

ORIGINAL ARTICLE

## Evaluation of arterial stiffness and subclinical atherosclerosis in patients with Behçet's disease without cardiovascular involvement

### Kardiyovasküler tutulumu olmayan Behçet hastalarında arter sertliği ve subklinik aterosklerozun değerlendirilmesi

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#### ABSTRACT

**Objective:** This study was conducted to evaluate arterial structure and function with assessment of pulse wave velocity (PWV) and carotid intima-media thickness (CIMT) in patients with Behçet's disease (BD).

**Methods:** Thirty patients (13 female, 17 male) with BD and 30 age, sex-matched healthy controls (12 female, 18 male) with no known cardiovascular disease were enrolled in this study. Carotid-femoral PWV and CIMT were measured.

**Results:** PWV was higher in BD patients compared with control group (6.35±1.05 vs. 5.75±0.83, respectively; p=0.017). There was no significant difference in maximum CIMT (0.751±0.077 mm vs. 0.735±0.079 mm, respectively; p=0.435), or mean CIMT (0.643±0.070 mm vs. 0.629±0.069, respectively; p=0.452). Maximum CIMT, mean CIMT, and PWV were positively correlated with duration of disease (r=0.410, p=0.025; r=0.404, p=0.027; and r=0.362, p=0.049, respectively).

**Conclusion:** Findings suggest that endothelial function is impaired in cases of BD before visible structural changes to arterial wall. PWV is more useful measurement than CIMT in determination of vascular damage in BD, especially in early stage of disease duration.

#### ÖZET

**Amaç:** Bu çalışmada Behçet hastalığı (BH) olgularında arter yapı ve fonksiyonları nabız dalga hızı (NDH) ve karotis intima-media kalınlığı (KİMK) ölçümleri değerlendirildi.

**Yöntemler:** Çalışmaya bilinen kardiyovasküler hastalığı bulunmayan 30 BH'li olgu (13 kadın, 17 erkek) ile yaş ve cinsiyet yönünden benzer olan 30 sağlıklı kontrol olgusu (12 kadın, 18 erkek) alındı. Karotis-femoral NDH ve KİMK ölçümleri yapıldı.

**Bulgular:** Nabız dalga hızı BH'de kontrol grubuna kıyasla daha yüksekti (sırasıyla, 6.35±1.05 ve 5.75±0.83, p=0.017). En yüksek KİMK (sırasıyla, 0.751±0.077 mm ve 0.735±0.079 mm, p=0.435), ortalama KİMK (sırasıyla, 0.643±0.070 mm ve 0.629±0.069, p=0.452) değerlerinde anlamlı farklılık yoktu. En yüksek KİMK, ortalama KİMK ve NDH ile hastalık süresi arasında pozitif korelasyon saptandı (sırasıyla, r=0.410, p=0.025; r=0.404, p=0.027 and r=0.362, p=0.049).

**Sonuç:** Bulgularımız BH'de arter duvarında görülebilir yapısal değişiklikler olmadan önce endotel fonksiyonlarında bozulma olduğunu göstermektedir. Özellikle hastalığın erken safhalarında NDH ölçümü, BH'deki damar hasarını saptamada KİMK ölçümünden daha yararlı bir incelemedir.

Behçet's disease (BD) is a multisystemic inflammatory disease of unknown cause, characterized by recurrent aphthous ulcers, genital ulcers, and uveitis.<sup>[1]</sup> In addition to classic triad, clinical manifestations in many other locations, including cardiovascular, pulmonary, gastrointestinal, nervous, and musculoskeletal systems have been described.<sup>[2]</sup>

Invasive or non-invasive methods may be used for determination of arteriosclerosis. Carotid intima-media thickness (CIMT) and pulse wave velocity (PWV) measurements are used to determine subclinical atherosclerosis non-invasively.<sup>[3]</sup> Endothelial dysfunction is a marker of vascular damage that can be observed before development of atherosclerotic

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plaque.<sup>[4]</sup> Reduced arterial elasticity and increased arterial stiffness occur as results of endothelial injury and dysfunction. Arterial stiffness may be assessed by degree of increase in PWV. It has been demonstrated in previous studies that increased arterial stiffness is a marker of increased cardiovascular mortality and morbidity.<sup>[5,6]</sup> Increased CIMT develops as a result of intimal smooth muscle proliferation and accumulation of atherogenic particles, and may be used in early diagnosis of atherosclerosis.<sup>[3]</sup>

Arterial compliance and elasticity changes have an important role in initiation and progression of atherosclerosis.<sup>[7]</sup> Early diagnosis of functional and/or structural arterial lesions, which can be identified with PWV and CIMT measurements, will be useful in identifying individuals at high risk for clinical cardiovascular events.<sup>[3,7]</sup> It has been demonstrated that arterial stiffness and increased CIMT are 2 independent markers of subclinical vascular damage in young adults.<sup>[8]</sup> In the literature, although there are a few studies evaluating CIMT and PWV separately in BD, there is only 1 study evaluating both CIMT and PWV in BD.<sup>[7,9-12]</sup> The present study was an investigation of functional and structural arterial lesions and tendency to atherosclerosis in patients with BD using PWV and CIMT measurements.

## METHODS

### Study population

A total of 30 patients who were examined in dermatology outpatient clinic between September 2014 and May 2015 and fulfilled criteria for BD developed by International Study Group for Behçet's Disease<sup>[13]</sup> were recruited prospectively. BD patients were divided into systemic disease group (vascular and/or ocular and/or central nervous system involvement) and mucocutaneous group. Thirty asymptomatic healthy individuals without cardiovascular disease who visited cardiology outpatient clinic for cardiovascular check-up between September 2014 and May 2015 were included as control group. Each subject was screened with complete history, physical examination, ultrasonographic investigation, and laboratory analysis. Exclusion criteria were as follows: (1) respiratory failure, pulmonary infection, congestive heart failure, or congenital heart disease; (2) known or suspected coronary artery disease; (3) valvular heart disease,

dysrhythmias such as atrial fibrillation, or atrioventricular block; (4) hypertension, diabetes, dyslipidemia (low-density lipoprotein [LDL] cholesterol >160 mg/dL, total cholesterol

>240 mg/dL, triglyceride >200 mg/dL), use of anti-hypertensives, antidiabetics, or lipid-lowering treatment; (6) malignancy, thyroid disorder, use of any vasoactive drugs; and (7) renal or hepatic insufficiency.

Written informed consent was obtained from each participant, and institutional ethics committee approved the study protocol, designed according to the Declaration of Helsinki.

After quiet resting in comfortable position for at least 15 minutes, all patients underwent physical examination. Blood pressure (BP) was measured in both arms in sitting position using sphygmomanometer.

### Pulse wave velocity measurement

SphygmoCor (AtCor Medical, West Ryde, Australia) device was used to record PWV measurements. Before the procedure, BP was measured. Distance between palpable point of femoral artery and sternal notch, and distance between most distal palpable part of carotid pulse and sternal notch were recorded. Applanation tonometry technique was used on these points sequentially. Once the most appropriate waveform amplitude and shape were obtained, measurements were recorded. Electrocardiography (ECG) recording continued during the procedure. Pulse wave velocity (PWV) was calculated automatically by SphygmoCor software based on time required for pulse wave to travel between arterial sites relative to ECG waveforms and measured distance between carotid and femoral pulse sites.

### Carotid intima-media thickness measurement

CIMT measurements were performed by a physician blinded to both patients and PWV values obtained. Both common carotid arteries were visualized using Toshiba Powervision 7500 ultrasound device (Toshiba Corp., Tokyo, Japan) with 7.5 MHz linear probe. Maximum and mean carotid intima-media thicknesses were calculated with M'Ath edge tracking software

#### Abbreviations:

BD	Behçet's disease
CIMT	Carotid intima-media thickness
ECG	Electrocardiography
FMD	Flow-mediated dilatation
LDL	Low-density lipoprotein
PWV	Pulse wave velocity
RA	Rheumatoid arthritis
SLE	Systemic lupus erythematosus
VEGF	Vascular endothelial growth factor

program version 2.0.1.0 (Intelligence in Medical Technologies, Paris, France). Measurements were performed in determined 1 cm segment of common carotid artery 2 to 3 cm distally located to carotid bulb.

### Statistical analysis

To calculate study sample size, difference of 0.9 m/sec in PWV and difference of 0.29 mm in CIMT were considered significant difference for BD and control groups.<sup>[9,11]</sup> According to these calculations, sample size of 24 patients per group would permit 2-sided significance level of 5% and 80% power.

All data were analyzed with SPSS for Windows software version 21.0 (SPSS Inc., Chicago, IL, USA). Numerical variables were defined as mean±SD or median (minimum-maximum); categorical variables were defined as counts and percentiles. Kolmogorov-Smirnov and Shapiro-Wilk tests were employed to evaluate whether distribution of variables was normal. In comparison of groups, if variables fit normal distribution, Student's t-test was used, and when not normally distributed, Mann-Whitney U test was used. Pearson's chi-square was used for comparison of categorical variables. Correlation analyses were conducted using Spearman and Pearson tests where appropriate. All probability values were 2-tailed and

calculated P-values were considered statistically significant when they were <0.05.

## RESULTS

### Baseline characteristics

Thirty patients with BD and 30 healthy volunteers who fulfilled inclusion criteria were enrolled in the study. Mucocutaneous involvement was seen in all patients with BD while systemic involvement had been identified in only 15 patients with BD: eye involvement was present in 11, gastrointestinal involvement was seen in 2, and neurological involvement was seen in 2 of the 15 patients. Mean time of disease duration was 9.2±9.4 years. Seven patients in BD group were smokers. Thirteen patients were using azathioprine, 11 patients were being treated with colchicine, 4 patients were taking corticosteroid, and 2 patients were receiving interferon therapy.

Basic characteristics including age, sex, smoking habit, systolic and diastolic BP, and heart rate were similar in patients with BD and control group. Laboratory parameters including creatinine, fasting blood glucose, LDL cholesterol, leukocyte, and platelet counts were similar in patients with BD and control group. Hemoglobin level was higher in control group (Table 1).

**Table 1. Demographic and biochemical characteristics of patients and controls**

	Behçet's disease (n=30)			Control (n=30)			p
	n	%	Mean±SD	n	%	Mean±SD	
Age, years			39.6±12.3			37.5±12.7	0.355
Female	13	43.3		12	40		0.793
Smoker	7	23.3		7	23.3		1.000
Systolic blood pressure (mmHg)			117.6±9.7			114.8±10.2	
			120 (100–130)			120 (90–130)	0.294*
Diastolic blood pressure (mmHg)			75.8±6.4			72.5±8.2	0.117*
			80 (60–85)			70 (60–85)	
Heart rate (beats/min)			71.7±8.3			68.1±6.1	0.063
LDL cholesterol (mg/dL)			105.4±28.2			100.4±21.3	0.953
Hemoglobin (gr/dL)			13.5±1.0			14.2±1.2	0.023
White blood cell (mm <sup>3</sup> /U)			7601±2554			7392±1790	0.807
Platelet counts (10 <sup>3</sup> /U)			234±61.7			247±49.1	0.348
Creatinine (mg/dL)			0.75±0.14			0.78±0.15	0.429
Glucose (mg/dL)			88.3±7.7			85.3±7.6	0.147

\*Mann-Whitney U test was used. LDL: Low-density lipoprotein; SD: Standard deviation.

**Table 2.** Comparison of groups in terms of carotid intima media thickness and pulse wave velocity

	Behcet's disease (n=30)	Controls (n=30)	p
	Mean±SD	Mean±SD	
Pulse wave velocity (m/second)	6.35±1.05	5.75±0.83	0.017
Maximum carotid intima media thickness (mm)	0.751±0.077	0.735±0.079	0.435
Mean carotid intima media thickness (mm)	0.643±0.070	0.629±0.069	0.452

SD: Standard deviation.

### Pulse wave velocity and carotid intima-media thickness

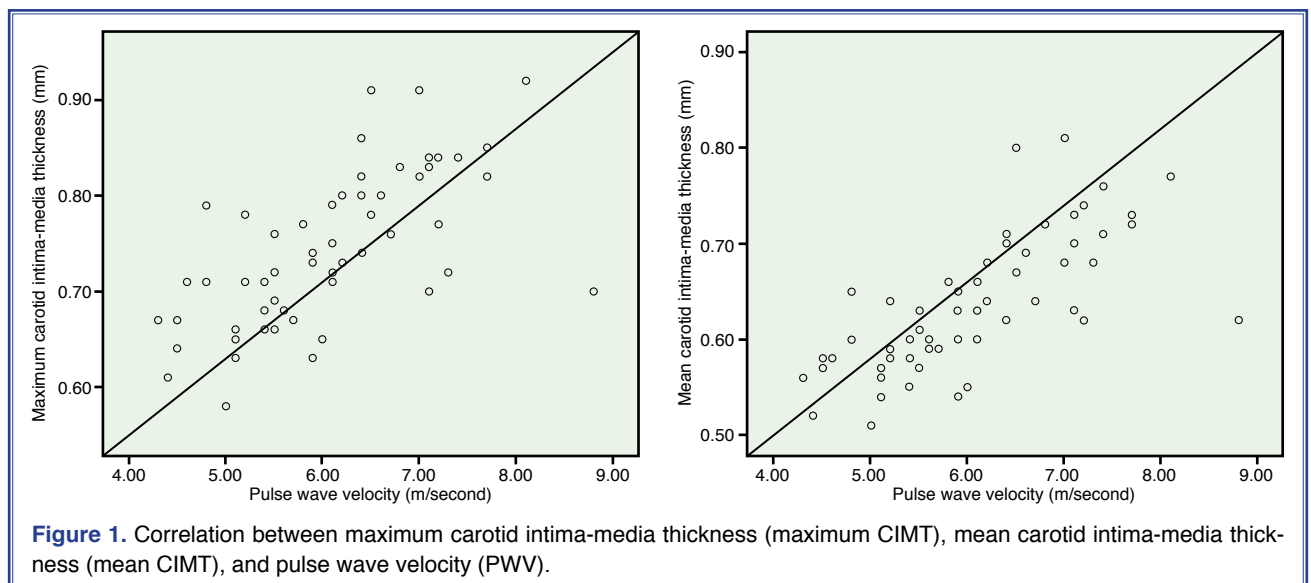
Maximum CIMT was  $0.751\pm 0.077$  mm in BD group and  $0.735\pm 0.079$  mm in control group. Mean CIMT was  $0.643\pm 0.07$  mm in BD group and  $0.629\pm 0.069$  mm in control group. Mean and maximum CIMT measurements were higher in BD group compared with control group, but they did not reach level of statistical significance. Measured PWV was  $6.35\pm 1.05$  m/sec in BD group and  $5.75\pm 0.83$  m/sec in control group, and difference between the 2 groups was statistically significant (Table 2). BD group was divided into 2 subgroups for further analysis: patients who had only mucocutaneous involvement (mucocutaneous involvement group; n=15) and who had mucocutaneous and systemic involvement (systemic involvement group; n=15). There was no significant difference in maximum CIMT ( $0.750\pm 0.092$  mm vs.  $0.752\pm 0.063$  mm, respectively;  $p=0.927$ ), mean CIMT ( $0.642\pm 0.081$  mm vs.  $0.644\pm 0.061$  mm, respectively;  $p=0.920$ ) or PWV ( $6.25\pm 0.95$  m/sec vs.  $6.46\pm 1.16$  m/

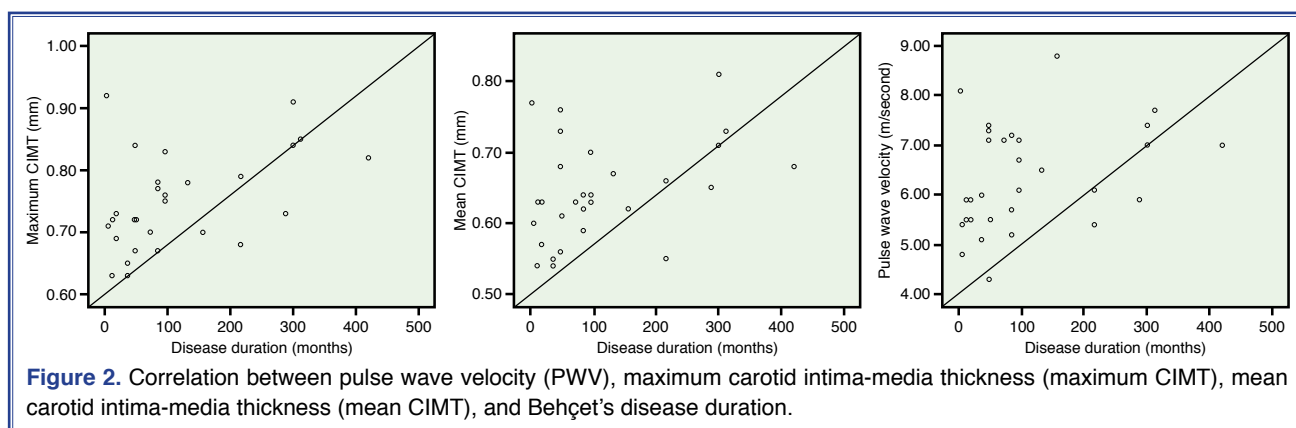
sec, respectively;  $p=0.600$ ) measurements between mucocutaneous and systemic involvement groups.

Maximum and mean CIMT values were found to increase with increment in PWV values ( $r=0.661$ ,  $p<0.001$ ;  $r=0.717$ ,  $p<0.001$ , respectively) (Figure 1). Maximum CIMT, mean CIMT, and PWV were positively correlated with duration of disease ( $r=0.410$ ,  $p=0.025$ ;  $r=0.404$ ,  $p=0.027$ ;  $r=0.362$ ,  $p=0.049$ , respectively) (Figure 2) and age ( $r=0.657$ ,  $p<0.001$ ;  $r=0.717$ ,  $p<0.001$ ;  $r=0.669$ ,  $p<0.001$ , respectively). PWV was positively correlated with systolic BP ( $r=0.319$ ;  $p=0.013$ ). Additionally, mean CIMT and PWV were positively correlated with serum fasting glucose levels ( $r=0.274$ ,  $p=0.034$ ;  $r=0.356$ ,  $p=0.005$ , respectively).

### DISCUSSION

This study aimed to assess tendency to atherosclerosis in patients with BD. Carotid-femoral PWV and CIMT were evaluated. Increase in PWV was determined in





patients with BD compared with control group. CIMT tended to be higher in patient group, but did not reach level of statistical significance.

Atherosclerosis is a progressive inflammatory process that can result in fatal vascular events. Initial mechanism of atherosclerotic process is endothelial injury.<sup>[3]</sup> Endothelial injury appears to play a key role in atherogenesis of BD.<sup>[14]</sup> Endothelial dysfunction demonstrated by brachial artery flow-mediated dilatation (FMD) is a feature of BD.<sup>[4]</sup> In a meta-analysis, Merashli et al. reported that FMD was impaired in BD.<sup>[15]</sup> PWV was demonstrated to be more sensitive than FMD for detection of vascular dysfunction.<sup>[16]</sup> Arterial stiffness is surrogate end-point for cardiovascular disease and can be determined with PWV measurement.<sup>[7]</sup> Increased PWV, as a reflection of arterial stiffness, is known to be an indicator of atherosclerosis and can be used to identify patients who have an increased cardiovascular risk.<sup>[3,7]</sup> Contradictory results have been reported in studies investigating PWV in BD. Kürüm et al. examined 14 BD patients and 28 healthy subjects without known cardiovascular disease and did not find any difference in PWV.<sup>[17]</sup> On the other hand, there are studies that demonstrated increased PWV in BD patients compared with healthy individuals. Caldas et al. found that PWV values of BD patients were higher than those of healthy individuals with statistical significance.<sup>[9]</sup> Kocabay et al. examined PWV in cases of BD and newly diagnosed chronic inflammatory diseases rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE), and found that PWV was higher in RA, SLE, and BD groups compared with healthy subjects.<sup>[7]</sup> In another study, Chang et al. found that patients with BD had significantly higher PWV values than controls.<sup>[10]</sup> Dif-

ferent or contradictory results in PWV values may be due to exclusion criteria. Kürüm et al. did not exclude cases of hyperthyroidism, hypothyroidism, or mild renal insufficiency.<sup>[17]</sup> Caldas et al. did not exclude mild renal insufficiency.<sup>[9]</sup> Similarly, Kocabay et al. did not exclude mild renal insufficiency or hypertension.<sup>[7]</sup> Chang et al. did not exclude thyroid disorders, renal or hepatic insufficiency, or dyslipidemia.<sup>[10]</sup> PWV values may have been influenced by these factors. In the present study, patients with thyroid disorders, renal or hepatic insufficiency, or dyslipidemia were excluded and PWV values were significantly higher in BD group compared with healthy subjects. In addition, significant correlation was demonstrated between PWV and BD duration in current study.

Increased CIMT is marker of subclinical atherosclerosis.<sup>[3]</sup> Recent studies have shown that increased CIMT was significantly correlated with endothelial dysfunction markers in BD patients, as was seen with PWV values.<sup>[11]</sup> However, contradictory results have been reported in studies examining CIMT in BD as well. Öztürk et al. investigated potential correlation between CIMT and serum vascular endothelial growth factor (VEGF) levels in patients with BD and found that CIMT and serum VEGF levels were higher in BD group compared with control group. They speculated that their findings might be indicative of subclinical atherosclerosis in patients with BD.<sup>[11]</sup> Öztürk et al., Hong et al., and Messedi et al. all found that CIMT was significantly higher in BD group than control group.<sup>[12,18,19]</sup> On the other hand, Seyahi et al. and Rhee et al. found no difference in terms of CIMT between BD patients and healthy controls.<sup>[20,21]</sup> Merashli et al. demonstrated that CIMT was greater despite a degree of statistical heterogeneity that reflects clinical

heterogeneity of BD.<sup>[15]</sup> In current study, CIMT levels were higher in BD group compared to control group, but difference was not significant. Positive correlation between CIMT level and disease duration was observed in our study. Also, it was considered that additional years of disease duration would produce a statistically significant increase in CIMT values.

Presence of subclinical atherosclerosis has been evaluated separately with CIMT or PWV measurements in most studies. In the literature, there is only 1 study that evaluated presence of subclinical atherosclerosis using both CIMT and PWV measurements. Caldas et al. found that BD patients had significantly higher PWV levels compared with healthy controls. CIMT levels were also higher in BD patients, but it did not reach level of statistical significance.<sup>[9]</sup> Similarly, there was a statistically significant difference in terms of PWV levels in BD patients and healthy subjects in our study, and CIMT levels were higher in BD group but difference was not statistically significant.

### Limitations of the study

The primary limitation of the study is small sample size. In addition, follow-up data was not available at the time of present analysis to compare results of this study with clinical end-points (cardiovascular mortality, cardiovascular morbidity, and clinical atherosclerosis).

### Conclusion

As a result of our study, it has been demonstrated that even in the absence of major atherosclerotic risk factors, BD patients might be at a higher risk for development of atherosclerosis and endothelial dysfunction compared with healthy subjects. In order to reduce cardiovascular morbidity and mortality, assessment of atherosclerotic risk factors and risk stratification are important as well as treatment of primary disease. Measurement of PWV, a non-invasive technique, is more useful tool than CIMT measurement to detect subclinical atherosclerosis, especially in early stage of disease duration.

**Conflict-of-interest issues regarding the authorship or article: None declared**

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**Keywords:** Atherosclerosis; Behçet's disease; carotid intima-media thickness; pulse wave velocity.

**Anahtar sözcükler:** Ateroskleroz; Behçet hastalığı; karotis intima-media kalınlığı; nabız dalga hızı.