Plasma Endothelin-1 Levels in Patients with Behçet's Disease: Association with Disease Activity

BEHÇET HASTALARINDA PLAZMA ENDOTELİN-1 DÜZEYLERİ: HASTALIK AKTİVİTESİ İLE İLİŞKİSİ

Aylin TÜREL ERMERTCAN, MD,[a] Serap ÖZTÜRKCAN, MD,[a] Mustafa Turhan ŞAHİN, MD,[a] Ece ONUR, MD,[b] Bekir Sami UYANIK, MD[b]

Departments of [a]Dermatology, [b]Biochemistry, Medical School of Celal Bayar University, MANİSA

Abstract

Objective: Behçet's disease is a multisystemic vasculitic disease associated with a vascular pathologic process. Endothelial dysfunction is thought to be responsible for these events. Endothelin-1, the most potent vasoconstructor peptide, is one of the substance derived from endothelium. In this study it was aimed to detect plasma endothelin-1 levels in patients with Behçet's disease and its relation with disease activity and systemic involvements.

Material and Methods: The study group included 46 patients with Behçet's disease (21 active, 25 inactive) and 17 age- and sex-matched healthy control subjects. Plasma endothelin-1 levels were measured by using Endothelin-1 ELISA kit in all patients and healthy controls. The results of active patients were compared with inactive patients and control subjects.

Results: Mean plasma endothelin-1 levels in patients with active Behçet's disease (0.55±0.062 pg/ml) were significantly higher than those in inactive patients (0.42±0.092 pg/ml) and healthy controls (0.41±0.036 pg/ml) ($p<0.05$). The difference in plasma endothelin-1 levels between patients with inactive Behçet's disease and control subjects was not significant ($p>0.05$). The elevation of endothelin-1 levels did not correlate with the disease duration, age and gender. Plasma endothelin-1 levels did not correlate with ocular, articular, dermatologic, or the other systemic involvements ($p>0.05$). Mean plasma endothelin-1 levels in patients with vasculitis were found to be higher compared with nonvasculitic patients, although the difference was statistically insignificant (0.54±0.13 pg/ml, 0.48±0.098 pg/ml).

Conclusion: Increased endothelin-1 production may play an important role in the pathogenesis of Behçet's disease, especially in the active stage of the disease.

Key Words: Behçet's disease; endothelin-1

Özet

Amaç: Behçet hastalığı vasküler patolojik proçesle ilişkili multisistemik vaskülit ile seyreden bir hastalıktır. Bu olaylardan endotelyal disfonksiyon sorumlu tutulmaktadır. Endotelin-1, en güçlü vazokonstruksiyon yapan peptid olup, endotelden salınan maddelerden biridir. Bu çalışmada Behçet hastalarında plazma endotelin-1 düzeylerinin tespit edilmesi ve bunun hastalık aktivitesi ve sistemik tutulumlar ile ilişkisinin saptanması amaçlanmıştır.

Gereç ve Yöntemler: 46 Behçet hastası (21'i aktif, 25'i inaktif) ve bunlarla yaş ve cinsiyet yönünden uyumlu 17 kişiden oluşan sağlıklı kontrol grubu çalışmaya dahil edildi. Tüm hastalarda ve sağlıklı kontrollerde plazma endotelin-1 düzeyleri Endothelin-1 ELISA kit ile ölçüldü. Aktif Behçet hastalarının sonuçları inaktif hasta grubu ve kontrol grubu ile karşılaştırıldı.

Bulgular: Plazma endotelin-1 düzeylerinin ortalaması, aktif Behçet hastalarında (0.55±0.062 pg/ml) inaktif hastalara (0.42±0.092 pg/ml) ve sağlıklı kontrol grubuna göre (0.41±0.036 pg/ml) anlamlı olarak yüksek bulundu ($p<0.05$). İnaktif Behçet hastaları ve kontrol grubunun ortalama plazma endotelin-1 düzeyleri arasındaki fark anlamlı değildi ($p>0.05$). Endotelin-1 düzeylerindeki artış hastalık süresi, yaş ve cinsiyet ile ilişkili bulunmadı. Plazma endotelin-1 düzeyleri göz, eklem, dermatolojik ve diğer sistemik tutulumlarla korele değildi ($p>0.05$). Vasküliti olan Behçet hastalarındaki ortalama plazma endotelin-1 düzeyleri vasküliti olmayan hastalara kıyasla, aradaki fark istatistiksel olarak anlamlı olmamasına rağmen, daha yüksek bulundu (0.54±0.13 pg/ml ve 0.48±0.098 pg/ml).

Sonuç: Artmış endotelin-1 salınımı Behçet hastalığı patogenezinde, özellikle de hastalığın aktif döneminde önemli rol oynayabilmektedir.

Anahtar Kelimeler: Behçet hastalığı, endotelin-1

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Yazışma Adresi/*Correspondence:* Aylin TÜREL ERMERTCAN
Celal Bayar Üniversitesi Tıp Fakültesi,
Dermatoloji ABD, MANİSA
draylinturel@hotmail.com

Behçet's disease is a chronic multisystemic disease with ulcerations of the oral and genital mucosa and iridocyclitis as its main features.[1] The etiology of disease is unknown; however, an immunogenic basis is suspected in view of the increased prevalence of human leucocyte antigen (HLA)-B5 and B51 anti-

gens that have been associated with the disease. It is characterized as a systemic vasculitic disorder associated with a vascular pathologic process. Affected endothelial cells in particular are thought to be responsible for these events.[2]

Endothelin-1 (ET-1), the most potent vasoconstructor peptide, is one of the substances derived from endothelium.[3] ET-1 may play an important pathogenetic role in the development or progression of vasculitis in Behçet's disease.[4] There are few reports indicating increased plasma ET-1 levels in patients with Behçet's disease.[3-7] In this study, we aimed to investigate the role of ET-1 in Behçet's disease and its correlation with the disease activity.

Material and Methods

A total of 46 patients (21 active and 25 inactive) with Behçet's disease attended to our outpatient clinic and 17 age- and sex-matched healthy control subjects were included to the study. All Behçet's disease patients fulfilled the criteria of the International Study Group for the disease.

The patients and control subjects with hepatic and renal diseases, hypertension, diabetes mellitus, thyroid disease, and who were on systemic therapy were excluded from the study. Clinical and laboratory findings were used to classify the patients as active or inactive. In clinical evaluation, the presence of at least 3 of the 5 major findings (oral aphthae, genital ulcers, uveitis, skin lesions and positive pathergy test) were considered to be in the active stage of the disease. In laboratory investigations, erythrocyte sedimentation rate (ESR), complete blood count (CBC), biochemical tests and C reactive protein (CRP) were performed.[8,9] Written informed consent was obtained from all participants in both groups.

Blood samples were drawn using a 25 gauge needle from a peripheral vein, avoiding haemolysis, into plain tubes in the morning after 30 minutes of supine rest. None of the subjects had received systemic or topical therapy for at least 2 weeks prior to blood collection. Blood samples were centrifugated at 800 g for 10 minutes at 4 ºC and then serum was stored at -70 ºC until using. At all serum samples, plasma endothelin-1 levels were measured by using an Endothelin-1 ELISA kit from BioSource company (BioSource Europe SA. Nivelles, Belgium, Catalog Number: KAPA0900-020). Analytic sensitivity was 0.14 pg/mL and intra-assay coefficient of variation values were 2.5% and 2.1% for 5.67 and 56.19 pg/mL respectively.

Statistical analysis was performed with Statistical Package for the Social Sciences for Windows (SPSS, version 11.0). Results were analysed statistically by using Kruskal-Wallis, Mann-Whitney U, Chi-square and Spearman correlation tests. Correlation between ET-1 levels and CRP levels was analysed by Pearson correlation coefficient and test.

Results

The study group included 46 patients with Behçet's disease (21 active, 25 inactive) and 17 healthy controls. The mean ages of active, inactive patients and control subjects were 38.7±12.8; 39.5±10.4 and 41.4±12.0 years, respectively. While 47.6% of active patients were male, it was 56.0% for inactive patients. 76.5% of control subjects were male. Active, inactive patients with Behçet's disease and control subjects were similar according to age and sex. Mean duration of the disease was 10.0±9.4 years for active patients. This was 9.5±8.1 years for inactive patients. There was no significant difference between active and inactive patients according to duration of the disease (Table 1).

Oral aphthous lesions were determined in all patients. 45 (97.8%) patients had genital ulceration. Erythema nodosum was seen in 18 (39.1%) patients. 28 (60.9%) patients had acneiform lesions and 4 (8.7%) had vasculitis. 29 of 46 patients (63.0%) had ocular involvement (anterior or posterior uveitis, panuveitis, iridocyclitis and synechiae). Articular symptoms were present in 29 (63.0%) patients. 6 (13.0%) patients had neurologic symptoms and 5 (10.9%) had gastrointestinal symptoms. Psychiatric disorders were found in 5 (10.9%) patients. Patergy test was positive in 34 cases (73.9%) (Table 2).

Table 1. Age, gender and disease duration of the patients with Behçet's disease (BD) and healthy controls.

	Active BD		Inactive BD		Healthy controls		P
Age (mean±SD)	**38.7±12.8**		**39.5±10.4**		**41.4±7.9**		**0.571***
Disease duration (year) (mean±SD)	**10.0±9.4**		**9.5±8.1**				**0.903****
Sex	**N**	**%**	**N**	**%**	**N**	**%**	
Male	10	47.6	14	56.0	13	76.5	0.187***
Female	11	52.4	11	44.0	4	23.5	

*Kruskal-Wallis analysis, **Mann-Whitney U test, ***Chi-square test

Complete blood count and biochemical tests were found to be normal in all patients and healthy controls. Sedimentation rates were high in 90.5% of active patients and in 8.0% of inactive patients. 5.9% of control subjects had high sedimentation rates. CRP was found to be high in 61.9% of active patients. It was high in 8.0% of inactive patients. All control subjects had normal CRP values (Table 3).

Mean plasma endothelin-1 levels were 0.55±0.062 pg/ml in active patients with Behçet's disease and the differences were significant when compared with inactive patients (0.42±0.092 pg/ml; p=0.000) and control subjects (0.41±0.036 pg/ml; p=0.000). No difference in plasma ET-1 level was observed between patients with inactive Behçet's disease and controls (p=0.529) (Table 4).

The elevation of ET-1 did not correlate with the disease duration, age and gender (Age-ET-1 levels; spearman r (spearman's coefficient)=0.06; p=0.6; spearman correlation test) (Disease duration-ET-1 levels; spearman r=0.04; p=0.7; spearman correlation test).

ET-1 levels statistically correlated with CRP levels (correlation coefficients were 0.82 (p=0.000) for all subjects, 0.67 (p=0.001) for active patients, 0.72 (p=0.000) for inactive patients and 0.62 (p=0.008) for control subjects).

In addition to that, ET-1 levels did not correlate with ocular, articular, dermatologic, or the other systemic involvements (Mann-Whitney U test, p>0.05). Mean ET-1 levels in the patients with vasculitis were found to be higher compared with nonvasculitic patients, although the difference was statistically insignificant (0.54±0.13 pg/ml, 0.48±0.098 pg/ml).

Table 2. Clinical findings of the patients with Behçet's disease (BD).

	Patients with BD	
Clin. findings	**N**	**%**
Oral aphthae	46	100
Genital ulceration	45	97.8
Erythema nodosum	18	39.1
Acneiform lesions	28	60.9
Vasculitis	4	8.7
Positive Pathergy test	34	73.9
Ocular manifestations	29	63.0
Articular symptoms	29	63.0
Neurological symptoms	6	13.0
Gastrointestinal symptoms	5	10.9
Psychiatric disorders	5	10.9

Discussion

Behçet's disease is a complex multisystem disease characterized by oral aphthae and by at least two of the following symptoms: recurrent genital ulcers, eye lesions, skin lesions, and positive pathergy test.[10] The Turkish dermatologist Hulusi Behçet (MD) first described Behçet's disease in 1937.[11] The prevalence is higher in Turkey, the Middle East, and Japan than in other countries and the disease is less common in northern Europe and the United States.[8] The etiology of Behçet's syndrome is uncertain.[12] Infectious, immunologic, and genetic causes have been postulated, but the evidence is still inconclusive for any of these.[13] The various HLA associations are striking: Muco-

Table 3. Laboratory findings.

	Active BD		Inactive BD		Healthy controls		
	N	%	N	%	N	%	p*
CBC							
Normal	21	100	25	100	17	100	1.0
Biochemistry							
Normal	21	100	25	100	17	100	1.0
ESR							
Normal	2	9.5	23	92.0	16	94.1	0.000
High	19	90.5	2	8.0	1	5.9	
CRP							
Normal	8	38.1	23	92.0	17	100	0.000
High	13	61.9	2	8.0	0	0	

CBC, Complete blood count
ESR, erythrocyte sedimentation rate
CRP, C reactive protein;
* Chi-square test

Table 4. Mean Endothelin-1 (ET-1) levels.

	Active BD	Inactive BD	Healthy controls
ET-1 (pg/ml) (mean±SD)	0.55±0.062†‡	0.42±0.092*	0.41±0.036

† Significantly different from the inactive stage by the Kruskal-Wallis analysis, p:0.000
‡ Significantly different from control subjects by the Mann-Whitney U test, p:0.000
* Insignificant from control subjects by the Mann-Whitney U test, p:0.529

cutaneous Behçet's syndrome is associated with HLA-B12, arthritic with HLA-B27, and ocular with HLA-B5, -DR-7.[1] Early and ongoing investigations have focused on cellular immune abnormalities due to primarily mononuclear cell nature of the infiltrate in developed aphthae and other lesions. Assessment of the early mucocutaneous lesions and of induced reveals a neutrophilic vascular reaction or leucocytoclastic vasculitis.[10]

The most prominent feature of Behçet's syndrome is systemic, dermal and ocular vasculitis with endothelial cell dysfunction.[9] The main factor responsible for the increased frequency of thrombosis is thought to be endothelial disfunction in Behçet's disease. Vascular endothelium regulates vascular tone by the roles of both vasoconstructor and vasodilator molecules. In subjects with endothelial dysfunction, vasodilator responses are lost, exacerbating the vasoconstructor response. ET-1 is a potent vasoconstructor peptide produced by vascular endothelium and participates in inflammation, cellular injury and vascular events.[5] In addition, ET-1 may indirectly enhance endothelial cell proliferation through stimulation of vascular endothelial growth factor (VEGF) production by other cell types. Furthermore, ET-1 potentiates the effect of several proangiogenic factors in vitro, including PDGF and VEGF.[14] Many cytokines and chemokines induce ET-1 release by endothelium.[5] It has been demonstrated that proinflammatory cytokines such as TNF-α, sIL-2R, IL-6 and chemokine IL-8 were increased in patients with Behçet's syndrome, especially in active disease.[15]

As elevated ET-1 levels are a well known marker of vascular endothelial dysfunction, we expected to find elevated plasma ET-1 levels in patients with Behçet's disease, especially in the active stage. Moreover, we investigated whether

patients with ocular Behçet's disease had higher ET-1 levels than nonocular subjects, as elevated ET-1 levels contribute to ocular pathological manifestations, promoting retinal capillary closure and ischaemia.

Er et al. reported a study in 43 patients (27 ocular, 16 nonocular) with Behçet's disease.[5] They found that mean serum homocysteine, ET-1 and nitric oxide levels were significantly higher in patients with Behçet's disease than in control subjects. Serum homocysteine, ET-1 and nitric oxide levels were significantly higher in active patients than in inactive patients and control subjects. In addition, among patients with ocular Behçet's disease, the mean homocysteine levels were significantly increased and correlated with ET-1 and nitric oxide levels when compared with nonocular disease and control subjects. Of the 27 ocular patients, 22 were defined as having retinal vascular occlusive disease. Uslu et al. measured plasma ET-1 concentrations in 27 patients with Behçet's disease and 18 healthy controls.[6] The plasma ET-1 concentrations were found to be significantly increased in patients with Behçet's disease compared with the healthy subjects. Elevation of ET-1 did not correlate with the disease duration, gender, ocular or articular involvement. Hamzaoui et al. investigated the production of ET-1 by the respiratory tract in active Behçet's disease.[7] Their study group included of 10 patients with active Behçet's disease with pulmonary manifestations and 10 control subjects. ET-1 levels were measured in bronchoalveolar lavages and in serum. All of Behçet's disease patients exhibited higher bronchoalveolar ET-1 levels than controls. But they reported that mean serum endothelin-1 levels were not significantly different in Behçet's disease patients and healthy controls. Ural et al. also measured plasma ET-1 levels in 30 patients with Behçet's disease and in 20 healthy subjects.[4] Mean ET-1 plasma concentrations were significantly increased in patients with active Behçet's disease compared to concentrations found in healthy volunteers. No difference in plasma ET-1 level was observed between patients with inactive Behçet's disease and volunteers. Cimşit et al. conducted a study to evaluate plasma levels of ET-1 before and after endothelial cell stimulation in patients with Behçet's disease.[3] The study group included 15 patients with Behçet's disease and 10 healthy volunteers. They observed no difference in basal ET-1 levels between patient and control groups. After the occlusion test, ET-1 levels in both groups were significantly increased. This change was not significantly different between patient and control groups.

In our study, plasma ET-1 levels were found to be significantly high in active patients with Behçet's disease compared with inactive patients and control subjects. Plasma ET-1 levels also statistically correlated with the CRP levels. Since ET-1 levels in patients with active Behçet's disease were significantly high and these ET-1 levels correlated with the CRP levels we concluded that ET-1 may be a useful serologic marker for assessment of the activity of Behçet's disease.

As in the study of Uslu et al, the elevation of ET-1 did not correlate with the disease duration, age and gender in our study. And also there were no significant correlation between ET-1 levels and systemic involvements. We did not find significant correlation between ET-1 levels and ocular involvement. This result may be due to our ocular patients did not have retinal vascular occlusive disease. Mean ET-1 levels in the patients with vasculitis were found to be higher compared with nonvasculitic patients, although the difference was statistically insignificant. Increased ET-1 production might have critical biologic activities relevant to vasculitic events and it may contribute to the functional and morphological abnormalities of the vasculature in Behçet's disease.

Whether the elevated ET-1 level is a direct result of its increased synthesis from injured vascular endothelial cells or whether its presence in high concentrations may be responsible in patients with Behçet's disease is uncertain. There are different thoughts about this subject.[3-5] Cimşit et al. and Ural et al. reported that ET-1 may play an important pathogenic role in Behçet's disease.[3,4] But, Er

et al. concluded that elevated ET-1 levels are a well known marker of vascular endothelial dysfunction and appear to be the result of the endothelial cell damage in Behçet's disease.[5]

Although the mechanisms for the increase in the peripheral venous plasma concentration of ET-1 in patients with Behçet's disease remain unknown, there may be several possible explanations: First, both thrombin and transforming growth factor-β (TGF-β) can induce ET-1 gene expression in cultured endothelial cells, so the increase in plasma ET-1 may result from the excessive local production of ET-1 induced by such substances, released from the aggregated platelets that adhere to the injured vasculature. Second; since the human ET-1 gene contains hexanucleotide concensus sequences for the acute-phase reactant regulatory elements, ET-1 production might be attributable to the stress-induced release of an acute-phase reactant during active Behçet's disease. Third; when polymorphonuclear leucocytes (PMN) accumulate at different sites within the circulation in some conditions such as myocardial infarction, hypertension, acute inflammation PMNs express the dual capacity to convert big-endothelin to ET-1 and to inactivate ET-1 by at least two different neutral proteases. A possible deficit of the PMN-derived elastase and cathepsin G or blocking of these enzymes with elgin-C, together with the endothelial damage, can represent a further mechanism leading to high levels of ET-1. And also, the raised plasma ET-1 levels in patients with Behçet's disease may be attributable to injured endothelial cells.[4]

As a result, we conclude that ET-1 levels are correlated with the activity of Behçet's disease and it may be used as a serologic marker for assessment of the active stage of the disease in the follow-up of clinical and therapeutic studies. Further studies should be performed to clearly establish the role of ET-1 and its association with ocular and other systemic involvements in Behçet's disease.

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