Original Article

Relation of Biochemical Parameters with Flow-mediated Dilatation in Patients with Metabolic Syndrome

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Abstract

Background: Metabolic syndrome (MetS) is one of the high cardiovascular (CV) situations. Endothelial dysfunction, which is a common finding in patients with MetS, is related with increased CV risk. In patients with MetS, the effect of the major CV risk factors, not included in the MetS definition, on endothelial dysfunction is not well known. The aim of this study was to determine the effect of major CV risk factors such as gender, smoking, family history, and biochemical parameters on endothelial dysfunction in patients with MetS.
Methods: The study was performed between December 2010 and August 2014. A total of 55 patients (15 females and 40 males) with MetS and 81 healthy controls (37 females and 44 males) with a body mass index <25 kg/m^2 were enrolled in the study. Endothelial dysfunction was measured by flow-mediated dilatation (FMD), oxidative stress parameters; high-sensitivity C-reactive protein (hs-CRP), oxidized low-density lipoprotein (ox-LDL), endothelial nitric oxide synthase (e-NOS), nitric oxide, and cell adhesion markers; von Willebrand factor, and e-selectin. Platelet aggregation (endothelial adenosine diphosphate), total platelet count, and mean platelet volume were additionally analyzed and demographic parameters were explored. Student's *t*-test, Mann-Whitney *U*-test, and Chi-square test were used to analyze the results.
Results: The fasting blood glucose ($z = 3.52$, $P = 0.001$), hs-CRP ($z = 3.23$, $P = 0.004$), ox-LDL ($z = 2.62$, $P = 0.013$), and e-NOS ($z = 2.22$, $P = 0.026$) levels and cardiac risk score ($z = 5.23$, $P < 0.001$) were significantly higher in patients with MetS compared with the control group. Smoking was correlated with decreased FMD ($\chi^2 = 9.26$, $P = 0.002$) in MetS patients but not in the control group.
Conclusions: Increased ox-LDL, hs-CRP, and e-NOS are likely to be a result of oxidative stress, a condition in which an imbalance occurs between the production and inactivation of reactive nitrogen and oxygen species. In addition, in patients with MetS, smoking is independently related to endothelial dysfunction.

Key words: Endothelial Dysfunction; Metabolic Syndrome; Oxidative Stress; Smoking

Introduction

Endothelial dysfunction is one of the key components of metabolic syndrome (MetS), which is characterized by an imbalance between endogenous vasodilatory substances and vasoconstrictive substances. Furthermore, endothelial dysfunction is an early functional disturbance in the natural progress of atherosclerosis and a powerful preclinic marker of future cardiovascular (CV) events.[1]

There are various techniques (invasive and noninvasive) for exploring of the pathobiology of the endothelium. Brachial flow-mediated dilatation (FMD) is a marker of endothelial function that reflects nitric oxide (NO) release from vascular endothelial cells and an accepted method for the assessment

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of individual CV risk.[2] Recently, studies have shown that oxidative stress plays an important role in the pathogenesis of vascular alterations by triggering the biochemical processes in endothelial dysfunction in MetS.[3-5] Still, comprehensive studies on the interaction between oxidative stress parameters and endothelial function evaluated by FMD in both genders in MetS are rare.

In this study, we have investigated the relationship between FMD and endothelial nitric oxide synthase (e-NOS), NO, e-selectin, von Willebrand factor (vWF), oxide low-density lipoprotein (ox-LDL), high-sensitivity C-reactive protein (hs-CRP), and thrombocyte aggregation as indicators of endothelial function in MetS patients and in a control group without MetS.

Methods

Ethical approval

The study was conducted in accordance with the *Declaration of Helsinki*. Ethical approval was received from the University Ethical Committee of Clinical Research (No: 268573). Informed consent was obtained from all patients.

Between December 2010 and August 2014, 136 patients admitted to the family medicine outpatient clinic run by our faculty were recruited into the study. MetS was present in 55 patients, and there were 81 healthy controls. The female/male ratio was 37/44 in the control group and 15/40 in the MetS group. The standard definition of the National Cholesterol Education Program's Adult Treatment Panel III report was used to select MetS patients.[6] Participants were classified as having MetS if they had three or more of the following criteria: abdominal obesity defined as waist circumference >102 cm in males and >88 cm in females; hypertriglyceridemia defined as triglycerides (TGs) >1500 mg/L; low high-density lipoprotein (HDL) cholesterol with an HDL <400 mg/L in males and <500 mg/L in females; hypertension defined as systolic blood pressure (SBP) >130 mmHg (1 mmHg = 0.133 kPa) or diastolic blood pressure (DBP) >85 mmHg; and impaired fasting glucose defined by fasting glucose >1010 mg/L. The control group selection criterion was the absence of MetS, CV disease, and diabetes.

Physical examination of the patients was conducted before testing. Patients with malignant disease, chronic inflammatory disease, chronic obstructive pulmonary disease, chronic renal disease, chronic liver disease, pregnancy, ischemic coronary artery disease, serious systemic infections, endocrine disease, or cerebrovascular disease were excluded from the study and the control groups. Patients taking medications affecting endothelial function (angiotensin converting enzyme inhibitors and antihyperlipidemics) were also excluded from the study. The international score system was used to predict their cardiac risk. All patients were asked about their family history, smoking, and concomitant diseases. Blood pressure was measured while patients were in the sitting position after a resting period of at least 5 min. Height was measured with a wall-mounted stadiometer to the nearest 0.1 cm. Weight was determined to the nearest 0.1 kg on the scale. Body mass index (BMI) was calculated from the measured body weight and height and expressed as kg/m^2. Waist circumference was measured as the horizontal distance around the abdomen at the level of the umbilicus.

Measurements of flow-mediated dilatation

Endothelial functions were assessed by measuring the FMD using Vingmed System V ultrasonography with a transducer (GE Healthcare, Little Chalfont, Buckinghamshire, UK) that has an appropriate frequency. All measurements were performed by the same operator. After 12 h of fasting, in a quiet room heated to 20°C–22°C, longitudinal images were taken from the proximal antecubital fossa of the brachial artery of the fixed arm. The base measurement of the artery diameter was performed after 10 min of rest. A sphygmomanometer was inflated to 300 mmHg on the arm for 4–5 min and the second measurement was performed 45–60 s after its deflation (reactive hyperemia). The last measurement was made after 15 min of rest, by giving 0.5 mg of sublingual glyceryl trinitrate and waiting for 3–4 min. FMD was calculated as the difference between the maximum diameter postocclusion and the average baseline diameter, which was expressed as a percentage relative to the average baseline diameter.[7] A normal healthy FMD response was accepted as 7–10%.[8]

Sample collection and preparation

Clinical parameters, including routine biochemical analysis, were measured using standard protocols. Blood samples were collected in ethylenediaminetetraacetic acid-containing tubes and anticoagulant-free tubes after an overnight fast. After immediate centrifugation (3000 g) for 10 min at 4°C, the plasma and serum were separated in Eppendorf tubes and frozen immediately at −80°C until analysis.

Laboratory analysis

Platelet aggregation method

Blood was obtained by venipuncture and collected into vacutainer tubes (BD) containing 3.2% sodium citrate to determine platelet function. Platelet functional studies were completed within 2 h of drawing blood. Platelet reactivity *ex vivo* was assessed with a platelet-rich plasma (PRP) aggregometer using a ChronoLog after stimulating samples with collagen (2 μg/ml), epinephrine (0.1 mmol/L), and adenosine diphosphate (ADP) (1 μmol/L). Peak aggregation within 5 min of agonist stimulation was recorded in ohms. PRP (500 μl) was placed in the tube in which 3×10^8 platelet/ml was included. Then, the tube was transferred to sample containers of the aggregometer and kept at 37°C for 3 min, and it was treated with 1 μmol/L ADP (ChronoLog) for 3 min. Platelet aggregation was observed and the aggregation curve taken from the aggregometer (ChronoLog 500, USA) was evaluated in terms of the slope and amplitude percentage.

The plasma ox-LDL, e-NOS, and e-selectin levels were measured using enzyme-linked immunosorbent assay

kit (ELISA, Wuhan EIAAB Science, Wuhan China). Plasma vWF levels were measured using ELISA kit (Assaypro, Missouri, USA). Plasma NO levels were measured using a colorimetric assay kit (Oxis International Inc., Foster City, USA). Deionized water was used for reconstitute of NADH and standard preparation. Sensitivity of the NO kit is 0.5 μmol/L. The total cholesterol (TC), HDL, LDL, TG, and hs-CRP levels and fasting blood glucose (FBG) were analyzed in the autoanalyzer of the Central Biochemistry Laboratory. The total platelet count and mean platelet volume were also measured.

Statistical analyses

Analysis of data was performed with SPSS statistical analysis software (version 20.0; SPSS Inc, Chicago, IL, USA). The results were expressed as the means ± standard deviation (SD) or median (interquartile range). The hs-CRP and e-NOS levels were logarithmically transformed to achieve normal distributions. General linear measurement analysis was used for comparing anthropometric parameters, and biochemical parameters were compared using Student's *t*-test, Mann-Whitney *U*-test, and Chi-square test. Pearson's correlation analysis was used for correlations. 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 $P < 0.05$ was considered statistically significant.

Results

The demographic and biochemical parameters in healthy individuals and MetS patients are displayed in Tables 1 and 2.

The hs-CRP ($z = 3.23$, $P = 0.004$), e-NOS ($z = 2.22$, $P = 0.026$), ox-LDL ($z = 2.62$, $P = 0.013$) levels, and cardiac risk score ($z = 5.23$, $P < 0.001$) were significantly higher in MetS patients [Table 2]. FMD was impaired in 58.3% of patients in the MetS group and 53% patients in the control group ($z = 0.03$, $P = 0.576$).

In the group with MetS, the percentage of smokers ($\chi^2 = 8.07$, $P = 0.004$) was significantly higher [Table 1]. In patients with MetS and endothelial dysfunction, the smoking frequency was significantly higher than that in patients with MetS and normal endothelial function ($z = 9.26$, $P = 0.002$) [Table 3].

In male patients with MetS, only hs-CRP levels were higher than the control group ($z = 2.41$, $P = 0.015$), whereas in female patients, the ox-LDL ($z = 2.03$, $P = 0.042$), e-NOS ($z = 2.12$, $P = 0.011$) levels, and platelets ($z = 2.95$, $P = 0.042$) were higher than that in the control group. In female patients, there were no differences in the demographic parameters and major CV risk factors between MetS and control groups. In male patients, the two groups were significantly different in terms of diabetes ($\chi^2 = 4.90$, $P = 0.033$), obesity ($\chi^2 = 7.42$, $P = 0.006$), and hypertension ($\chi^2 = 5.18$, $P = 0.020$).

Endothelial dysfunction was related to the hs-CRP and e-NOS levels in MetS patients ($z = 2.65$, $P = 0.008$; $z = 2.58$, $P = 0.010$, respectively). Endothelial dysfunction was only related to the hs-CRP level in the control group ($z = 2.77$, $P = 0.006$) [Tables 4 and 5].

There were no differences between the NO ($z = 1.26$, $P = 0.207$), vWF ($z = -0.34$, $P = 0.394$), e-selectin ($z = -0.02$, $P = 0.555$), and endothelial adenosine diphosphate ($z = 1.17$, $P = 0.241$) values between the MetS patients and control group.

Table 1: Demographic parameters of the study (*n*)

Parameters	MetS (−), *n* = 81	MetS (+), *n* = 55	χ^2	*P*
Gender				
Female/male	44/37	15/40	9.32	0.002
Smoking	31	35	8.07	0.004

MetS: Metabolic syndrome.

Discussion

In the present study, the hs-CRP and ox-LDL levels in MetS patients were significantly elevated due to inflammation and oxidative stress. The e-NOS level was also paradoxically high which contrasts with the general knowledge. On the other hand, NO, cell adhesion markers (vWF and e-selectin), and thrombocyte aggregation parameters (ADP[e]) did not have a significant difference. FMD was not significantly different between the MetS patients and control group. Endothelial dysfunction was significantly affected by smoking in MetS patients, but smoking did not affect endothelial dysfunction in the control group.

Smoking is a strong, dose-dependent risk factor for atherosclerosis and CVD. Smokers have abnormalities in endothelial function.[9] Kang and Song[10] in a Korean study stated that the presence of MetS was significantly higher in smokers than nonsmokers for both men and women. They proposed that the relationship between MetS and smoking was mainly due to the association between smoking and dyslipidemia.

In a different study by Tsai *et al.*,[11] the authors concluded that the plasma zinc α2-glycoprotein level, which is an independent risk factor for MetS, significantly increased with smoking in men. In the present study, smoking was a significantly more common trait in MetS patients than that in the control group.

The relationship between FMD and MetS has been investigated in other studies.[12-16] In the Finns study,[12] there was a direct correlation between the number of MetS components and FMD. The FMD response was not impaired in young individuals with MetS. Another three studies by Wendelhag *et al*., Title *et al.*, and Lind[13-15] reported no association between the brachial FMD and MetS, similar to this study. Lin *et al*. and Golledge *et al.*[16,17] demonstrated a relationship between FMD and MetS in peripheral artery disease patients and hypertensive patients.

Table 2: Changes in demographic and biochemical parameters of MetS

Variables	MetS (−), *n* = 81	MetS (+), *n* = 55	Statistics	*P*
Age (years)	46.74 ± 7.13	47.07 ± 29.98	−0.25*	0.782
BMI (kg/m²)	26.40 ± 3.04	6.44 ± 2.74	−6.93*	0.001
SBP (mmHg)	116.54 ± 18.77	128.82 ± 18.18	−3.80*	0.001
DBP (mmHg)	74.01 ± 11.97	83.00 ± 10.70	−4.43*	0.001
HDL cholesterol (mg/L)	540 (426–680)	420 (350–490)	−5.63†	0.001
Triglycerides (mg/L)	1020 (730–1290)	1760 (1410–2130)	6.24†	0.001
Glucose (mg/L)	820 (790–882)	915 (820–1037)	3.52†	0.001
hs-CRP (mg/L)	1.21 (0.68–2.23)	2.83 (1.33–4.35)	3.23†	0.004
ox-LDL (ng/ml)	2.70 (1.91–4.03)	3.55 (2.35–6.11)	2.62†	0.013
e-NOS (pg/ml)	95.50 (51.30–200.10)	145.10 (57.50–278.80)	2.22†	0.026
NO (pg/ml)	43.20 (34.40–47.90)	45.20 (37.80–52.70)	1.26†	0.207
ADP (e)	60 (53.75–65.00)	62.50 (55.60–70.60)	1.17†	0.241
e-selectin (ng/ml)	1.70 (0.72–2.68)	117 (0.64–3.63)	−0.02†	0.555
vWF (mU/ml)	1352 (842–1704)	1249 (793–1753)	−0.34†	0.394
Risk score	3 (1.25–7.75)	9 (6–15)	5.23†	0.001

Values are mean ± SD or median (interquartile range). *Student's *t*-test and †Mann-Whitney *U*-test are used to analyze variables. MetS: Metabolic syndrome; BMI: Body mass index; NO: Nitric oxide, ox-LDL: Oxide LDL; vWF: von Willebrand factor; ADP (e): Adenosine diphosphate (endothelial); SBP: Systolic blood pressure; DBP: Diastolic blood pressure; LDL: Low-density lipoprotein; HDL: High-density lipoprotein; hs-CRP: High-sensitivity C-reactive protein; SD: Standard deviation; e-NOS: Endothelial nitric oxide synthase.

Table 3: Relationship between FMD and major CV risk factors in patients with MetS, *n* (%)

Risk factors	FMD		χ^2	*P*
	Normal	Impaired		
Family history	13 (59.1)	22 (73.3)	1.37	0.241
Smoking	8 (36.4)	24 (80.0)	9.26	0.002
High LDL (>1300 mg/L)	15 (68.2)	17 (56.7)	0.30	0.583

FMD: Flow-mediated dilatation; CV: Cardiovascular; MetS: Metabolic syndrome; LDL: Low-density lipoprotein.

The relationship between smoking and brachial FMD has been investigated in previous publications. In 2511 Chinese subjects,[18] the relationship between FMD and CV risk factors was analyzed and multivariate analysis revealed that the FMD was negatively correlated with age ($\beta = -0.29$, $P < 0.001$), gender ($\beta = -0.12$, $P < 0.001$), BMI ($\beta = -0.12$, $P = 0.001$), SBP ($\beta = -0.12$, $P < 0.001$), FBG ($\beta = -0.04$, $P = 0.009$), TC ($\beta = -0.04$, $P = 0.014$), smoking ($\beta = -0.05$, $P = 0.003$), and baseline brachial artery diameter ($\beta = -0.35$, $P < 0.001$). Skaug *et al*.[19] reported that endothelial dysfunction was more prevalent and FMD was lower in women with MetS and they stated that smoking and hyperglycemia contributed to this result.

Another study by Karahan *et al*.[20] analyzed the relationship between oxidative stress index (OSI) and FMD in 80 smoking healthy male volunteers. The SBP and DBPs, hs-CRP, LDL, and OSI were predictive of an abnormal FMD response. The results of this study supported this finding and the OSI, hs-CRP, and e-NOS levels were related to an abnormal FMD response in MetS patients.

The current study demonstrated that the e-NOS levels were paradoxically increased in patients with MetS, which is supported by the results of some previous studies.[21-23] This effect was thought to be partially mediated by limiting the availability of NO, thereby exerting a negative feedback on NOS expression through activation of nuclear factor-κB.[24] Zhao *et al*.[25] also recently reported that endothelial dysfunction was associated with an increase rather than a decrease in e-NOS expression. The present study indicated that in women with MetS, the OSI and e-NOS levels are higher, whereas in men with MetS, the hs-CRP level is higher than that in the control group.

In a cohort study on 1702 Turkish participants,[26] who were followed up for 10 years, 546 of them developed MetS. In males, abdominal obesity, hs-CRP, and gamma glutamine transferase were related to MetS; in females, only hs-CRP was related to MetS and current smoking tended to be protective, which was a result different from this study.

Li *et al*.[27] observed that the hs-CRP levels were significantly high in the MetS patients in agreement with our results. The hs-CRP level has been found to be a powerful independent predictor of increased CV risk. This study further indicated that elevated hs-CRP levels were associated with reduced basal and stimulated NO release from arterial endothelial cells through various mechanisms. In the study, hs-CRP was the strongest indicator of MetS after controlling for demographic parameters such as gender and smoking.

ox-LDL plays an important role in endothelial dysfunction. It induces endothelial injury and inhibits apoptosis, monocyte adhesion, platelet aggregation, and e-NOS expression/activity, contributing to atherosclerosis.[28] A study by Bae *et al*.[29] showed that the ox-LDL ($P < 0.05$) and hs-CRP ($P < 0.01$) levels increased with the number of MetS risk factors in 108 MetS patients and 91 controls. Kosola *et al*.[30] reported that the ox-LDL levels were elevated in Finnish young men with MetS (odds ratio: 1.118). In two

Table 4: Changes in biochemical parameters due to MetS in patients with endothelial dysfunction

Endothelial dysfunction (+)	MetS (−), *n* = 81	MetS (+), *n* = 55	*Z*	*P*
ox-LDL (ng/ml)	263 (1.87–3.44)	3.30 (2.26–5.06)	1.81	0.070
e-selectin (ng/ml)	1.76 (0.70–2.44)	1.43 (0.72–3.70)	1.19	0.234
vWF (mU/ml)	1368.80 (948.90–1576.20)	1296.50 (777.40–1743.60)	−0.71	0.476
hs-CRP (mg/L)	1.15 (0.51–1.77)	2.61 (1.31–3.53)	2.65	0.008
e-NOS (pg/ml)	70.20 (36.70–150.20)	190.90 (54.82–301.55)	2.58	0.010
NO (pg/ml)	41.88 (33.32–46.95)	44.26 (36.28–50.99)	0.84	0.415

Values are median (interquartile range). MetS: Metabolic syndrome; ox-LDL: Oxide LDL; vWF: von Willebrand factor; hs-CRP: High-sensitivity C-reactive protein; e-NOS: Endothelial nitric oxide synthase; NO: Nitric oxide.

Table 5: Changes in biochemical parameters due to MetS in patients with normal endothelial function

Endothelial dysfunction (−)	MetS (−), *n* = 81	MetS (+), *n* = 55	*Z*	*P*
ox-LDL (ng/ml)	2.59 (1.58–4.43)	3.33 (2.28–5.74)	1.32	0.186
e-selectin (ng/ml)	2.15 (1.04–3.50)	1.54 (0.64–3.75)	−0.23	0.817
vWF (mU/ml)	1373.30 (838.60–1849.80)	1323.00 (932.90–1889.50)	0.21	0.832
hs-CRP (mg/L)	1.10 (0.78–2.20)	2.78 (1.40–4.46)	2.77	0.006
e-NOS (pg/ml)	104.90 (59.11–244.60)	113.02 (56.10–306.20)	0.17	0.862
NO (pg/ml)	45.50 (34.80–52.40)	48.09 (38.810–55.30)	1.07	0.295
ADP (e)	57.50 (52.50–65.00)	61.20 (47.50–73.70)	0.62	0.536

Values are median (interquartile range). MetS: Metabolic syndrome; ox-LDL: Oxide LDL; vWF: von Willebrand factor; hs-CRP: High-sensitivity C-reactive protein; e-NOS: Endothelial nitric oxide synthase; NO: Nitric oxide; ADP (e): Adenosine diphosphate (endothelial).

other studies,[31,32] elevated ox-LDL levels were associated with MetS ($P < 0.001$). This study showed that ox-LDL levels were elevated in women but not men with MetS when compared to a healthy control group.

Platelet aggregability in response to ADP and collagen did not change in MetS patients when compared to the control group. Impaired coronary microvascular function with MetS has been suggested in a study by Lanza *et al*.[33] Platelet functions are influenced by some physical and chemical factors, including sample collection, handling, and processing.

Visceral fat tissue produces proatherogenic adipokines that are important contributors to oxidative stress and chronic inflammation, and these factors have an important harmful effect on endothelial function. Strategies that aim to reduce central obesity improve endothelial function and ameliorate low-grade inflammation.[34] In a study by Suboc *et al*.,[35] the authors found that FMD was lower in women with BMI ≥30 kg/m^2 than that in women with BMI <30 kg/m^2 ($P = 0.020$) and that BMI was correlated with hs-CRP ($P = 0.006$). This study further suggested that obesity-associated arteriolar endothelial dysfunction in women was primarily related to impaired eNOS activity and/or NO bioavailability. In addition, potential for greater adverse impact of obesity on systemic inflammation and endothelial function in women relative to men was observed.

In this study, there was a significant decrease in the brachial FMD in smokers compared to nonsmokers for patients with MetS. After controlling for smoking and gender, the significant difference between the MetS and control groups in the oxidative stress parameters disappeared. This finding might support the notion that endothelial dysfunction is an important mechanism of gender-specific effects on CVD in MetS patients.[36]

In MetS patients, increases in ox-LDL, e-NOS, and hs-CRP were likely due to oxidative stress, a condition in which there is imbalance between the production and inactivation of reactive nitrogen and oxygen species. Smoking cessation is especially important in MetS patients and it combines with other therapeutic strategies to prevent further CV risk. Women seem to be more affected by MetS in terms of their biochemical markers of endothelial dysfunction.

The limitations of this study might be that the sample size of MetS patient was lower than the control group and this could be the reason for not finding any significant differences between the groups in FMD and some biochemical parameters such as vWF and NO. Moreover, the sample size of women with MetS might not be large enough to detect the effect of gender on the searched parameters. Large-scale studies investigating endothelial dysfunction and prothrombotic alterations in MetS for both genders are needed to clarify the findings in the study.

In conclusion, FMD is not different from the control group in both genders of MetS patients. Smoking effects endothelial dysfunction independently. hs-CRP and e-NOS are related to endothelial dysfunction in MetS patients. Oxidative stress parameters (ox-LDL and eNOS) are elevated in females, and inflammation markers (hs-CRP) are elevated in males when compared with the control group in Mets patients.

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Conflicts of interest

There are no conflicts of interest.

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