ENDOTHELIAL DYSFUNCTION MARKERS IN LOW CARDIOVASCULAR RISK INDIVIDUALS: COMPARISON OF MALES AND FEMALES

MARKERI ENDOTELNE DISFUNKCIJE KOD OSOBA SA NISKIM KARDIOVASKULARNIM RIZIKOM: POREĐENJE MUŠKARACA I ŽENA

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Summary

Background: Cardiovascular diseases (CVD) account for approximately 50% of the total deaths in Turkey. Most of them are related with atherosclerotic coronary heart disease. Predictive value of endothelial dysfunction markers related with the earliest stage of atherosclerosis has been getting more attention. We hypothesized that differences in endothelial dysfunction biochemical markers among genders would aid to capture proatherogenic activity that was not diagnosed by conventional risk assessment scoring systems.

Methods: We assessed the endothelial dysfuntion markers in 92 Turkish adults who were in the »low CV risk group« according to ESC (European Society of Cardiology)-Score Risk Charts. We compared the males and females.

Results: We observed higher endothelial dysfunction rates in males, with higher median and mean levels of e-NOS, ox-LDL before and after adjustment for HDL lowness and obesity (P=0.018, P=0.036 for NOS; P=0.000, P=0.004 for ox-LDL, respectively). Men had higher hs-CRP levels than females before adjustment (P=0.021). Decreased e-NOS levels were related with FMD for females before adjustment for confounders (P=0.028). We also found significant correlation between e-NOS and ox-LDL levels both before (r=0.360, P<0.001) and after adjustment (r=0.366, P<0.01) for confounders which pointed out the nitrosative stress. In multivariate regression

Kratak sadržaj

Uvod: Kardiovaskularne bolesti (KVB) odgovorne su za približno 50% ukupnog broja smrtnih slučajeva u Turskoj. Većina njih povezana je sa aterosklerotskom koronarnom bolešću srca. Sve više pažnje pridaje se prediktivnoj vrednosti markera endotelne disfunkcije koji su povezani s najranijim stupnjem ateroskleroze. Naša teza je da razlika u biohemijskim markerima endotelne disfunkcije između polova može pomoći da se otkrije proaterogena aktivnost koja nije dijagnostikovana uobičajenim sistemima za procenu rizika.

Metode: Odredili smo markere endotelne disfunkcije kod 92 odraslih Turaka koji su spadali u grupu sa »niskim rizikom od KVB« prema tabelama za bodovanje rizika Evropskog društva za kardiologiju (EDK). Uporedili smo muškarce i žene.

Rezultati: Uočili smo višu stopu endotelne disfunkcije kod muškaraca, sa višim medijanama i srednjim nivoima e-NOS, ox-LDL pre i posle prilagođavanja za nizak HDL i gojaznost (P=0,018, P=0,36 za NOS; P=0,000, P=0,004 za ox-LDL). Muškarci su imali više nivoe hs-CRP nego žene pre prilagođavanja (P=0,021). Sniženi nivoi e-NOS bili su povezani sa protokom uzrokovanom dilatacijom kod žena pre prilagođavanja za druge relevantne faktore (P=0,028). Takođe smo utvrdili postojanje značajne korelacije između e-NOS i ox-LDL nivoa kako pre (r=0,360, P<0,001) tako i posle prilagođavanja (r=0,366, P<0,01) za druge relevantne faktore, što je ukazalo na nitrozativni stres. U analizama multivarijantne regresije, posle prilagođavanja za druge markere

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analyses, after adjusting for other endothelial dysfunction markers which were not included in the ESC-risk scoring system, decreased e-NOS levels were independently asssociated with impaired flow mediated dilatation for females (odds ratio 0.3; P=0.038).
Conclusions: Our results underline the importance of gender in evaluating endothelial dysfunction biochemical markers to assess cardiovascular risk for low CV risk indivuals.

Keywords: low cardiovascular (CV) risk, endothelial dysfunction, flow mediated dilatation (FMD), gender, oxidized LDL (ox-LDL)

endotelne disfunkcije koji nisu uključeni u sistem za bodovanje rizika EDK, sniženi nivo e-NOS bio je kod žena nezavisno povezan sa smanjenom dilatacijom uzrokovanom protokom (odnos mogućnosti 0,3; P=0,038).
Zaključak: Naši rezultati podržavaju važnost uloge pola u određivanju biohemijskih markera endotelne disfunkcije sa ciljem procene kardiovaskularnog rizika kod osoba sa niskim rizikom od KVB.

Ključne reči: nizak kardiovaskularni rizik, endotelna disfunkcija, protokom uzrokovana dilatacija, pol, oksidovani LDL (ox-LDL)

Introduction

The mechanism of atherosclerosis has been well investigated since 1950 in different aspects such as lipid mechanism (1), role of oxidative stress (2), and endothelial function (3, 4). It is well characterized by chronic oxidative stress and inflammatory changes in the vascular tissue play a crucial role in coronary atherosclerosis pathogenesis (2). Endothelium maintains the balance between vasodilation and vasoconstriction, inhibition and stimulation of smooth muscle cell proliferation, migration, thrombogenesis and fibrinolysis (5, 6). When the vasomotor functions of endothelium are impaired, endothelial dysfunction occurs and causes damage to the wall. Damage to the endothelium promotes substantial events and provokes atherosclerosis by increasing endothelial permeability, platelet aggregation, leukocyte adhesion (2).

Endothelial cells, circulating platelets and proteins of the coagulation and fibrinolytic systems are known to contribute to the hemostatic processes. Activation level of platelet is shown by the variation of platelet activity and functions in inflammatory diseases, specifically in CVD. Platelets localized in intravascular compartment and platelet-specific secretory granule products are also increased in CVD. This situation shows the intravascular platelet activation.

There has been growing interest in the links between endothelial dysfunction and CV risk factors. Several studies have shown that CV risk factors can promote development of endothelial dysfunction in persons with no clinical evidence of coronary disease. Traditional risk factors such as hypercholesterolemia, hypertension, smoking, family history, diabetes, obesity which predispose a person to the development of atherosclerosis are also associated with endothelial dysfunction (7–9).

Among the traditional risk factors, sex has a crucial importance in the progression of cardiovascular diseases due to preponderance of mortality and morbidity rates across genders. The progression rate of vascular diseases also differs among genders. These differences may also suggest gender-based mechanisms underlying cardiovascular diseases. Differences in coronary flow reserve and atheroma burden have also been observed between males and females.

Some studies reported that women had slightly lower coronary flow reserves even with normal angiographic results (10). Beside, the role of gender in atherosclerosis still remains unclear.

In some studies, brachial FMD has been stated as an independent predictor of cardiovascular events (11, 12). Despite the data linking FMD and cardiovascular diseases, most of them are limited primarily to subjects with high risk for CVD events. In daily practice we use traditional CV risk factors for predicting CVD risk, however, impact of untraditional CV risk factors such as endothelial dysfunction markers is not clear.

Current approaches support the assumption that combinations of non-invasive vascular indices with traditional and untraditional CV risk factors may serve as cumulative risk markers for the assessment of subclinical vascular diseases. To contribute the current approach, we assessed the relationship between FMD and endothelial dysfunction biomarkers among genders in low CV risk indivuals.

Material and Methods

One hundred thirty-six patients applied to Cerrahpasa Medical School Department of Family Practice at University of Istanbul and were enrolled in this study. The study protocol was previously reviewed and approved by the Ethics Committee of University of Istanbul, Cerrahpasa Medical School (Issue Number: 12793, April 5, 2011). Written informed consent was obtained from all participants. All blood samples were collected in accordance with the Declaration of Helsinki. Patients with chronic liver disease, chronic renal failure, cancer, serious systemic infections, chronic lung disease, or any endocrine disease were excluded. CV risk of the patients was evaluated according to the European Society of Cardiology (ESC) risk score system which includes age, blood pressure, smoking, total cholesterol and gender (13, 14). Among 136 patients, 92 low CV risk individuals were selected. Total cholesterol (TC), high density lipoprotein (HDL), low density lipoprotein (LDL), triglyceride (TG) and high sensitive C-reactive protein (hs-CRP) levels were analyzed in the autoanalyzer of the faculty's central biochemistry laboratory.

Plasma oxidized LDL levels (Wuhan EIAAB Science, Wuhan China), ADMA levels (Wuhan EIAAB Science, Wuhan China), E-Selectin levels (Wuhan EIAAB Science, Wuhan China), vWF levels (Assaypro, Missouri, USA), endothelial nitric oxide synthase (e-NOS) levels (Wuhan EIAAB Science, Wuhan China) were measured. Platelet function was evaluated by an ADP-induced platelet aggregation method conventionally measured by optical density (15). Blood was obtained from venipuncture and collected into vacutainer tubes containing 3.2% sodium citrate for platelet function. Platelet functional studies were completed within 2 hours of blood drawing.

Platelet reactivity *ex vivo* was assessed by PRP (platelet rich plasma) aggregometer using a Chrono-Log after stimulating samples with arachidonic acid (0.5 mmol/L), collagen (1 μg/mL), or adenosine diphosphate (ADP) (1 μmol/L). Peak aggregation within 5 minutes of agonist stimulation was recorded in ohms and 5 μL od ADP was added to 495 μL sample for a final concentration of 10 μmol/L. PRP (500 μmol/L) was put in tube 3×10^8 platelet/mL was included. Then, the tube was transferred to the sample containers of the aggregometer and kept at 37 °C for 3 minutes, afterwhich it was treated with 1 μmol/L ADP (Chrono log) for 3 minutes. Platelet aggregation was observed and the aggregation curve taken from the aggregometer (Chronolog 500, USA) was evaluated in terms of slope and amplitude percentage (slope Ω, amplitude activity %) (16, 17). Sialic acid was measured by a colorimetric method (18).

We evaluated endothelial function based on the measurement of flow-mediated dilatation (FMD) using brachial artery ultrasonography. Brachial artery ultrasonography was performed in the Echocardiography Laboratory, Department of Cardiology, Cerrahpasa Medical School. A transducer connected to a Vingmed System V ultrasound instrument (GE Healthcare, Little Chalfont, Buckinghamshire, UK) at the appropriate frequency was used to achieve this aim. After 12 hours of fasting, examination was performed by two experienced practitioners in a quiet room at 20−24 °C. Longitudinal images of the brachial artery were taken from the antecubital fossa. Baseline brachial artery diameter measurements of patients were made after being rested for at least 10 minutes. A sphygmomanometer cuff was inflated to 300 mmHg and this pressure was maintained for 4−5 minutes. The second measurement was made 45−60 seconds after removing the inflated cuff (reactive hyperemia). Patients had 15 minutes rest. Then, 0.5 mg diluted glyceryl trinitrate (GTN or nitroglycerine) was administered and the final measurement was made after 3−4 minutes. Vessel diameters and flow rates measured after reactive hyperemia and administration of GTN were compared with resting values. FMD results were calculated according to the method described by Celermajer et al. (19, 20). Normal healthy FMD % response was accepted as 7–10 (21, 22).

Analysis of data was done with SPSS statistical analysis software (version 20.0; SPSS Inc, Chicago, IL, USA). Results were expressed as means ± SD or median and interquartile range. Hs-CRP, ADMA, e-NOS, levels were logarithmically transformed to achieve normal distributions. General linear measurement analyses were used for anthropometric and metabolic parameters before and after adjustments for obesity, HDL lowness or group means were compared using analysis of covariance (ANCOVA). Power analysis was done by the General Linear Model where observed powered > 0.8 is defined high power, > 0.14 is defined large effect. Cut-off values for HDL cholesterol < 1.01 mmol/L for men and < 1.27 mmol/L for females were defined as low; LDL cholesterol > 3.36 mmol/L was defined as high; triglyceride level > 2.26 mmol/L was defined as high; hypertension was defined as SBP/DBP ≥ 140/90 mmHg; obesity was defined as BMI > 30 kg/m^2 (14). Because of the variability in HDL levels and obesity ratio among genders, analysis was done before and after adjustment for the confounders. Correlation analysis was done by Pearson correlation analysis after adjustment for obesity and HDL lowness. Multiple stepwise regression analysis was applied to predict the variables that independently and significantly contributed to the dependent variable (FMD). All analyses were two-tailed, and *P*-values less than 0.05 were considered statistically significant.

Results

Clinical characteristics of 92 subjects according to the ESC- risk score system were summarized in *Table I*. Mean age of female and male subjects was similar (45±6 vs 47±7, P>0.05). Incidence of hypertension was 12% for females and 33% for males (P>0.05). Thirty-six percent of females and 54% of males were smoker (P=0.035). Total cholesterol levels of females and males were 5.61±1.03 mmol/L and 5.48±1.14 mmol/L respectively, P>0.05).

Patient characteristics among genders which were not included in the ESC risk score system are seen in *Table II*; the prevalence of family history, LDL and triglyceride highness were 59.2%, 44.9%, 10.6% for women and 53.5%, 62.8%, 18.6% for men, respectively. There was a significant difference for HDL lowness with 23.4% for women and 34.9% for men, respectively (X^2=90.000, P=0.000). Obesity ratio differed between genders with a higher ratio for males (16.3% vs 45.2%, respectively). The prevalence of impaired FMD was 51% for females and 46.5% for men, respectively. The distributions of e-NOS, ox-LDL, ADMA, CRP and e-selectin are seen in *Figure 1*. Endothelial nitric oxide synthase (e-NOS), oxidized LDL (ox-LDL) and high sensitive CRP (hs-CRP) levels significantly differed between genders. Males had higher median and mean levels of e-NOS (P=0.018), OX-LDL (P=0.000) and hs-CRP

Table I Patient characteristics among genders according to the ESC risk score system.

	Female N=49	Male N=43	*P*-Value
Age	45±6	47±7	NS
Hypertension (%)	12.2	32.6	0.023
Smoking (%)	32.7	53.5	0.035
Total Cholesterol (mmol/L)	5.61±1.03	5.48±1.14	NS

Data are means ± SD. P values were calculated using student's t test analysis. Noncategorical parameters were compared by chi-square test. NS: not significant, significance level: P<0.05

Table II Patient characteristics among genders which are not included in the ESC risk score system.

	Female N=49	Male N=43	*P*-Value
Family history (%)	59.2	53.5	NS
HDL lowness			X2=90.000/0.000
< 1.27 mmol/L (%)	23.4	–	
< 1.01 mmol/L (%)	–	34.9	
LDL > 3.36 mmol/L (%)	44.9	62.8	NS
Triglyceride > 2.26 mmol/L (%)	10.6	18.6	NS
Obesity (BMI > 30 kg/m^2) (%)	16.3	45.2	X^2=9.059 /0.003
First diameter (mm)	3.57±0.6	4.14±0.8	0.000
Second diameter (mm)	3.89±0.7	4.50±0.7	0.000
FMD (mm)	3.97±0.7	4.64±0.8	0.000

Noncategorical parameters were expressed as number or percentage and compared by chi-square test. NS: not significant

Table III Endothelial dysfunction biomarkers gender adjusted for obesity, HDL levels.

	Female	Male	P-Value[a]	P-Value[b]
CRP (mg/L)	1.35 (0.50–2.20)	1.45 (0.92– 3.33)	0.021	NS
Oxidized LDL (ng/mL)	2.35±1.16	3.64±1.67	0.000	0.004
ADMA (µmol/L)	0.15 (0.07–0.45)	0.12 (0.06–0.35)	NS	NS
E-Selectin (ng/mL)	2.09±1.31	3.00±1.24	0.065	NS
vWF (mU/mL)	1499.9±700.2	1362.2±682.7	NS	NS
e-NOS (pg/mL)	60.81 (44.4–131.9)	145.01 (58.1– 276.3)	0.018	0.036
ADP E (%)	61.12±9.05	58.55±11.74	NS	NS
ADP A (%)	77.98±13.01	72.23±13.23	0.056	NS
Sialic acid (mmol/L)	1.50±0.7	1.51±0.8	NS	NS

Data are presented as means ±SD or as median (interquartile range) for non-normally distributed variables. *P* values were calculated using ANCOVA, and values for CRP, ADMA and NOS levels were logarithmically transformed before analysis. Non-transformed values are shown. [a] represents unadjusted P – Value across gender, [b] represents P – Value adjusted for obesity and HDL levels. NS: not significant, significance level: P<0.05

(P=0.021). However, there were no significant differences for ADMA, e-selectin, sialic acid, vWF, ADP slope and ADP amplitude % among genders (*Table III*). e-NOS activity and ox-LDL levels remained significant after adjustment for HDL lowness and obesity (P=0.036, P=0.004, respectively).

We next analyzed whether the presence of obesity and HDL lowness would modulate endothelial function. To test this hypothesis, first, we compared the levels of endothelial dysfunction biomakers among genders before and after adjustment for obesity and HDL levels (*Table III*). Male subjects had significantly higher CRP (P=0.021), OX-LDL
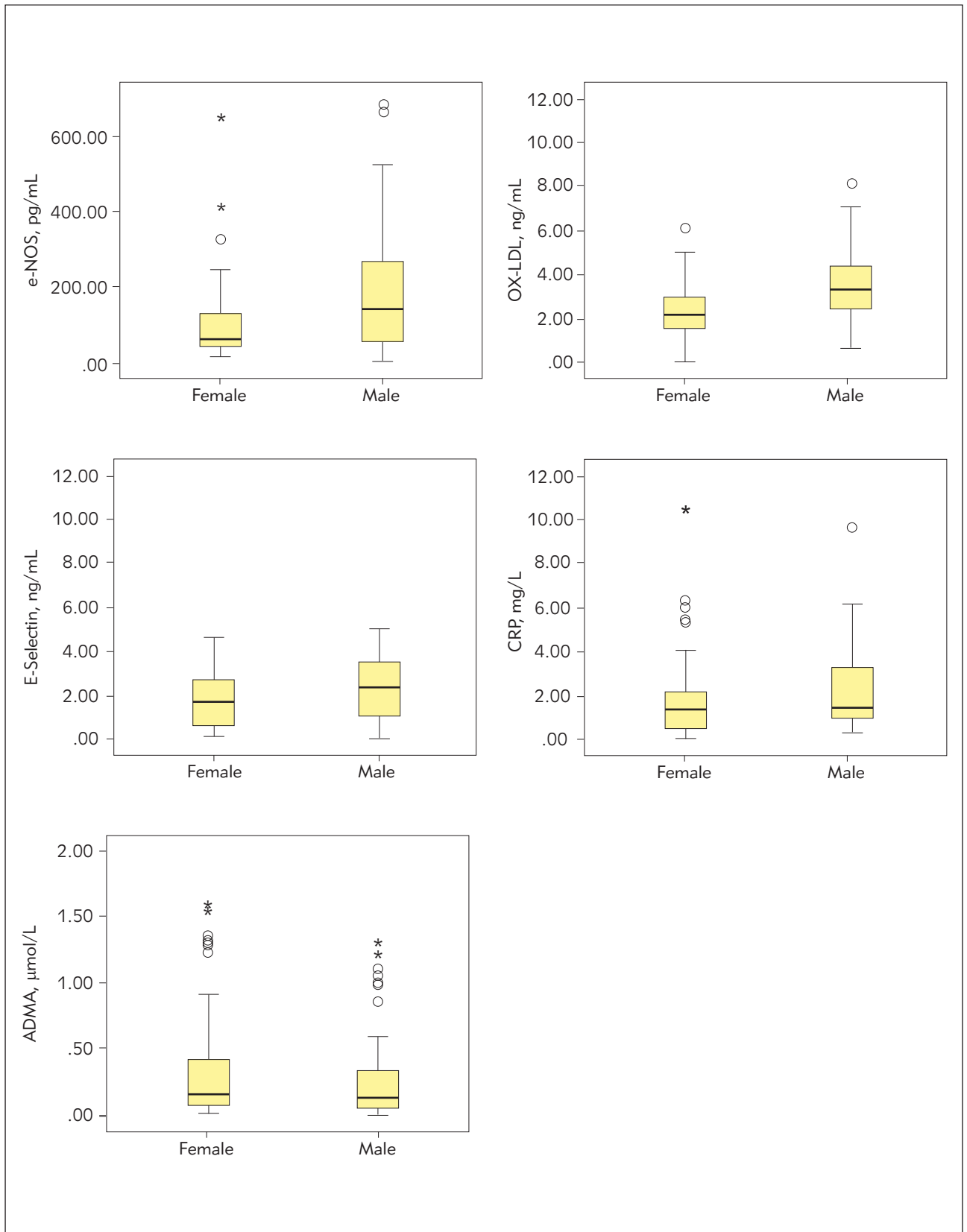
Figure 1 Distribution of e-NOS, ox-LDL, ADMA, CRP and E-Selectin across gender. Results were based on 45 female and 42 male subjects. NS, Not significant. Data represent median and interquartile range. o, Outliners. P values were calculated using Student's t test and values for ADMA, NOS, CRP levels were logarithmically transformed before analysis. Nontransformed values are shown in the graphs.

Table IV Endothelial dysfunction biomarkers gender adjusted for obesity, HDL levels.

	Female	P-Value[a]	Male	P-Value[b]
Endothelial function normal				
CRP (mg/L)	1.12 (0.55–2.72)	NS	1.79 (1.09–2.98)	NS
Oxidized LDL (ng/mL)	2.41±1.21	0.004	3.85 ±1.88	NS
ADMA (µmol/L)	0.36 (0.09–0.61)	NS	0.12 (0.06–0.33)	NS
E-Selectin (ng/mL)	2.18±1.43	NS	2.72±1.58	NS
vWF (mU/mL)	1559.13±646.62	NS	1300.16±748.91	NS
e-NOS (pg/mL)	77.7 (55.9–218.8)[a]	NS	145.1 (58.1–281.8)	NS
ADP E (%)	60.69±8.98	NS	60.36±12.05	NS
ADP A (%)	80.96±10.59	NS	74.05±14.45	NS
Sialic acid (mmol/L)	45.13±18.29	NS	41.52±21.69[d]	
Endothelial dysfunction				
CRP (mg/L)	1.43 (0.46–2.04)	NS	1.31 (0.71– 4.11)	NS
Oxidized LDL (ng/mL)	2.29±1.13	0.008	3.40±1.40	0.019
ADMA (µmol/L)	0.11 (0.06–0.20)	NS	0.14 (0.05–0.89)	NS
E-Selectin (ng/mL)	1.54±1.13	NS	2.04±1.13	NS
vWF (mU/mL)	1438.03 ±761.60	NS	1433.48±608.9	NS
e-NOS (pg/mL)	50.4 (30.8–94.7)[a]	NS	135.7 (54.0–239.9)	NS
ADP E (%)	61.60±9.32	NS	56.17±11.25	NS
ADP A (%)	74.69±14.81	NS	69.84±11.43	0.020
Sialic acid (mmol/L)	52.78±25.48	NS	52.80±24.70[d]	NS

Data are presented as means ± SD or as median (interquartile range) for non-normally distributed variables.
P values were calculated using student's t test analysis, and values for CRP, ADMA and NOS levels were logarithmically transformed before analysis. Nontransformed values are shown. [a]represents comparison of e-NOS levels among endothelial function for female gender (P=0.028 before adjustment for obesity and HDL, P=0.065 after adjustment for confounders), [b] represents P Value for unadjusted levels, [c] represents P, Value after adjustment for obesity and HDL, [d]represents comparison among endothelial function for male gender (P=0.038 for adjusted level). NS: Not significant, significance level: P<0.05

(P=0.000), e-NOS (P=0.018) levels than female counterparts for the unadjusted model; only ox-LDL and e-NOS levels remained significant after adjustment for obesity and HDL lowness (P=0.004, P= 0.036 respectively). ADP E and ADP A which show platelet function were decreased in men as compared to female counterparts but this decline did not reach any significance. To check our significant results, we conducted a power analysis and the effect size to show the statistical power. The effect of this size (.417 partial eta squared) to be detected (99% chance) as significant at the (P=0.001).

We next examined whether the difference in endothelial dysfunction biomarkers between genders might be confounded by FMD, obesity and HDL lowness. To argue this hypothesis, we compared the same endothelial dysfunction biomarkers across FMD between genders before and after adjustment for confounders (*Table IV*). In subjects with normal FMD, males had higher ox-LDL as compared to females before adjustment (P=0.004); sialic acid levels were significantly lower for same counterparts in an adjusted model (P=0.045). In subjects with abnormal

FMD, males had significantly higher ox-LDL than females in both unadjusted and adjusted models (P= 0.008, P=0.019 respectively). Platelet aggregation marker ADP A showed a significant decrease in males compared to females only after an adjustment model (P=0.020). Then, we compared the same biomarkers among FMD for each gender. In females, e-NOS levels were significantly lower for subjects with endothelial dysfunction before adjustment (P=0.028) and the significance did not remain after adjustment. In males, sialic acid levels were significantly higher for subjects with endothelial dysfunction only after adjustment for confounders (P=0.038).

The intercorrelations between endothelial dysfunction biomarkers with and without adjustments for obesity and HDL lowness are shown in *Table V*. E-selectin/e-NOS (r=.366, P < 0.001) and e-NOS/ox-LDL (r=0.360, P < 0.001) significantly and positively correlated with each other before adjustment. ADMA significantly but negatively correlated with e-NOS (r=–0.298, P<0.01), e-selectin (r=–0.361, P < 0.001) and ox-LDL (r=–0.351, P < 0.001) before adjustment. Notably, a relationship between e-selectin/e-NOS

Table V Correlations (r) between endothelial dysfunction biomarkers with and without adjustment for confounders[a].

	e-NOS	E-Selectin	ox-LDL	CRP	ADMA
Unadjusted					
e-NOS	1.000	0.366**	0.360**	0.144	-0.298*
E-Selectin	0.366**	1.000	0.136	0.218*	-0.361**
ox-LDL	0.360**	0.136	1.000	0.033	-0.351**
CRP	0.144	0.218*	0.033	1.000	-0.017
ADMA	-0.298*	-0.361**	-0.351**	-0.017	1.000
vWF	0.224	0.038	0.061	0.140	-0.125
ADP E	0.025	-0.013	-0.117	0.070	0.206
ADP A	-0.036	0.017	-0.203	0.047	0.182
Sialic acid	-0.082	-0.206	0.085	-0.130	0.079
FMD	0.122	0.213*	0.225*	0.146	-0.010
Adjusted[a]					
e-NOS	1.000	0.344*	0.366*	0.077	-0.204
E-Selectin	0.344*	1.000	0.459**	0.180	-0.266*
ox-LDL	0.366*	0.459**	1.000	0.085	-0.312*
CRP	0.077	0.180	0.085	1.000	0.115
ADMA	-0.204	-0.266*	-0.312*	0.115	1.000
vWF	0.158	0.104	-0.095	0.127	0.009
ADP E	0.054	-0.103	-0.088	0.040	0.232
ADP A	-0.052	-0.061	-0.103	-0.007	0.199
Sialic acid	-0.055	-0.158	-0.096	-0.035	0.069
FMD	-0.001	0.223	-0.034	-0.012	-0.046

Values for CRP, ADMA and e-NOS levels were logarithmically transformed before analysis. [a]Obesity, HDL lowness. ** $P < 0.001$, * $P < 0.01$

Table VI Multiple stepwise logistic regression analysis relating e-NOS level to the presence of FMD across gender.

Models	Female		Male	
	OR (%95 CI)	*P* – Value	OR (%95 CI)	*P* – Value
A. e-NOS level		0.038	0.8 (0.4–1.5)	NS
Model 1A: e-NOS level only	0.3 (0.1–0.9)			
e-NOS level		0.030	0.93 (0.42–2.07)	NS
Model 2A: Other endothelial	0.15 (0.03–0.83)	NS	0.35 (0.05–2.45)	NS
dysfunction factors	0.96 (0.06–15.19)	0.050	0.09 (0.005–1.46)	NS
e-NOS level	74.7 (0.99–5589.5)	NS	0.85 (0.10–7.34)	NS
Obesity	0 (0–0)	NS	0.73 (0.31–1.71)	NS
Triglyceride	0.91 (0.33–2.53)	0.016	1.92 (0.84–4.39)	NS
HDL lowness	0.24 (0.08–0.77)	NS	0.77 (0.35–1.72)	NS
Oxidized LDL	0.53 (0.20–1.41)			
ADMA				
E-Selectin				

CI, Confidence interval; NS, not significant

(r=0.344, P < 0.01), ox-LDL/e-NOS (r=0.366, P < 0.01) and negative relationships between e-selectin/ADMA (r=−0.266, P < 0.01), ox-LDL/ADMA (r=−0.312, P < 0.01) remained significant after adjustments for the confounders.

Finally, we performed a stepwise multiple logistic regression analysis to identify variables that independently and significantly contributed to the presence of endothelial dysfunction which was detected by FMD (*Table VI*). In a univariate analysis, decreased levels of e-NOS were associated with FMD in the female gender [odds ratio (OR) 0.3, P < 0.05]. Further, in a multivariate logistic regression model, after adjustment for other risk factors, e-NOS was independently associated with FMD among females [odds ratio (OR) 0.13, P <0.05].

Discussion

In the present study, we investigated the association of endothelial dysfunction markers among genders in low CV risk individuals. Males have higher CRP, oxidized LDL, e-selectin and e-NOS levels than females. The positive relationship between e-selectin/e-NOS, ox-LDL/e-NOS, ox-LDL/e-selectin and negative relationship between ADMA/e-selectin and ADMA/ox-LDL were not affected by BMI and HDL lowness. However, the negative relationship between ADMA and e-NOS was confounded by BMI and HDL lowness in our study group. We also noted several important differences and associations among genders with respect to FMD. First, ox-LDL levels differed between genders in subjects with endothelial dysfunction and this difference was irrelevant of BMI and HDL lowness. Second, sialic acid levels (indicator of antioxidant defense system) were higher for males in endothelial dysfunction group compared to normal endothelial function group. Third, decrease in NOS activity was independently associated with FMD only for female gender. Our results underline that these biomarkers might capture different aspects of traditional predictors for each gender. Furthermore, untraditional cardiovascular risk factors affect each sex divergently even for low CV risk individuals which were not captured by the conventional risk score systems.

The prevalence of CVD is lower for women than men, and the effects of traditional biomarkers on CVD risk differ among them. Further, both of these factors might have an impact on CVD risk. The incidence of first cardiovascular events in men is higher for young men and is increasing very fast along with age, whereas women are 10 years behind them (23). A meta-analysis of 23 prospective studies revealed that apolipoproteins had moderately strong associations with risk of CHD, and these associations differed among men and women (24).

Although CVD has been known as a male disease, there is accumulating evidence on the different impact of major CV risk factors leading to a worse outcome in women, and female-specific risk factors are of influence in the onset of CVD (25). One of the modifiable risk factors of CVD is smoking. The global prevalence of smoking is almost 5 times higher for men than in women (48% vs 10%) (26). Recent researches showed that women who smoked had a 50% greater risk compared to male counterparts (27). Other prospective studies have shown that the prevalence of overweight in men and women depends on the development of the country and levels of BMI are mostly higher in men than women, however, the association between BMI and coronary heart disease is similar between men and women (28).

There are also some biological differences between men and women related to the size of arteries, coronary flow reserve and atheroma burden. Women have smaller carotid arteries (29, 30), with less plaque but more apparent stenosis (31) which may relate to differences in remodeling. Furthermore, it has been suggested that men have greater atheroma burden, more eccentric atheroma, and more diffuse epicardial endothelial dysfunction than women (32). However, women had slightly lower coronary vasodilatory reserves even with normal coronary angiographic results (33).

Current evidence suggests that endothelium has a crucial impact on CV risk and CV risk factors, including traditional or untraditional factors which were also related with endothelial dysfunction. Further, many of them are associated with overproduction of reactive oxygen species or increased oxidative stress (34) and contribute to atherosclerosis development and progression (34–37). Reactive oxygen species may also react with NO, reduce NO bioavailability and improve vascular damage (37). In agreement with this study and others, we observed significantly higher ox-LDL and e-NOS levels for men, before and after adjustment for confounders. Within male individuals, we stratified subjects according to FMD. Subjects with impaired FMD had higher sialic acid levels irrelevant of BMI and HDL lowness. In the same counterparts, ox-LDL levels and e-NOS level were decreased but this decline did not reach any significance. Increase in sialic acid levels which accompanied the decline in ox-LDL levels and e-NOS level might be the consequence of antioxidative and nitrosative defence systems. Iijima et al. stated that sialic acid consumes toxic hydrogen peroxide (H_2O_2) under physiologic conditions and acts as a radical scavenger (38). Other studies suggest that mucin – a sialic acid – storage-synthesis is induced by oxidative stress (39).

To the best of our knowledge, traditional CV risk factors affect both genders differently, and endothelial dysfunction is another contributor of atherosclerosis which is as a systemic disorder (40). It can be detected noninvasively by flow-mediated dilatation (FMD) in

the brachial artery that is closely associated with endothelium dependent vasomotion in the coronary circulation (40).

In our study, impaired vascular tone defined by FMD differed between sexes which did not reach significance. We performed multiple logistic regression analyses to determine whether e-NOS would independently contribute to the presence of FMD beyond traditional and untraditional risk factors among genders. In a univariate analysis, we observed a significant association of e-NOS level with the presence of impaired FMD only in females. When taking other risk factors into account, the picture did not change for both genders and the e-NOS level remained significant for females. There are lots of studies demonstrating that estrogen markedly improves endothelium-dependent vasodilator responses to various physiological stimuli. This effect is likely mediated through the activation of endothelial nitric oxide synthase and the antioxidant effect of estrogen (41–42). Our female individuals are in their late forties and might have decrease in estrogen levels. There were also significant correlations between ox-LDL and e-NOS levels, and negative significant correlations between ox-LDL and ADMA levels before and after adjustment for confounders which support the relationship between oxidative stress and vascular function.

There were also several abnormalities which might favour platelet activation that have been reported in CVD, including endothelial dysfunction (43) and increased oxidative stress (35). Platelet activation resulting from disrupted plaque might be another marker for atherosclerosis. Gremmel et al. stated that females express significantly more pronounced formation of leukocyte-platelet aggregates than males (44). Cowman et al. showed that age related changes in platelet function were more profound in women than in men indicating that age and gender significantly impact on platelet interactions with VWF. Some researchers showed that vWF levels increased during the acute phase of endothelial dysfunction (45). In our study, ADP amplitude was higher in female than male subjects. Further, ADP amplitude was also higher in females than males for the endothelial dysfunction group after adjustment for confounders. We also found vWF level higher in females as compared to male counterparts but this increase remained insignificant. vWF showed an inverse pattern across genders among endothelial function; it decreased for female whereas increased for male which was not significant.

We acknowledge some of the limitations of this study. Patients taking any medication that could affect FMD and biomarker measurements including statins and antihypertensive agents were excluded from the study. Samples were collected from apparently healthy healthcare workers from Cerrahpasa Medical School who were in the same age range. Our sample size is small. Female individuals menapausal status were not evaluated. We defined endothelial dysfunction by FMD and determined risk score according to European Society of Cardiology score Risk Charts.

Conclusion

Endothelial dysfunction biochemical markers differ between genders in low CV risk indivuals and the difference pointed out male gender had more risk for CVD than female that were not captured by ESC-score and other conventional risk score systems; however, additional studies are needed to verify these results in big populations. Our results underline the different medical practice for each gender might reduce chronic vascular diseases in early stages which may also decrease further healthcare costs.

Conflict of interest statement

The authors stated that they have no conflicts of interest regarding the publication of this article.

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