# Relationship between endothelial dysfunction and cardiovascular risk factors and the extent and severity of coronary artery disease

## Endotel fonksiyonu bozukluğu ile kalp damar hastalığı risk faktörleri ve koroner arter hastalığı yaygınlığı ve ciddiyeti arasındaki ilişki

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## ABSTRACT

***Objectives:*** Endothelial dysfunction (ED) is associated with coronary artery disease (CAD) and cardiovascular risk factors. The relationship between cardiovascular risk factors, ED and the presence, extent and severity of CAD, was evaluated in patients with and without angiographically defined CAD in our study.

***Study design:*** Eighty patients with CAD and 20 subjects with normal coronary arteries were included. Endothelial function was evaluated by endothelium-dependent, flow-mediated dilatation (FMD) and nitroglycerine-mediated dilatation (NMD) measurements, using brachial artery Doppler ultrasonography (USG). Cardiovascular risk factors were identified. The extent and severity of CAD was determined via vessel and modified Gensini scores.

***Results:*** FMD% and NMD% were significantly decreased in the CAD(+) group compared with the CAD(–) group (p=0.0001). In the CAD(+) group, the cut-off values of FMD% and NMD% in distinguishing between single-vessel and multi-vessel diseases were 8.5% (sensitivity: 95%, specificity: 62%) and 13.6% (sensitivity: 91%, specificity: 62%), respectively. Additionally, a modified Gensini score was significantly correlated with both FMD and NMD (r=-0.825, r=-0.778, respectively; p=0.0001) in the CAD(+) group.

***Conclusion:*** ED was more prevalent in the CAD(+) group, and the degree of ED correlated well with the extent and severity of CAD.

## ÖZET

***Amaç:*** Endotel fonksiyonu bozukluğu (EFB), kalp damar hastalığı risk faktörleri ve koroner arter hastalığı (KAH) ile ilişkilidir. Çalışmamızın amacı, anjiyografik olarak saptanmış KAH'sı olan ve olmayan hastalarda, KAH risk faktörleri ile EFB ilişkisinin değerlendirilmesi ve KAH varlığı, yaygınlığı ve ciddiyeti ile EFB arasındaki ilişkinin gösterilmesidir.

***Çalışma planı:*** Koroner arter hastalığı saptanan 80 hasta çalışma grubuna, normal koroner arterleri olan 20 hasta kontrol grubuna alındı. EFB, brakiyal arter ultrasonografisi ile "endotel bağımlı vazodilatatör yanıt" (EBVY) ve "nitrat bağımlı vazodilatatör yanıt" (NBVY) ölçümleri yapılarak değerlendirildi. KAH risk faktörleri kaydedildi. KAH yaygınlığı ve ciddiyeti, hasta damar sayısı ve Gensini skoru ile belirlendi.

***Bulgular:*** Koroner arter hastalığı (+) grupta KAH (–) gruba göre EBVY ve NBVY anlamlı olarak daha düşük bulundu (p=0.0001). KAH (+) grupta, EBVY için; %8.5 cut-off değerinin %95 duyarlılık ve %62 özgüllükle, NBVY için ise; %13.6 cut-off değerinin %91 duyarlılık ve %62 özgüllükle tek damar ve çok damar hastalığı ayrımını sağlayabileceği bulundu. KAH (+) grupta Gensini skor ile EBVY ve NBVY arasında çok güçlü düzeyde korelasyon saptandı (sırasıyla, r=-0.825, r=-0.778; p=0.0001).

***Sonuç:*** Çalışmamızda KAH (+) grupta EFB'nin daha fazla olduğunu ve KAH yaygınlığı ve ciddiyeti ile EFB derecesinin arttığını tespit ettik.

The endothelium is an autocrine, paracrine, and endocrine organ, which plays a key role in the regulation of vascular tone, thrombogenesis, lipid metabolism, inflammation, and vessel growth.[1-3] Endothelial dysfunction (ED), a hallmark of vascular diseases, often leads to atherosclerosis. Traditional risk factors such as hypertension (HT),[4-6] cigarette smoking,[7] diabetes mellitus (DM),[8] age,[9-12] and hypercholesterolemia[13] may induce ED in the coronary arteries, due to decreased bioavailability of nitric oxide (NO). A dysfunctional endothelium loses its ability to protect the vascular system as a result of the reduction of anti-atherosclerotic and anti-thrombotic properties. ED promotes the development and progression of atherosclerosis.

The aim of the present study was to evaluate the relationship between ED and the presence, extent and severity of coronary artery disease (CAD) in patients with and without angiographically defined CAD. The potential risk factors that might affect ED in the CAD(+) group were also analyzed. Among the various methods used to assess endothelial function was the brachial artery Doppler ultrasonography (USG), which measured endothelium-dependent, flow-mediated dilatation (FMD) and nitroglycerin-mediated dilatation (NMD) in the brachial artery. This is a noninvasive, highly reproducible, and simple method to use, which is based on high-sensitivity ultrasound waves.[14]

## PATIENTS AND METHODS

### Study Population

This study included 100 patients seen between February and May 2008 with suspected CAD due to typical chest pain or ischemic findings on a treadmill exercise test or myocardial scintigraphy who underwent coronary angiography. Eighty patients (62.4±9.9 years, 65% male) who had angiographically proven CAD were defined as the study group. Twenty subjects who had normal coronary arteries (57.7±10.5 years; 30% male) served as the control group. Informed consents were obtained from all the patients in each group. The study was approved by the local ethics committee. Patients with acute coronary syndrome, severe left ventricular dysfunction (left ventricular ejection fraction <35%) and a history of coronary angioplasty or coronary artery bypass surgery were excluded from the study. CAD was defined as a visually estimated percent diameter stenosis of greater than 25% in any of

the major epicardial coronary arteries. Clinically important CAD was defined as greater than 50% diameter stenosis in any of the major epicardial coronary arteries by consensus of two observers who were blinded to the results of the ultrasound studies. All study subjects underwent a complete ph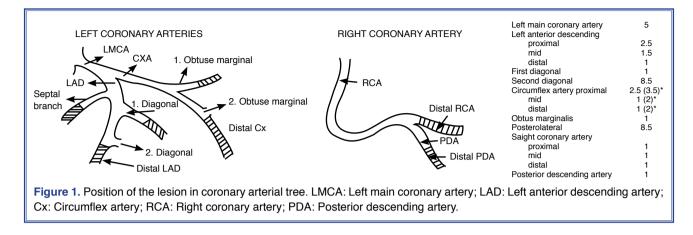ysical examination. Biochemical and electrocardiographic measurements were also performed. The following risk factors for atherosclerosis were assessed: smoking, HT, hyperlipidemia (HL), DM, obesity, and a positive family history of CAD. Obesity was defined as a body mass index (BMI) above 30 kg/m². HT was defined as blood pressure ≥140/90 mmHg or use of anti-hypertensive drugs. DM was defined as fasting blood glucose level ≥126 mg/dl, in two separate measurements, or the use of anti-diabetic agents. Endothelial function was evaluated by brachial artery Doppler USG. The extent and severity of CAD was determined according to the number of diseased vessels and a modified Gensini score.[15]

### Assessment of vascular function

The noninvasive determination of ED was performed according to the method described by Corretti et al.[16] A high-resolution echocardiography Doppler ultrasound (General Electric Vivid I) with an 8.0 MHz linear array transducer was used to measure the flow velocity and diameter of the left brachial arteries.

Each subject was studied in the morning, after abstaining from food, alcohol, caffeine, and tobacco for 8h-12h before the examination.[17] All ultrasound images were recorded for subsequent blinded analysis. Recordings of both B-mode and pulsed Doppler spectral curve were taken at rest, during reactive hyperemia and following the sublingual application of isosorbide dinitrate. After baseline 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 5 minutes to occlude arterial flow. To verify that suprasystolic compression of the brachial artery caused adequate increase in blood flow, flow velocity was measured at

| Abbreviations: | |
| --- | --- |
| BMI | Body mass index |
| CAD | Coronary artery disease |
| DM | Diabetes mellitus |
| ED | Endothelial dysfunction |
| FMD | Flow-mediated dilatation |
| HDL-C | High-density lipoprotein cholesterol |
| HL | Hyperlipidemia |
| HT | Hypertension |
| NMD | Nitroglycerin mediated dilatation |
| NTG | Nitroglycerin |
| ROC | Receiver operator characteristic |
| USG | Ultrasonography |

**Figure 1.** Position of the lesion in coronary arterial tree. LMCA: Left main coronary artery; LAD: Left anterior descending artery; Cx: Circumflex artery; RCA: Right coronary artery; PDA: Posterior descending artery.

rest and again within 15 seconds after cuff deflation. Blood flow, pressure and end-diastolic diameter were recorded at 30-second intervals for 300 seconds after cuff release and at six, eight and ten minutes until recovery to baseline values. After re-establishing baseline conditions 15-20 minutes later, measurements of the arterial diameter and flow velocity were repeated, followed by sublingual isosorbide dinitrate administration at a dose of 5 mg in order to assess endothelium-independent vasodilatation. Four minutes later, measurements of arterial diameter and flow velocity were 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. The maximum FMD and NMD diameters were calculated as the average of the three consecutive maximum diameter measurements after hyperemia and nitroglycerin, respectively. FMD% was calculated as the percent change from the basal diameter value during reactive hyperemia. NMD% was calculated as the percent change in diameter from the resting value to the value after nitroglycerin administration.

## Assessment of coronary atherosclerosis

All patients underwent selective coronary angiography after brachial artery Doppler USG. After femoral artery cannulation, the Judkins technique was used for the cannulation of the left and right coronary arteries. All angiograms were evaluated by two experienced invasive cardiologists blinded to the study. The severity of CAD in major epicardial coronary arteries was evaluated according to the modified Gensini scoring system.[15] Modified Gensini scores grade the degree of narrowing of the coronary arteries as: 1: 1-25% narrowing, 2: 26-50% narrowing, 4: 51-75% narrowing, 8: 76-90% narrowing, 16: 91- 99% narrowing, and 32: total occlusion. The score is multiplied by a factor that reflects the importance of the position of the lesion in the coronary arterial tree (Figure 1).

The extent of CAD was assessed by the vessel score. The number of vessels with significant stenosis (presence of at least one lesion causing at least ≥50% diameter stenosis) was determined to be the vessel score. Scores ranged from 0 to 4, depending on the number of vessels involved. Left main artery stenosis was scored as a one-vessel disease. CAD involving only one vessel was defined as 'single-vessel disease' while, when two or more coronary arteries were involved, it was defined as 'multi-vessel disease'.

## Statistical analysis

Statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS-15) software package. Descriptive statistics were calculated, including means and standard deviations for continuous variables, frequencies for qualitative variables, and percentages for categorical variables. To test the normal distribution of the samples, a Kolmogorov-Smirnov test was performed. Parameters normally distributed were compared by Student's t-test, whereas uneven distributions were compared by Mann-Whitney U test. Chi-square or Fisher's test was used to compare qualitative variables, and the Student's t-test was used to compare quantitative variables. Pearson's correlation test was used to test the correlations between variables. Multivariate analysis was performed using multiple linear regression models. Multiple linear regression analysis was used to assess the relation-

ship between coronary risk factors and FMD% and NMD%. Variables with a p<0.1 value in the univariate analysis were included in the multivariate analysis. Smoking status, age, gender, DM, HL, triglyceride (TG), and high-density lipoprotein cholesterol (HDL-C) levels and the presence of CAD were analyzed in the multivariate model. A receiver operator characteristics (ROC) curve analysis was performed to identify the predictive index value of FMD and NMD for single-vessel disease or multi-vessel disease. Statistical significance was defined as p<0.05.

## RESULTS

### Patient population

Clinical and demographic characteristics of the study population are presented in Table 1. The number of male patients were significantly higher in the CAD(+) group (p=0.005). The number of smokers were lower in the CAD(−) group compared with the CAD(+) group, which was not statistically significant (p=0.052). Serum HDL-C level was decreased in the CAD(+) group compared with the other group (p=0.007); however, TG level was found to be higher in the CAD(+) group compared with the CAD(−) group (p=0.006). There was no significant difference between the groups in terms of serum total cholester-

ol (TC) and LDL-C levels. Other cardiovascular risk factors were similar between the groups. There were no significant differences between the two groups with regard to medical therapy.
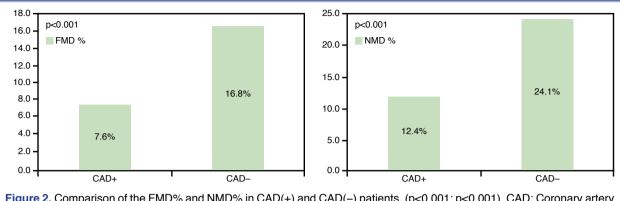
### Brachial artery Doppler ultrasound measurements

In the study population (n=100), mean FMD% was 9.5±4.5% and mean NMD% was 14.8±5.6%. There was a significant correlation between FMD% and NMD% (r=0.950, p<0.001). In the CAD(+) group, mean FMD% was 7.6±2.8% and mean NMD% was 12.4±3.4%. In the CAD(−) group, mean FMD% was 16.8±1.8% and mean NMD% was 24.1±2%. FMD% and NMD% values were markedly reduced in the CAD(+) group compared to the CAD(−) group (p<0.001 and p<0.001, respectively) (Figure 2).
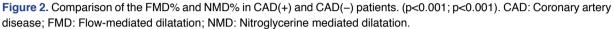
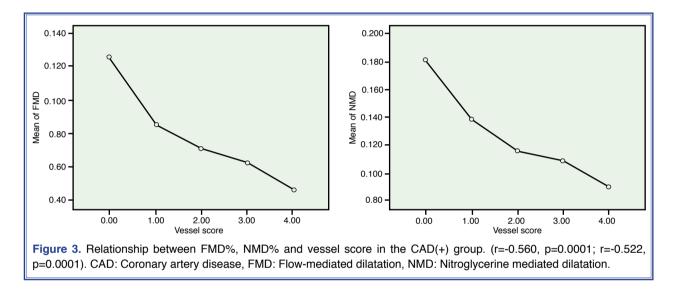### Cardiovascular risk factors and brachial artery Doppler ultrasonography measurements

In the univariate analysis, it was observed that diabetic patients had significantly lower FMD% and NMD% values than non-diabetic patients in the CAD(+) group (p=0.037 and p=0.035, respectively) (Table 2). However, when we used the Bonferroni adjustment, there was no significant difference between diabetic patients and non-diabetic patients. No significant relationship was observed between the other risk factors and FMD% and/or NMD% in the CAD(+) group.

**Table 1.** Clinical and demographic characteristics

| Characteristics | CAD(+) (n=80) | | | CAD(−) (n=20) | | | *p* |
|---|---|---|---|---|---|---|---|
| | n | % | Mean±SD | n | % | Mean±SD | |
| Age (years) | | | 62.4±9.9 | | | 57.7±10.5 | 0.06 |
| Male | | | | | | | |
| Gender | 52 | 65 | | 6 | 30 | | 0.005 |
| Diabetes mellitus | 35 | 43.8 | | 4 | 20 | | 0.072 |
| Hypertension | 57 | 71.3 | | 12 | 60 | | 0.331 |
| Hyperlipidemia | 60 | 75 | | 11 | 55 | | 0.078 |
| Smoking | 27 | 33.8 | | 2 | 10 | | 0.052 |
| Family history | 35 | 43.8 | | 5 | 25 | | 0.201 |
| Body mass index (kg/m²) | | | 28.7±4.0 | | | 28.9±5.0 | 0.761 |
| Total cholesterol (mg/dL) | | | 197.5±46.3 | | | 188.7±49.7 | 0.453 |
| Triglycerides (mg/dL) | | | 148.1±75 | | | 99.9±35.4 | 0.006 |
| HDL-C (mg/dL) | | | 45.3±10.9 | | | 53.6±15.7 | 0.007 |
| LDL-C (mg/dL) | | | 126.9±35.8 | | | 118.1±36.4 | 0.327 |

CAD: Coronary artery disease; HDL: High density lipoprotein cholesterol; LDL: Low density lipoprotein-cholesterol.

**Figure 2.** Comparison of the FMD% and NMD% in CAD(+) and CAD(−) patients. (p<0.001; p<0.001). CAD: Coronary artery disease; FMD: Flow-mediated dilatation; NMD: Nitroglycerine mediated dilatation.



**Figure 3.** Relationship between FMD%, NMD% and vessel score in the CAD(+) group. (r=-0.560, p=0.0001; r=-0.522, p=0.0001). CAD: Coronary artery disease, FMD: Flow-mediated dilatation, NMD: Nitroglycerine mediated dilatation.
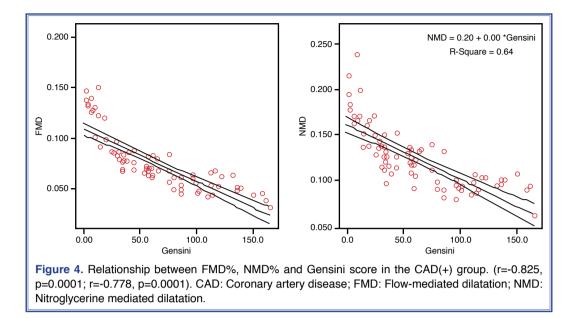
In the multiple linear regression analysis, lower HDL-C (B=0.001, 95% confidence interval (CI) (0.000, 0.001); p=0.016) and the presence of CAD (B= -0.082, 95% CI (-0.099, -0.066); p<0.001) were determined as independent risk factors for FMD ($R^2$=0.76). For NMD, lower HDL-C (B=-0.001, 95% CI (0.000, 0.001); p<0.003), and the presence of CAD (B=-0.104, 95% CI (-0.124, -0.084); p<0.001) were established as independent risk factors ($R^2$=0.79).

**Table 2.** Brachial artery Doppler ultrasound measurements, vessel scores and modified Gensini scores in diabetic and non-diabetic patients

| | CAD(+) Group (n=80) | | p |
| --- | --- | --- | --- |
| | DM(+) (n=35) | DM(−) (n=45) | |
| Brachial artery diameter (mm) | 4.2±0.7 | 4.5±0.6 | 0.062 |
| Flow-mediated dilatation (%) | 6.9±2.3 | 8.2±3 | 0.037 |
| Nitroglycerine mediated dilatation (%) | 11.5±2.7 | 13.1±3.8 | 0.035 |
| Vessel score | 2.4±0.9 | 1.9±1.1 | 0.026 |
| Gensini score | 76.4±45 | 51.5±33.3 | 0.010 |

CAD: Coronary artery disease; DM: Diabetes mellitus.

**Figure 4.** Relationship between FMD%, NMD% and Gensini score in the CAD(+) group. (r=-0.825, p=0.0001; r=-0.778, p=0.0001). CAD: Coronary artery disease; FMD: Flow-mediated dilatation; NMD: Nitroglycerine mediated dilatation.

## Endothelial dysfunction and the extent and severity of coronary artery disease

In the CAD(+) group, 18 patients had single-vessel disease, 56 patients had multi-vessel disease, and 6 patients had noncritical lesions. The mean number of diseased vessels was 2.1±1.2 and the mean modified Gensini score was 62.4±43.4. We obtained a strong, negative correlation between the number of diseased vessels and both FMD and NMD in the CAD(+) group (r=-0.560, p<0.001; r=-0.522, p<0.001, respectively) (Figure 3). Moreover, a strong, positive correlation was observed between a modified Gensini score and the mean number of diseased vessels (r=0.601, p<0.001).

## Predictive Value of Percent Change in FMD and NMD in Distinguishing Single-Vessel from Multi-Vessel Disease

The cut-off value for FMD% for predicting multi-vessel disease was <8.5% (area under ROC curve = 0.838; CI 0.71-0.96%) with 95% sensitivity and 62% specificity. The positive predictive value and negative predictive value for FMD were 89% and 84%, respectively. For NMD%, the cut-off value was <13.6% (area under ROC curve = 0.84; CI 0.70-0.97%) with 91% sensitivity and 62% specificity. The positive predictive value and negative predictive value for NMD were 89% and 89%, respectively.

## Relationship between Modified Gensini Score and Percent Change in FMD and NMD

We obtained a strong, negative correlation between modified Gensini scores and both FMD and NMD in the CAD(+) group (r=-0.825, p<0.001; r=-0.778, p<0.001, respectively) (Figure 4).

## DISCUSSION

Atherosclerosis is a progressive, inflammatory process that initially involves ED. Growing evidence indicates that ED may also influence the progression of atherosclerotic lesions.[18] Many studies have investigated the relationship between coronary risk factors and endothelial function. Benjamin et al.[19] studied the relationship between coronary risk factors and endothelial function in 2,883 participants in the Framingham study. They revealed that in stepwise multivariable linear regression models, FMD% was inversely correlated to age, systolic blood pressure, BMI, lipid-lowering medication, and smoking and positively related to female gender, heart rate, and prior walk test. Kirma et al.[20] investigated the relationship between endothelial function and coronary risk factors in patients with angiographically proven CAD. In univariate analysis, they found a negative correlation between FMD% and age, BMI, and DM. In multiple stepwise regression analysis, FMD% was found to be correlated with DM and advanced age, but not with BMI. However, FMD% was not associated with HL, family history of premature atherosclerosis, HT, or smoking.

We investigated the relationship between coronary

risk factors and brachial artery Doppler USG measurements in 80 patients with angiographically proven CAD. We also investigated the relationship between ED and modified Gensini and vessel scores. In our study, in the multiple linear regression analysis for FMD%, lower HDL-C and the presence of CAD were established as independent risk factors. For NMD%, lower HDL-C and the presence of CAD were determined as independent risk factors.

Gender differences in endothelial function have been the focus of considerable research interest. In a previous study, Uehata et al.[21] observed increased FMD% in young healthy females compared with males. Urstad et al.[22] revealed that FMD% was similar in men and women at about 55 years, while FMD% and NMD% values were similar in men of both age groups. However, women at 55 years had lower FMD% than women at 35 years. Taddei et al.[23] demonstrated an age-related decline in men, but endothelial function was preserved until the onset of menopause in women. In our study, the number of males was significantly higher than the number of females in the CAD(+) group. However, no significant gender difference was obtained regarding FMD% and NMD%. The possible reason may be attributed to the mean ages of female patients being high in both groups in our study.

Diabetes mellitus (DM) is associated with accelerated atherosclerosis and an increased prevalence of cardiovascular disease. Simova II et al.[24] revealed in their study that diabetic patients had a significantly lower FMD compared with non-diabetic patients. In our study, modified Gensini scores and vessel scores were significantly higher in diabetic CAD(+) patients. Also, diabetic patients had lower FMD% and NMD% values in the CAD(+) group. However, due to the limited number of patients, when the Bonferroni adjustment was used, there was no significant difference between diabetic patients and non-diabetic patients.

Elevated serum levels of TC and blood pressure has been shown to impair endothelial function.[25,26] Anti-hypertensive and lipid-lowering therapy improve endothelial function and decrease cardiac event rates. [27,28] In our study, we did not observe any significant relationship between HT, HL and FMD% or NMD%. This may have resulted from the fact that nearly all of the patients were under optimal anti-hypertensive and lipid-lowering therapy.

Cigarette smoking is strongly associated with CAD and atherosclerosis. While smoking causes ED[29] as a result of impairment in endothelium-dependent vasorelaxation, the exact mechanisms involved are not completely understood.[30] In our study, the number of smokers was lower in the CAD(−) group compared with the CAD(+) group, which was not statistically significant (p=0.052). In contrast to previous studies, we found no relationship between smoking and ED in the CAD(+) group. Benjamin et al.[19] found that FMD% was increased in smokers who did not smoke within six hours prior to testing than in those who did. In our study, all participants abstained from tobacco from 8h-12h before the study..

The extent and severity of CAD is of particular importance in prognosis and treatment. Neunteufl et al.[31] observed a strong correlation between FMD and the number of diseased vessels and maximum percent diameter stenosis. In our study, we found a strong correlation between vessel score, modified Gensini score and FMD% and NMD%.

Consequently, cardiovascular risk factors may cause ED in both healthy individuals and patients with CAD. Noninvasive techniques such as exercise electrocardiogram (ECG), stress echocardiography, myocardial perfusion scintigraphy, positron emission tomography, and cardiac magnetic resonance imaging allow earlier diagnosis of CAD in order to prevent cardiovascular diseases. However, new diagnostic tests for the early detection of disease that are lower in cost and higher in sensitivity and specificity are required. Brachial artery Doppler USG is a non-invasive and reproducible method that elicits diagnosis of premature atherosclerosis in a subclinical stage.[32-34] Additionally, impairment in endothelial function, assessed by the brachial artery Doppler USG method, is closely related to the angiographic extent and severity of CAD. It is important to detect subclinical atherosclerosis and prevent cardiovascular diseases.

## Study limitations

The major limitation of our study is the small number of patients in the study population. Brachial artery Doppler USG is a widely used, reproducible and reliable method, based on the current literature. Nevertheless, long-term studies involving larger study populations are warranted to establish the beneficial effect of brachial artery Doppler USG technique in predicting

cardiovascular events at a subclinical stage.

In conclusion, we observed that ED was more prevalent in the CAD(+) group and that the degree of ED correlated well with the extent and severity of CAD. Based on these results, we suggest that evaluation of ED with a non-invasive ultrasonographic method may be beneficial for the diagnosis and prediction of the severity of CAD.

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

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