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HYPOPROTHROMBINEMIA IN THE COMPENSATED FORM OF HEPATOSPLENIC SCHISTOSOMIASIS: FURTHER STUDIES.

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SUMMARY

Coagulation abnormality is frequently observed in schistosomiasis patients but its pathophysiology has not been established. We measured, by immunodiffusion, the prothrombin-antigen concentration in 56 individuals; of these 19 with demonstrated compensated form of hepatosplenic schistosomiasis, 17 with cirrhosis and 20 were control subjects. Transaminases, albumin, transthyretin, prothrombin time, antithrombin III, factor VII, and fibrinogen were also evaluated.

All parameters were altered in the cirrhotic group but only albumin, prothrombin and antithrombin III levels were altered in the schistosomiasis group. Ninety percent of the patients with cirrhosis and sixty percent of the patients with schistosomiasis had abnormal plasma levels of albumin, transthyretin, prothrombin-antigen, and/or antithrombin III; an impaired hepatic synthesis was responsible for these results.

Conversely forty percent of the schistosomiasis patients with normal plasma concentrations of both albumin and transthyretin had decreased mean plasma levels of both prothrombin and antithrombin III. These results suggest that either proth rombin and antithrombin III are more sensitive markers of impaired hepatic synthesis in schistosomiasis than are levels of albumin and transthyretin combined, or a low grade chronic consumption of clotting proteins also occurs. Considering the latter hypothesis it is possible that the thrombin formed would be inhibited by antithrombin III with the complexed thrombin-antithrombin III being cleared by the liver. Consequently the plasma levels of both prothrombin and antithrombin would be decreased, but the level of fibrinogen would be preserved.

KEY WORDS: Prothrombin; Antithrombin III; Schistosomiasis; Coagulopathy.

INTRODUCTION

Over 200 million people worldwide suffer from schistosomiasis. This parasitic infection affects about 10% of the Brazilian population of 135 million inhabitants. Alterations in the plasma coagulation system are frequently detected in this disease, although the precise etiology is not well defined.[2, 12, 13] These alterations may, however, be partially corrected by heparinization or surgical procedures which correct portal hypertension[2].

Schistosomiasis and cirrhosis are the main causes of liver fibrosis in Brazil. In hepatic cirrhosis the alterations of the coagulation tests are usually attributed basically to the deficiency in the synthesis of coagulation factors. Other

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purposed causes for these alterations have been suggested and these are deficiency in γ carboxylation of vitamin K dependent proteins, disfibrinogenemia, and chronic or slow consumption of coagulation factors[8].

Unlike cirrhosis, in schistosomiasis portal hypertension is pre-sinusoidal and vascular alterations of the portal area may recede spontaneously or after chemotherapy[1, 17]. In the hepatosplenic form of schistosomiasis the evident portal hypertension is accompanied by preservation of hepatic function[15] whereas in cirrhosis, portal hypertension is accompanied in most cases with hepatocytic insuficiency. Thus, the study of coagulation in schistosomiasis is important because, in this disease there is, to a certain extent, a dissociation between hepatocytic function and portal hypertension.

In order to study alterations of coagulation in the hepatosplenic form of schistosomiasis, we measured the plasmatic levels of prothrombin-antigen, antithrombin III (the main thrombin inhibitor) and fibrinogen (thrombin physiological substrate). We studied prothrombin because: 1) it is a protein synthesized by the liver; 2) it is not an acute phase protein; 3) it influences decisively the results of the coagulation tests used in daily practice (prothrombin and partial thromboplastin times); 4) and it has a central role in the coagulation cascade because on its activation depends not only the hydrolysis of fibrinogen but, also, in the activation of factors V, VIII, XIII, and protein C.

Hepatic protein synthesis was assayed through determination of serum transthyretin and albumin. Only non-alcoholics (less than 10g ethanol daily intake) and HBsAg negative (RIA) patients with schistosomiasis were chosen in order to avoid interference of the main factors which may lead to liver cirrhosis.

Two other groups of individuals, normal controls, and others with cirrhosis, were included for the sake of comparison in the study. All individuals were not in an acute phase situation, as determined by the serum acid α-1-glycoprotein concentration, and were well nourished.

CASES AND METHODS

A total of 56 well nourished individuals (with an adequate weight-height relationship) and from the same socio-economic class were divided into 3 groups (control, schistosomiasis, and cirrhosis) and studied.

The control group (CON) consisted of 20 healthy volunteers (14 females) age 16-67 years (median 34 years); they were all non-alcoholics, HBsAg negatives and non-schistosomotics. The group with schistosomiasis (SCH) consisted of 19 individuals (12 males) aged 15-65 years (median 35 years). Clinical and epidemiological diagnosis of schistosomiasis was confirmed by the presence of eggs of **S. mansoni** in the faeces or by biopsy of rectal valves. The size of the liver varied between 1 and 8 cm (starting from the border of the last right rib), and that of the spleen between 1 and 20 cm (starting from the border of the last left rib). All these patients presented the compensated form of hepatosplenic schistosomiasis, with no jaundice, ascites, edema or signs of bleeding.

The cirrhotic (CIR) group consisted of 17 individuals (14 males), age 30-63 years (median 46 years). Twelve of these patients were alcoholics with a mean ethanol intake of 142 g/day for a period varying from 7 to 35 years (median 25 years). The 5 other cirrhotics were non-alcoholics, but HBsAg positives. The clinical diagnosis of hepatic cirrhosis was confirmed in all cases by liver histological findings. None of the patients presented ascites, edema, signs of encephalopathy or bleeding, and could be classified as Child A[16].

Blood was collected using the double-syringe technique. Following this technique, after venous puncture the first syringe containing 1 to 2 ml of blood was substituted by a second syringe adapted to the same needle. This technique aims to avoid the presence of thromboplastic material from the puncture itself into the plasma that is to be assayed.

Prothrombin time and prothrombin-antigen, antithrombin III, fibrinogen, and factor VII levels, were determined in citrated plasma (9 parts of blood; 1 part of sodium citrate 0,109M). Albumin was determined in serum by the bromocresol purple technique[7] and the total protein content by the biuret method[5]. Serum transaminases and γ-glutamyltransferase were determined by kits (nº 124419, 124567, 125931, respec-

tively) from Boehringer Mannheim. Serum transthyretin, α_1 acid glycoprotein, plasma fibrinogen, prothrombin-antigen were assayed by radial immunodiffusion, utilizing M. Partigen plates (Boehringwerke A.G., Marburg, W. Germany), employing standards from the same source. The antibody used for prothrombin-antigen recognizes both forms of prothrombin (γ and non γ carboxilated forms). Antithrombin III was assayed through residual thrombin activity after incubation with an excess of thrombin using the synthetic substrate Tos-Gly-Pro-Arg-pNA (Boehringer Mannhein GmBH, W. Germany). Factor VII was assayed with the use of human factor VII deficient plasma; 40 to 160 U/ml were considered normal values[14]. A pool of plasma from the control group was used to establish the reference curve. Prothrombin time was measured using human brain thromboplastin[13] with the results being expressed as a ratio. In all 56 individuals HBsAg was tested by radioimmunoassay (RIA).

Comparison of the results of the different determinations in the 3 groups was performed by analysis of variance at a level of significance $p < 0,05$. Scheffe's test was used when there was a statistically significant difference.

Cut-off values for each determination were established using the results of the control group in order to discriminate these individuals from those of the two groups[18]. This procedure allowed for the classification of the results of the assays either as normal or altered.

RESULTS

The results (mean ± SE) of the different determinations are shown in Table 1. The cirrhotic group results were statistically different from those of the control and schistosomiasis groups. Results for albumin, prothrombin and antithrombin III of the schistosomiasis group were sta-

TABLE 1
Comparison of clotting and biochemical tests.

Tests	Group	Control n = 20 M ± SE	Schistosomiasis n = 19 M ± SE	Cirrhotic n = 17 M ± SE	Variance Analysis
Aspartate aminotransferase U/L		17 ± 2	24 ± 3	60 ± 7	CIR > SCH = CON*
Alanine aminotransferase U/L		14 ± 1	24 ± 4	39 ± 6	CIR > SCH = CON
Total bilirubin mg/dl		0,7 ± 0,05	1,0 ± 0,06	2,6 ± 0,57	CIR > SCH = CON
γGlutamyl-transferase U/L		20 ± 2,5	44 ± 6,1	142 ± 36,7	CIR > SCH = CON
Albumin g/L		39,2 ± 0,7	35,9 ± 0,9	28,6 ± 1,3	CIR < SCH < CON
Transthyretin mg/L		257 ± 6,7	229 ± 12,4	175 ± 18	CIR < SCH = CON
Fibrinogen g/L		3,4 ± 0,8	3,5 ± 0,13	2,8 ± 0,17	CIR < SCH = CON
Prothrombin time ratio		1,00 ± 0,03	1,08 ± 0,04	1,33 ± 0,08	CIR < SCH = CON
Prothrombin-antigen mg/L		94 ± 4,4	52 ± 2,9	37 ± 2,8	CIR < SCH < CON
Antithrombin III U/ml		23 ± 0,7	19 ± 0,9	14 ± 1,7	CIR < SCH < CON
Factor VII U / ml		—	78 ± 3,1	51 ± 4,9	CIR < SCH

* CIR: Cirrhotic group; SCH: Schistosomiasis group; CON: Control group.

tistically different from those of the control group.

On the basis of the established cut-off values (Table 2), it was observed that the proteins which were altered in the greatest number of patients were albumin, transthyretin, prothrombin-antigen, and antithrombin III.

The schistosomiasis group may be thus divided into two sub-groups: one showed altered albumin and/or transthyretin values (10 patients) and other with normal albumin and transthyretin values. It is noteworthy that even this latter group presented lower mean prothrombin and antithrombin III values than the control group (Table 3).

TABLE 2
Critical levels for each test and number of patients showing altered results.

	Critical Levels	Number of Schistosomotics With Altered Results	Number of Cirrhotics With Altered Results
Albumin	33 g/L	6	13
Transthyretin	220 mg/L	7	12
Albumin and Transthyretin	—	10	15
Fibrinogen	3,4 g/L	0	1
Prothrombin-time	1,00	3	10
Prothrombin-antigen	95 mg/L	11	17
Antithrombin III	23 U/mL	11	12
Factor VII	40 U/mL	0	5

TABLE 3
Comparison of antithrombin III and prothrombin mean values in 9 schistosomiasis patients with normal albumin and trans thyretin.

	Control n = 20 Mean ± SE	Schistosomiasis n = 9 Mean ± SE	Student "t" Test
Prothrombin antigen mg/L	95 ± 4,5	76 ± 2,9	$p < 0,05$
Antithrombin III U/mL	23 ± 0,7	20 ± 0,8	$p < 0,05$
Albumin g/L	39 ± 0,7	38 ± 0,8	NS
Transthyretin mg/L	257 ± 6,7	272 ± 7,7	NS

DISCUSSION

In the present work we studied prothrombin-antigen as well as other clotting plasma proteins in schistosomiasis patients. In order to minimize the possibility of including patients with cirrhosis in the schistosomiasis group we excluded from it patients who may be carriers of HBsAg as well as alcoholics. The reason for this is the fact that there's a high incidence of alcoholism and schistosomiasis in the Brazilian population, and of virus B carriers in hepatosplenic schistosomiasis patients[11].

Transaminases and γ-glutamyltranferase as well as bilirubins showed the expected values in the schistosomiasis infected and cirrhotic subjects.

In order to evaluate hepatic protein synthesis we included in the study only well nourished individuals and those that were not in acute phase situation because it is well known that both factors interfere with normal protein synthesis. The liver function of protein synthesis was evaluated through serum content of albumin (half-life 20 days) and transthyretin (half-life 48 hours)[6]. The latter has a half-life similar to that of antithrombin III (42 hours), but being shorter than that of prothrombin (86 hours), fibrinogen (92 hours) and longer than that of factor VII (5 hours)[20].

On analysis of the mean values found for each kind of determination the schistosomiasis group could be differentiated from the control only by comparing the levels of albumin, prothrombin, and antithrombin III; the cirrhotics differed from the other two groups in regard to all assays.

Values obtained in the control group allowed the establishment of a reference range and, thus, each patient could be individually evaluated. Prothrombin-antigen was the protein found to be lowered in the greatest number of schistosomiasis patients. Its value was shown to be altered in all cirrhotics.

Prothrombin time, being a less sensitive assay than the immunologic determination of prothrombin, differentiated fewer patients in both groups.

Despite the fact that factor VII has a shorter half-life than does prothrombin, its values were altered in only 5 cirrhotics but in none of the schistosomiasis patients. This low sensitivity of factor VII determination can be explained as a consequence of the biological method used.

Decrease in fibrinogen was only found in one patient with cirrhosis. This agrees with findings cited in the literature stating that fibrinogen values are altered only in severe liver disease[4].

Regarding hepatic protein synthesis it was observed that transthyretin values discriminated the greatest number of patients in the schistosomiasis group in spite of the fact that the mean values of transthyretin were not statistically different from that of the control group. This may be due to the great dispersion of the values found in the schistosomiasis infected group. It should be noted that in this group both mean albumin and transthyretin values were decreased by 10% in relation to that of the control group.

Besides discriminating a greater number of schistosomiasis patients, transthyretin could be more specific in evaluating hepatic protein synthesis because nonspecific decreased albumin content, secondary to increase in γ - globulin, has been described[9]. In fact we could observe an inverse significant correlation ($r = -0,672$, $p < 0,05$) between serum albumin and globulins in schistosomiasis patients.

On the basis of the results of albumin and transthyretin determinations it can be said that in at least 90% of the patients with cirrhosis and in 60% of the schistosomiasis patients, the alterations of the coagulation parameters were secondary to deficiency of hepatocytic protein synthesis. Conversely, 40% of these schistosomiasis patients with apparent normal synthesis (normal values of albumin and transthyretin) showed a decrease of the mean plasmatic levels of prothrombin-antigen and antithrombin III. This finding can be interpreted in at least two ways: firstly prothrombin-antigen and antithrombin III are more sensitive than are albumin and transthyretin in evaluating hepatic protein synthesis. It should be noted however, that transthyretin has a shorter half-life than does prothrombin-antigen, being similar to that of antithrombin III; secondly a qualitatively altered synthesis or increased consumption of coagulation factors might account for the results. In cirrhosis an altered synthesis of fibrinogen with consequent decrease in its half-life has been described[3]. No reference to disfibrinogenemia in hepatosplenic schistosomiasis has been reported but we have previously shown indirectly the existence of non-γcarboxylated prothrombin in this disease[12]. Another mechanism which could explain a decrease in coagulation factors would be a slow and chronic consumption which would surpass the liver functional reserve.

VERSTRAETE et al.[19] suggest that the slowness of the spleno-portal flow in cirrhotics would favour activation of the contact system of coagulation. In an earlier paper[13] we showed a decrease of pre-kallirein in hepatosplenic schistosomiasis patients with normal albumin and transthyretin values.

A slow and chronic prothrombin activation would lead to formation of thrombin which would be inhibited by antithrombin III, with the resulting complex being cleared by the liver[10] before hydrolyzing fibrinogen. This hypothesis could explain the preservation of fibrinogen plasma levels concomitant with decrease in cir-

culating levels of prothrombin and antithrombin III.

RESUMO

Hipoprotrombinemia na forma compensada da esquistossomose hepatoesplênica: novos estudos

Alterações na coagulação são frequentemente observadas em portadores da esquistossomose, mas sua fisiopatologia ainda não foi estabelecida. Medimos, por imunodifusão, a concentração da protrombina-antígeno em 56 indivíduos: 19 com a forma compensada da esquistossomose hepatoesplênica 17 com cirrose e 20 sadios. Foram também determinadas transaminases, albumina, transtiretina, tempo de protrombina, antitrombina III, fator VII e fibrinogênio.

As médias de todos os parâmetros estavam alteradas no grupo cirrótico mas apenas as de albumina, protrombina e antitrombina III no grupo esquistossomótico. Noventa por cento dos portadores de cirrose e 60% dos portadores de esquistossomose tinham níveis plasmáticos diminuídos de albumina, transtiretina, protrombina e/ou antitrombina III; síntese hepática diminuída pode explicar estes resultados.

Por outro lado, em 40% dos portadores de esquistossomose com concentrações plasmáticas de albumina e transtiretina normais as concentrações médias de protrombina e antitrombina III estavam diminuídas. Estes resultados sugerem que a protombina e antitrombina III são marcadores mais sensíveis de síntese hepática do que o conjunto albumina/transtiretina ou que um consumo crônico das proteínas da coagulação ocorra. Considerando esta última possibilidade é possível supor-se que a trombina formada seja inibida pela antitrombina III e o complexo trombina-antitrombina III depurado pelo fígado. Consequentemente os níveis plasmáticos de protrombina e antitrombina III diminuem mas o do fibrinogênio é preservado.

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