Coagulation activity in liver disease

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ABSTRACT: Patients with advanced hepatic failure may present with the entire spectrum of coagulation factor deficiencies. This study was designed to determine laboratory abnormalities in coagulation in chronic liver disease and the association of these abnormalities with the extent of chronic hepatitis and cirrhosis. Coagulation markers were assayed in 60 participants: 20 patients with chronic hepatitis, 20 patients with cirrhosis, and 20 healthy individuals (control). Plasma levels of anti-thrombin III were determined by a chromogenic substrate method, and plasma concentrations of fibrinogen were analyzed by the Rutberg method. Commercially available assays were used for laboratory coagulation tests. The levels of coagulation activity markers in patients with chronic liver disease were significantly different in comparison to those in healthy participants. These results indicate the utility of measuring markers for coagulation activity in determining which cirrhosis patients are more susceptible to disseminated intravascular coagulation.

KEY WORDS: Liver disease; Coagulopathy; Disseminated intravascular coagulation

INTRODUCTION

Coagulation and fibrin formation may be viewed as two opposing processes. The first is a procoagulant process that is triggered by the binding of tissue factor to factor VII to form a complex, which in turn initiates a series of reactions ultimately leading to thrombin generation and fibrin clot formation. The latter is an anticoagulant process that originates from thrombin directly via plasma antithrombin (AT) III. A balance between these procoagulant and anticoagulant processes is essential to prevent unwanted thrombin generation under normal physiological conditions. In patients with chronic liver disease, this balance is disrupted. Hemostatic abnormalities in these patients reflect the degree of hepatic dysfunction and can include impaired synthesis of coagulation factors, production of abnormally functioning clotting factor, vitamin K deficiency, thrombocytopenia, qualitative platelet dysfunction, consumptive coagulopathy, and impaired clearance of circulating activation complex.

In patients with advanced liver disease, bleeding and thrombosis are dangerous complications, particularly those who are challenged by infection or who require surgery. It is clear that disseminated intravascular coagulation (DIC) involves the activation of the extrinsic coagulation pathway, which is critically dependent on tissue damage specifically to the endothelium. This activation triggers inflammation, increases the circulating levels of a variety of cytokines, and impairs physiological mechanisms of anticoagulation. DIC presents a diagnostic and therapeutic challenge in patients with liver disease. Various theories propose liver necrosis as a probable triggering event for DIC, because the coagulation profile in DIC is

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virtually indistinguishable from that seen in advanced liver disease\(^9\).

We studied the correlation between laboratory measurements of coagulation activity in patients with chronic hepatitis, chronic cirrhosis, or no known liver disease, to determine the utility of these biomarker tests in determining whether patients with cirrhosis are more susceptible to DIC.

**MATERIALS AND METHODS**

The present study included 40 patients who were 30 years of age or older. Patients were categorized in two groups: 20 patients with chronic hepatitis C virus infection (group I), 20 patients with cirrhosis due to viral etiology (group II), and 20 healthy participants (group III). None of the patients were receiving anticoagulant therapy at the time of the study. This study was approved by the local ethics committee. All samples that were collected in the Department of Gastroenterology of the Shalimov Institute (Kiev, Ukraine) were tested in a biochemical laboratory for determination of platelet count (PLT), plasma fibrinogen (Fib), prothrombin time (PT), partial thromboplastin time (PTT), thrombin time (TT), and AT III levels. Hepatic infection in group I was confirmed by enzyme linked immunosorbent assay (ELISA), and cirrhosis in group II was confirmed by liver biopsy. Plasma was obtained from fasting blood samples drawn by venipuncture with Vacutainer\(^7\) tubes: for AT III levels, blood was drawn into heparinized tubes, and for coagulation test it was drawn into tubes containing sodium citrate 3.8%.

AT III levels were measured using a chromogenic substrate method with a reactive test-standard, according to the manufacturer’s instructions (Technology Standard, Barnaul, Russia). In this assay, thrombin was first added to a diluted plasma solution containing excess heparin; after incubation, a thrombin-specific chromogenic substrate was used to determine AT III concentration photometrically at 405 nm with a photometer (Screen Master Plus, Germany)\(^8\). Fibrinogen levels were determined by the gravimetric method of Rutberg, a principle method based on Wight clotting after thrombin generation by thromboplastin. Clots were then dried on filter paper and incubated at room temperature for 10-15 minutes\(^9\).

PT was measured by the Quik method\(^10\), and PTT was measured using the method of Langdell et al\(^11\) with a reactive test-standard (P. Z. Cormay, Lublin, Poland). TT was measured by a clotting-based assay with a reactive test-standard (Human, Wiesbaden, Germany). The thrombin time is simple test, a low potency thrombin is added to undiluted plasma and clot formation is timed\(^12\).

Data are expressed as means ± standard deviation (SD). Statistical significance between individual samples was determined by Student’s \(t\)-test, and linear regression analysis was used to examine correlations between various measurements. Statistical analysis was performed using the Statistica Demo version 8 for Microsoft Windows XP Professional (Tulsa, OK, USA). \(P<0.05\) was considered statistically significant.

**RESULTS**

Group I consisted of 8 male and 12 female patients with a mean age of 44.8 years (range 35-60 years); Group II consisted of 11 male and 9 female patients with a mean age of 43 years (range 38-63 years); group III consisted of 10 male and10 female patients with a mean age of 45.8 years (range 38-52 years). The levels of marker coagulation activity measured in each study group are presented in Table 1. The physiologically normal ranges for each marker, based on reference values from the laboratory, are 75-140% for AT III, 24-33 sec for PTT, 12-14 sec for PT, and 8-14 sec for TT. The normal plasma concentration range of fibrinogen is 2-4 g/l. In patients with chronic hepatitis, PT and TT were not significantly different from those of healthy participants. All of the other tested measures of coagulation activity were significantly different between the patients in both Groups I and II, and the healthy participants. Table 2 shows statistical tests of means against reference constants, regression and correlations for the coagulation activity in each study group.

**DISCUSSION**

Chronic liver disease is a cause of abnormal hemostasis tests, with results that may include thrombocytopenia and impaired platelet function, or low plasma levels of coagulation factors. There is also evidence that fibrinolytic activity is heightened in chronic liver disease, with the implication that the fibrinolytic system contributes to the derangement of hemostasis through an increased tendency to lyse formed clots. Evidence shows that abnormalities in hemostasis tests are associated with an increased bleeding tendency; however, patients with liver disease do not have bleeding patterns like those of patients who have coagulation factor deficiencies\(^14\). Ewe\(^15\) and Dillon et al\(^16\) concluded that abnormal bleeding
after liver biopsy is a random event that cannot be predicted by the laboratory methods currently used to explore the hemostatic system. In addition, studies by McVay and Toy\textsuperscript{17} and Caturelli et al\textsuperscript{18} indicate little or no association between the risk of bleeding after liver biopsy and the degree of abnormal hemostasis tests. In a study of patients with decompensated liver disease, Boks et al. concluded that variceal bleeding was not related to the impairment of an array of coagulation and fibrinolysis tests\textsuperscript{19}. The liver is the cornerstone of the coagulation system, and patients with liver disease are at a substantially increased risk of both thrombosis and hemorrhage. The interaction between the coagulation and fibrinolytic mechanisms affords numerous opportunities for dysfunction resulting in DIC. The fibrinolytic system is responsible for the degradation and removal of fibrin clots by plasmin, while anticoagulant mechanisms, such as the action of AT III, regulate the extent of thrombin formation and inhibit fibrin formation. In DIC, the generation of large amounts of thrombin may result in fibrin deposition in the microvasculature, leading to tissue ischemia. Depletion of platelets, fibrinogen, prothrombin, and other hemostatic proteins may lead to a consumption coagulopathy, and, if severe enough, bleeding\textsuperscript{8}. Many patients with DIC have low levels of AT III, due to consumption\textsuperscript{20}. In this study, the level of AT III in plasma was lower in patients with liver disease in comparison to those in healthy participants (P<0.05).

**Table 1. Coagulation marker measurements in each group of patients.**

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Chronic Hepatitis n=20</th>
<th>Cirrhosis n=20</th>
<th>Control n=20</th>
</tr>
</thead>
<tbody>
<tr>
<td>PT (sec)</td>
<td>17.6 ± 1.4</td>
<td>20.9 ± 2.5</td>
<td>12.5 ± 0.6</td>
</tr>
<tr>
<td>PTT (sec)</td>
<td>38.4 ± 1.1</td>
<td>47.4 ± 2.9</td>
<td>29.4 ± 2.3</td>
</tr>
<tr>
<td>TT (sec)</td>
<td>8.5 ± 0.5</td>
<td>9.8 ± 1.0</td>
<td>8.4 ± 0.5</td>
</tr>
<tr>
<td>Fibrin (g/l)</td>
<td>2.4 ± 0.1</td>
<td>1.8 ± 0.1</td>
<td>2.6 ± 0.4</td>
</tr>
<tr>
<td>PLT</td>
<td>177.9 ± 8.7</td>
<td>146 ± 25.3</td>
<td>274 ± 26.8</td>
</tr>
<tr>
<td>AT III (%)</td>
<td>70.4 ± 2.4</td>
<td>61.4 ± 3.4</td>
<td>97.2 ± 1.5</td>
</tr>
</tbody>
</table>

Data are presented as mean ± SD.

**Table 2 statistical tests of means against reference constants, regression and correlations for the coagulation activity in each study group**

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>P value Group I</th>
<th>P value Group II</th>
<th>r 1</th>
<th>r 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>PT</td>
<td>0.195</td>
<td>0.001*</td>
<td>-0.07</td>
<td>-0.2</td>
</tr>
<tr>
<td>PTT</td>
<td>0.000*</td>
<td>0.000*</td>
<td>-0.3</td>
<td>-0.3</td>
</tr>
<tr>
<td>TT</td>
<td>0.186</td>
<td>0.000*</td>
<td>0.02</td>
<td>-0.28</td>
</tr>
<tr>
<td>Fibrin</td>
<td>0.000*</td>
<td>0.000*</td>
<td>0.3</td>
<td>0.2</td>
</tr>
<tr>
<td>PLT</td>
<td>0.000*</td>
<td>0.000*</td>
<td>0.43*</td>
<td>-0.48*</td>
</tr>
<tr>
<td>AT III</td>
<td>0.0071*</td>
<td>0.0087*</td>
<td>0.07</td>
<td>0.17</td>
</tr>
</tbody>
</table>

*P values of 0.05 or less are considered statistically different.

A 1989 report evaluated platelet counts, fibrinogen degradation products (FDPs), specifically D-dimer, by latex agglutination, fibrinogen concentration, and TT in a prospective study of patients with DIC\textsuperscript{21}. Further, the absence of thrombocytopenia causes portal hypertension in liver disease\textsuperscript{22} and excludes the diagnosis of DIC. On the other hand, a low platelet count lacks specificity, as it may be due to an underlying disease, such as sepsis\textsuperscript{8}. In our study, platelet counts in patients with either liver disease were comparable to those in healthy participants. As pointed out by Lisman et al, defects in platelet number and function may be accompanied by decreased levels of coagulation factors\textsuperscript{23}.
Many patients with DIC do not present with hypofibrinogenemia, and patients with chronic DIC may have normal fibrinogen levels, because fibrinogen is an acute phase reactant. However, in our study, patients with liver disease had significantly different fibrinogen levels compared to those of the healthy participants. Liver disease, vitamin K deficiency, warfarin intoxication, and primary fibrinolysis may be associated with prolonged PT and TT values, but in our study, PT and TT values in patients with chronic hepatitis were similar to those in healthy participants. Because PT is a measure of vitamin K dependent factors and TT is recognized as an accurate predictor of liver damage and the likelihood of progression to end stage liver failure, all vitamin K dependent coagulation factors are low in patients with chronic hepatitis depending on liver dysfunction. These tests have, thus, been incorporated into the commonly used prognostic indices for chronic hepatitis or other chronic liver diseases, such as Child-Pugh or Mayo End-Stage Liver Disease.

In patients with chronic hepatitis, PTT was prolonged, possibly due to a procoagulant effect. In states of liver disease, plasma concentrations of natural anticoagulants have been demonstrated to be considerably reduced. While PTT only measures the formation of fibrin from thrombin and does not assess the effects of fibrinolytic factors, hyperfibrinolysis is frequently reported in patients with cirrhosis and is thought to contribute to the increased bleeding patterns exhibited by these patients. However, available tests do not entirely mimic the process of thrombin generation as it occurs in vivo, particularly the balance between procoagulant and anticoagulant processes. Tests such as PT and PTT are responsive only to procoagulant factors. Thus, in patients with liver disease, standard coagulation test results are abnormally prolonged. Tripodi et al showed that in patients with cirrhosis, PT is similar to control values when thrombomodulin is added to the thrombin generation test. Although the thrombin generation test was used by Tripodi as a more global test of coagulation than the routine tests such as the PT, their test measures thrombin generation in platelet rich plasma, which more closely represents physiological conditions.

**CONCLUSION**

Attempts have been made to evaluate the role of hemostatic agents in the management of the most frequent problems of patients with severe liver disease: bleeding and thrombosis. Laboratory diagnosis of these conditions is difficult, because many laboratory tests lack sensitivity and specificity. However, our data show that results of coagulation activity tests were significantly lower in patients with either liver disease were comparable to those in healthy participants and suggest that cirrhosis patients may be more susceptible to DIC. Thus, the measurement of markers for coagulation activity may be valuable in evaluating the risk of DIC in patients with cirrhosis.

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**REFERENCES**