Evaluation of Coagulation Disorder in Patients with Liver Disease in Bangladesh

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ABSTRACT

In liver disorder, prothrombin time (PT) is elevated, and fibrinogen level decreased which are considered predictors of increased bleeding risk. We aimed at determining whether increased PT and fibrinogen value reflect the haemostatic potential and bleeding risk in patients with liver disorder. The study comprises a total of 135 subjects including patients with liver disorder (n=75) and healthy volunteer (n=60) matched by age and sex. The PT was measured by STart 4 haemostasis analyzer and fibrinogen was measured by ELISA based method. The PT was significantly (p<0.001) increased in patients with liver disorder compared to the controls. On the other hand, the fibrinogen level was significantly decreased (p<0.001) in patients with liver disorder. There was a negative correlation (r = -0.6; p<0.001) of fibrinogen with PT. Coagulation abnormalities were profound in liver disorder. Thus PT and fibrinogen level may be used as a useful tool for diagnosis and treatment of liver disorder.

Key words: Coagulation disorder, Fibrinogen, Haemostasis, Liver disorder, Prothrombin time.

1. Introduction

The liver plays a central role in the maintenance of haemostasis. It serves as the site of synthesis of all clotting factors and their inhibitors. In patients with liver disease, substantial changes in the haemostatic system are frequently found [1]. These changes include thrombocytopenia and platelet function defects, decreased circulating levels of coagulation factors and inhibitors, and decreased levels of proteins involved in fibrinolysis. These coagulation abnormalities can predispose patients from minor localized bleeding to massive life threatening haemorrhage or thrombosis formation [2]. Coagulation abnormalities in acute and chronic liver disease usually measured through the prolongation of first-line global screening tests such as the prothrombin time (PT) and the activated partial thromboplastin time (aPTT) [3]. The PT consists of the time needed for the platelet-poor plasma to clot after the addition of tissue extracts (thromboplastin) and calcium chloride. PT determines vitamin K dependent extrinsic factors VII, X, II, V and fibrinogen.

The prolonged PT is related to the severity of liver failure and is one of the parameter used in commonly used prognostic indices of liver disease such as Child-Pugh or Mayo End-Stage Liver Disease (MELD) scores [4, 5]. The PT is considered as a simple, inexpensive, qualitative and accurate prognostic marker of liver impairment and also a predictor of bleeding [6]. The degree of PT impairment an expression of decreased liver synthesis predicts the severity of portal hypertension and the presence of esophageal varices [4]. PT is related both to bleeding risk and mortality. Patients with moderately or severely prolonged PT have 5 to 10 fold higher mortality rates than patients with normal PT [7]. Fibrinogen level is also considered as an evidence of increased fibrinolytic activity. Fibrinogen increases bleeding tendency in patients with liver disease. Fibrinogen levels are within normal range in patients with stable chronic liver disease but decreased levels are found in patients with advance cirrhosis [6]. In Bangladesh, there is very few information available on this issue. Therefore, in this study coagulation disorder among patients with liver disease was investigated using PT and plasma fibrinogen level.

2. Materials and Method

2.1 Study Subjects

The study was a case-control study conducted on 135 subjects. The case group comprises 75 patients with liver disease (40 men, 35 women; mean age 46.1 ± 17.0). The patients were recruited from the outpatient department of Dhaka Medical College Hospital (DMCH), Dhaka. The patients were clinically diagnosed as suffering from liver disease (acute and chronic), and treated using different therapeutic regimens. Patients with other haemostatic abnormalities (aplastic anaemia, leukaemia, lymphoma, sepsis and vasculitis), alcohol intake, pregnancy were excluded from the study. A total of 60 healthy controls (35 men, 25 women; mean age 44.1 ± 15.0) with no history of liver disease were recruited from different hospitals of Dhaka city where they came for regular health check up.

All participants were given an explanation of the nature of the study and informed consent was obtained. They completed a structured questionnaire covering information on age, gender, medical and family history of chronic diseases. This study was approved by the ethical committee of University of Dhaka and Dhaka Medical College Hospital. All the analyses were done in the Department of Clinical Pathology, DMCH, Dhaka and Department of Biochemistry and Molecular Biology, University of Dhaka, Dhaka-1000, Bangladesh.

2.2 Sample Collection

About 5.0 mL of venous blood was drawn from each individual following all aseptic precautions with the help of a trained person, using a disposable syringe. The blood sample was taken in an EDTA coated tube for the estimation of study parameters. The plasma was separated by centrifugation at 3000 rpm for 10 minutes and kept at -20°C until analysis.

2.3 Clinical Analysis

Estimation of PT value was performed by STart 4 haemostasis analyzer (Diagnostica Stago Inc. USA), and kits were procured by Diagnostica Stago Inc. Plasma fibrinogen level was assayed through clauss method (which uses thrombin to convert fibrinogen to fibrin, followed by measurement of fibrin in the clot) by using a commercial kit (Hemostat fibrinogen kit), manufactured by HUMAN GmbH, Wiesbaden, Germany [8].

2.4 Statistical Analysis

All the results were expressed as mean ± SEM. The statistical analysis of the data was carried out with Statistical Package of Social Science (SPSS), version 17 and Graph pad

Prism version-5. The comparisons between two groups were tested by unpaired t-test. A 95% confidence interval was used. *p* values less than 0.05 were considered as statistically significant. Correlation between two continuous outcomes among patients was evaluated using Pearson correlation coefficient.

3. Results

Statistically significant differences among patients and controls are indicated in Table 1 and in Figure 1 along with their significant values.

As shown in Table 1 the PT was significantly (p<0.001) higher among the patients compared to the controls. On the other hand, the fibrinogen level was significantly (p<0.001) lower in patients when compared to the healthy controls.

Table 1 P-Time and fibrinogen levels in study subjects.

Study Parameters	Control (n=60)	Patients (n=75)	*p* vaule
P-Time (Sec.)	13.50 ± 0.2	23.4 ± 1.2	<0.001
Fibrinogen (g/L)	2.90 ± 0.04	1.56 ± 0.07	<0.001

Results are expressed as mean ± SEM. Unpaired t-test was done as the test significant. p<0.05 was taken as the level of statistical significant.

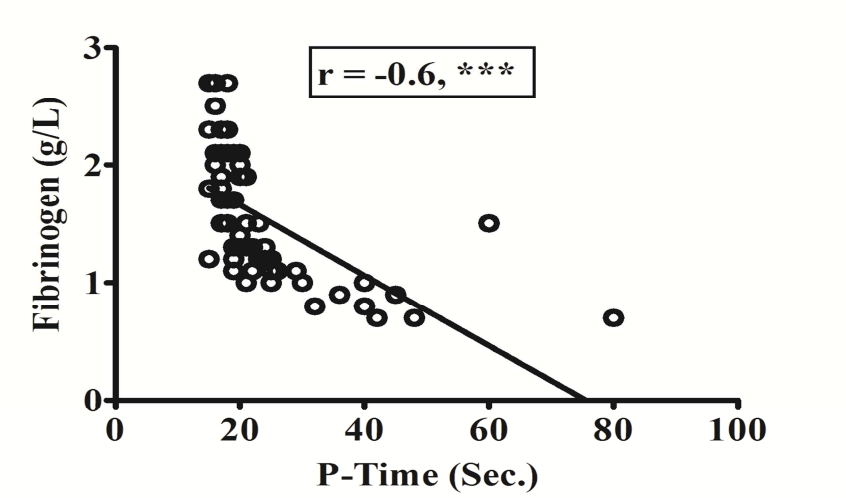

Fig. 1. Correlations of fibrinogen levels with P-Time. ***; p<0.001.

Fig. 1 shows the correlation of fibrinogen level with PT. The fibrinogen level is strongly associated with estimated PT in patients. There is a significant negative

correlation (r = -0.6, p>0.001) of fibrinogen level with PT.

4. Discussion

The liver synthesizes almost all clotting factors and their inhibitors, though the endothelium also acts as an additional source of FVIII while also synthesizing VWF. PT has been used to assess coagulability of plasma in liver disease since the time of the test's inception by Quick [9]. As PT is only affected by five clotting factors (factors VII, FX, FV, FII and fibrinogen), it does not take into account anticoagulant pathways and effects of platelet and endothelial contributions. In the last decade, global assays of coagulation have received much attention. Several investigators explored the potential haemostatic mechanisms by describing thrombin generation, role of endogenous heparinoids, microparticles and the relative role of platelets and fibrinogen in modulating the coagulation disturbances in patients with liver disease [10, 11]. In cirrhosis, patients with acute liver failure exhibit a similar but much magnified reduction in pro- and anticoagulant proteins, and elevation of factors VIII and VWF levels [11].

Prolong PT has been associated with an increased risk of gastrointestinal bleeding in chronic liver disease [12-14]. Siddiqui et al. reported that majority of chronic liver disease patients had raised PT and presented with GI bleeding. Our present study also showed increased PT in patients with liver disease. The likely causes of prolonged PT may be oral anticoagulation, vitamin K deficiency, heparin contamination and non-specific inhibitors [15]. This parameter does give a good estimate of the synthetic function of the liver and may still be used as a prognostic marker [4, 5]. Decrease plasma fibrinogen level related with liver disease was also reported in the study. Siddiqui et al. and Agarwal et al. reported decreased level of fibrinogen in patients with liver disease [10, 11]. In our recent study we have got a negative correlation of fibrinogen with PT. This may be due to impaired synthesis of fibrinogen, loss into extravascular spaces (ascites), and increased catabolism or due to massive haemorrhage [15].

5. Conclusions

In summary, present study showed that PT significantly increased in patients with liver disease while fibrinogen level significantly decreased. Therefore, evaluation of PT and fibrinogen is very simple, rapid and

sensitive can be used as a useful tool for diagnosis and treatment of liver disorder. However, our study has a small sample size resulting in low power to detect minor to modest associations, therefore further study with large sample size is required.

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