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Fibronectin in the ascitic fluid of cirrhotic patients: correlation with biochemical risk factors for the development of spontaneous bacterial peritonitis

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Abstract

Cirrhotic patients (23 with alcoholic cirrhosis, 5 with posthepatitic cirrhosis and 2 with cryptogenic cirrhosis) with ascites and portal hypertension were studied and divided into two groups corresponding to high or low risk to develop spontaneous bacterial peritonitis (SBP) related to the concentration of total protein in the ascitic fluid (A-TP): group I (high risk): A-TP≤1.5 g/dl and group II (low risk): A-TP>1.5 g/dl. Fibronectin (FN), C3 and C4 concentrations were measured by radial immunodiffusion while total protein was measured by the biuret method. The mean values (group I *vs* group II) of C3 (12.59 ± 4.72 *vs* 24.53 ± 15.58 mg/dl), C4 (4.26 ± 3.87 *vs* 7.26 ± 4.14 mg/dl) and FN (50.47 ± 12.49 *vs* 75.89 ± 24.70 mg/dl) in the ascitic fluid were significantly lower (P<0.05) in the group considered to be at high risk for SBP. No significant difference was observed in the plasma/ascites fibronectin ratio (3.91 ± 1.21 *vs* 3.80 ± 1.26) or gradient (131.46 ± 64.01 *vs* 196.96 ± 57.38) between groups. Fibronectin in ascites was significantly correlated to C3 (r = 0.76), C4 (r = 0.58), total protein (r = 0.73) and plasma FN (r = 0.58) (P<0.05). The data suggest that the FN concentration in ascites is related to the opsonic capacity of this fluid, and that its concentration in the ascitic fluid may be a biochemical risk factor indicator for the development of spontaneous bacterial peritonitis.

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- Fibronectin
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- Ascites

Introduction

Many studies have shown that cirrhotic patients present a deficiency in the opsonic activity of the ascitic fluid (1-3), which is related to low levels of total protein, C3, and C4 (2-4), thus predisposing the patient towards spontaneous bacterial peritonitis (SBP). While fibronectin (FN) is a glycoprotein involved in the mechanism of non-specific opsonization of a number of endogenous substances and bacteria (5,6), its diagnostic significance as well as its physiopathological role in the ascitic fluid are not

yet completely understood (7,8).

The ascitic fluid of cirrhotic patients displays lower fibronectin concentration when compared with that of noncirrhotic patients (7,9), which may be related to the higher infection risk of these patients (7,10). However, few studies have measured the concentration of this glycoprotein in the ascitic fluid of cirrhotic patients who developed SBP and the results have been inconsistent (4,7,10), as was also the case for studies determining the correlation between FN and biochemical risk factors for SBP, in particular total protein (8,11).

Therefore, we determined the fibronectin concentration in the ascitic fluid and plasma of cirrhotic patients without a previous history of SBP and correlated it with biochemical parameters considered to be risk factors for development of SBP, in particular total protein and the C3 and C4 fractions of the complement.

Patients and Methods

Thirty patients, 25 males and 5 females (mean age, 51.2 years), presenting liver cirrhosis (23 with alcoholic cirrhosis, 5 with posthepatitic cirrhosis and 2 with cryptogenic etiology) and ascites detected by physical examination were studied. Cirrhosis was diagnosed by hepatic biopsy and clinical and biochemical criteria (12), as well as by imaging and endoscopy.

None of the patients had been submitted to paracentesis, had a history of SBP, presented clinical or bacteriological evidence of systemic infection, or had received antibiotic therapy for thirty days prior to entering the study. Only those patients whose ascitic fluid presented cellularity levels lower than 250 polymorphonuclear neutrophils/mm^3 with negative culture and neoplastic cell assay and a plasma-ascites albumin gradient greater than 1.1 g/dl were included in the study.

The patients were divided into two groups according to the total protein concentration in the ascitic fluid (A-TP) (13,14): group I with A-TP≤1.5 g/dl (N = 16) and group II with A-TP>1.5 g/dl (N = 14).

The parameters of the Child-Pugh (15) classification were used for comparison of the two groups. Ascitic fluid was considered to be tense when the viscera could not be felt during the physical examination of the abdomen, and moderate when the viscera were directly palpated or felt by ballottement (16).

Ascitic fluid was obtained by means of sterile paracentesis, and stored as aliquots at -70°C for less than 6 months; 9 ml of blood in 1 ml of 3.8% sodium citrate was collected from fasting patients from a peripheral vein on the same day of the paracentesis procedure. Plasma was centrifuged at 2,500 rpm and stored in 2-ml aliquots at -70°C.

Plasma and ascitic fluid total protein was determined by biuret reagent colorimetry. The simple radial immunodiffusion technique with NOR-Partigen C3c and C4 plates (Behring, Marburg, Germany) was used to determine C3 and C4 levels, using human serum with known C3c and C4 concentrations (Behring) as a standard. The same technique was used to determine plasma and ascitic fluid fibronectin concentration using human fibronectin antibodies (Sigma Chemical Co., St. Louis, MO). Agar plates were prepared using anti-fibronectin antibodies at 1/100 concentration and placing 20 µl of blood or ascitic fluid in 4-mm diameter orifices. Standard human plasma protein dilutions (Behring) containing a known fibronectin concentration (250 mg/l) were used to obtain a reference curve for the glycoprotein concentrations.

The Student *t*-test was used to compare mean values between groups; the chi-square test complemented by the Fisher exact test was used to compare frequencies within each group, and the Pearson correlation coefficient was used to correlate the studied variables (17). In all tests, the level of significance was set at $P<0.05$.

Results

No significant differences in age, race, sex, etiology, Child-Pugh classification, or the use of diuretics were observed between the groups studied (Table 1). Spironolactone was the diuretic used with a maximum daily dose of 200 mg.

Based on the parameters used for the Child-Pugh classification, only the ascitic fluid tension differed between groups (Table 2).

The mean concentration of C3, C4, and fibronectin (A-FN) in the ascitic fluid and fibronectin in plasma (P-FN) was significantly lower in the patients of the group at higher risk for SBP (Table 3).

A statistically significant correlation was observed between A-TP and A-FN (r = 0.73, Figure 1) and the C3 fraction (r = 0.71) of the ascitic fluid complement. A lower, but significant correlation was observed between A-TP and the C4 fraction (r = 0.52) of the complement and with P-FN (r = 0.50).

Ascitic fluid fibronectin was also significantly correlated with the C3 (r = 0.76, Figure 2) and C4 (r = 0.58) fractions of the complement and with P-FN (r = 0.58).

Since A-FN and P-FN concentrations differed between groups and showed a significant positive correlation, we determined the P-FN and A-FN ratio and gradient, which did not differ between groups.

Patients classified as having tense ascitic fluid presented significantly lower fibronectin levels than patients classified as having moderately tense ascitic fluid.

Table 1 - Age, color, sex, etiology, Child-Pugh classification and use of diuretics among cirrhotic patients grouped according to total protein concentration in the ascitic fluid (A-TP).

N = Number of patients; [a]others = viral or cryptogenic. There were no significant differences between groups when the Student *t*-test was used for age and the chi-square test was used for all other parameters.

	Group I A-TP≤1.5 g/dl (N = 16)	Group II A-TP>1.5 g/dl (N = 14)
Age (mean ± SD)	51.12 ± 11.40	51.14 ± 8.31
Color (white/others)	11/5	9/5
Sex (masculine/feminine)	14/2	11/3
Etiology (alcohol/others[a])	12/4	11/3
Child-Pugh (A/B/C)	1/8/7	1/8/5
Use of diuretics (yes/no)	13/3	12/2

Table 2 - Clinical and biochemical parameters used for Child-Pugh classification of cirrhotic patients grouped according to total protein concentration in the ascitic fluid (A-TP).

There were no significant differences between groups according to the [a]Student *t*-test or [b]chi-square test, except for ascites. *P<0.05 compared to group I.

	Group I A-TP≤1.5 g/dl (N = 16)	Group II A-TP>1.5 g/dl (N = 14)
Total bilirubin (mg/dl)[a]	3.21 ± 4.37	2.06 ± 1.47
Prothrombin activity (%)[a]	48.05 ± 12.76	47.73 ± 11.25
Serum albumin (g/dl)[a]	3.16 ± 0.41	3.23 ± 0.50
Ascites (tense/moderate)[b]	13/3	4/10*
Encephalopathy (absent/present)[b]	12/4	11/3

Discussion

Spontaneous bacterial peritonitis is a serious cirrhosis complication because of its frequency and mortality rates (2,18) and because of its unclear clinical picture. For cirrhotic patients to develop SBP in their ascitic fluid, a translocation of the intestinal bacteria due to portal hypertension should occur (13,18), and a reduction in the ascitic fluid opsonic activity (2,4,19) should occur as indicated by the low-protein concentration in this fluid (2,3,13,20). Since fibronectin is active in the opsonic process (5,21), its involvement in the defense mechanisms protecting against infection has been investigated. Several investigators have observed an association between low fibronectin levels in the ascitic fluid and the risk of SBP in cirrhotic patients (7,10), although others have not confirmed these findings (4,8). Although

the correlation between fibronectin levels in the ascitic fluid and clinical and biochemical risk factors for developing SBP has not been widely investigated in these patients, it is well known that fibronectin levels in the ascitic fluid are significantly correlated with total protein and the opsonic activity in the fluid (4,11).

Table 3 - C3, C4, and fibronectin concentration in the ascitic fluid (A-FN) and plasma (P-FN) of patients with liver cirrhosis.

Data are reported as means ± SD. *P<0.05 compared to group I (Student *t*-test).

	Group I A-TP≤1.5 g/dl (N = 16)	Group II A-TP>1.5 g/dl (N = 14)
Ascites C3 (mg/dl)	12.59 ± 4.72	24.53 ± 15.58*
Ascites C4 (mg/dl)	4.26 ± 3.87	7.26 ± 4.14*
A-FN (mg/l)	50.47 ± 12.49	75.89 ± 24.70*
P-FN (mg/l)	193.75 ± 64.17	270.71 ± 65.12*

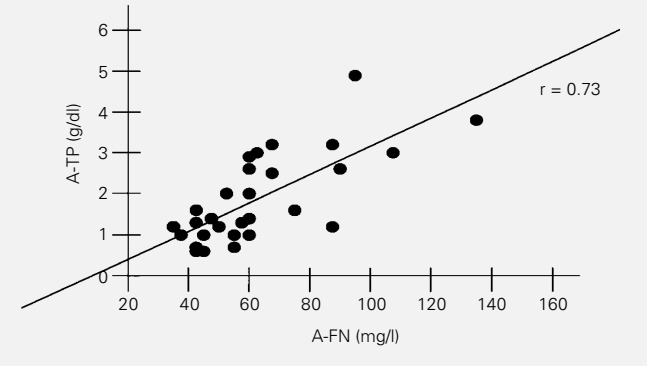

Figure 1 - Correlation between fibronectin (A-FN) and total protein (A-TP) in the ascitic fluid.

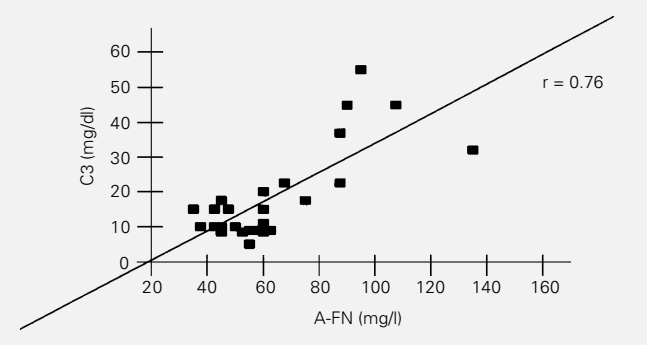

Figure 2 - Correlation between fibronectin (A-FN) and C3 concentrations in the ascitic fluid.

The present study investigated the fibronectin concentration in the ascitic fluid of cirrhotic patients who were separated into two groups according to high or low levels of total protein concentration in the ascitic fluid. Total protein in the ascitic fluid is the most widely used biochemical parameter for identifying cirrhotic patients at risk for SBP. Its methodological simplicity and high prognostic value have been demonstrated by prospective studies (3,13,14,20).

Significant differences in the C3 and C4 values and the degree of tension of the ascitic fluid were observed between the groups which were divided according to total protein values higher or lower than 1.5 g/dl (13,14). The present findings confirmed that these groups have different opsonic activities in the ascitic fluid and therefore different risk levels for developing SBP. The higher frequency of tense ascitic fluid in cirrhotic patients with lower levels of A-TP indirectly shows that the dilution factor determines not only the low levels of total protein but also of C3 and C4 in the ascitic fluid (2,18).

Fibronectin concentration was significantly lower in patients biochemically characterized as being at risk for SBP when compared with patients with higher ascites total protein levels, suggesting that this parameter may also be a criterion for the risk of SBP. This possibility was further strengthened by the calculation of the correlation between individual fibronectin values and biochemical SBP risk factors. The positive and highly significant correlation between fibronectin and the C3 and C4 fractions of the complement and A-TP further emphasizes the possible role of this glycoprotein in local defense mechanisms of ascitic fluid, corroborating the findings of Runyon (11) and Mal et al. (4) who found a significant correlation between A-FN and the opsonic activity of the fluid.

The low fibronectin levels found in the

ascitic fluid may only reflect its low level in plasma observed in the advanced stages of cirrhosis of the liver (14,22). Thus, we evaluated the relationship between the fibronectin concentration in the ascitic fluid and in the plasma of these patients by studying the gradient and ratio of these variables. The comparison of plasma and ascitic fibronectin gradient and ratio, unlike the isolated measurement of each one, did not show any difference between groups, indicating that changes in plasma and ascitic fluid levels of fibronectin are related. These observations corroborate the findings of Hafter et al. (23) that plasma fibronectin is the main factor determining the levels of this glycoprotein in ascitic fluid.

The present data show that fibronectin concentrations may be used as a risk indicator for SBP in cirrhotic patients. Further prospective studies on a larger number of subjects, correlating fibronectin levels in the ascitic fluid with SBP, are needed to validate these findings.

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