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Hyperfibrinolysis in hepatosplenic schistosomiasis

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Abstract

***Aim*—To evaluate the nature of accelerated fibrinolysis in hepatosplenic schistosomiasis.**

***Methods*—The biological activity of plasminogen (Plg), plasminogen activators (PA), α_2-antiplasmin (α_2-AP) and plasminogen activator inhibitor-1 (PAI-1) was determined by photometric analysis in 15 compensated and 35 decompensated patients with endemic Egyptian hepatosplenomegaly. Quantitative measurement of plasma concentrations of tissue t-PA, t-PA-PAI-1 complex, α_2-antiplasmin-plasmin complex (α_2-APP), fibrinogen degradation products (FbDP), D-dimers (D-D), thrombin-antithrombin complex (TAT) and prothrombin fragment (F 1+2) complexes, using double antibody sandwich enzyme linked immunosorbent assays and grading of the degree of hepatic insufficiency according to the Child-Pugh classification, were also carried out.**

***Results*—The progressive deterioration of liver function in schistosomal patients, which matched the severity of the disease, led to simultaneous defects in profibrinolytic (decreased Plg and increased PA and t-PA) and antifibrinolytic (decreased α_2-AP and PAI-1) factors—the latter defects being the most prominent—resulting in significant generation of plasmin (increased APP complexes) and therefore enhanced fibrinolysis (increased FbDP and D-dimer). The raised concentrations of FbDP, D-D, TAT and F 1+2 established its secondary nature.**

***Conclusion*—These findings suggest that the amount of PAI-1 available to bind and neutralise circulating t-PA may be a critical factor in the progress of hyperfibrinolysis observed in hepatosplenic schistosomiasis, and that the pronounced reduction in its plasma concentration may be regarded as a potential warning indicator of haemostatic imbalance in decompensated schistosomal patients at high risk of variceal bleeding.**

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Keywords: hyperfibrinolysis, fibrinolytic activity, haematemesis, hepatosplenic schistosomiasis.

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Hepatosplenic schistosomiasis is a chronic liver disease that constitutes a major health problem in endemic areas. Bleeding oesophageal varices[1] and accelerated fibrinolysis[2 3] are common and serious complications of *Schistosoma mansoni* infection. Hyperfibrinolysis is an important risk factor[4] in liver cirrhosis, because cirrhotic patients with bleeding have a higher frequency of accelerated fibrinolysis[5–7] and those with hyperfibrinolysis are at high risk of gastrointestinal bleeding.[4 8] A multifactorial pathogenesis for accelerated fibrinolysis in liver cirrhosis has been presumed,[9–11] but there have been few similar studies in hepatosplenic schistosomiasis.

This study was designed to assess the impact of bilharzial hepatic fibrosis (BHF) on the balance between profibrinolytic and antifibrinolytic proteins—the balance between plasminogen and α_2-antiplasmin (α_2-AP) and that between tissue-type plasminogen activator (t-PA) and plasminogen activator inhibitor-1 (PAI-1)—and to determine whether, and to what extent, in addition to synthetic reduction, the consumption of fibrinolytic components contributes to haemorrhagic diathesis in schistosomal patients.

Methods

Fifty patients with biopsy confirmed endemic Egyptian hepatosplenic schistosomiasis were included in this study. Diagnosis of patients with severe liver failure or those with acute haematemesis was based on liver biopsy performed during previous hospital admission. Fifteen healthy volunteers were selected from the medical and paramedical staff and served as controls; their age ranged from 22 to 48 years (nine men and six women). None of the subjects under study gave a history of alcohol intake or had taken drugs with known effects on coagulation or fibrinolysis, specially β-blockers and oral contraceptives; and each gave informed consent.

The cause of liver disease was *Schistosoma mansoni* infection either alone (11 subjects) or coexistent with viral hepatitis (B and/or C) in 39 patients. Assessment of BHF, including bilharzial portal fibrosis and associated parenchymal changes, and mixed hepatic lesions showing histopathological features of both BHF and chronic active hepatitis (CAH), was based on several morphological criteria, as described before.[12] Patients were studied when they first presented and were grouped into compensated (n = 15) or decompensated, either hepatocellular (n = 15) or vascular (n = 20), if ascites and/or acute haematemesis from ruptured oesophageal varices (defined as vomiting of fresh red blood and diagnosed by emergency endoscopy) superimposed. The degree of hepatic insufficiency was determined using Child-Pugh criteria.[13 14]

Except for patients with acute haematemesis, blood was collected by venepuncture between 0830 and 1000 hours from resting individuals who had fasted overnight. Blood

Table 1 Clinical and histopathological parameters in schistosomal patients

Characteristic	*Compensated hepatosplenomegaly (n = 15)*	*Decompensated ascites (n = 15)*	*Decompensated haematemesis* (n = 20)*
Age in years (range)	18–51	26–63	27–60
Sex (M/F)	10/5	9/6	13/7
Child-Pugh category			
Grade A (n)	13	–	–
Grade B (n)	2	7	8
Grade C (n)	–	8	12
Pure BHF			
Slight (n)	1	–	–
Mild/moderate (n)	1	1	1
Marked (n)	2	2	3
Mixed cases of BHF and CAH with			
Minimal parenchymal damage (n)	3	2	2
Mild/moderate parenchymal damage (n)	8	5	6
Mixed cases with severe changes suggestive of cirrhosis (n)	–	5	8

*Only patients during acute attack of haematemesis from ruptured oesophageal varices were selected; nine of these patients had ascites.

samples from the former patients were withdrawn without any medication, blood transfusion, component therapy, endoscopic ligation or injection sclerotherapy. Blood (9 volumes) was mixed with buffered citrate, 0.1 mol/l, pH 4.5 (1 volume) (Behring Institute, Behringwerke AG, Marburg, Germany), centrifuged (1500 × *g*, at 4°C for 10 minutes), aliquoted, and stored at −70°C until assayed.

Fibrinogen was quantitated in plasma by a functional assay according to the method of Clauss[15] using a Multifibrin test kit (Behringwerke AG). Functional plasminogen activators,[16] PAI-1 activity,[17] functional plasminogen,[18] and α_2-AP[19] were evaluated using chromogenic substrate assays—Stachrom PA (Diagnostica Stago, Asnière, France), Berichrom PAI, Berichrom Plasminogen and Berichrom α_2-Antiplasmin (Behringwerke AG), respectively. Antigen titres of t-PA,[20] tissue-plasminogen activator-plasminogen activator inhibitor-1 complex (t-PA-PAI-1),[21] α_2-antiplasmin-plasmin complex (APP),[22] fibrin degradation products (FbDP),[23 24] D-dimer (D-D),[25] thrombin-antithrombin III complex (TAT),[26] and prothrombin fragment F 1+2 (F 1+2)[27] were measured in plasma by sandwich enzyme immunoassays using Asserachrom t-PA and Asserachrom t-PA-PAI-1 (Diagnostica Stago), EIA APP micro (Behringwerke AG), Fibrinostika FbDP (Organon Teknika, Boxtel, Holland), Asserachrom D-Di (Diagnostica Stago), Enzygnost TAT and Enzygnost F 1+2 micro-test kits (Behringwerke AG), respectively.

Data are presented as median and range. Differences between groups were tested for significance using the Mann-Whitney U test. Regression analysis to determine significant correlations among different parameters was performed using the Spearman rank correlation coefficient. A 95% and 99% confidence interval was quoted.

Results

The clinical and histopathological characteristics of schistosomal patients are shown in table 1. Different degrees of hepatic dysfunction according to the Child-Pugh classification were detected. While Child A grade was prevalent (100%) in compensated patients, 47% and 41% of patients with Child B grade were found among cases with haematemesis and ascites, respectively. Twenty patients were graded as Child C; 40% and 60% of them were found among the ascites and haematemesis groups, respectively. Histopathological examination of liver biopsy specimens revealed the presence of three morphological features: (a) pure BHF; (b) mixed BHF and CAH; and (c) mixed lesions merging into cirrhosis. Seventy eight per cent (39/50) of patients had mixed BHF and CAH, which may explain the high incidence of clinical and hepatic decompensation in schistosomal patients.

Table 2 shows that the plasma concentrations of fibrinogen, plasminogen, α_2-AP and PAI-1 were significantly decreased ($p < 0.01$) in diseased groups compared with controls. The decrease in these parameters matched the severity of the disease as patients with ascites and/or acute haematemesis had significantly lower ($p < 0.01$) concentrations than compensated patients. Conversely, PA, t-PA, t-PA-PAI-1 and α_2-APP complexes, FbDP, D-D, TAT and F 1+2 complexes were significantly increased ($p < 0.01$) in diseased groups than in controls, and these concentrations progressively increased as the disease progressed. The highest values were mostly encountered in patients during acute attacks of haematemesis. Decompensated patients with acute haematemesis had significant increases in TAT ($p < 0.05$) and F 1+2 ($p < 0.01$) concentrations along with significant reductions ($p < 0.05$) in PAI-1 concentrations than patients with ascites. Although a decrease in plasminogen and α_2-AP concentrations, and an increase in free t-PA concentration were also detected in patients with haematemesis, the differences were not significant ($p > 0.05$).

A highly significant direct correlation was found between t-PA and t-PA-PAI-1 complex in ascites ($r = 0.9692$, $p < 0.001$) and haematemesis ($r = 0.9216$, $p < 0.001$) groups. Although no significant correlation ($p > 0.05$) was detected between PA activity and either t-PA or t-PA-PAI-1 complex concentrations in ascites ($r = 0.3314$ and $r = 0.1245$, respectively) and haematemesis ($r = 0.3938$ and $r = 0.1970$, respectively) groups, a highly significant correlation was found between its activity and free t-PA in patients with ascites ($r =$

Table 2 Fibrinolytic parameters in schistosomal patients

Parameters	*Controls*	*Hepatosplenomegaly*	*Ascites*	*Haematemesis*
PA	0.4	0.8[a]	1.5[a]	1.4[a b]*
(IU/ml)	0.3	1.4	3.4	3.3
t-PA	3.5	5.2	19[a b]	26.3[a b]
(ng/ml)	6	29	48.8	51.8
PAI-1	3.1	2.4[a]	1.7[a b]	1.1[a b c]*
(U/ml)	1.3	2.4	2.1	1.8
t-PA-PAI-1	2.1	2.2	16.5[a b]	15.8[a b]
(ng/ml)	3.05	20	43.6	48
Plg	105.91	87.1[a]	62.4[a b]	56.4[a b]
(%)	55.21	62.1	44.5	65.9
α_2-AP	110	78.5[a]	58.7[a b]	47.4[a b]
(%)	33.22	39.4	43.6	51
APP	186	300	570[a b]*	535[a b]*
(µg/l)	310	750	1110	1720
Fbg	314	274[a]*	214[a b]	166.5[a b]
(mg/dl)	173	274	212	163
FbDP	220	750[a]	2460[a b]	3240[a b]
(ng FE/ml)	170	6155	9660	19580
D-D	135	537[a]	1340[a b]	1401[a b]
(ng/ml)	170	835	1134	1019
TAT	2.4	5.4[a]	6.3[a b]*	10.5[a b c]*
(µg/l)	2.3	4.4	16	30.3
F 1+2	0.8	1.4[a]	1.4[a]	3[a b c]
(nmol/l)	1	2	2.9	5.9

Data are expressed as median and range. [a]Controls *vs* other groups, $p < 0.01$. [b]Compensated *vs* decompensated groups, $p < 0.01$. [c]Ascites *vs* haematemesis group, $p < 0.01$. *$p < 0.05$.
PA = plasminogen activator; t-PA = tissue-plasminogen activator; PAI-1 = plasminogen activator inhibitor-1; t-PA-PAI-1 = tissue-plasminogen activator-plasminogen activator inhibitor-1 complex; Plg = plasminogen; α_2-AP = α_2-antiplasmin; APP = α_2-antiplasmin-plasmin complex; Fbg = fibrinogen; FbDP = fibrin degradation products; D-D = D-dimers; TAT = thrombin-antithrombin complex; F 1+2 = prothrombin fragment 1+2.

0.8606, $p < 0.001$) and acute haematemesis ($r = 0.8702$, $p < 0.001$). This direct correlation may help to verify that free t-PA is a major mediator of the hyperfibrinolytic process that results in high concentrations of the fibrin breakdown products, FbDP and D-D. Furthermore, a direct correlation was found between TAT and D-D in patients with ascites ($r = 0.6284$, $p < 0.01$) and haematemesis ($r = 0.7316$, $p < 0.01$) which may reflect their intimate relation. Both may be affected by similar factors such as increased production and/or decreased hepatic clearance.

Discussion

Increased concentrations of α_2-APP and increased release of fibrin degradation products (high concentrations of FbDP and D-D) were detected in hepatosplenic schistosomal patients compared with controls, which is consistent with a hyperfibrinolytic state. Patients with acute haematemesis from ruptured oesophageal varices had the highest APP concentrations which indicates suppression of control mechanisms by the fibrinolytic stimulus. This finding is similar to that of Booth and Bennet,[28] who detected an association between raised APP concentrations and pathological bleeding. High concentrations of activated coagulation (TAT and F 1+2) and fibrinolysis (FbDP and D-D) markers show a simultaneous increase in both thrombin and plasmin generation which suggest the secondary nature of accelerated fibrinolysis in hepatosplenic schistosomiasis. Furthermore, the correlation between TAT and D-dimer level probably reflects increased haemostatic turnover rather than the possible saturation of the hepatic balance clearance capacity as additional routes of clearance by endothelial cells[29] exist for TAT complexes.

Our data reveal the presence of high concentrations of enzyme-inhibitor complexes, t-PA-PAI-1 complexes, in schistosomal patients compared with healthy subjects and show that both t-PA and PAI-1 antigen titres concomitantly increase with the severity of disease. Similar results were reported in cirrhosis by Leebeek *et al*[8] and Leiper *et al*[30] Our results support earlier studies,[31 32] that impaired liver function may lead to diminished hepatic clearance and an increase in both t-PA antigen and t-PA-PAI-1 complexes in the circulation.[33] Furthermore, endotoxaemia inducing increased plasminogen activator production[34] has been suggested as a further contributing factor in schistosomal patients.[35] However, PAI-1 activity was significantly reduced.

Raised specific functional activity of PA was detected in schistosomal patients, but this activity did not correlate with either t-PA or t-PA-PAI-1 complex, but correlated strongly with free t-PA. Moreover, the ratios of t-PA antigen and PAI-1 activity were concomitantly increased with worsening liver damage. The fact that PAI-1 is partly synthesised by the liver[36] and partly by endothelial cells,[37] whereas t-PA is synthesised only by endothelial cells,[38] may account for this change in t-PA/PAI-1 balance. These findings are consistent with data from other studies[10] which attributed the increased fibrinolytic activity in liver cirrhosis to increased plasminogen activators, causing consumption of their inhibitors and resulting in bleeding episodes. Patients with acute haematemesis mostly had a pronounced reduction in PAI-1 activity compared with those in the ascites group; the increase in free t-PA and PA activity in the former group, however, was not significant.

Significant decreases in plasminogen and α_2-AP concentrations were detected in hepatosplenic schistosomal patients compared with controls. Impaired hepatic synthesis may account for this reduction in their concentrations as it is correlated with the degree of liver insufficiency and the presence of mixed infection. The decrease in their concentrations may also be attributable to high t-PA activity, resulting in their increased consumption. The fact that α_2-AP concentration was lower than that of plasminogen in hepatosplenic patients, especially those with acute haematemesis, may potentiate the tilting of the balance towards hyperfibrinolysis and thence haemorrhage and imply a contributory role for α_2-AP in its pathogenesis. Similar findings[8 9] have been reported in liver cirrhosis.

Our data show that the progressive deterioration of liver function in patients with hepatosplenic schistosomiasis increasingly impacts on the plasma concentrations of fibrinolytic components and, therefore, on the whole fibrinolytic system. In decompensated patients

these changes may be the direct result of massive liver injury. Increased t-PA relative to PAI-1 can result in extremely high t-PA activity; this in turn can stimulate the generation of plasmin, which is inadequately inhibited by the decreased α_2-AP, and therefore may lead to enhanced fibrinolysis. In compensated patients inhibitory activity (α_2-AP and PAI-1) seems to be present in sufficient quantities, possibly limiting the incidence of bleeding due to enhanced fibrinolysis in these patients. Our findings suggest that PAI-1 may be a potentially valuable indicator for the prediction of occurrence, exacerbation, or monitoring of the well known enhancement of fibrinolysis which may precipitate and/or aggravate bleeding complications in hepatosplenic schistosomiasis.

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