Coagulation Profile and its Correlation with Severity of Liver Dysfunction and Gastrointestinal Bleed in Alcoholic Liver Disease Patients

Niraj Bohania¹, Aparna Agrawal², Anupam Prakash³*, Anita Nangia⁴, Abhishek Kumar⁵

Abstract

Introduction: The study aimed to determine coagulation factor abnormalities in alcoholic liver disease (ALD) and correlate these with severity of liver dysfunction (Child’s class) and gastrointestinal (GI) bleeding.

Methods: 60 patients of ALD (alcohol intake >10 years and clinical, biochemical or radiological evidence of chronic liver disease) were included. Patients with Hepatitis B, Hepatitis C, HIV infection, DIC, low platelet count due to other causes, or on drugs which affect coagulation profile were excluded.

Observations: Age was 44.42 ± 10.26 years (100% males), 53% in Childs class C. Severity of liver dysfunction showed a significant association (p<0.05) with prolongation of prothrombin time (PT), activated partial thromboplastin time (aPTT) and thrombin time (TT), increasing factor VIII and D-Dimer level, low platelet counts, low protein S and factor VII activity; as well as decreasing fibrinogen levels, protein C and antithrombin (AT) III. GI bleed is associated significantly (p<0.05) with PT >20 sec and decreased plasma fibrinogen levels, while normal protein C, normal AT III, normal factor VII, normal factor VIII, normal TT, increased plasma fibrinogen levels, normal PT and normal platelet count appeared to be protective.

Conclusions: Several coagulation parameters are altered in ALD variably. Alterations in PT, aPTT, TT, factor VIII, D-Dimer, fibrinogen, protein C and AT III levels can be used for grading severity of liver disease. Decreased fibrinogen, protein C activity, AT III activity, factor VII activity, and increased factor VIII activity, are associated with GI bleed.

Introduction

The liver plays several key roles in blood coagulation being involved in both primary and secondary hemostasis.¹ It is the site of synthesis of all coagulation factors and their inhibitors except for von Willebrand factor.² The hemostatic system is in a delicate balance between prothrombotic and antithrombotic processes. Liver failure is accompanied by multiple changes in hemostatic system, because of reduced plasma levels of procoagulant and anticoagulant clotting factors synthesized by hepatocytes and sinusoidal cells and also reduced capacity to clear activated hemostatic proteins and protein inhibitor complexes from circulation. Thus, the global effect of liver disease with regard to hemostasis is complex.³

Studies have been conducted on these procoagulant and anticoagulant factors in chronic liver disease patients. There is paucity of literature on the combined role of these factors in alcoholic liver disease (ALD) alone, which is a major health problem in our country. The aim of this research is to study these procoagulant and anticoagulant factors in alcoholic liver disease patients and to correlate these factors with severity of liver dysfunction and gastrointestinal bleed (GI bleed).

Materials and Methods

This cross-sectional observational study was approved by the Institutional Ethics Committee. Sixty patients with history of alcoholism (>10 years) and clinical, biochemical or radiological findings suggestive of chronic liver disease were included from the medical out-patient department. Patients with hepatitis B, hepatitis C, HIV infection, low platelet count due to extrahepatic cause, DIC or on drugs which affect coagulation profile were excluded. A detailed history and clinical examination were performed, blood was collected and plasma stored in deep freeze to assess full coagulation profile detailed as follows.

Coagulation screening tests

Prothrombin Time (PT) (using STA – NEOPLASTINE® CI Plus kit), Activated partial thromboplastin time (aPTT) (using STA® C.K. CREST® 5), Thrombin Time (TT) (using NEOPLASTINE® CI PLUS 5) and plasma fibrinogen (using STA-FIB 2 kit) levels were estimated.

Natural coagulation inhibitors

Protein C and protein S assay (using STA- STACLOT®) and Antithrombin III colorimetric assay (using STA-STACHROM® AT III) were done.

D-dimer was estimated using immuno-turbidimetric assay (using STA-LIATEST® D-DI), Factor VII and factor VIII by clot base assay.

Severity of liver disease was classified on basis of Child Turcotte Pugh (CTP) class A (score 5 and 6), B (score 7-9) or C (score 10-15). Cirrhosis with a Child-Pugh score of ≥7 indicated decompensation. Presence of GI bleed

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Table 1: Coagulation parameters according to severity of liver disease

<table>
<thead>
<tr>
<th>Parameters</th>
<th>CTP A (n=9)</th>
<th>CTP B (n=19)</th>
<th>CTP C (n=32)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>PT (sec)</td>
<td>13.48±1.40</td>
<td>19.40±10.88</td>
<td>22.63±5.46</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>aPTT (sec)</td>
<td>29.50±4.34</td>
<td>35.76±8.77</td>
<td>64.67±46.67</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>TT (sec)</td>
<td>16.20±1.33</td>
<td>19.17±4.41</td>
<td>21.33±2.96</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Fibrinogen (mg/dl)</td>
<td>486.4±126.3</td>
<td>441.6±210.1</td>
<td>247.8±136.1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Protein C (% activity)</td>
<td>92.22±26.47</td>
<td>62.84±24.91</td>
<td>30.88±19.72</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Protein S (% activity)</td>
<td>91.33±22.39</td>
<td>74.68±25.75</td>
<td>63.97±22.77</td>
<td>0.016</td>
</tr>
<tr>
<td>AT III (%activity)</td>
<td>100.6±17.6</td>
<td>75.5±15.2</td>
<td>40.63±17.50</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>D-dimer (µg/ml)</td>
<td>1.15±0.87</td>
<td>4.82±2.40</td>
<td>4.60±4.00</td>
<td>0.004</td>
</tr>
<tr>
<td>Factor VIII (% activity)</td>
<td>92.44±21.50</td>
<td>66.00±12.98</td>
<td>253.4±119.2</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Data was analysed using IBM SPSS software version 19. Results were expressed as mean ± standard deviation. The t test and the Mann–Whitney U test were used to compare normally distributed data and non-normally distributed data, respectively.

Table 2: Correlation of coagulation screening test groups with CTP classes

<table>
<thead>
<tr>
<th>Parameters</th>
<th>CTP A (n=9)</th>
<th>CTP B (n=19)</th>
<th>CTP C (n=32)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>PT (seconds)</td>
<td>12 – &lt;16 (N)</td>
<td>16 – &lt;18 (N)</td>
<td>18 – 20 (N)</td>
<td>&gt; 20 (N)</td>
</tr>
<tr>
<td>Factor VIII (% activity)</td>
<td>&lt; 55 (N)</td>
<td>55 – 170 (N)</td>
<td>&gt; 170 (N)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Fibrinogen (mg/dl)</td>
<td>&lt; 180 (N)</td>
<td>180 – 360 (N)</td>
<td>&gt; 360 (N)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Protein C</td>
<td>&lt; 60 (N)</td>
<td>60 – 135 (N)</td>
<td>&gt; 135 (N)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>AT III</td>
<td>&lt; 75 (N)</td>
<td>75 – 125 (N)</td>
<td>&gt; 125 (N)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Factor VII (% activity)</td>
<td>&lt; 55 (N)</td>
<td>55 – 170 (N)</td>
<td>&gt; 170 (N)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>factor VIII (% activity)</td>
<td>&lt; 60 (N)</td>
<td>60 – 150 (N)</td>
<td>&gt; 150 (N)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Platelet count (lac/mm³)</td>
<td>&lt; 5.0 (N)</td>
<td>5.0 – 10.0 (N)</td>
<td>&gt; 10.0 (N)</td>
<td>0.006</td>
</tr>
</tbody>
</table>

Association between coagulation profile parameters with several variables were tested using Kruskal-Wallis (non-normally distributed data) and ANOVA for continuous variables. Chi square test was used for categorical variables. A p value <0.05 was taken as statistically significant.

Results

All enrolled subjects were male, average age was 44.42 ± 10.26 years (n=60), 43.33% belonged to 30-40 years age group, 35% in 41-50 years, 15% in 51-60 years and only 6.67% patients >60 years age. Almost all patients (>95%) in our study consumed country-made liquor (alcohol content- 42.5%). Mean duration of alcohol intake was 18.2 ±6.7 years. Daily alcohol intake ranged from a quarter (180 ml) to a full bottle (720 ml) i.e. >60-80 gm/day. CTP class A and B patients constituted 15% and 31.67%, while the remaining (53.33%) belonged to CTP class C. The mean values of different coagulation screening tests, natural anticoagulants, fibrinolytic markers and other parameters are shown in relation to the severity of liver disease (CTP class) in Table 1.

PT, aPTT, TT and D-dimer levels increased as the severity of liver disease worsened (CTP class A to C), while a greater reduction of serum fibrinogen, factor VII levels, and platelet counts was observed with worsening liver disease, although factor VIII levels were variable.

The coagulation parameters were stratified according to the degree of alteration (elevation/reduction), and the same in relation to the severity of liver diseases, has been outlined in Table 2. Greater degree of elevation of PT, aPTT, TT, D-dimer is seen with greater class of CTP (severity of CLD), while greater degree of reduction in fibrinogen, protein C, antithrombin III levels, factor VII activity, and platelet counts was seen with greater severity of CLD. Factor VIII activity was also greater in Child Class C compared to Child Class A, but 68.4% Child Class B patients had normal Factor VIII activity. Protein S levels were reduced in only 11% of Child Class A, as against 42% and 50% respectively of Class B and C, however, the degree of reduction in protein S levels did not correlate significantly with degree of severity of liver disease (p=0.112).
Evidence of GI bleeding was present in 19 (32%) patients, 18 of which belonged to CTP class C (94.74%) and 1 belonged to class B. Serum fibrinogen levels, protein C, antithrombin III and factor VII activities are lower, while factor VIII activity is higher in patients with Gastrointestinal bleed, compared to non-bleeders (Table 3).

Average PT and aPTT were statistically comparable between the group with and without presence of GI bleed (Table 3). When stratified in to four ranges of PT, significant association was seen with normal PT range (12 to <16 seconds) and absence of GI bleed, while PT >20 seconds was associated with GI bleed. There was significant association between higher TT and presence of GI bleed (Table 3). While patients with decreased fibrinogen levels showed statistically significant association with presence of GI bleed, but interestingly, patients with increased fibrinogen levels were protected from GI bleed. Notably, protein C activity was reduced in all patients and antithrombin III activity was reduced in 90% who had GI bleed, compared to 53.7% and 56.1% individuals respectively who did not have active bleed. Similarly, significantly low factor VII activity and high factor VIII activity levels were noted in the GI bleed group compared to no active GI bleed group (Table 4).

Discussion

All 60 patients with alcoholic liver disease (ALD) in the present study were men, apparently because of the inclusion criteria of minimum 10 years history of daily alcohol intake, which is uncommon in Indian females specially in the socio-economic strata of patients coming to our institution. The male preponderance and average age of our study is similar to other studies.6,14 There is paucity of literature on study of coagulation parameters exclusively in ALD patients, therefore, we are comparing our results with those of others studies in cirrhosis patients irrespective of etiology.

Coagulation screening tests

Prolongation of PT was observed in 41 (68.33%) and of aPTT in 37 (61.67%) ALD patients, which is similar to 51% and 52% respectively reported by Devrajani et al7 in 118 patients with liver cirrhosis, but much lower than 88% and 71% reported by Siddiqui et al.8 Both PT and aPTT were raised in 115 (67%) cases. In our study, both PT and aPTT were raised in 35 patients (58.33%). A lower proportion of patients having prolonged PT and aPTT has also been reported, as by Nwokediuko et al9 (36.6% and 22.6%). Several studies showed prolongation of PT and aPTT in patients of liver disease.5-18 TT was prolonged in 32 (53.33%) of our patients. 19 out of 60 patients had fibrinogen levels within normal range (180-360 mg/dl) in our study. 14 patients had values <180 mg/dl and 27 patients had values >360 mg/dl (Table 2). No consistent pattern of serum fibrinogen levels was observed earlier, with some studies reporting decreased levels10,13 and some increased levels.14

Natural coagulation inhibitors (protein C, protein S and AT III)

In the present study, patients with decrease in % activity of protein C (68.33%) and AT III (66.67%) were more as compared to patients with decrease in protein S % activity (41.67%). This can be explained by the fact that protein C and AT III are synthesized in the liver whereas protein S is synthesized in the endothelium. Results in our study are consistent with those of other studies.11,15-18

Fibrinolytic marker and other parameters

Majority of patients (86.67%) in our study had raised serum D-dimer levels (>0.5 µg/ml). This is consistent with the results of other studies.9,13,19 Factor VII activity was decreased and factor VIII was increased in our study. These results are also consistent with those of other studies.15,16 Similar to other studies5,6,7,12,13 platelet count was decreased (70% cases) in our study.

Correlation with severity of liver disease (CTP class)

Similar to other studies,6-9 majority of patients in our study belonged to CTP class C (53.33%). Our study had...
more class C patients compared to other studies because in other studies majority of cirrhotic patients had viral etiology while progression is rapid in alcoholic liver disease as compared to viral hepatitis. Indian cirrhotic patients usually present in the late stages of the disease and most of our enrolled patients were in-patients.

**Correlation of PT, aPTT, TT and fibrinogen levels with severity of liver disease (CTP classes)**

PT and aPTT values were significantly higher (p≤0.001 for each) as the CTP class progressed from A to C i.e. most of CTP class C patients showed markedly prolonged values of both PT and aPTT. This result is consistent with that reported in many other studies.\(^{5,6,21}\) The mean TT increased significantly (p<0.001) with increasing CTP score whereas the mean plasma fibrinogen levels decreased significantly (p<0.001) with increasing CTP class. The proportion of patients with prolonged TT increased from 0% to 78.13% and those with decreased plasma fibrinogen increased from 0% to 40.63% as we progressed from Child’s class A to C. Majority of class A patients on the other hand had increased plasma fibrinogen levels. This result can be explained by the fact that plasma fibrinogen is an acute phase reactant, and remains normal or increased in patients with liver disease. Low concentrations are due to decreased synthesis by the liver, yet values above 100 mg/dl, are only seen with very severe liver disease. Similar results were seen in study conducted by Arif et al\(^{14}\) and Kleinegris et al.\(^{21}\)

**Correlation of natural coagulation inhibitors with severity of liver disease (CTP classes)**

When mean of natural anticoagulants were compared in each CTP class, statistically significant correlation was seen between low levels of natural anticoagulants and higher CTP class. When proportion of patients in each CTP class were compared, it was found that patients with lower protein C activity increased from 11.1% to 93.7% as we moved from CTP class A to C. Similarly, when proportion of patients with decreased AT III activity in different CTP classes were compared, again a significant association between lower AT III activity and higher CTP class was found. Similar results have been reported in other studies.\(^{5,18,20-22}\)

**Correlation of D-dimer (fibrinolytic marker) and other parameters with severity of liver disease (CTP classes)**

Statistically significant difference was seen in our study, when mean D-dimer in each CTP class were compared (high D-dimer levels with higher CTP class). Similar results were seen in study by Kleinegris et al.\(^{21}\) But when proportion of patients with raised D-dimer levels in each CTP class were compared, even though the percentage of patients increased from 67% (in class A) to 91% (in class C), it did not reach statistical significance. Statistically significant correlation (p=0.016) was also seen in our study, when mean factor VII activity in each CTP class was compared (low factor VII activity in higher CTP class). Significant correlation was also seen between mean factor VIII activity with different CTP classes i.e. high factor VIII activity in higher CTP class. The above results can be explained by the fact that factor VII is synthesized by liver exclusively and hence decreased activity with increasing severity of liver disease. However, factor VIII is synthesized by both liver and endothelial cells, hence the variable response. The proportion of patients with decreased factor VII activity increased from 0% in class A to 84% in class C. Similarly, the proportion of patients with increased factor VIII activity increased from 67% in class A to 91% in class C. The mean platelet count as well as the proportion of patients with normal platelet count decreased significantly as we progressed from class A to class C. This result in our study is consistent with that reported in many other studies.\(^{5,21}\)

**Correlation with GI bleeding**

19 (31.66%) out of 60 ALD patients in our study had GI bleeding on admission or during hospitalization and 18 out of 19 of these patients belonged to child’s class C. In the study by Siddiqui et al\(^{8}\) 72% of the patient had GI bleeding, whereas in the study by Shah Shaila et al\(^{8}\) 56% of 50 cirrhotic patients had GI bleed.

**PT and aPTT**

Average PT and aPTT in our study were comparable in patients with and without GI bleed, however, grossly prolonged PT (>20 seconds) was associated with presence of GI bleed, while normal range PT was seen in patients without GI bleed. Siddiqui et al\(^{8}\) showed that 108 (72%) cases of prolonged PT and 86 (70%) cases of prolonged aPTT had GI bleeding. On the other hand, 15 (71%) out of 21 normal PT cases and 37 (75%) out of 49 normal aPTT cases had GI bleeding i.e. the percentage of patients with GI bleed in group with normal and prolonged PT or aPTT were similar. The numbers in our study were 16 (39%) bleeds in prolonged PT patients and 14 (38%) in prolonged aPTT and 3 (16%) bleeds in normal PT patients and 5 (26%) bleeds in normal aPTT patients, because we had fewer patients with GI bleed in our series.

**TT**

Although the average TT values were similar in patients with or without GI bleed, only 14% patients with normal TT had GI bleed compared to 47% with prolonged TT. We could not lay our hands on any study correlating TT with GI bleed in patients of liver disease.

**Fibrinogen**

Patients without GI bleed had higher mean plasma fibrinogen levels, but normal plasma fibrinogen levels did not show a correlation with either presence or absence. However, low plasma fibrinogen levels predisposed to GI bleed whereas higher plasma fibrinogen levels seemed protective in our study. Siddiqui et al\(^{8}\) also showed increased risk of bleeding in patients with low levels of plasma fibrinogen.

There are only a few studies on protein C, protein S and AT III activity in patients of ALD and their correlation with GI bleed these patients are even fewer. In some studies, low levels of these natural coagulation inhibitors favoured thrombosis (Senzolo et al\(^{19}\)) but hardly any studies described their association with GI bleed. In our study while correlating these natural coagulation inhibitors with GI bleed in ALD patients, statistically significant association was found between low protein C and AT III activity and presence of GI bleed. Patients with normal protein C activity were found to be protective for GI bleed since all patients with normal protein C activity did not have GI bleed. Also, patients with normal AT III activity were found to be protective for GI bleed since 18 out of 20 patients with normal AT III activity did not have GI bleed. All the 19 patients with GI bleed had protein C activity less than lower limit of normal
(<61% activity) whereas 17/19 patients with GI bleed had AT III activity less than lower limit of normal (<75% activity). Association between protein S and GI bleed was not significant.

D-dimer

D-dimer levels in our study did not correlate with the presence of GI bleed. No study could be traced correlating D-dimer with GI bleed in patients of liver disease.

Factor VII and Factor VIII

Reduced factor VII activity and increased factor VIII activity correlated with the presence of GI bleed. Whereas half of the patients with factor VII activity less than lower limit of normal (<55% activity) had GI bleed, 26/30 patients with normal factor VII activity had no GI bleed (p=0.001). On the other hand, 18/19 patients with normal factor VIII activity had no GI bleed (p=0.001). In a study by Senzolo et al in low level of factor VII favoured bleeding, otherwise hardly any studies showed significant association of these factors with GI bleed in ALD patients.

Platelet count

Statistically significant correlation was not found when mean platelet count was compared with the presence of GI bleed. Even though 16/19 of patients with GI bleed had low platelet count (<1.5 lac/mm³), it did not achieve statistical significance because it formed only 38% (16/42) of patients with low platelet count (p=0.051). This result was in contrast with many studies which showed increased risk of bleeding with decreased platelet count. In a study by Siddiqui et al in 171 CLD cases, a positive relationship between thrombocytopenia and presence of GI bleeding was found. Out of 63 cases of decreased platelet counts, 52 (83%) had GI bleeding (RR = 1.96; 95% CI, 1.08-3.56). In another study conducted by Shah Shaila et al, decreased platelet count showed statistically significant correlation with bleeding tendency (p=0.02).

Conclusions

In the present study, patients with alcoholic liver disease demonstrated prolonged PT, aPTT, TT; increased D-Dimer levels and Factor VIII activity; decreased Platelet count, Protein C, Protein S, AT III, factor VII activity and variable plasma fibrinogen levels. Severity of liver dysfunction showed a highly significant correlation with prolonged PT, aPTTT and TT, increasing factor VIII activity and D-Dimer levels as well as decreasing fibrinogen levels, protein C and AT III activity. So, these parameters i.e. prolonged PT, aPTT and TT; increasing factor VIII activity and D-Dimer levels; decreasing fibrinogen levels, protein C and AT III activity can probably be used as a marker of severity of liver dysfunction. These parameters demonstrating positive correlation can be used to develop new scoring system just like CTP scoring for assessment of severity of liver disease.

GI bleed correlates significantly with grossly prolonged PT (>20 sec) and decreased plasma fibrinogen levels. Highly significant (p<0.005) factors protective for GI bleed are normal protein C activity, normal AT III activity, normal factor VII activity, normal factor VIII activity, normal TT and increased plasma fibrinogen levels. Other significant (p<0.05) factors which are protective for GI bleed are normal protein S activity, decreased AT III activity, decreased factor VII activity, increased factor VIII activity, prolonged TT, decreased plasma fibrinogen levels and PT prolongation >20 seconds can be used as predictors of GI bleed.

Limitations of this study are a small sample size of 60 patients, and being a cross-sectional study, correlation of abnormal coagulation parameters with long-term incidence of GI bleed is not possible.

References