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RAPID COMMUNICATION

Does endothelium agree with the concept of idiopathic hepatic vessel thrombosis?

Ozgur Harmanci, Yahya Buyukasik, Serafettin Kirazli, Ferhun Balkanci, Yusuf Bayraktar

Ozgur Harmanci, Yusuf Bayraktar, Hacettepe University Faculty of Medicine, Department of Gastroenterology, 06100 Sihhiye, Ankara, Turkey
Yahya Buyukasik, Serafettin Kirazli, Hacettepe University Faculty of Medicine, Department of Hematology, 06100 Sihhiye, Ankara, Turkey
Ferhun Balkanci, Hacettepe University Faculty of Medicine, Department of Radiology, 06100 Sihhiye, Ankara, Turkey
Supported by Hacettepe University Office of Scientific Research Center
Co-first-authors: Yusuf Bayraktar
Correspondence to: Dr. Ozgur Harmanci, Department of Gastroenterology, Hacettepe University Faculty of Medicine, 06100 Sihhiye, Ankara, Turkey. ozgurmd@hacettepe.edu.tr
Telephone: +90-312-3051713
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Abstract

AIM: To investigate the major steps of thrombogenesis and to identify the differences in these steps between idiopathic patient group and control group.

METHODS: Fibrinogenesis was studied by measuring the activated factor VII, total and free levels of tissue factor pathway inhibitor (TFPI). The fibrinolysis step was investigated by determining the global fibrinolytic capacity. The endothelial function was assessed by measuring the levels of soluble adhesion molecules, namely soluble intercellular adhesion molecule 1 (sICAM-1), soluble vascular cell adhesion molecule 1 (sVCAM-1) and soluble E-selectin molecule. The exclusion criteria from "idiopathic" patient group were abdominal surgery, pregnancy, use of oral contraceptives, anti-phospholipid syndrome, Behçet's disease, cancer, myeloproliferative diseases. The congenital factors like mutations of factor-V Leiden and prothrombin, deficiencies of proteins C and S, antithrombin, hyperhomocysteinemia and hyperfibrinogenemia were ruled out. The total number of patients was reduced from 96 to 9 (7 with portal vein thrombosis, 2 Budd Chiari syndrome) by exclusion criteria.

RESULTS: The levels of adhesion molecules sICAM-1, sVCAM-1, free TFPI levels and global fibrinolytic capacity were significantly different ($P < 0.05$) in the patient group indicating an endothelial dysfunction and a lower fibrinolytic activity.

CONCLUSION: These results show that this patient group should be tested by means of endothelial dysfunction and managed differently.

Key words: Portal vein thrombosis; Budd-Chiari syndrome; Endothelial dysfunction; Soluble adhesion molecules; Fibrinolysis

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INTRODUCTION

Thrombosis in the hepatic vascular system (hepatic veins and portal veins) is an established risk factor for both morbidity and mortality due to inevitable consequences of disturbed liver vascular physiology and anatomy. The primary mechanism resulting in this condition may be clinically evident (ie, a mass-forming lesion adjacent to a major vessel resulting in compression and occlusion, which is called a "secondary" thrombosis) and by definition these patients are treated with therapeutic techniques to eliminate the cause. On the other hand, in some patients there may be a thrombophilic condition resulting in a "primary" thrombosis which usually is the case in this patient population. The clinical importance of presence or absence of a thrombophilic condition lies in the fact that the presence of a thrombophilic condition increases the mortality by a factor of 4[1].

Studies investigating the etiology of thrombosis in hepatic vasculature indicate that the most commonly-found predisposing thrombophilic conditions are deficiencies of proteins C and S as well as antithrombin (AT), mutations of factor V Leiden and prothrombin followed by myeloproliferative diseases and other less commonly-observed disorders including Behçet's disease[2-6]. On the other hand, there seems to be a patient population in whom there is not any obvious thrombophilic condition that can be found utilizing routine techniques but mortality risk increases still in the high risk group due to undiagnosed thrombophilic condition. This unique population is called "idiopathic" thrombosis group and their contribution to the main patient population is about

Table 1 Applied exclusion criteria

Exclusion criteria
Acute thrombosis of portal or hepatic veins
Any ultrasonographic (including Doppler studies) finding compatible with mass occupying lesion in liver
Positivity of viral hepatitis markers
Any finding in the liver biopsy suggesting parenchymal liver disease (including autoimmune liver diseases and metabolic liver diseases)
Acquired hematological abnormality leading to thrombophilia (myeloproliferative diseases, paroxysmal nocturnal hemoglobinuria, history of use of oral contraceptive or estrogen replacement treatment, anti-phospholipid syndrome, history of cancer, history of hepatobiliary surgery, history of pancreatitis)
Genetic hematological abnormality leading to thrombophilia (mutation of factor V Leiden, mutation of prothrombin, deficiencies in proteins C and S or antithrombin, hyperfibrinogenemia, hyperhomocysteinemia)
Severe and widespread atherosclerosis, uncontrolled or newly diagnosed malignancy elsewhere, systemic inflammatory diseases, history of recent operation

7%-22%[7,8]. In the current knowledge, hematological changes of this remaining "idiopathic" group is unclear, hence giving opportunity to investigate this patient population with advanced markers of thrombosis formation.

According to the current concept of pathologic thrombus formation, a thrombus is the result of convergence of multiple factors which may be either genetic or acquired. Therefore, it seems crucial to clarify the underlying possible pathogenetic factors or predisposing conditions in the "idiopathic" patient population in terms of tests not routinely used for the investigation of thrombogenesis.

In our study, we aimed to investigate the general characteristics of patients with idiopathic portal or hepatic vein thrombosis, to compare the "idiopathic" patient group with healthy adults in terms of major steps of thrombogenesis and fibrinolytic potentials, and to reconcile the "thrombophilia theory" in etiology of idiopathic portal hypertension with idiopathic portal vein thrombosis. The advanced tests that we used to investigate possible thrombophilia included activated factor VII (FVIIa) which is the most potent initiator of thrombin formation via tissue factor and its potent controller called tissue factor pathway inhibitor (TFPI; total and free fractions), adhesion molecules, namely soluble vascular cell adhesion molecule-1 (sVCAM-1), soluble intercellular adhesion molecule-1 (sICAM-1) and soluble E-selectin molecule as markers of endothelial dysfunction and global fibrinolytic capacity (GFC) to assess the steps of fibrinolysis.

This study was designed to investigate the endothelial dysfunction, steps of fibrinogenesis and fibrinolytic capacity in a patient group named as "idiopathic" after use of current techniques to investigate thrombophilia.

MATERIALS AND METHODS

Patient and control groups

All the patients diagnosed to have chronic portal vein or hepatic thrombosis with any appropriate diagnostic technique (Doppler-ultrasound, conventional angiography, computerized tomographic angiography or magnetic resonance angiography) between January 2000 and December 2003 were evaluated for possible inclusion to the study. One splenoportal angiography of our patient group showed portal vein cavernous transformation (Figure 1). The exclusion criteria (as shown in Table 1) were applied to eliminate chronic parenchymal liver diseases including cirrhosis, mass occupying lesion around great vessels and any genetic or acquired abnormality leading to thrombophilia.

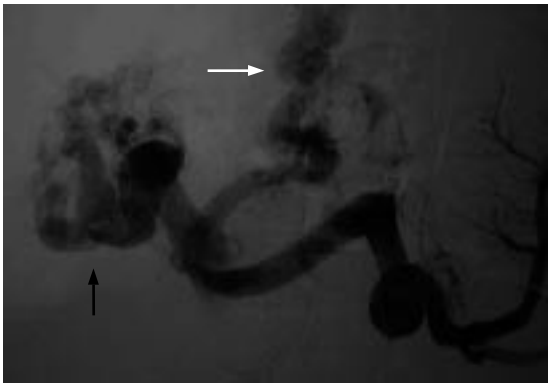

Figure 1 Splenoportal angiography showing cavernous transformation (bold arrow), eso phageal varices (white arrow) indicating severe portal hypertension.

All the patients underwent a series of evaluation comprised of viral markers of hepatitis, screening tests of metabolic liver diseases (Wilson's disease, hemochromatosis), liver biopsy, red blood cell count, full biochemical tests, reticulocyte counts and peripheral blood smear in order to rule out silent liver diseases or myeloproliferative blood diseases. Any increased hematocrit more than 50% indicated a scintigraphic total red cell mass count, whereas any abnormality in reticulocyte count or any suspicious finding in peripheral smear indicated a bone marrow aspiration. Biopsy studies were carried out to exclude silent or latent hematological diseases.

Genetic and other thrombophilic risks were evaluated by determining the levels of factor V Leiden and prothrombin mutations, proteins C and S, antithrombin, IgG and IgM classes of anti-cardiolipin and anti-phospholipid antibodies, homocysteine and fibrinogen. CD 55 and CD 59 clusters in peripheral blood were studied in all patients to rule out paroxysmal nocturnal hemoglobinuria after all hematological investigations.

In view of potential interference with the tests to be used in the study, patients with a history of uncontrolled diabetes and hypertension, severe and widespread atherosclerosis, oral contraceptive use or estrogen replacement treatment, use of anticoagulation drugs, uncontrolled or newly diagnosed malignancy elsewhere, systemic inflammatory diseases and recent (last 3 mo) major operation were excluded. Application of the exclusion criteria reduced the total number of 96 patients (portal vein involvement in 70, hepatic vein involvement in 26) to 9 patients comprised of 7 cases of portal vein thrombosis (PVT) including cavernous transformation of the portal vein, and 2 cases of hepatic vein thrombosis (HVT). After all these investigations, bone marrow biopsies were taken from patients with

Table 2 General characteristics of the patients (results were given as minimum, maximum and median)

Test	Result
Age	28-74 (48)
Female/Male	6/3
PVT (*n*)	7
HVT (*n*)	2
Hemoglobin (gr/L)	9.5-16.6 (13.5)
Platelet (count/mL)	62.000-358.000 (168.000)
ALT (U/L)	13-38 (23)
AST (U/L)	18-38 (27)
ALP (U/L)	87-355 (200)
GGT (U/L)	16-123 (34)
Total Bilirubin (mg/L)	0.24-2.08 (0.65)
INR	1.06-1.47 (1.14)
Protein C (%)	55-137 (87)
Protein S (%)	58-113 (85)
AT (%)	75-109 (90)
Homocysteine (umol/L)	9-19 (15)
Fibrinogen (mg/L)	181-420 (271)

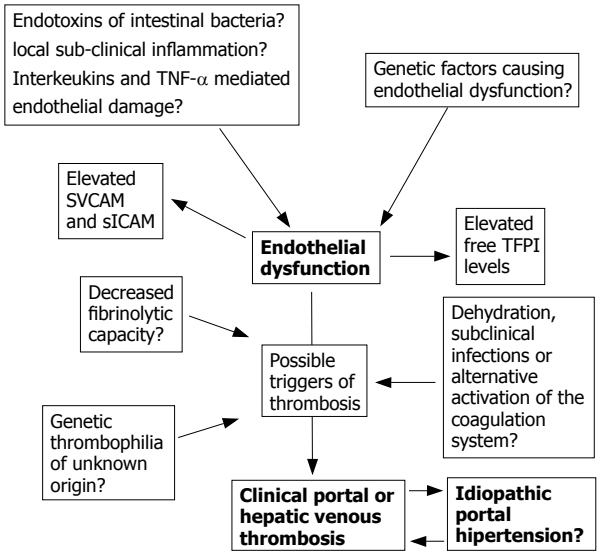

Figure 2 Results from our study and possible associations with other processes.

a diagnosis of idiopathic thrombosis to rule out any silent hematological diseases.

In excluded patients, the most common thrombophilic conditions were deficiency in proteins C and S and antithrombin III (28 patients, 29.1%), followed by myeloproliferative disorders (10 patients, 10.4%), factor V Leiden mutations (10 patients, 10.4%), prothrombin mutations (6 patients, 6.25%). The other conditions related with possible thrombophilia included cyst hydatid disease of the liver, Behcet's disease, metastasis of cancer, mastocytosis invasion of the liver, tuberculosis of the choleduct and abdominal surgery.

The control group comprised of 14 healthy adults (6 males, 8 females) with no history of cigarette smoking, hypertension, atherosclerotic vascular disease, systemic inflammatory condition and no use of medication (oral contraceptives and estrogen replacement treatment). An informed consent was obtained from each patient and control subject. The study protocol recieved approval of Ethical Committee of Hacettepe University.

Blood samples

Blood samples from patient and control groups were drawn from antecubital veins with a 20 G needle at 08:00 am after an overnight fasting. All samples were collected in 0.129 mol/L trisodium citrate tubes and centrifuged at 3000 r/min for 10 min, while plasma samples were stored at -30 ℃ for studies.

Evaluation of endothelial function

The endothelial function was tested by measuring serum concentrations of adhesion molecules, namely sVCAM-1, sICAM-1 and soluble E-selectin. The concentrations of these adhesion molecules were evaluated quantitatively by sandwich enzyme immunoassay (Parameter®, R&D systems, UK).

Evaluation of inital step of thrombogenesis and its control

The most potent initial step during formation of thrombin, tissue factor-(released from damaged endothelium) related activation of factor VII was evaluated by quantitative measurement of activated factor VII (Staclot® VIIa-TF, Diagnostica Stago, Asnieres, France). The major controlling step of this pathway, namely the TFPI (both total and free fractions) level, was tested quantitatively by one-step ELISA (Diagnostica Stago, Asnieres, France).

Fibrinolytic potential

Fibrinolytic capacity of each sample was evaluated by GFC, a semi-quantitative method designed to detect low potentials of given samples. This technique uses the measurement of generated D-dimer in the given sample when a standard amount of fibrin is added into it. The generated D-dimer was measured using macrolatex agglutination (Diagnostica Stago, Asnieres, France). Therefore, the results from this test were semi-quantitatively expressed as "normal GFC", "moderately low GFC" and "severely low GFC".

Statistical analysis

All the data were presented as minimum, maximum and median unless otherwise specified. Statistical tests used for evaluation of significant differences were Mann-Whitney U test and Fisher's exact test.

RESULTS

The general characteristics of the patients are shown in Table 2 and Figure 2. Four patients had portal vein cavernous transformation and 3 patients had pure portal vein thrombosis in the portal vein group, whereas HVT group had only large hepatic vein involvement. As the blood cell count was taken into account, patient group showed a general tendency towards thrombocytopenia due to presence of increased portal venous pressure leading to mild degree of hypersplenism. Liver enzymes were all normal or very slightly elevated owing to the strict exclusion criteria. One important fact was that the international normalized ratio (INR) of the patient group was normal, thus not affecting the hematological tests

Table 3 Comparison of patient and control groups by advanced tests (results were given as minimum, maximum and median)

Test (normal limits)	Patient group (*n*)	Control group (*n*)	Significance
FVIIa (25.5-96.9 mU/mL)	27-1232 (96)	39-325 (129)	NS[1]
Total TFPI (81.2±30.4 ng/mL)	22-114 (87.2)	43-112 (79)	NS
Free TFPI (10.0±4.8 ng/mL)	5-41 (19)	9-20 (13)	[a]$P<0.05$
sVCAM (395-714 ng/mL)	489-1890 (915)	410-890 (517)	[c]$P<0.05$
sICAM (115-306 ng/mL)	148-400 (252)	24-260 (164)	[b]$P<0.05$
E-Selectin (29.1-63.4 ng/mL)	14-68 (20)	11-64 (29)	NS

[1]NS: Not significant; [a]$P<0.05$, [c]$P<0.05$, [b]$P<0.05$ *vs* control group.

Table 4 Results and comparisons of GFC (global fibrinolytic capacity)

Group	Severely low GFC	Moderately low GFC	Normal GFC
Patient group	0	4	5
Control group	0	0	14
	Fisher's exact test		$P<0.05$

during the study. Proteins C and S showed that the values near the lower normal limits and even mildly low values were due to the decreased synthesis from liver as a result of abnormal blood flow dynamics of liver rather than a congenital deficiency.

The results of hematological evaluation of patient and control groups with advanced tests are shown in Table 3. The comparisons of FVIIa in both groups were similar, so were total TFPI levels. The results obtained from comparisons of free TFPI and adhesion molecules sVCAM-1 and sICAM-1 were striking. Levels of free TFPI, sVCAM-1 and sICAM-1 were significantly higher in patient group than in control group. sE-selectin levels did not differ between the two groups.

The results obtained from patient and control groups by GFC are shown in Table 4. The patient group showed a significant tendency towards moderately low fibrinolysis whereas fibriolysis was normal in control group.

DISCUSSION

The overall results and our proposals are shown in Figure 2. Firstly, the significant differences in vascular adhesion molecules indicated that there was an overall activation of endothelium comparable to normal subjects, suggesting that there is an unknown abnormality in endothelial functions. Adhesion molecules are synthesized from endothelium in response to bacterial endotoxins, interleukins and inflammatory mediators like tumor necrosis factor-α[9-11], indicating that this endothelial dysfunction is a pure result of endothelial dysfunction of splenoportal or hepatic venous systems. Since other external risk factors affecting endothelial function and our tests such as diabetes mellitus, hypertension, cigarette smoking or atherosclerosis were excluded in both patient and control groups, this finding may be of importance.

The comparable difference in free TFPI could be explained also by this endothelial dysfunction. TFPI is a protein molecule formed of three Kunitz particles synthesized and secreted from endothelium. Its main function is to inhibit the activated FVII-tissue factor complex before coagulation cascade becomes uncontrollable[12,13]. Severe deficiency in TFPI may lead to overt thrombosis in veins[14]. After synthesized from endothelium, the active component of this protein circulates in free form accounting for approximately 2.5 % of total TFPI pool and it is this free form that exhibits the main TFPI activity whereas the low density lipoprotein-bound TFPI shows no activity at all[12]. Our findings (a significant difference in free TFPI levels between two groups but no difference in total TFPI levels) were parallel to the findings of endothelial dysfunction confirmed by adhesion molecules, since the increased free TFPI levels indicated a stimulated synthesis from malfunctioning endothelium.

The results obtained from GFC studies indicated that in patient group there was a significant tendency towards moderately low GFC. This in a way is consistent with the recent thrombogenesis theory which accepts that thrombogenesis is not attributable to only one factor. In fact, thrombogenesis requires more than one risk factor for potent initiation of the pathway. From this point, we propose that either low GFC or endothelial dysfunction forms an appropriate milieu for development of this kind of "idiopathic" thrombosis.

The study presented can be questioned in some ways. Firstly, the number of patients studied was small to be conclusive about the hypothesis proposed before. But as discussed before, this patient population was a very rarely confronted group and similar studies are few. Secondly, the patient group had two different vessel involvements and could not be studied as they were homogenous. But if the liver functions and the patterns of thrombus formation were examined in detail, these two different vessel involvements would be similar in terms of idiopathic thrombus formation in milieu of normal liver function. Thirdly, the laboratory tests applied were not informative enough for our assumptions. Formation of thrombus can be evaluated in many ways including quantification of thrombin-antithrombin complexes or prothrombin fragments 1+2 or many other sophisticated tests. However, our hypothesis is not to test the presence of abnormal activation of prothrombin, but to find any possible factors triggering coagulation cascade, resulting in abnormal clot formation. Therefore we tested the most potent initiator of coagulation cascade. It can be argued to study the endothelial functions with other tests like plasminogen activator inhibitor-1 (PAI-1), von-Willebrand factor or thrombomodulin.

To our knowledge, there is no evidence that these tests with adhesion molecules can show endothelial dysfunction. These tests have no superiority over each other for estimation of the degree of endothelial dysfunction.

There is an ongoing debate over the presence of portal vein thrombosis in case of idiopathic portal hypertension (IPH). The pro-thrombogenesis theoreticians believe that there is a tendency towards thrombosis which manifests itself in any life time of a patient with IPH[15]. The anti-thrombogenesis theoreticians believe that although thrombophilia and repeated micro-thrombi over a long time may be one of the possible etiological factors, the presence of portal thrombosis is a finding against diagnosis of IPH[16]. We believe that thrombogenesis and silent or unknown risk factors may contribute to the development of IPH[17].

In conclusion, major idiopathic hepatic thrombosis (hepatic or portal veins) is not as rare as it was thought and our study may be an important clue to further studies in respect of endothelial dysfunction and other possible accompanying coagulation control defects like deficient fibrinolysis.

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