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Plasma Thrombin Activatable Fibrinolysis Inhibitor Levels in Behçet's Disease

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Aim: The precise pathogenetic mechanisms causing thrombotic complications in Behçet's disease (BD) are still not known. To explain the pathogenesis with coagulation induction or a defective fibrinolysis superimposed on endothelial dysfunction, various hemostatic parameters were studied. Thrombin activatable fibrinolysis inhibitor (TAFI), downregulating plasmin generation and fibrinolysis, is a novel risk factor for thrombotic disorders. We studied plasma TAFI levels in BD in comparison with healthy controls.

Materials and Methods: Twenty-three patients with BD (mean age: 38.3 ± 10.83, M/F: 5/18) diagnosed according to the criteria of the International Study Group and 20 healthy volunteers (mean age: 38.05 ± 6.29, M/F: 9/11) were enrolled in this study. Patients with liver or renal disease, diabetes mellitus, coronary artery disease, hemophilia, antiphospholipid antibody positivity or using oral contraceptive drugs were excluded from the study. Plasma TAFI levels were determined by using an ELISA test.

Results: The mean TAFI antigen levels were 8.40 ± 1.81 µg/ml in BD patients and 7.30 ± 0.64 µg/ml in healthy volunteers. A statistically significant difference was found between TAFI antigen levels of these two groups (P = 0.01).

Conclusions: TAFI antigen levels were found to be increased in BD, regardless of thrombotic events. To clarify the exact role of TAFI in thrombotic complications of the disease, future studies including more patients with and without thrombosis are needed.

Key Words: Behçet's disease, TAFI, fibrinolysis, coagulation

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Behçet Hastalığında Plazma Trombin ile Aktive Edilebilen Fibrinoliz İnhibitör (TAFİ) Seviyesi

Amaç: Behçet hastalığında görülen trombotik komplikasyonların patogenetik mekanizmaları henüz net değildir. Patogenezi endotel disfonksiyonu, koagülasyon ve fibrinolitik sistemlerdeki bozukluklar ile açıklayabilmek için pek çok hemostatik parametre çalışılmıştır. Trombin ile aktive edilebilen fibrinoliz inhibitörü (TAFİ) plazmin oluşumunu ve fibrinolizi inhibe eden, trombotik bozukluklarda etkisi son yıllarda keşfedilmiş bir risk faktörüdür. Bu çalışmada Behçet hastalığında ve kontrol grubunda TAFİ plazma seviyeleri araştırılmıştır.

Yöntem ve Gereç: Erkek kadın oranı 5/18, ortalama yaşları 38.3 ± 10.83 olan 23 Behçet hastası (Uluslararası çalışma kriterlerine göre tanı almış) ile, erkek kadın oranı 9/11 ortalama yaşları 38.05 ± 6.29 olan sağlıklı kontroller çalışmaya alındı. Karaciğer, böbrek hastalığı, diyabeti, koroner hastalığı, antifosfolipid antikor pozitifliği olan, oral kontraseptif kullanan hastalar çalışma dışı bırakıldı. Plazma TAFİ seviyesi ELİZA testi kullanılarak ölçüldü.

Bulgular: TAFİ antijen plazma seviyeleri Behçet hastalarında 8.40 ± 1.81 µg/ml, sağlıklı gönüllülerde 7.30 ± 0.64 µg/ml olarak bulundu. İki grup arasında TAFİ seviyeleri açısından istatistiksel anlamlı fark mevcuttu (P = 0.01).

Sonuç: Trombotik olay varlığından bağımsız olarak Behçet hastalığında TAFİ antijen seviyesi yüksekti. Ancak hastalığın etiolojisinde TAFİ'nin yerinin daha net anlaşılabilmesi için trombotik komplikasyonu olan grubun da bulunduğu, daha fazla sayıda olgu içeren çalışmalara gerek vardır.

Anahtar Sözcükler: Behçet Hastalığı, TAFİ, koagülasyon, fibrinoliz

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Introduction

Behçet's disease (BD), firstly described by Hulusi Behçet in 1937, is a chronic multisystem disorder of unknown etiology. The disease is characterized by recurrent aphthous oro-genital ulcers, ocular inflammations and skin lesions. The main histopathology found in BD is vasculitis involving all types and sizes of blood vessels (1). Systemic vascular lesions include arterial aneurysms, small vessel vasculitis, and arterial and venous thrombosis (2). Thrombotic complications have been reported in approximately 12-40% of patients with this disease. The precise pathogenetic mechanisms causing thrombotic complications are still not known (1). Endothelial dysfunction due to vascular inflammation is considered to be an important factor of thrombosis. Endothelial injury itself cannot be the only factor for development of thrombosis, because other vasculitic syndromes do not increase the risk of thrombosis (3). In order to explain the pathogenesis with coagulation induction or a defective fibrinolysis superimposed on endothelial dysfunction, various hemostatic parameters were studied. Protein C, protein S and antithrombin (AT) deficiencies, presence of antiphospholipid antibodies and factor V Leiden and prothrombin 20210A mutations, and high homocysteine level have been considered to be related with hypercoagulable state in BD (4-9). Low values of plasminogen activator activity, impaired fibrinolytic kinetics or relative hypofibrinolytic state were also suggested in the prothrombotic state of BD (10-12).

Thrombin activatable fibrinolysis inhibitor (TAFI) is a novel risk factor for thrombotic disorders. It is a plasma procarboxypeptidase B-like proenzyme (zymogen) synthesized in the liver. TAFI is converted to an active carboxypeptidase (TAFI a) enzyme to downregulate plasmin generation and fibrinolysis (13). The role of TAFI in thrombotic events has been studied. Increased plasma TAFI antigen levels were found to be related to thrombotic tendency in the literature (14-18).

Considering the role of TAFI in downregulation of fibrinolysis, we aimed to investigate the relationship between plasma TAFI levels and BD.

Materials and Methods

Twenty-three patients with BD (mean age: 38.3 ± 10.83 , M/F: 5/18) diagnosed according to the Criteria of the International Study Group and 20 healthy volunteers (mean age: 38.05 ± 6.29 , M/F: 9/11) were enrolled in this study. Informed consent was obtained from all individuals. Patients with liver or renal disease, diabetes mellitus, coronary artery disease, hemophilia, antiphospholipid antibody positivity or using oral contraceptive drugs were excluded from the study.

The patients were evaluated with respect to current clinical activity. They were included in the active group if they had at least two of the following clinical findings: mouth ulcers, genital ulceration, active uveitis, recent arthritis, thrombophlebitis and large vessel involvement.

Blood Collection

Blood samples were obtained from antecubital vein into citrated tubes (trisodium-citrate 0.129 mol/L, whole blood ratio 1:9) and centrifuged at 1500 xg for 15 min. All plasma samples were then divided into aliquots and frozen at -80°C until test performance. At the same time blood samples were obtained, whole blood count, liver function tests, renal function tests, erythrocyte sedimentation rate (ESR), and serum C-reactive protein (CRP) were also studied in each subject.

TAFI Antigen Assay

TAFI antigen was determined using an ELISA kit from Affinity Biologicals Inc, Ontario, Canada, which consists of affinity purified sheep anti-TAFI IgG capture antibody and pre-diluted HRP-conjugated affinity purified sheep anti-TAFI detecting antibody. This test measures total TAFI antigen, including pro-TAFI (procarboxypeptidase U), TAFI (carboxypeptidase U), and TAFIi (inactivated carboxypeptidase U). All necessary buffers and reagents were prepared according to manufacturer's instructions and all samples were studied as soon as they were thawed at room temperature. Test plasma samples were diluted 1/200. All results were given as $\mu\text{g/ml}$.

Statistics:

All data were collected at 'Excel XP for Windows' program and statistical analysis was performed using the SPSS 10.0 statistical package. Mann-Whitney U test was used to compare the two groups, and all p-values were two-sided. A value of $P < 0.05$ was considered as significant.

Results

Demographic data and main clinical features of the patients and healthy volunteers are given in Table 1 and Table 2. The two groups were sex-matched ($P = 0.104$) and there was also no significant difference between the two groups according to age. The characteristic features of the disease in BD patients are also given in Table 1 (at the time of diagnosis and at the time of the study). Thirteen (56.5%) patients were clinically active and none of the patients had deep vein thrombosis (DVT) at the time of the study. Only two (8.7%) patients who were clinically inactive had a history of DVT.

The mean TAFI antigen levels were 8.40 ± 1.81 $\mu\text{g/ml}$ in BD patients and 7.30 ± 0.64 $\mu\text{g/ml}$ in healthy volunteers. A statistically significant difference was found between TAFI antigen levels of the two groups ($P = 0.01$) (Figure 1).

The mean TAFI antigen levels were also studied according to activity of the disease. Although it seems that TAFI antigen levels were higher in patients with active disease compared with the inactive group (9.04 ± 1.61 $\mu\text{g/ml}$ vs 7.56 ± 1.78 $\mu\text{g/ml}$) (Figure 2), there was no significant difference between the two disease groups ($P = 0.088$).

Discussion

TAFI plays an important role in hemostatic balance between coagulation and fibrinolysis and affects clot stability (18,19). It is activated by many factors including thrombin, plasmin and trypsin. After activation, it is converted to an active carboxypeptidase (TAFIa) enzyme. TAFIa removes the carboxy-terminal lysyl and arginyl residues of partially degraded fibrin, which serve as plasminogen binding sites, and plasminogen activation is inhibited. Hence, TAFIa is a potent inhibitor of tissue plasminogen activator-induced fibrinolysis (19). The role of TAFI has been studied in many diseases to explain disease pathology (14-16,20). Due to thrombotic tendency in BD, many factors that may underlie this pathology were investigated (4-11). Deficiencies in protein C, protein S, and antithrombin or the presence of antiphospholipid antibodies and factor V Leiden and prothrombin 20210A mutations have been considered. While some studies found an association between these abnormalities and thrombosis in BD (4-10), others did not (21,22). In light of all these literatures, questions remain regarding the cause of the majority of the thrombosis in this disease, so one may speculate that TAFI as a novel candidate may also be involved in the pathology of thrombotic events in BD.

Table 1. Activity parameters in patients with Behçet's disease.

	BEHÇET'S PATIENTS (n: 23)
Female/Male	5/18
Oral ulcer	17/23(73.9%)
Genital ulcer	5/23 (21.7%)
Skin lesions	10/23 (43.5%)
Arthritis	3/23 (13.0%)
Eye lesion	2/23 (8.7%)
Pathergy positivity	3/9 (14 cases missing)
ENLL (erythema nodosum-like lesion)	2/23 (8.7%)
Superficial thrombophlebitis	1/23 (4.3%)
Deep vein thrombosis	0/23
Clinical activity	13/23 (56.5%)

Table 2. Characteristics of patients and healthy volunteers.

	Behçet's Patients n:23	Control Group n:20
Age	38.3 ± 10.83	38.05 ± 6.29
Female/Male	5/18	9/11
Duration of disease (month)	8.62 ± 9.65	
Hb (g/dl)	14.33 ± 1.56	
WBC ($\times 10^9/L$)	7.62 ± 1.85	
Platelet ($\times 10^9/L$)	289.36 ± 76.39	
Erythrocyte sedimentation rate (mm/hr)	18.5 ± 20.13	
C-reactive protein (CRP)	8.69 ± 15.71	

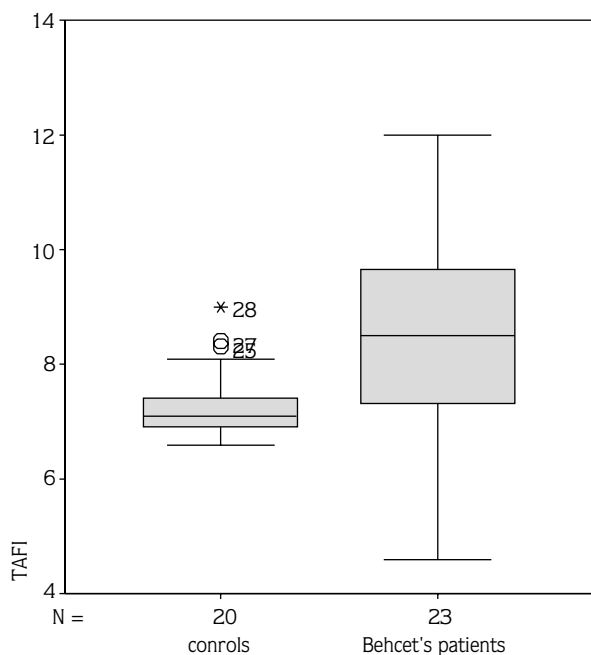


Figure 1. Thrombin activatable fibrinolysis inhibitor antigen levels in patients and volunteers.

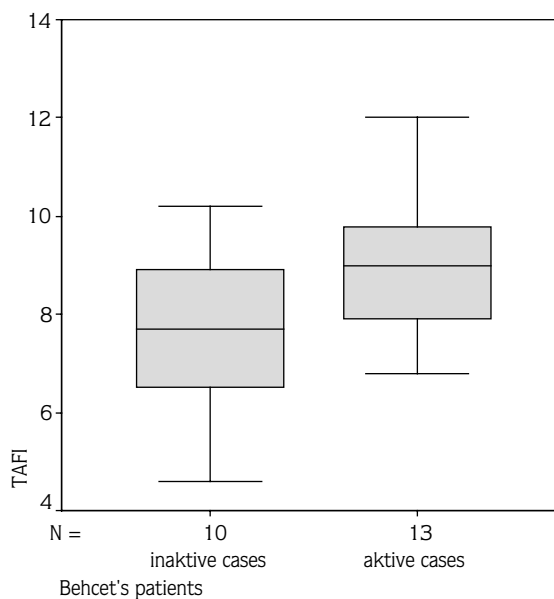


Figure 2. Thrombin activatable fibrinolysis inhibitor antigen levels in active and inactive Behçet's patients.

The first study about TAFI levels in BD was made by Donmez et al. (23). The main aim of their study was to assess plasma TAFI levels in this disease, compared with healthy controls, and BD subgroups with and without thrombosis. They evaluated TAFI variations in 105 BD (55 with thrombosis, 50 without thrombosis, 29 clinically active, 76 clinically inactive) patients and 53 healthy volunteers. They found that plasma TAFI levels in all subgroups of BD were significantly higher than in healthy controls; however, there was no positive correlation of TAFI antigen levels with thrombosis or with clinical activity.

In our study, we enrolled 23 patients with BD and 20 healthy volunteers. 56.5% of patients were clinically active at the time of clinical assessment. They had no thrombotic events at the time of the study. Since during thrombotic events, coagulation and the fibrinolytic system would be active and affect the interpretations of the results, we did not include the patients with thrombosis in this study. But 8.7% of patients (2 patients) had DVT history. The number of patients in this group was not enough to make a comment on the relationship between TAFI levels and BD subgroups (with/without thrombosis). There were no significant age or

sex differences between the two groups. TAFI antigen levels in patients with BD were significantly higher than in healthy volunteers ($P = 0.01$).

The mean TAFI antigen levels were also studied according to disease activity because TAFI is accepted as an acute phase reactant. TAFI antigen levels were found higher in patients with active disease compared with the inactive group, but the difference was not significant ($P = 0.088$). This may be due to the small numbers of the study subgroups.

In this study, we did not evaluate other fibrinolytic parameters in BD. We only investigated whether or not TAFI was involved in thrombotic tendency in BD. High TAFI levels, which reached a level of significance, may support this idea. In the previous study, which was the first case-control study to show higher TAFI levels in the disease, subgroups with and without thrombosis were studied and no differences between them were determined. In our study, there were no such subgroups for comparison. But it is known that BD itself has thrombotic complications. The patients may manifest such complications in the future. They are still followed at regular intervals. Sole measurement of plasma TAFI

antigen levels but not functional TAFI activity is also a limitation of our study.

In conclusion, many factors have a role in thrombotic tendency in BD. TAFI antigen levels were found to be increased in BD, regardless of thrombotic events, in this

study. To clarify the exact role of TAFI in thrombotic complications of the disease, future studies including more patients with and without thrombosis are needed, and functional TAFI activity and TAFI gene polymorphisms need to be evaluated as well.

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