liver damage and likelihood of progression to end stage liver failure. It has thus been incorporated into the commonly used prognostic indices of chronic liver disease such as the Child–Pugh or Mayo End-Stage Liver Disease (MELD) scores.[4]

The reticuloendothelial system (RES) of the liver greatly participates in the clearance of breakdown products of activated clotting factors such as fibrin-related products, thrombin-antithrombin complex (TAT), plasmin–plasmin- inhibitor complex (PPIC), and activated platelets.[5]

Thus, a variety of haemostatic abnormalities can occur in patients with severe liver disease, including impaired synthesis of clotting factors, excessive fibrinolysis, DIC, thrombocytopenia, and platelet dysfunction. The haemostatic abnormalities in liver disease also predispose to hypercoagulable state [6]

The routinely practised tests in coagulation laboratories i.e. prothrombin time (PT), activated partial thromboplastin time (APTT) and thrombin time (TT) which measure impairment in extrinsic, intrinsic and common pathways of coagulation respectively have shown significant alterations in liver diseases.[7] Similarly the level of fibrinogen in circulating blood is also altered in liver disease.[8] However, some studies have suggested that conventional tests of the clotting cascade such as the PT and APTT correlate poorly with procedure-related bleeding in patients with cirrhosis.[9]

The present study is therefore designed to assess the values of various haemostatic parameters like prothrombin time (PT), activated partial thromboplastin time (APTT) and thrombin time (TT), fibrinogen level and platelet counts in liver disease like acute and chronic liver diseases and primary hepatic malignancies.

2. Material and Methods

The present study is a prospective observational study carried out in the haematology division of the Department of Pathology, over a period from January 2010 to July 2011.

The material for the study was obtained from the patients of liver diseases admitted in the hospital wards or attending the outpatient departments. Study – Consisted of cases and controls

The inclusion criteria were the cases with diagnosed liver disease except those which were specifically excluded because of the below mentioned **exclusion criteria:**

All primary (Genetic) and secondary conditions that lead to hypercoagulable states were excluded. The relevant secondary hypercoagulable conditions that are excluded from the study are

i. **High risk for thrombosis** – Prolonged bed rest or immobilization, Myocardial Infarction, Atrial fibrillation
ii. **Low risk for thrombosis-** Cardiomyopathy, Nephrotic syndrome, Hyperestrogenic states (pregnancy), Oral contraceptive use, Sickle cell anaemia
iii. **Extrahepatic malignancies with hepatic metastasis** (secondary conditions that may lead to hypercoagulable states)

The selected cases for study were categorised as under –

1. With acute liver disease or acute hepatitis
2. With Chronic liver disease including
   a. Chronic hepatitis
   b. Cirrhosis of liver
3. Primary hepatic malignancy

"Controls" comprised of age and sex matched healthy individuals

The informed consent of the patient was obtained prior to the collection of blood. The study obtained the ethical clearances from the ethical committee.

The amount of anticoagulant (3.2% tri-sodium citrate) and blood sample were maintained to give 1:9 ratio and the desired amount of blood was collected to get 5 ml anticoagulated blood sample; This blood sample was immediately centrifuged at 3000 rpm (approximately 2000 g) for 15 minutes and the supernatant plasma was transferred to a clean polystyrene tube. This plasma sample was used for studying Prothrombin time (PT), Activated partial thromboplastin time (APTT), Thrombin time (TT) and Fibrinogen levels. The tests were carried out within 3 hrs of collection of blood sample. The control blood samples were collected from healthy individuals in 3.2 % tri-sodium citrate with ratio of anticoagulant and blood as 1:9 and were processed similarly, Additional 2 ml blood sample was collected in EDTA anticoagulant and was processed for complete blood count on Beckman coulter make automated blood cell counter.

Statistical analysis of patients with liver diseases (acute, chronic and primary hepatic malignancies) with that of controls without any liver disease was done. The mean values along with standard deviations of all the included parameters and predictive values (p values) were computed using 2x2 tables (EPI 6).

3. Results

In the present study we have assessed the haemostatic alterations in liver diseases in 96 patients comprised of 90 males and 6 females. Similarly 70 age and sex matched healthy individuals were included in the study as controls. The platelet count was done using three part Beckman coulter make automated blood cell counter. As shown in Table I, the '**platelet count'** of the cases of liver diseases and controls were

Table I: Showing platelet counts in cases of liver disease and in controls

Sr. No	Diagnosis	Total Cases	Platelet count x 10^9/L	
			Range	Mean ±SD
I	**Cases**			
1	Acute liver diseases	19	45-490	269.31±143.52
2	Chronic Liver diseases	74	47-608	182.1±104.67
	i Alcoholic Cirrhosis	62	47-608	188.17±106.32
	ii Non- alcoholic cirrhosis	12	55-390	150.75±93.47
3	Primary Hepatocellular carcinoma	3	229-297	271.33±36.93
Total Cases		**96**	**45-608**	**202.15±117.11**
II	**Controls**	**70**	**187-441**	**303.78±59.16**

On comparing the mean platelet count in cases of liver disease with that in the controls, it was found to be significantly decreased in liver diseases ($p<0.001$).

In the subgroups of liver diseases, the mean platelet count in acute liver disease (269.31±143.52 x10^9/L) and in hepatocellular carcinoma (271.33±36.93 x10^9/L) were found to be comparable with that in the controls (303.78±59.16x10^9/L). However statistically, the decrease in platelet count in both acute and chronic liver diseases were found to be significant ($p=0.04$ and $p<0.001$).

In the present study PT, APTT, TT and fibrinogen levels were calculated using semi-automated coagulometer of Behnk German make. As shown in Table II, the '**prothrombin time (PT) and activated partial thromboplastin time (APTT)'** of the cases of liver diseases and controls were -

Table II – Showing results of Prothrombin time (PT) test and Activated partial thromboplastin time (APTT) test in cases of liver disease and in controls

Sr. No	Diagnosis	Total Cases	Prothrombin Time (PT) in Seconds		Activated partial thromboplastin time (APTT) in seconds	
I	**Cases**	**19**	**Range**	**Mean ±SD**	**Range**	**Mean ±SD**
1	Acute liver diseases		13.4-43.8	22.10±8.42	31.5-52.8	39.73±5.65
2	Chronic Liver diseases	**74**	13.1-41.0	21.44±8.16	32.0-68.7	39.15±8.3
	i Alcoholic Cirrhosis	62	13.1-40.5	21.23±7.62	32.0-68.7	39.33±8.44
	ii Non-alcoholic Cirrhosis	12	14.2-41.0	22.51±10.89	32.2-60.5	38.19±7.91
3	Primary Hepatocellular carcinoma	**3**	13.5-60.3	29.13±26.99	33.1-85.3	50.7±29.87
Total		**96**	**13.1-60.3**	**21.81±9.04**	**31.5-85.3**	**39.65±9.04**
II	**Controls**	**70**	**12.5-13.2**	**13.02±0.22**	**30.0-30.2**	**30.16±0.04**

On comparing the **mean prothrombin time** in cases of liver disease with that in the controls, it was found to be significantly ($p<0.001$) increased in liver disease. When the mean prothrombin time in subgroups of liver diseases were compared with that in the controls, the increase in prothrombin time in acute liver disease was more than that in chronic liver disease and both values as compared to the controls were statistically significant ($p=0.04$, $p<0.001$).

On comparing the **mean activated partial thromboplastin time (APTT)** in cases of liver disease with that in the controls, it was found to be significantly increased in liver disease ($p<0.001$). When the mean APTT in subgroups of liver diseases were compared with that in the controls, the increase in APTT in both acute liver disease (39.73±5.65 seconds) and in chronic liver disease (39.15±8.3 seconds) were statistically significant ($p=0.04$ and $p<0.001$) whereas it was not in primary hepatic malignancy.

As shown in Table III, the '**thrombin time (TT) and fibrinogen level'** of the cases of liver diseases and controls were -

Table III – Showing results of Thrombin time (TT) test and Fibrinogen level assay in cases of liver diseases and in controls

Sr. No	Diagnosis	Total Cases	Thrombin Time in seconds		Fibrinogen level in mg/dl	
			Range	Mean ±SD	Range	Mean ±SD
I	**Cases**					
1	Acute liver diseases	**19**	8.6-26.3	16.55±3.86	30-320	113.15±64.18
2	Chronic Liver diseases	**74**	8.1-36.5	15.34±4.72	30-530	175.17±96.57
	i Alcoholic Cirrhosis	62	8.1-36.5	15.30±4.80	30-530	176.45±100.3
	ii Non- alcoholic Cirrhosis	12	10.8-20.2	15.58±4.49	60-320	168.58±77.76
3	Primary Hepatocellular carcinoma	**3**	15.5-23.4	18.36±4.37	30-90	60±30
	Total cases	**96**	**8.1-23.4**	**15.68±4.56**	**30-530**	**159.32±94.34**
II	**Controls**	**70**	**10-10.2**	**10.11±0.08**	**180-350**	**249.07±48.29**

On comparing the mean thrombin time in cases of liver diseases with that in the controls, it was found to be significantly increased in cases of liver disease ($p<0.001$). When the thrombin time in subgroups of liver diseases were compared with that in the controls, the increased thrombin time was found to be significant in both acute liver disease (16.55±3.86 seconds), and in chronic liver disease (15.34±4.72 seconds) however in hepatocellular carcinoma (18.36±4.37 seconds) the findings were statistically insignificant.

The normal range of **'fibrinogen'** in healthy individuals is 150-400 mg/dl.

Although the present study observed the mean fibrinogen level in both cases and controls within normal range, it was found much lower in cases of liver disease than in controls as shown in Table III.

The difference of mean fibrinogen level in cases of liver diseases as compared to that in the controls was found to be statistically significant ($p<0.001$).

In the present study, the mean fibrinogen level observed in subgroups of liver diseases were 113.15±64.18 mg/dl, 175.17±96.57 mg/dl and 60±30 mg/dl in acute liver disease, chronic liver disease and hepatic malignancy respectively. On comparing these values in the subgroups of liver diseases with that in the controls, maximum decrease of fibrinogen level was in hepatic malignancy followed by that in acute liver diseases, whereas the decrease in fibrinogen level was less in chronic liver diseases. Statistically these findings in all the three subgroups of liver diseases when compared with controls were found to be significant ($p<0.001$ in each).

4. Discussion

In the present study total 96 cases of liver diseases were studied for platelet counts, basic or first line screening tests of hemostasis [Prothrombin time (PT), Activated Partial Thromboplastin Time (APTT). Thrombin Time (TT)], and fibrinogen level assay to find out the abnormalities in these tests associated with liver diseases. Seventy age and sex matched healthy individuals were also studied similarly as controls.

Present study observed the mean '**Platelet count'** in liver diseases was significantly decreased as compared to that in the controls.

The thrombocytopenia in liver diseases is explained by different mechanisms as under –

1. Increased splenic sequestration as a result of congestive splenomegaly related to portal hypertension.[10]
2. Decreased production of platelets in alcoholic cirrhosis due to folic acid deficiency or due to toxic effects of ethanol on megakaryopoiesis.[11]
3. Due to decreased thrombopoietin production by cirrhotic liver.[10]
4. Antiplatelet autoimmunity plays a role. Circulating B cells producing platelet associated and circulating anti GpIIb-IIIa antibodies are detected in cirrhosis of liver.[12]
5. Bone marrow suppression by virus or interferon based antiviral treatment may play a role.[6]

The prothrombin time is commonly increased in liver diseases because liver is unable to manufacture adequate amount of clotting factors including those involved in extrinsic pathway.Out of factors II, V, VII and X, prothrombin time is crucially dependent on factor VII.It is the rate limiting factor in extrinsic pathway and thus has the greatest influence on prothrombin time. Factor VII has shortest half life (6 hrs) and its fall is associated with bad prognosis.[7]

The APTT is a simple test of coagulation. It is a measure of factors involved in intrinsic pathway (Prekallikrein, HMWK and factors XII, XI, IX and VIII) and in the common pathway (factors X, V, Prothrombin and fibrinogen). It is more sensitive to the deficiencies of factor VIII and IX than to deficiencies of factor XI or XII or factors involved in common pathway. It is also used to monitor heparin anticoagulation. It yields abnormal results, if the plasma level of any of the essential factor is <15-30% of the normal value. In liver diseases, prolongation of APTT is because of impaired synthesis of factors IX, XI, XII and fibrin stabilizing factor by the diseased liver.

Thrombin time test is a measure of the rate at which fibrin forms. It yields abnormal results when the fibrinogen level is <70-100 mg/dl and is unaffected by the levels of any of the other coagulation factors.

The increased thrombin time in liver diseases is because of –

i. Defective aggregation of fibrin monomers into a polymer[13] and
ii. It strongly correlates with increased sialic acid content in Bβ and γ chains of fibrinogen molecule[14]

Fibrinogen is a glycoprotein synthesized by liver and by megakaryocytes. Liver diseases alter not only the level of circulating fibrinogen but also make it functionally abnormal. In 50% cases of advanced cirrhosis and nearly 100% cases of fulminant hepatic failure, the structure of fibrinogen is abnormal although the levels of fibrinogen may be normal. Abnormalities in fibrinogen structure may impair fibrin polymerization and clot formation despite its normal level[15].

The functional abnormalities of fibrinogen are due to –

i. Increased antithrombin activity in the plasma because of fibrinogen/fibrin degradation products whose clearance by the diseased liver is delayed[16]
ii. Dysfibrinogenemia due to abnormal fibrinogen synthesis by damaged liver in cirrhosis[13] and aggressive chronic hepatitis and in hepatocellular carcinoma[17]

The structural abnormalities in fibrinogen are because of –

(a) Increase in sialic acid content in Bβ and γ chains of fibrinogen molecule[14].
(b) An alteration of the glucide fraction with elevation of sialic acid content[18].

These structural changes result in slow fibrin formation, giving rise to functional dysfibrinogenemia and abnormalities in fibrinogen level[8]; thus, the fibrinogen level can be normal, decreased or increased in liver diseases.

5. Conclusions

- Thrombocytopenia with mild to moderate decrease in platelet count is frequently seen.
- The values of baseline screening tests i.e. PT, APTT and TT are increased in liver diseases.
- Fibrinogen level may be decreased, normal or even increased in liver diseases.
- PT and APTT are inadequate to reflect the balance of coagulation as occur in vivo. Therefore alternative tests mimicking more closely what occurs in vivo like platelet function analysers, thrombin generation test and thromboelastography should be developed and investigated.

References

1. Mammen EF. Coagulopathies of liver disease. *Clin Lab Med* 1994; 14:769–80.
2. Amitrano L, Guardascione MA, Brancaccio V, Balzano A. Coagulation disorders in liver disease. *Semin Liver Dis* 2002; 22:83–96.
3. Sherlock S. Nutritional complications of biliary cirrhosis: chronic cholestasis. *Am J Clin Nutr* 1970; 23:640–43.
4. Pugh RN, Murray-Lyon IM, Dawson JL, Pietroni MC, Williams R. Transection of the oesophagus for bleeding oesophageal varices. *Br J Surg* 1973; 60:646–9.
5. Wada H, Usui M, and Sakuragawa N. Hemostatic Abnormalities and Liver Diseases. *Seminars in thrombosis and Hemostasis* 2008; 34:772-78.
6. Peck-Radosavljevic M. Review article: coagulation disorders in chronic liver disease. *Aliment Pharmacol Ther* 2007; 26:21-28.
7. Ahmadhameed, Samina Naeem, A. Saeed Shaikh Irfan Khursheed, Ambreen Hamid, Naveed IA: An assessment of coagulation parameters in liver cirrhosis. *Biomedica* 2006; 22:74-77.
8. Williams EC. Disseminated intravascular coagulation. In Loscalgo J. Schafer A I, eds. Thrombosis and haemorrhage. Inded osten; Black well Sceintific. 1994: pp 921-32.
9. Ewe K. Bleeding after liver biopsy does not correlate with indices of peripheral coagulation. *Dig Dis Sci* 1981; 26:388-93.
10. Goulis J, Chau TN, Jordan S, Mehta AB, Watkinson A, Rolles K, and Burroughs AK: Thrombopoietin concentrations are low in patients with cirrhosis and thrombocytopenia and are restored after orthotopic liver transplantation. *Gut* 1999; 44:754–58.
11. Levine RF, Spivak JL, Meagher RC, Sieber F: Effect of ethanol on thrombopoiesis. *Br J Haematol* 1986; 62:345–54.
12. Kajihara M, Kato S, Okazaki Y, Kawakami Y, Ishii H, Ikeda Y, Kuwana M: A role of autoantibody- mediated platelet destruction in thrombocytopenia in patients with cirrhosis. *Hepatology* 2003; 37:1267–76.
13. Palascak J, Martinez J. Dysfibrinogenemia associated with liver disease. *J Clin Invest* 1977; 60:89.
14. Martinez J, Palascak J, Kawasniak D. Abnormal sialic acid content of the dys- fibrinogenemia associated with liver disease. *J Clin Inves* 1978; 61:535.
15. Ratnoff OD, Forman WB. Criteria for the differentiation of dysfibrinogenemia states. *Semin Hematol* 1976; 13:141-57.
16. Lane DA, Scully MF, Thomas DP, Kakkar VV, Woolf IL. Williams R. Acquired dysfibrinogenemia in acute and chronic liver disease. *British.J Haematol* 1977; 35:301.
17. Barr RD, Ouna N, Simpson JC, Bagshave AF. Dysfibrinogenemia and primary hepatocellular carcinoma. *Quart J Med* 1986; 45:647.
18. Gralnick H, Gilvelber H, Abrams E. Dysfibrinogenemia associated with hepatoma, increased carbohydrate content of fibrinogen molecule. *New Engl J Med* 1978; 299: 221.