Vitamin K dependent coagulation proteins in endemic hepatosplenomegaly in Egypt

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Abstract

Aims—To evaluate the role of bilharzial hepatic fibrosis—whether pure or combined with chronic hepatitis B virus infection—on the functional activity of vitamin K dependent coagulation proteins.

Methods—Coagulation screening using prothrombin time (PT), partial thromboplastin time (PTT), and thrombin time (TT) was carried out on 31 patients with endemic Egyptian hepatosplenomegaly and on 14 healthy controls. The functional activities of factors II, VII, IX and X and protein C were measured. Patients were classified as pure hepatosplenic schistosomiasis (n = 17) and schistosomiasis with concomitant chronic hepatitis B virus infection (n = 14).

Results—Prolongation of the PT and PTT were noticed in bilharzial patients compared with the controls. The increase in the TT remained within the upper range of normal. Factors II, VII, IX and X and protein C functional activities were significantly reduced in all groups studied.

Conclusion—The decreased activity of vitamin K dependent coagulation factors might be partially offset by the reduced activity of circulating protein C which tends to establish a haemostatic balance at a lower level in endemic Egyptian hepatosplenomegaly. No significant difference could be shown between the pure and mixed cases. Schistosomal coagulopathy is therefore not necessarily aggravated by chronic hepatitis B virus infection.

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Schistosomiasis is a chronic liver disease and is a major health problem in Egypt. Bleeding is one of its most dangerous complications. It has been reported that hepatitis B surface antigen is commonly found in *Schistosoma mansoni* infection.¹ This association is claimed by some authors to worsen liver function in schistosomal patients.² But other authors have failed to prove if either disease is a risk factor for the other,^{3,4} or that both can act synergistically to damage the liver.^{5,6}

Methods

This study was conducted on 14 healthy men with no history of schistosomiasis, thrombo-

sis, or haematemesis, (age range 28 to 36 years) and 31 bilharzial males were studied. Seventeen of the patients had pure hepatosplenic schistosomiasis (age range 13 to 60 years) and 14 had schistosomiasis and hepatitis (age range 12 to 55 years). The presence of disease was confirmed by urine and stool analysis, rectosigmoidoscopy, rectal biopsy, ultrasonography, detection of hepatitis B surface antigen, liver function tests and liver biopsy. The patients included in this study were admitted to hospital, and adequate dietary intake was assured to avoid nutritional deficiencies.

Those with an alcohol or drug intake which might have interfered with the blood coagulation profile were excluded.

Blood (9 volumes) was collected in sodium citrate solution (0.11 mol/l) (1 volume) under aseptic conditions by clean venepuncture, without frothing, and with minimal venous stasis. Citrated plasma was prepared, kept at 4°C, and tested within four hours. Special haemostatic tests were carried out according to the manufacturer's instructions (Behring Institute Mannheim, Germany) using the Fibrintimer (Behring Institute). These include measuring the prothrombin time (PT) using Thromborel reagent, the partial thromboplastin time (PTT) using Pathromtin reagent, and the thrombin time (TT) using Test Thrombin reagent, as well as assay of the vitamin K-dependent coagulation proteins.

Assays of coagulation factors II, VII, and X were based on comparison of the degree of correction of the long PT of the factor deficient plasma after the subsequent addition of dilutions of the test plasma using Clotting Factor II Deficient Plasma, Clotting Factor VII Deficient Plasma, and Clotting Factor X Deficient Plasma reagents, respectively. Assay of coagulation factor IX was based on a comparison of the degree of correction of the prolonged PTT of factor IX deficient plasma after the addition of dilutions of the test plasma using Clotting Factor IX Deficient Plasma reagent.

Assay of protein C was done by activating it in the test plasma with a specific snake venom protein C activator reagent, thus inhibiting factors V and VIII in the added Protein C Deficient Plasma reagent and prolonging the subsequent PTT test. The prolongation of PTT is a measure of the protein C concentration in the patient's plasma.

The study protocol conformed with the ethical guidelines of the 1975 Declaration of Helsinki as reflected. Informed consent was obtained from each patient.

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Tested parameters in controls and schistosomal groups

Group	Controls	Pure schistosomiasis (17 cases)	Mixed schistosomiasis and hepatitis (14 cases)
	Range (Mean (SD))	Range (Mean (SD))	Range (Mean (SD))
PT (%)	102-138	37-125	33-138
TT (%)	121-14 (10.24)	90-21 (25.67)	85-96 (28.85)
TT (seconds)	15-20-22.65	16-55-30.50	17-30-26.50
PTT (seconds)	17-03 (2.16)	22-84 (4.07)	21-37 (2.52)
Factor II (%)	32-20-43.70	30-20-50.30	32-05-65.60
Factor VII (%)	36-16 (3.73)	38-04 (5.97)	40-84 (8.06)
Factor IX (%)	60-120	28-120	30-120
Factor X (%)	99-93 (18.76)	72-00 (26.92)	75-07 (30.51)
Protein C (%)	80-110	48-104.5	67-110
	99-43 (9.87)	86-71 (17.11)	92-86 (13.67)
	65-120	36-120	21-115
	86-18 (19.04)	77-94 (27.41)	66-36 (26.04)
	79-170	44-150	25-170
	128-00 (30.70)	90-29 (24.95)	94-86 (38.39)
	69-130	40-85	31-75
	97-39 (22.60)	59-94 (11.71)	53-00 (14.32)

Results

There was a progressive decrease in percentage PT with a prolongation in PTT and TT in the bilharzial groups. The functional activities of coagulation factors II, VII, IX and X and protein C concentrations were severely depleted in the bilharzial groups, and this was more pronounced the more advanced the disease (table).

Statistical analysis of the data from the different groups was conducted using the Kruskal-Wallis one way ANOVA test. A significant increase in PT and TT ($p < 0.05$) and a significant decrease in factors II, VII, and X, and protein C concentrations ($p < 0.05$) was found in the group with pure schistosomiasis compared with the control group. A significant lengthening in PT, PTT, and TT ($p < 0.05$) and a significant decline in factors II, IX, and X concentrations ($p < 0.05$) was found between the group with mixed infection and the controls. A comparison of the pure and the mixed infection groups showed that there was no significant difference ($p > 0.05$) in any of the parameters studied.

Discussion

Some alteration in both the extrinsic and intrinsic blood coagulation mechanisms was observed in the groups studied, as monitored by prolonged PT and PTT. This finding was detected in all bilharzial patients and became more evident as the disease progressed.

TT was slightly increased in the infection groups studied compared with the controls, but its level remained within the upper range of normal. Previous studies have reported a prolonged TT at different stages of hepatosplenic schistosomiasis.⁷ This could be explained on the basis of the rise in fibrinogen/fibrin degradation products and the slight depletion in fibrinogen usually encountered in hepatosplenic schistosomiasis.⁸

Although coagulation factors II, IX, and X were substantially reduced as the disease progressed no significant reduction was noted between the pure and mixed groups.

A substantial reduction in factor VII activity was detected in the pure infection group, with the mixed infection group showing only a slight decrease. This agrees with the findings of Orlando and co-workers⁹ who reported normal factor VII values in early liver cirrhosis, indicating that at this stage liver protein synthesis was not affected.¹⁰ This could have been due to either increased cell mass or accelerated synthesis with normal cell volume.¹¹ Moreover, factor VII was the last coagulation protein to be influenced by fibrinolysis and disseminated intravascular coagulation.⁹ Enhanced fibrinolysis⁸ and chronic disseminated intravascular coagulation⁸ were found in patients with hepatosplenic schistosomiasis.

Several mechanisms could be responsible for the decreased values of the vitamin K dependent factors in hepatosplenic schistosomiasis: reduced hepatic synthesis,¹² increased consumption,¹³ impaired carboxylation of the precursor molecule either as a result of premature release of the protein from damaged hepatocytes, or vitamin K dependent carboxylase deficiency¹⁴ and production of abnormal proteins.¹⁵

Despite the fact that protein C was substantially and progressively reduced as the disease progressed, the difference between the pure and mixed infection groups was not significant. This finding agreed with that of Bassily and co-workers⁵ and that of van den Bosch,⁶ who reported no significant deterioration in liver function in simultaneous infection with schistosomiasis and hepatitis. Concomitant hepatitis, therefore, might not necessarily aggravate schistosomal coagulopathy.

In conclusion, the reduced plasma activity of vitamin K dependent proteins—namely, factors II, VII, IX and X, and protein C—might have a role in the defective haemostatic mechanism encountered in hepatosplenic schistosomiasis. Nevertheless, this decreased activity might be offset, even in part, by the parallel reduction in protein C activity, thereby establishing a haemostatic balance in endemic Egyptian hepatosplenomegaly.

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