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

Zeynep B. Gungor¹, Nurver Sipahioglu², Huseyin Sonmez¹, Hakan Ekmekci¹, Sait Toprak¹, Gulsel Ayaz², Cigdem Bayram Gurel³, Tugba Mutlu³, Turgut Ulutin³, Fikret Sipahioglu⁴, Bars Ilerigelen⁵

¹Department of Biochemistry, ²Department of Family Medicine, ³Department of Medical Biology, ⁴Department of Internal Medicine, ⁵Department of Cardiology, Cerrahpasa Medical School, University of Istanbul, Istanbul, Turkey

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 dysfunction 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. Veštače pažnje pridaje se prediktivnoj vrednosti markeri 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 Tursaka 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: Očitili smo višu stopu endotelne disfunkcije kod muškaraca, sa višim medijanima i srednjim nivooma e-NOS, ox-LDL pre i posle prilagođavanja za nizak HDL i inesne faktore (P=0.018, P=0.036 za NOS; P=0.000, P=0.004 za ox-LDL). Muškarci su imali više nivoa hs-CRP nego žene pre prilagođavanja (P=0.021). Sniženi nivoi e-NOS bili su povezani sa protokom uzrokovanim dilatacijom kod žena pre prilagođavanja za druge relevantne faktore (P=0.021). Sniženi nivoi e-NOS bili su povezani sa protokom uzrokovanim 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 nitroazitivni stres. U analizama multivarijantne regresije, posle prilagođavanja za druge markere...
analyses, after adjusting for other endothelial dysfunction markers which were not included in the ESC-risk scoring system, decreased e-NOS levels were independently associated with impaired flow mediated dilatation for females (odds ratio 0.5; 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 individuals.

Keywords: low cardiovascular (CV) risk, endothelial dysfunction, flow mediated dilatation (FMD), gender, oxidized 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 vasodilatation 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. Endothelial dysfunction which predispose a person to the development of cardiovascular 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.

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 individuals.

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 vacuum-tainer 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 of 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, after which 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, Cerrahpasıa 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 power > 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).

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 the General Linear Model where observed power > 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 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
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 biomarkers 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.
(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 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=−.298, P<0.01), e-selectin (r=−.361, P < 0.001) and ox-LDL (r=−.351, P < 0.001) before adjustment. Notably, a relationship between e-selectin/e-NOS

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

| Endothelial function normal | Female | P-Value\(^a\) | Male | P-Value\(^b\) |
|-----------------------------|--------|--------------|------|--------------|
| 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) | 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 \(^d\) | NS |

| Endothelial dysfunction     | Female | P-Value\(^a\) | Male | P-Value\(^b\) |
|-----------------------------|--------|--------------|------|--------------|
| 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) | 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.
### Table V Correlations (r) between endothelial dysfunction biomarkers with and without adjustment for confounders.

|                | 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    |          |
| ADMA           | -0.298*   | -0.361**   | -0.351**  | -0.017   |          |
| vWF            | 0.224     | 0.038      | 0.061     | 0.140    | 1.000    |
| 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     | 0.215*     | 0.225*    | 0.146    | -0.010   |
| **Adjusted**   |           |            |           |          |          |
| 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    | 0.099    |
| 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. *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** |            |          |            |          |
| Model 1A: e-NOS level only | 0.3 (0.1–0.9) | 0.058    | 0.8 (0.4–1.5) | NS       |
| Model 2A: Other endothelial dysfunction factors |            |          |            |          |
| e-NOS level     | 0.15 (0.03–0.83) | 0.050    | 0.93 (0.42–2.07) | NS       |
| Obesity         | 0.96 (0.06–15.19) | NS      | 0.35 (0.05–2.45) | NS       |
| Triglyceride    | 74.7 (0.99–5589.5) | 0.050    | 0.09 (0.005–1.46) | NS       |
| HDL lowness     | 0 (0–0)     | NS      | 0.85 (0.10–7.34) | NS       |
| Oxidized LDL    | 0.91 (0.33–2.53) | NS      | 0.73 (0.31–1.71) | NS       |
| ADMA            | 0.24 (0.08–0.77) | 0.016    | 1.92 (0.84–4.39) | NS       |
| E-Selectin      | 0.53 (0.20–1.41) | NS      | 0.77 (0.35–1.72) | NS       |

CI, Confidence interval; NS, not significant
...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 levels 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 (H₂O₂) 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 decreased 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 favor 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 individuals 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.

**Acknowledgements.** This work was supported by the grant of University of Istanbul Research Foundation. Project number is 5322. We thank Prof. Dr. Orkide Donma for assistance in the statistical analysis.

**Conflict of interest statement**

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

1. Duff GL, McMillan GC. Pathology of atherosclerosis. Am J Med 1951; 11: 92–108.
2. Ross R. Atherosclerosis–an inflammatory disease. N Engl J Med 1999; 340: 15–26.
3. Steinberg D. Research related to underlying mechanisms in atherosclerosis. Circulation 1979; 60: 59–65.
4. Parthasarathy S, Quinn MT, Steinberg D. Is oxidized low density lipoprotein involved in the recruitment and retention of monocyte/macrophages in the artery wall during the initiation of atherosclerosis? Basic Life Sci 1988; 49: 75–80.
5. Lüscher TF, Barton M. Biology of the endothelium. Clin Cardiol 1997; 20 (suppl II): II-S-II-10.
6. Kinlay S, Libby P Ganz P. Endothelial function and coronary artery disease. Curr Opin Lipidol 2001; 12: 3–9.
7. John S, Schlaich M, Langenfeld M, Weihprecht H, Scharrer I, Zeiher AM. A positive family history of premature coronary artery disease is associated with impaired endothelium-dependent coronary blood flow regulation. Circulation 1999; 100: 1–6.
8. Vita JA, Treasure CB, Nabel EG, McLenachan JM, Fish RD, Yeung AC, Vekshtein VI, Selwyn AP, Ganz P. Coronary vasomotor response to acetylcholine relates to risk factors for coronary artery disease. Circulation 1990; 81: 1–7.
9. Schächeringer V, Britten MB, Elsner M, Walter DH, Scharrer I, Zeiher AM. A positive family history of premature coronary artery disease is associated with impaired endothelium-dependent coronary blood flow regulation. Circulation 1999; 100: 2–8.
10. Kern MJ, Bach RG, Mechem CJ, Caracciolo EA, Aguirre FV, Miller LW, Donohue TJ. Variations in normal coronary vasodilatory reserve stratified by artery, gender, heart transplantation and coronary artery disease. J Am Coll Cardiol 1996; 28: 54–60.
11. Brevetti G, Silvestro A, Schiano V, Chiariello M. Endothelial dysfunction and cardiovascular risk prediction in peripheral arterial disease: additive value of flow-mediated dilatation to ankle-brachial pressure index. Circulation 2003; 108: 2093–8.
12. Gokce N, Keaney JF, Jr. Hunter LM, Watkins MT, Menzoian JO, Vita JA. Risk stratification for postoperative cardiovascular events via noninvasive assessment of endothelial function: a prospective study. Circulation 2002; 13: 67–72.
13. Conroy RM, Pyorala K, Fitzgerald AP, Sans S, Menotti A, De Backer G, De Bacquer D, Ducimetière P, Jousilahti P, Keil U, Njølstad I, Oganov RG, Thomsen T, Tunstall-Pedoe H, Tverdal A, Wedel H, Whincup P, Williams L, Graham IM. SCORE project group. Estimation of ten-year risk of fatal cardiovascular disease in Europe: the SCORE project. Eur Heart J 2003; 24: 987–1003.
14. Authors/Task Force Members: Reiner ZE, Catapano AL, Backer GD, Graham Riitta Taskinen IMR, Wiklund O, Agewall S, Alegria E, Chapman MJ, Durrington P, Erdine S, Halcox J, Hobbs R, Kjekshus J, Filardi PP, Riccardi G, Storey RF, Wood D. ESC/EAS Guidelines for the management of dyslipidaemias. The Task Force for the management of dyslipidaemias of the European Society of Cardiology (ESC) and the European Atherosclerosis Society (EAS). European Heart Journal 2011; 32: 769–818.
15. Born GVR. Aggregation of blood platelets by adenosine diphosphate and its reversal. Nature 1962; 194: 7–9.
16. Tutluoglu B, Gurel CB, Ozdas SB, Musellim B, Er turan S, Anakkaya AN, Kilinc G, Ulutin T. Platelet function and fibrinolytic activity in patients with bronchial asthma. Clin Appl Thromb Hemost 2005; 11: 77–81.
17. Melanie McCabe, White BA. Platelet protocol research and clinical laboratory procedures printed in the United States of America, Academic Press Limited, 1999.
18. Warren L. The thiobarbituric acid assay of sialic acids. J Biol Chem 1959; 234: 1–5.
19. Celermajer DS, Sorensen KE, Bull C, Robinson J, Deanfield JE. Endothelium-dependent dilation in the systemic arteries of asymptomatic subjects relates to coronary risk factors and their interaction. J Am Coll Cardiol 1994; 24: 68–74.
20. Celermajer DS, Sorensen KE, Gooch VM, Spiegelhalter DJ, Miller OL, Sullivan ID, Lloyd JK, Deanfield JE. Noninvasive detection of endothelial dysfunction in children and adults at risk of atherosclerosis. Lancet 1992; 340: 1–5.
21. Corretti M, Anderson TJ, Benjamin EJ, Celermajer D, Charbonneau F, Creager MA, Deanfield J, Drexler H, Gerhard-Herman M, Herrington D, Vallance P, Vita J, Vogel R. International Brachial Artery Reactivity Task Force. Guidelines for the Ultrasound Assessment of Endothelial-Dependent Flow-Mediated Vasodilation of the Brachial Artery. A Report of the International Brachial Artery Reactivity Task Force. JACC 2002; 39: 257–65.
22. Ryan A, Harris Steven K, Nishi yama D, Walter WR, Richardson S. Ultrasound Assessment of Flow-Mediated Dilation. Hypertension 2010; 55: 75–85.
23. Mosca L, Benjamin EJ, Berra K, et al. Effectiveness-based guidelines for the prevention of cardiovascular disease in women – 2011 update: a guideline from the American Heart Association. Circulation 2011; 123: 43–62.
24. Thompson A, Danesh J. Associations between apolipoprotein B, apolipoprotein Al, the apolipoprotein B/Al ratio and coronary heart disease: a literature-based meta-analysis of prospective studies. J Intern Med 2006; 258: 81–92.
25. Appelman Y, van Rijn BB, Ten Haaf ME, Boersma E, Peters SA. Sex differences in cardiovascular risk factors and disease prevention. Atherosclerosis 2015; 241: 1–8.
26. Hitchman SC, Fong GT. Gender empowerment and female-to-male smoking prevalence ratios. Bull World Health Organ 2011; 89: 195–202.
27. Huxley RR, Woodward M. Cigarette smoking as a risk factor for coronary heart disease in women compared
with men: a systematic review and meta-analysis of prospective cohort studies. Lancet 2011; 378: 297–305.

28. Prospective Studies Collaboration, Whitlock G, Lewington S, Sherliker P, Clarke R, Emberson J, Halsey J, Qizilbash N, Collins R, Peto R. Body-mass index and cause-specific mortality in 900 000 adults: collaborative analyses of 57 prospective studies. Lancet 2009; 373: 1083–96.

29. Schulz UG, Rothwell PM. Sex differences in carotid bifurcation anatomy and the distribution of atherosclerotic plaque. Stroke 2001; 32: 25–31.

30. Krejza J, Arkuszewski M, Kasner SE, Weigele J, Ustymowicz A, Hurst RW, Cucchiara BL, Messe SR. Carotid artery diameter in men and women and the relation to body and neck size. Stroke 2006; 37: 3–5.

31. Lemolo F, Martiniuk A, Steinman DA, Spence JD. Sex differences in carotid plaque and stenosis. Stroke 2004; 35: 77–81.

32. Han SH, Bae JH, Holmes Jr DR, Lennon RJ, Eekhout E, Barsness GW, Rihal CS, Lerman A. Sex differences in atheroma burden and endothelial function in patients with early coronary atherosclerosis. Eur Heart J 2008; 29: 59–69.

33. Trifunović D, Stanković M, Marinković J, Banović M, Đukanović N, Vasić M, Vujisić-Tešić B, Petrović M, Stepanović J, Dorđević-Dikić A, Beleslin B, Nedeljković I, Tešić M, Ostojić M. Oxidized low density lipoprotein and high sensitive c-reactive protein in non-diabetic, pre-diabetic and diabetic patients in the acute phase of the first myocardial infarction treated by primary percutaneous coronary intervention. J Med Biochem 2015; 34: 60–69.

34. Van Deel ED, Octavia Y, De Boer M, Juni RP, Tempel D, Van Haperen R, De Crom R, Moens AL, Merkus D, Duncker DJ. Normal and high eNOS levels are detrimental in both mild and severe cardiac pressure-overload. J Mol Cell Cardiol 2015; 88: 45–54.

35. Di Pietro N, Formoso G, Pandolfi A. Physiology and pathophysiology of oxLDL uptake by vascular wall cells in atherosclerosis. Vascul Pharmacol 2016; 84: 1–7.

36. Stancel N, Chen CC, Ke LY, Chu CS, Lu J, Sawamura T, Chen CH. Interplay between CRP, Atherogenic LDL, and LOX-1 and Its Potential Role in the Pathogenesis of Atherosclerosis. Clin Chem 2016; 62(2): 320–7.

37. Besedina A. NO-synthase activity in patients with coronary heart disease associated with hypertension of different age groups. J Med Biochem 2016: 35: 3–9.

38. Iijima R, Takahashi H, Namme R, Ikegami S, Yamazaki M. Novel biological function of sialic acid (N-acetylneuraminic acid) as a hydrogen peroxide scavenger. FEBS Lett 2004; 561: 13–6.

39. Takeyama K, Dabbagh K, Jeong Shim J, Dao-Pick T, Ueki IF, Nadel JA. Oxidative stress causes mucin synthesis via transactivation of epidermal growth factor receptor: role of neutrophils. J Immunol 2000; 164: 46–52.

40. Thijsen DH, Black MA, Pyke KE, Padilla J, Atkinson G, Harris RA, Parker B, Widlansky ME, Tschakovsky ME, Green DJ. Assessment of flow-mediated dilation in humans: a methodological and physiological guideline. Am J Physiol Heart Circ Physiol 2011; 300: H2–12.

41. Duckles SP, Miller VM. Hormonal modulation of endothelial NO production. Pflugers Arch 2010 May; 459(6): 841–51.

42. Sack MN, Rader DJ, Cannon RO III. Oestrogen and inhibition of oxidation of low-density lipoproteins in postmenopausal women. Lancet 1994; 343: 69–70.

43. Andreas S, Daniela F, Lennart W, Johann B. Guanylyl cyclase activator ataciguat improves vascular function and reduces platelet activation in heart failure. Pharmacological Research 2010; 62: 2–8.

44. Gremmel T, Kopp CW, Eichelberger B, Koppensteiner R, Panzer S. Sex differences of leukocyte-platelet interactions and on-treatment platelet reactivity in patients with atherosclerosis. Atherosclerosis 2014; 237: 2–5.

45. Lip GY, Blann A. von Willebrand factor: a marker of endothelial dysfunction in vascular disorders? Cardiovasc Res 1997; 34: 55–65.

Received: July 29, 2016
Accepted: November 10, 2016