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The Importance of Anticardiolipin Antibody and Antiplatelet Antibody in the Development of Thrombocytopenia in Cirrhosis

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Abstract: **Objective:** The purpose of the present study was to investigate the effects of the presence of hypersplenism, antiplatelet antibody and anticardiolipin antibody, which are thought to be important factors, on the development of thrombocytopenia in patients with cirrhosis with viral etiology.

Methods: This study includes 44 patients with cirrhosis related to HBV and HCV, and 12 healthy control subjects. Spleen size was evaluated by ultrasonography, the presence of antiplatelet antibody by flow cytometry, and IgG and IgM isotypes of anticardiolipin antibody by enzyme immunoassay.

Results: There was a significant difference in ultrasonographic spleen size between the patients with and without thrombocytopenia ($p<0.05$). The presence of antiplatelet antibody was demonstrated in 19 patients (43%) with cirrhosis, whereas none of the control subjects had antiplatelet antibody. In our study we found no relationship between the presence of antiplatelet antibody and platelet count or severity of thrombocytopenia. Of 31 patients with cirrhosis, six (19%) had anticardiolipin antibody. There was no relationship between IgG and IgM isotypes and anticardiolipin antibody platelet count, presence and severity of thrombocytopenia.

Conclusions: Our results support the idea that spleen size is an important factor development of thrombocytopenia in patients with cirrhosis with viral etiology. Antiplatelet antibody and anticardiolipin antibody are often found in patients with cirrhosis. However, we obtained no data regarding any role which these antibodies play in the immune degradation of platelets.

Key Words: Cirrhosis, thrombocytopenia, anticardiolipin antibody, antiplatelet antibody

Introduction

Thrombocytopenia due to liver disease and portal hypertension is a well-recognized finding and its prevalence in cirrhosis varies from 15 to 55% (1). The mechanisms responsible for thrombocytopenia in cirrhosis include hypersplenism, immune-mediated platelet destruction and platelet production defect in bone marrow (e.g., synthesis abnormality of thrombopoietin and nutrional folate deficiency). It is known that increased sequestration of cells in the spleen due to splenomegaly is only one of the causes of thrombocytopenia (2-4). It has been suggested that the thrombocytopenia found in cirrhotic patients may be developed in the presence of antibodies to platelets and the resultant immune-mediated platelet destruction or in the presence of anticardiolipin antibodies as well as the existence of hypersplenism (3-7). The purpose of this study was to investigate the effects of spleen size and the presence of anticardiolipin antibodies (ACA) and of antiplatelet antibodies (APA) on the development of thrombocytopenia in cirrhotic patients.

Material and Methods

Forty-four chronic liver patients with biopsy-proved liver cirrhosis related to HBV (19 patients) or HCV (25 patients), and 12 healthy control subjects (seven males) were studied. Criteria for inclusion in the study were as follows: absence of a history of receiving therapy that could suppress bone marrow, absence of a history of blood transfusion during the past three months, absence of a secondary disease except cirrhosis, or the use of a drug that could affect coagulation parameters, and normal levels of serum B_{12} and folate. In our study, Child-Pugh classification was used in all cirrhotic patients for evaluation of the severity of the disease. Levels of serum albumin and bilirubin, prothrombin time, the presence and severity of ascites and encephalopathy were parame-

ters for Child Pugh classification, and a compatible score was given for each parameter. Each patient had a score of between 5 and 15. A score below 7 was considered Child-Pugh A, a score between 7-9 was Child-Pugh B and a score above 9 was Child-Pugh C (8). The severity of hepatocellulary dysfunction was assessed by Child-Pugh classification with 12 being Child-Pugh A, 17 Child-Pugh B, and 15 Child-Pugh C. Platelet count was measured using a Coulter SKTS instrument. Thrombocytopenia was defined as a platelet count below $150x10^3$/µl. Platelet count was made by automatic counter, but a peripheral blood smear evaluation was made for every patient to exclude miscount of platelets. Pseudothrombocytopenia was excluded via peripheral smear evaluation.

While antiplatelet antibodies were investigated in all patients, anticardiolipin antibodies were evaluated in 31 of the 44 patients.

The presence of antiplatelet antibodies in the cirrhotics and control subjects was studied by flow cytometry using goat anti IgG FITC, goat anti IgM FITC, anti-human CD41 FITC and mouse IgG1 FITC to demonstrate nonspecific staining. The results were evaluated by flow cytometry using a Becton Dickinson Facscalibur instrument. The cells in the lower right-hand position of the diagram show the platelets contain anti IgG and anti IgM antibodies, and the ratio of cells in this position was estimated by computer. The results were evaluated by flow cytometry using a Becton Dickinson Facscalibur instrument (9-10).

Anticardiolipin antibody measurement was made via enzyme immunoassay methods and cardiolipin was used as an antigen in the test. The test was read on a multiwell strip reader and results were calculated by a formula: adsorbance level/calibrator adsorbance level x calibrator concentration.

The results of IgG and IgM examinations were determined quantitatively as GPL and MPL, respectively. The positivity of IgG and IgM was defined as the values equal to or greater than 23 GPL unit/ml and 11 MPL unit/ml, respectively.

Abdominal ultrasonographic studies were carried out using a General Electric RT-x200 with a 3.5 MHz convex probe. Spleen size was examined in the supine and right lateral positions by measuring the longitudinal distance of the spleen.

Statistical analysis was performed with the Pearson product moment correlation test and Student's t test as appropriate. Data are expressed as mean ± standard error of mean (SEM). A level of $p<0.05$ was considered as significantly different.

Results

Some demographic and clinical features of the patient and control groups are shown in Table 1. Thrombocytopenia was detected in 91% of the 44 cirrhosis patients. The platelet count in the patients (82.688 ± 5.201 platelets/µl) was significantly lower than that of the control subjects (255.333 ± 15.072 platelets/µl) ($p<0.001$)

Some important clinical characteristics of the patients with cirrhosis are shown in Table 2. Positivity for APA was found in 19 patients (43%), positivity for ACA of the IgG in 6 (19%) and ACA of the IgM in 6 (19%). The control population did not demonstrate either positivity for

Table 1. Some Demographic and Clinical Features of the Patient and Control Groups.

	Patients (n=44)	Controls (n=12)
Age (years)	58+2	51+4
Sex: Male (n)	26	7
Female (n)	18	7
Platelet (µL)	82688+5201***	255333+15072
APA positive (n) (%)	19 (43)**	0 (0)
Anticardiolipin antibody IgG (GPL)	17+2**	10+1
Anticardiolipin antibody IgM (MPL)	8+2	5+1
Anticardiolipin antibody IgG positive (n) (%)	6 (19)*	0 (0)
Anticardiolipin antibody IgM positive(n) (%)	6 (19)	1 (8)

(Difference is significant:***p<0.001, **p<0.01, *p<0.05)

Table 2. Some Important Clinical Characteristics of the Patients with Cirrhosis.

	n	%
Etiology		
HBV	19	43
HCV	25	57
Child-Pugh grade		
A	12	27
B	17	39
C	15	34
Platelet count		
<150x10^3/µml	40	91
>150x10^3/µl	4	34
Platelet count		
<100x10^3/µl	31	71
100-150x10^3/µl	9	20
Antiplatelet antibody		
Positive	19	43
Negative	25	57
Anticardiolipin antibody IgG		
Positive	6	19
Negative	25	81
Anticardiolipin antibody IgM		
Positive	6	19
Negative	25	81

APA or positivity for ACA of the IgG isotype, whereas one control subject (8%) demonstrated positivity for ACA of the IgM isotype. The proportion of positivity for APA and positivity for ACA of the IgM isotype in the cirrhotics was significantly different from those of the controls ($p<0.01$ for APA, and $p<0.05$ for ACA of the IgG). The mean values for ACA of the IgG were 17 ± 2 GPL in the patients with cirrhosis and 10 ± 2 GPL in the control subjects. The mean values for ACA of the IgM were 8 ± 2 MPL in the patients with cirrhosis and 5 ± 1 MPL in the control subjects. There was a significant difference between the patient group and the controls with regard to the level of ACA of the IgG ($p<0.05$).

When the thrombocytopenic patient population was divided into two subgroups according to platelet count (<100x10^3/ml and 100x10^3-150x10^3/ml), no significant difference was observed between these two groups with regard to the positivity of APA, ACA of the IgG and ACA of the IgM, and the levels of serum IgG, IgA, IgM and globulin. However, in the cirrhotic patients whose platelet counts were below 100x10^3/ml, the average ultrasonographic spleen size was found to be significantly greater than that of other subgroup (15±3 cm. and 11±1 cm, respectively; $p<0.05$).

Of the 44 patients with cirrhosis, 19 (43%) had positivity for APA. No significant correlation was found between the positivity for APA in the cirrhotics and age, platelet count, spleen size measured ultrasonographically, the presence of splenomegaly or serum levels of globulin, IgG, IgA and IgM. The mean Child-Pugh score in the patients who had positivity for APA was significantly greater than that of the patients without APA (9.4±0.5 and 7.7±0.4, respectively; $p<0.05$).

Of the 31 cirrhotic patients, 6 (19%) had positivity for ACA of the IgG isotype. No significant correlation was found between the positivity for ACA of the IgG or IgM isotype and platelet count, the presence of ultrasonography-proved splenomegaly or the level serum total globulin ($p>0.05$). A positive linear correlation was observed between the level of ACA of the IgG and the level of serum IgG ($p<0.05$). Similarly, there was a positive linear correlation between the level of ACA of the IgM and the level of serum IgM ($p<0.05$).

When the cirrhotic patients were divided into three groups according to the severity of hepatocellulary dysfunction by Child-Pugh classification, no significant difference was observed among these three groups with regard to platelet count, and levels of APA, and ACA of the IgM and IgG. The mean level of ACA of the IgG in Child-Pugh A patients (11±2 GPL) was likely to be lower than in Child-Pugh B and Child-Pugh C patients (21±4 and 18±3 GPL, respectively), and there was a weak correlation between Child-Pugh A, Child-Pugh B and Child-Pugh C patients ($p=0.08$).

No significant difference was found in platelet count and levels of APA, and ACA of the IgG and IgM between the patients with splenomegaly detected ultrasonically and those without splenomegaly ($p>0.05$).

Discussion

Thrombocytopenia is a common laboratory finding in subjects with cirrhosis. In these patients, hypersplenism is considered the main factor in the development of thrombocytopenia (2-4). However, the fact that platelet lifespan in some cirrhotic patients has been demonstrated to be markedly shortened in several platelet survival studies, and that the high frequency of antiplatelet antibodies and

antiphospholipid antibodies has been determined in cirrhotic patients, suggests that the immune-mediated platelet destruction could be effective in the development of thrombocytopenia, as well (3-7).

In this study, we aimed to investigate the effects of factors, such as hypersplenism, the presence of antiplatelet antibodies and anticardiolipin antibodies, thought to be responsible for the development of the thrombocytopenia, on the occurrence of thrombocytopenia.

In our study, spleen size measured by ultrasound was used to study the effect of spleen volume on thrombocytopenia. We found a significant difference in the spleen size measured by ultrasound between subjects with or without severe thrombocytopenia. These findings are in agreement with the studies indicating that spleen size and increased splenic sequestration have an effect on the development of thrombocytopenia (11-14).

It was demonstrated that 19 (43%) of the 44 cirrhotic patients had antiplatelet antibodies. Our finding was in agreement with previous studies report the prevalence of APA to be 40-80% in cirrhotic patients (3, 5, 15). We also found a relationship between the presence of APA and the Child-Pugh score, indicating the severity of the disease, in our study. The level of sialic acid of the components of platelet membrane in the patients with chronic liver disease is lower in the cirrhotic subjects. Therefore, a new antigenic process of changing develops in the surface of the platelet membrane. It is claimed that the changes in the level of sialic acid in the platelet membrane increases with the severity of disease and facilitates the adherence of antibodies to the surface of the platelet membrane (5, 16).

No significant correlation was found between positivity for APA and the levels of serum IgG, IgA and IgM. Some controversial results have been reported about this relationship. While it has been suggested, especially in previous reports, that increased serum globulin and immunoglobulin fractions are nonspecifically adsorbed to the platelet membrane in cirrhotics (16-18), recently it was reported that no relationship was found between serum globulin and immunoglobulin fractions and the presence of APA, and that previous findings were due to a methodic problem resulting from measuring nonspecific antibodies to the platelet membrane (15). Our results support this view. We believe that there was no relationship between the increase of polyclonal globulin observed in the cirrhotic patients and the occurrence of APA.

In our study, the fact that no relationship was found between the severity of thrombocytopenia and positivity for APA seems to be a finding which supports the idea that immune-mediated platelet destruction has no role in the development of the thrombocytopenia. Other possibilities may include failure of production response in bone marrow to immune-mediated platelet destruction and further impairment of reticuloendothelial system function in these patients (20-21).

It has been suggested that the major determinant of the fact that APA may lead to platelet destruction is the functions of the reticuloendothelial system in the subjects (23-24). It is known that the functions of the reticuloendothelial system are markedly impaired according to the severity of the disease in cirrhotic patients (2-3, 25). Impaired reticuloendothelial system function may also be a factor which prevents the clearence of the platelets from circulation. Our findings demonstrate that patients with positivity for APA had higher Child-Pugh scores (i.e., had more severe hepatocellulary dysfunction) than those without APA, and so it is indicated indirectly that these patients with APA would have markedly impaired reticuloendothelial system function. The main reason that there was no difference in platelet count between the patients with and without APA may be the marked impairment of reticuloendothelial system function in patients with APA.

In our study, six (19%) of the 31 cirrhotic patients evaluated for ACA had positivity for ACA. The level of ACA of the IgG in the cirrhotic patients was significantly higher than that of the controls.

A statistically significant correlation was found in our study between ACA of the IgG and serum level of IgG, and between ACA of the IgM and serum level of IgM. According to a hypothesis that describes the occurrence of ACA in the cirrhotic patients, the activation of polyclonal B cell in these cases triggers the occurrence of ACA (26). Another factor which determines the subtype of ACA in the cirrhotics may be the increasing fraction of serum immunoglobulin.

No significant correlation was found between the presence of ACA of the IgG and ACA of the IgM, and platelet count, and presence and severity of thrombocytopenia. The prevalence of the thrombocytopenia has been reported to be from 10-40% in the cases with pos-

itivity for ACA (21, 27-30). In some published studies, it has been reported that the binding of ACA to the platelets is not associated with the occurrence of thrombocytopenia, and that the major factor determining the platelet count in cases with ACA is the balance between production and clearence of the platelets, and this picture was defined as a compensated thrombocytolytic situation (20-22).

In conclusion, our study supports the idea that the spleen size was an effective factor in the development of thrombocytopenia in patients with cirrhosis with viral etiology. Splenic sequestration probably increases in patients with splenomegaly. Although ACA and APA are frequently found in cirrhotic patients, we did not obtain enough data indicating that these two kinds of antibody play a role in immune-mediated platelet destruction. In order to elucidate the effects of these antibodies in the development of the thrombocytopenia in chronic liver patients, new studies including thrombopoietin, an effective growth factor in the production of the platelets, and platelet kinetic, are necessary.

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