

Evaluation of endothelial functions in patients with Behcet's disease without overt vascular involvement

Klinik olarak vasküler tutulumu olmayan Behçet hastalarında endotel fonksiyonlarının değerlendirilmesi

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ABSTRACT

Objectives: Behcet's disease (BD) is a chronic inflammatory disease characterized by recurrent oral and genital ulcerations and ocular lesions. This multisystemic disorder primarily affects the vascular system. In the present study, we aimed to evaluate vascular endothelial function in patients with Behcet's disease without vascular involvement.

Study design: We studied 40 patients with BD (44.9±5.4 years) and 20 healthy controls (45.4±8.2 years). Brachial artery Doppler ultrasonography (USG) and bilateral carotid artery intima-media thickness measurements were performed.

Results: Basal diameter of the brachial artery were similar between the two groups. However, flow-mediated dilation was markedly impaired in patients with Behcet's disease (p=0.03). Nitrate-induced dilation values were similar between the two groups (p=0.16). Carotid artery intima-media thickness was slightly increased in the patient group compared to the control group, but the difference did not reach statistical significance (0.69±0.15 to 0.59±0.09 respectively; p=0.06).

Conclusion: Vascular endothelial function is impaired in BD. Brachial artery Doppler USG is a reliable and reproducible method to establish changes in vascular functions.

ÖZET

Amaç: Behçet hastalığı (BH), tekrarlayıcı oral ve genital aftlar, deri lezyonları ve üveit ile kendini gösteren kronik bir hastalıktır. Bu multisistemik hastalık öncelikle vasküler sistemi etkilemektedir. Bu yazıda, damar tutulumu olmayan Behçet hastalarında vasküler endotel fonksiyonlarının değerlendirilmesi amaçlandı.

Çalışma planı: Çalışmaya BH tanısı konmuş olan 40 hasta (ortalama yaş: 44.9±5.4) ve 20 sağlıklı kontrol (45.4±8.2 yaş) alındı. Brakiyal arter Doppler ultrasonografi (USG) ve bilateral karotis arter intima-media kalınlığı ölçümleri yapıldı.

Bulgular: Hasta ve kontrol gruplarında bazal brakiyal arter çapı değerleri benzer bulundu. Hasta grubunda akım aracılı dilatasyon değerlerinin kontrol grubuna göre anlamlı olarak düşük olduğu görüldü (p=0.03). Nitratla indüklenmiş dilatasyon değerleri ise iki grup arasında benzerdi (p=0.16). Ortalama karotis intima-media kalınlığı değerinin hasta grubunda kontrol grubuna göre arttığı saptandı, bu artış anlamlı değildi (sırasıyla 0.69±0.15, 0.59±0.09, p=0.06).

Sonuç: Vasküler endotel fonksiyonu BH'de bozuktur. Brakiyal arter Doppler USG'si vasküler fonksiyonlardaki değişiklikleri saptamada güvenilir ve tekrarlanabilir bir yöntemdir.

Behcet' disease (BD) is a systemic inflammatory disorder with a classical triad of symptoms: oral ulcers, genital ulcers, and uveitis.^[1] The underlying etiology is still uncertain, though immune system dysregulation, genetic factors, and endothelial dysfunction are often assumed to be possible mechanisms.^[1] The most important complications of the disease

are associated with the vascular system, presenting as vasculitis, thromboembolism, and pulmonary artery aneurysm. Vascular manifestations of the disease may involve all vessels^[2]

Abbreviations:

BD	Behcet' disease
CCA	Common carotid arteries
CAD	Coronary artery disease
CIMT	Carotid artery intima media thickness
FMD	Flow-mediated dilatation
USG	Ultrasound

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and related complications represent the leading cause of death in BD.

Vascular involvement in BD is recognized as an unclassified vasculitis. There is controversy about the underlying mechanism of vascular injury in patients with BD. In several studies it has been suggested that increased inflammatory response may account for endothelial dysfunction, which results in vasculopathy.^[3]

Impaired flow-mediated dilatation (FMD) assessed by ultrasonographic determination of reduced vasodilator influence during reactive hyperemia is considered to be a reliable indicator of endothelial dysfunction in human resistance arteries. Endothelial dysfunction, which is regarded as the initial lesion in developing atherosclerosis, has been demonstrated by measurement of carotid artery intima media thickness (CIMT) in several studies.^[4]

In this study, we aimed to assess endothelial functions using brachial artery Doppler ultrasound (USG) and CIMT, in patients with BD and without vascular involvement.

PATIENTS AND METHODS

Study design and patient population

The study included 40 patients (44.9±5.4 years and 59% male) with Behcet's disease and 20 age- and sex-matched control subjects (45.4±8.2 years and 60% male). All the patients fulfilled the criteria of the International Study Group for the diagnosis of the disease.^[5] Inclusion criteria were^[1] the presence of Behcet's disease,^[2] Class I functional capacity according to New York Heart Association (NHYA),^[3] sinus rhythm, and^[4] normal LV EF (≥55%). Patients with vascular involvement (venous or arterial thrombus and/or disease), LV EF <60%, known coronary artery disease (CAD), diabetes mellitus, hypertension, smoking history, alcohol consumption, using any vasoactive drug, steroid or immunosuppressive therapy, having atrioventricular conduction abnormalities or atrial fibrillation were excluded from the study. None of the patients had clinical signs of CAD and the electrocardiograms revealed no signs of ischemia.

The study protocol was approved by the local ethics committee of our institute, and a detailed written informed consent was obtained from each patient. The study was conducted according to the Declara-

tion of Helsinki.

Brachial artery Doppler ultrasound measurements

The noninvasive determination of vascular endothelial dysfunction was performed according to the method described by Celermajer et al.^[6] Imaging studies of the brachial artery were performed using a high-resolution ultrasound machine (Siemens, Sequoia, C256; Mountainview, CA, USA) equipped with a 10-MHz linear-array transducer. All vasoactive medications were withheld for 24 hours before the procedure. The subjects remained at rest in the supine position for at least 15 minutes before the examination started. The subject's left arm was comfortably immobilized in the extended position to allow consistent recording of the brachial artery 2-4 cm above the antecubital fossa. Recordings of B-mode images were taken at rest, during reactive hyperemia (endothelium-dependent vasodilatation), and following the sublingual application of isosorbide dinitrate (endothelium-independent vasodilatation). After taking baseline brachial artery diameter measurements, a sphygmomanometer cuff, placed around the left upper arm proximal to the imaged artery segment, was inflated to the pressure of 240 mmHg for 4.5 minutes. Subsequently, the cuff was deflated and the brachial artery diameter was measured at 60 seconds after deflation. After re-establishing baseline conditions 15 to 20 minutes later, 5 mg sublingual isosorbide dinitrate was administered in order to assess endothelium-independent vasodilatation. Four minutes later, measurement of arterial diameter was repeated. The arterial diameter was measured in millimeters as the distance between the anterior wall media-adventitial interface ("m" line) and the posterior wall intima-lumen interface at end-diastole, coincident with the R wave on the continuously recorded electrocardiogram at two sites along the artery. All measurements were calculated as the average of the values obtained during three consecutive cardiac cycles. The FMD and NID were calculated as the percent change in diameter compared with baseline resting diameters.

CIMT and plaques were measured in the left and right common carotid arteries (CCA) using high-resolution B-mode USG (Siemens, Sequoia, C256; Mountainview, CA, USA) with a linear transducer at a frequency of 11 Mhz. The left and the right CCAs were imaged and focused on the near and far walls of the distal 2 cm of the CCA proximal to its bifurcation. Intima media thickness measurements were made at 3

points of the far walls of both distal CCAs. The distance between the leading edge of the first echogenic line and the leading edge of the other echogenic line was measured, as defined by Pignoli.^[7] Three measurements were obtained for each side of the CCAs. Separate mean values were calculated and recorded as the mean CIMT of the left and right CCA. Carotid artery plaques were defined either as a distinct protrusion of >1.5 mm into the lumen of the artery or as definite echogenicity with a posterior echogenic shadow.^[8]

Statistical analysis

Statistical analysis was performed with Statistical Package for the Social Sciences 16.0 (SPSS, Chicago, IL, USA) program. Results were expressed as mean and standard deviation. Independent samples t-test was used for comparisons of each group. The results were considered significant when the *p* value was less than 0.05.

RESULTS

Clinical characteristics and demographic data

Age, gender, heart rate, body surface area, systolic and diastolic blood pressure indices were similar both

in control and patient groups. There was no significant difference between patient and control groups, in terms of serum levels of glucose, total cholesterol, and low-density lipoprotein cholesterol. The mean disease duration among patients was 5.9 years. Clinical features of the patient group are presented in Table 1.

Brachial artery Doppler USG

Brachial arterial basal diameter measurements were similar in both the study and the control groups (*p*=0.867). We revealed a significant decrease in FMD measurement in the patient group compared to the control group (*p*=0.03), whereas NID measurements were similar between the two groups (*p*=0.16) (Table 2).

Carotid artery Doppler USG

Carotid artery intima-media thickness was slightly increased in the patient group, compared to the control group, approaching statistical significance (0.69 ± 0.15 to 0.59 ± 0.09 respectively; *p*=0.06).

DISCUSSION

In the present study, we revealed that endothelial functions, assessed by brachial artery Doppler USG evalu-

Table 1. Clinical and demographic characteristics

Variable	Behcet's disease group (n=40)			Control group (n=20)			<i>p</i>
	n	%	Mean±SD	n	%	Mean±SD	
Age (year)			44.9±5.4			45.4±8.2	0.345
Male gender (%)		59			60		0.618
Heart rate (per min)			73.1±1.6			73.7±1.3	0.84
BSA (m ²)			1.75±0.18			1.81±0.16	0.241
SBP (mmHg)			130±12.3			129±13.6	0.77
DBP (mmHg)			69±12			70.1±12.4	0.89
Fasting glucose (mg/dl)			95±10			96±11	0.90
Total cholesterol (mg/dl)			185±34			190±52	0.89
LDL-cholesterol (mg/dl)			100±23			104±24	0.75
Oral aphthous ulceration	40						
Genital ulceration	20						
Uveitis	10						
Skin lesions	32						
Arthralgia/arthritis	20						
Major vascular involvement	0						
Disease duration (years)	5.9						

BSA: Body surface area; SBP: Systolic blood pressure; DBP: Diastolic blood pressure; LDL: Low density lipoprotein.

Table 2. Brachial artery Doppler ultrasound measurements

	Basal diameter	FMD (%)	NID (%)
Patients with Behcet's disease (n=40)	3.49±0.48	13.34±4.92	16.31±2.45
Control group (n=20)	3.46±0.52	16.36±4.62	20.05±1.57
<i>p</i>	0.867	0.03	0.16

FMD: Flow-mediated dilation, NID: Nitrate induced dilation.

ation, are significantly impaired in patients with BD having no vascular involvement. We also performed carotid artery Doppler USG to measure CIMT, however we failed to find a marked difference between the patients and healthy subjects.

Based on the published literature, it is evident that Behcet's disease primarily involves the major vascular structures, the coronary arteries, the conduction system, endocardium and myocardium.^[9,10] The characteristic vasculitis of BD is recognized as an unclassified form, affecting all types of vessels but preferentially affecting the venous system.^[3] There is controversy about the underlying mechanism of vascular injury in BD. Chronic inflammatory disease and immune-mediated vascular injury has been suggested as the major pathology leading to ED.

Some studies have previously reported that FMD, assessed by brachial artery Doppler USG, is reduced in patients with BD.^[11,12] In the present study, we were able to demonstrate impairment of endothelium-dependent vasodilation even among patients without vascular disorder due to BD. We propose that impaired endothelial function in patients with BD is the manifestation of a systemic inflammatory process, irrespective of overt vascular involvement. As prior studies have suggested, ED may develop in patients with BD before overt vascular lesions appear.^[13]

Kayikçioğlu et al.^[14] have published supporting results, evaluating endothelial functions in patients with BD. Distinct from our study, 27 of 65 patients in their group had vascular involvement due to BD. Similarly, Caliskan et al.^[15] demonstrated that endothelial functions, assessed by FMD, as well as coronary microvascular function based on coronary flow reserve measurement, are deteriorated in patients with BD without vascular involvement. However, they detected more severe impairment of endothelial functions in active disease.

Chronic inflammation is closely related to atherogenesis.^[16] It is widely known that endothelial dysfunction, which is evaluated by both brachial artery Doppler USG and carotid IMT measurements, is a manifestation of atherosclerosis. In the present study, CIMT measurements in our patient group were increased when compared to the healthy controls; however the difference was not statistically significant. In contrast to our results, there are some studies in which higher CIMT values were observed in patients with BD.^[17,18] There are conflicting data regarding the association between vascular injury and atherosclerosis in BD. In several studies, ED was introduced as a marker of cardiovascular risk in patients with BD.^[19]

Endothelial dysfunction is widely accepted as the initial lesion of atherosclerosis.^[20] According to our findings, we propose that patients with BD are more prone to atherosclerotic risk. However it is still a matter of debate whether the endothelial dysfunction in BD precludes atherosclerosis. To overcome the discrepancies between several conflicting studies and to verify the different observations, prospective studies involving large cohorts are warranted.

Limitations

The major limitation of our study is that we were not able to exclude CAD according to coronary angiography. However, none of the patients had clinical features or signs of CAD based on ECG and echocardiography. Additionally, we were able to eliminate cardiovascular risk factors in order to evaluate the independent effects of vascular injury.

In the present study, we demonstrated impaired endothelial functions in patients with BD and without vascular involvement. We suggest that the lack of evidence-based data about the mechanism of vascular injury in BD should stimulate clinical research to advance our knowledge of this disease.

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Key words: Behçet syndrome/ultrasonography; carotid intima-media thickness; echocardiography, Doppler, pulsed; endothelium, vascular; ventricular dysfunction.

Anahtar sözcükler: Behçet sendromu/ultrasonografi; karotis intima-media kalınlığı; ekokardiyografi, Doppler, atımlı; endotel, vasküler; ventrikül disfonksiyonu.