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Thrombin activation and increased fibrinolysis in patients with chronic liver disease

J. A. Páramo, J. Rifón, J. Fernández, B. Cuesta and E. Rocha

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The respective roles of intravascular coagulation (DIC) and fibrinolysis were assessed in severe chronic liver disease by measuring thrombin-antithrombin (TAT) complexes, tissue-type plasminogen activator antigen (tPA Ag) and fibrinogen and fibrin degradation products (FgDP and FbDP respectively) in 66 patients with liver disease caused by cirrhosis ($n = 34$) or chronic hepatitis ($n = 32$) as compared to findings in a control group ($n = 30$). There was a significant increase of TAT complexes ($P < 0.01$), tPA Ag ($P < 0.002$), FDP and FbDP ($P < 0.001$) in patients as compared to controls. FbDP increase was more evident in patients with cirrhosis than in those with hepatitis ($P < 0.01$). Significant correlations between these parameters with some liver function tests were also demonstrated. Thus, in patients with severe liver disease, an increased thrombin activity, as demonstrated by high TAT levels; followed by hyperfibrinolysis suggest that a low grade DIC may occur.

Key words: Liver disease, disseminated intravascular coagulation, thrombin-antithrombin, tissue-type plasminogen activator, fibrinogen degradation products, fibrin degradation products.

Introduction

Liver disease is a frequent cause of haemostatic abnormalities leading to a clinically important bleeding tendency. The pathogenesis of the haemostatic disorder includes decreased synthesis of clotting factors, thrombocytopenia, disseminated intravascular coagulation (DIC), failure of the hepatic clearance mechanisms and abnormalities of fibrinolysis.[1–4]

Patients with severe liver disease often demonstrate several alterations characteristic of DIC, although the trigger initiating the activation of blood coagulation is not known and controversy still exists, because in most cases, it is difficult to attribute the haemostatic alteration to DIC alone.[5–8] On the other hand, accelerated fibrinolysis is a recognized complication in liver disease, because the liver is the site of synthesis of main zymogens and inhibitors of fibrinolysis and also the site of clearance of blood plasminogen activators.[3,4,9] In addition, increased fibrinogen/fibrin degradation products (FDP) measured by latex agglutination and fibrinogen-fibrin fragment E have been found in patients with liver disease.[10,11]

This study was undertaken to determine the roles of intravascular coagulation and fibrinolysis in the pathogenesis of haemostatic abnormalities that invariably occur in severe chronic liver disease by using specific assays to measure thrombin generation, tissue plasminogen activator (tPA) and the presence of fibrin(ogen) degradation products.

Patients and methods

Blood was obtained by venepuncture from 30 healthy volunteers (mean age 49 ± 18 years) and from 60 patients with hepatic insufficiency divided into two categories if they showed biopsy evidence of cirrhosis ($n = 34$, mean age 56 ± 11 years) or chronic hepatitis ($n = 32$, mean age 43 ± 18 years). The degree of hepatic insufficiency according to liver function tests such as total bilirubin, prothrombin time and aspartyl aminotransferase (AST) is shown in Table 1. The blood was anticoagulated by addition of 0.1 vol of 0.1 M sodium citrate pH 4.5 and centrifuged for 15 min at 2500 *g* and 4 °C. The platelet-poor plasmas were stored at − 70 °C. No blood products

The authors are with the Haematology Service, University Clinic, University of Navarra, Pamplona, Spain. Address correspondence to J. A. Páramo.

Table 1. Liver function tests in patients and controls (Median and 95th percentile)

	Cirrhosis (n = 34)	Chronic hepatitis (n = 32)	Controls (n = 30)
Total bilirubin (mg/dl)	1.4 (1.0 – 1.6)	1.2 (0.7 – 1.5)	0.8 (0.3 – 1.3)
Prothrombin index (%)	70.7 (63 – 78)	78.3 (76 – 94)	90.6 (78 – 120)
AST (U/l)	54.1 (49 – 83)	63.5 (45 – 124)	16.4 (0 – 30)

were administered preceding the date of plasma sampling.

Thrombin-antithrombin (TAT) complexes were measured with an enzyme-immunoassay (Enzygnost TAT, Behring Institute, Germany).[12] Diluted samples were added to each tube coated with a polyclonal antibody to human thrombin to capture the plasma TAT. After washing, peroxidase-conjugated anti-human antithrombin III (AT III) was added. Following incubation each tube was washed and then filled with a substrate solution containing hydrogen peroxide. The reaction was stopped by addition of sulphuric acid and absorbance was read in a spectrophotometer at 492 nm. Results were expressed in Ug/l.

Tissue plasminogen activator (tPA) was quantitated immunologically with an ELISA kit purchased from Biopool (Umea, Sweden). Results were expressed in ng/ml.

Fibrinogen degradation products (FgDP) and fibrin degradation products (FbDP) were both measured with a microELISA system using specific monoclonal antibodies FDP-Y18 (Fibrinostika FgDP) and FDP-DD13 (Fibrinostika FbDP) (Organon Teknika, The Netherlands). Diluted samples were first incubated in the wells of polystyrene microELISA strips which had been coated with monoclonal FDP-14 specific for degradation products of both fibrin and fibrinogen. In the next step the peroxidase-conjugated specific monoclonal antibody was added. After incubation, a tetra methylbenzidine solution was added and the reaction stopped by addition of sulphuric acid. Results were expressed in Ug/l.

Prothrombin time, AST, and total bilirubin were performed by standard methods.

The significance of differences between patient groups and normal subjects was assessed using the Mann-Whitney test. The correlation coefficient (r) was calculated by regression analysis.

Results

The study was carried out in 34 patients with liver cirrhosis and 32 with chronic hepatitis, both showing hepatic insufficiency (Table 1). The mean values in the plasma concentration of TAT complexes, tPA antigen, FgDP, and FbDP are shown in Figures 1 and 2.

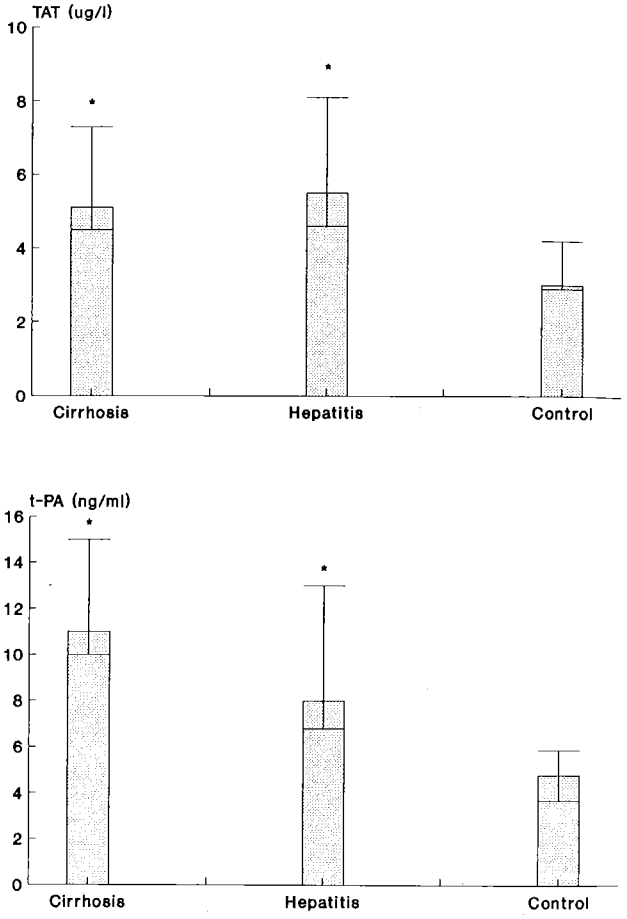

Figure 1. Concentrations of TAT complexes and tPA Ag in patients with cirrhosis and hepatitis and in normal subjects. (Median and 95th percentile) *P < 0.01 with respect to control group.

TAT levels were significantly higher in both patients groups than in controls ($P < 0.01$), but no significant differences between cirrhosis and hepatitis were observed. tPA was also significantly elevated ($P < 0.002$) in each patient group with respect to normal subjects (Figure 1). As shown in Figure 2, increased FgDP and FbDP concentrations were demonstrated in patients as compared to controls ($P < 0.001$), although the D-dimer increase

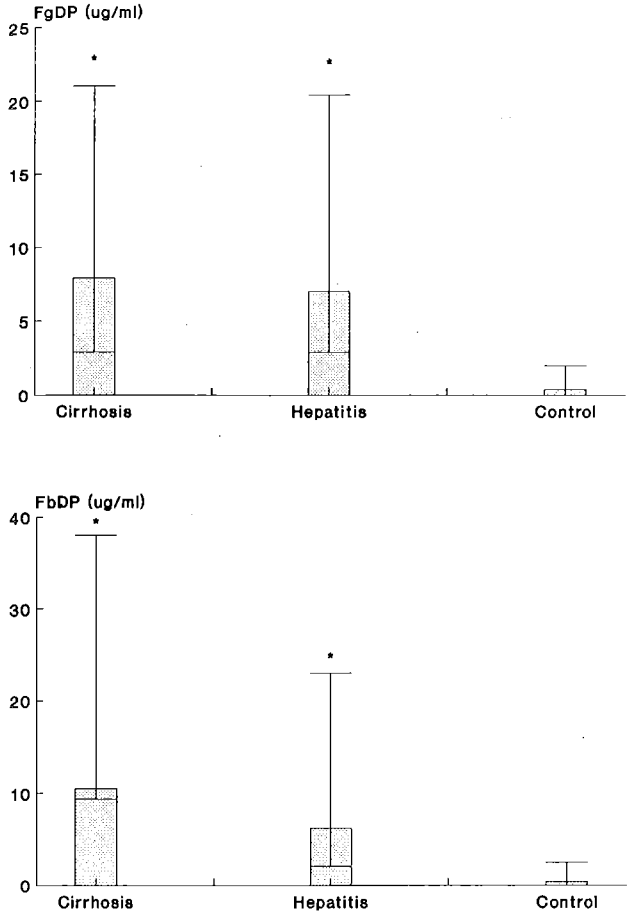

Figure 2. Concentrations of fibrinogen (FgDP) and fibrin (FbDP) degradation products in patients with cirrhosis and hepatitis and in normal subjects. (Median and 95th percentile) *P < 0.001 with respect to control group.

was greater in patients with cirrhosis than in those with chronic hepatitis ($P < 0.01$).

Correlations of TAT, tPA, FgDP and FbDP with liver function tests are shown in Table 2. In general, a good positive correlation between TAT complexes and the increase of bilirubin ($P < 0.008$) and a negative correlation between these complexes and the prolongation of prothrombin time ($P < 0.002$) was observed in the patient group. A positive correlation between both tPA ($P < 0.002$) and FbDP ($P < 0.01$) with bilirubin was also observed. Interestingly, plasma TAT value correlated with tPA levels ($P < 0.006$).

Discussion

Patients with severe liver disease often show several alterations characteristic of DIC and increased fibrinolytic activity, which both contribute to haemorrhagic complications. The interpretation of results of coagulation/fibrinolysis tests in liver disease is, however, controversial. Although increased FDP levels and reduced coagulation factors and platelets may indicate DIC, this may not always be the case since the liver is the main site of synthesis and catabolism of many coagulation and fibrinolysis proteins.[5-7]

Table 2. Correlation of TAT, tPA, FgDP and FbDP with liver function tests

	TAT	tPA	FgDP	FbDP
TAT	–	0.33**	0.11	0.18
tPA	0.33**	–	0.08	0.23
FgDP	0.11	0.08	–	0.81*
FbDP	0.18	0.23	0.81*	–
Bilirubin	0.32**	0.38**	0.14	0.32**
Prothrombin index	−0.36**	0.23	−0.04	−0.18

*P < 0.001 **P < 0.01

We found a marked increase of TAT levels in both patient groups. The measurement of TAT seems to be a sensitive marker of coagulation activation. Elevated plasma levels have been found in patients with deep venous thrombosis, pulmonary embolism, acute promyelocytic leukemia and other malignancies and DIC.[13-17] Two recent studies have also demonstrated high TAT levels in patients with liver disease.[11,18] Since generation of thrombin represents a central event within the coagulation cascade, determination of TAT complexes may be relevant in the diagnosis of clotting activation in these patients. Although Bauer *et al.*[19] have shown age-related elevations in levels of prothrombin fragment 1 + 2 and fibrinopeptide A, indices of prothrombin and thrombin activation respectively, this cannot explain our findings since we studied age-matched controls.

The significant increase in the fibrinolysis parameters analyzed observed in the patients compared to the controls agrees with a previous study.[20] tPA was significantly elevated in liver disease confirming previous studies by Hersch *et al.*[21], suggesting that circulating tPA is an important factor in the development of accelerated fibrinolysis.

Moreover, FgDP and FbDP levels, assessed with specific monoclonal antibodies, were also significantly higher in both patient groups, indicating plasmin generation and enhancement of fibrinolytic activity. Although some authors have observed that patients with liver disease had no evidence of plasmin-cleaved products,[22] others have shown significant correlation between serum FDP and plasma D-dimer levels in patients with liver disease, suggesting that elevated FDP levels in this disease are a direct consequence of plasmin activity.[18,23] According to Carr *et al.*[24] the finding of elevated FDP

confirmed by elevated D-dimer concentrations has high predictive value in the detection of DIC in patients at risk. Therefore, our data confirm that accelerated fibrinolysis is a common complication of severe liver disease. The fact that fibrin degradation products were higher in patients with cirrhosis than in those with hepatitis indicates that fibrinolysis plays a more important role than fibrinogenolysis in these patients and may contribute to the severe haemorrhagic complications.

Interestingly, we found strong correlations between TAT, tPA and FbDP with liver function tests, indicating that these parameters may be good markers of severe liver disease. Whether TAT may also be an early marker of liver defects remains to be evaluated.

Thus, in patients with severe liver disease, an increased thrombin activity followed by plasmin activity suggests that a low grade DIC may occur.

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