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Serum paraoxonase 1 activity in women with metabolic syndrome

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Abstract

Background: Metabolic syndrome (MetS) is a leading risk factor for coronary artery disease (CAD) in women. Reduced paraoxonase 1 (PON1) activity may play a role in the pathogenesis of atherosclerosis through increased susceptibility to lipid peroxidation in patients with MetS.

Aim: To examine whether there is a relationship between serum PON1 activity and MetS in women.

Method: The study group consisted of 54 women with MetS. The NCEP ATP III guidelines were used to define MetS. The control group consisted of 65 women without MetS and CAD. All patients from the MetS group underwent coronary angiography.

Results: The PON1 activity and salt-stimulated PON1 activity were not significantly altered in women with MetS when compared to controls ($p = 0.902$, $p = 0.877$, respectively). There was no significant difference in PON1 activity ($p = 0.159$), and salt-stimulated PON1 activity ($p = 0.139$) between diabetics and non-diabetics. In the MetS group, patients with CAD ($n = 16$) had significantly reduced PON1 activity and salt-stimulated PON1 activity compared to MetS patients without CAD ($p = 0.008$ and $p = 0.004$, respectively).

Conclusions: Serum PON1 activity is significantly reduced in women with CAD and MetS. MetS per se does not alter serum PON1 activities.

Key words: paraoxonase activity, metabolic syndrome, coronary artery disease

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INTRODUCTION

Metabolic syndrome (MetS) is a common and complex disorder. It is characterised by insulin resistance, hyperglycaemia, abdominal obesity, arterial hypertension, atherogenic dyslipidaemia, a prothrombotic state, and a proinflammatory state. The presence of these risk factors carries an increased risk for development of type 2 diabetes mellitus (DM) and cardiovascular (CV) diseases [1].

The oxidative modification of low-density lipoprotein (LDL) plays an important role in the development of atherosclerosis. Recent data suggest that high-density lipoprotein (HDL) inhibits the oxidation of LDL [2]. Paraoxonase 1 (PON1) is an HDL-associated enzyme and probably has a role in protecting LDL from oxidative stress. The PON1 activity varies among individuals and populations [3].

Hyperglycaemia, a fundamental component of MetS, adversely affects vascular function and directly correlates with CV events. In patients with DM, the lipoproteins may be glycated, resulting in an abnormal function [4]. Chronic hyperglycaemia causes modification of protein structure and func-

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tion due to non-enzymatic glycation of amino acid residues [4], and LDL cholesterol containing glycated apolipoprotein B100 [5] Glycated LDL cholesterol is more readily oxidised [6], resulting in accelerated macrophage uptake. Decreased levels of PON1 activity have been found in patients with overt DM in several studies [7, 8]. Therefore, in patients with MetS, the protective effects of PON1 against peroxidation of LDL can be important. Reduced PON1 activity may play a role in the pathogenesis of atherosclerosis through increased susceptibility to lipid peroxidation in patients with MetS.

Based on the data from the WISE study, MetS is a leading and major risk factor in women. The risk factor assessment and the risk factor profiles in women that are associated with coronary artery disease (CAD) may be different [9]. Accordingly, a recent study describing the prevalence of MetS in acute myocardial infarction (AMI) demonstrated that almost half of AMI patients also suffered from MetS. Additionally, it has been found that MetS was more prevalent in women [10–13]. The association between hypertension, CAD and early mortality has been reported to be stronger in women than in men [14]. The prevalence of MetS is surprisingly higher in Turkey than in other European countries [12]. It is even higher among Turkish women than Turkish men (45.2% *vs* 27%) [15]. With respect to these findings, we measured serum PON1 activity in women with MetS in this special population.

METHODS

Subjects

We recruited 54 women (age 53.6 ± 9.9 years) with MetS. The NCEP ATP III guidelines define five components of MetS and at least three of the following five criteria are required to diagnose MetS: abdominal obesity; waist circumference > 88 cm in women, > 102 cm in men; triglyceride ≥ 150 mg/dL; HDL < 50 in women, < 40 in men; blood pressure ≥ 130/ /85 mm Hg; and fasting glucose ≥ 110 mg/dL [1]. Diabetes mellitus and impaired fasting glucose were defined according to American Diabetes Association criteria [16]. In the MetS group, 18 patients had previously diagnosed DM. Impaired fasting glucose was defined as fasting plasma glucose 100 mg/dL to 125 mg/dL.

In the MetS group, 16 patients had CAD. We defined the presence of CAD in patients with MetS as greater than 50% stenosis in one of the major segments of the coronary arteries. All patients with MetS had symptoms suggestive of CAD and were referred for coronary angiography by their attending physician. Exclusion criteria were: pregnancy, cardiomyopathy, recent myocardial infarction, significant valvular or congenital heart disease, recent coronary angioplasty or coronary bypass surgery.

The control population consisted of 65 women (mean age 51.1 ± 10.2 years) without MetS and with no CAD. Lack of CAD in the control population was assessed using a health questionnaire, physical examination and electrocardiography. All had no history of CAD. In subjects with chest discomfort or possible angina, equivalents such as dyspnea and fatigue, exercise stress testing, myocardial perfusion imaging and echocardiography were performed when necessary. Coronary angiography was performed for symptomatic patients with positive test results only. Subjects with diabetes, renal and hepatic disease were excluded. All subjects consented to participate in the study, which was approved by the local Ethics Committee.

Laboratory analysis

Serum samples were obtained by venipuncture with vacutainer tubes after 12 hours fasting to measure the serum lipid and biochemical profile. Serum total cholesterol, LDL, HDL, and triglycerides were measured by automated enzymatic methods.

Baseline and salt-stimulated PON1 activity was measured by the hydrolysis of paraoxon using a modified method of Eckerson [17]. The PON1 activity from serum samples was estimated with and without the addition of 1 M NaCl in order to determine salt-stimulated and basal activity. The rate of hydrolysis of paraoxon was assessed by following the liberation of p-nitrophenol measured by the increase of absorbance at 412 nm on a spectrophotometer (Cary100, Varian) at 25°C. The basal assay mixture included 1 mM paraoxon and 1 mM $CaCl_2$ in 0.05 M glycine-buffer (pH 10.5) and that of salt stimulated PON1 assay included 1 M NaCl in addition to this mixture. One unit was defined as the amount of PON1 producing 1 nmol of p-nitrophenol per minute per millilitre of serum.

Statistical analysis

Statistical calculations were performed using SPSS 13 program for Windows. Values are expressed as mean ± SD. For comparing clinical and laboratory results between the groups, we used Student *t*-tests or Mann-Whitney U test. Chi square or Fisher's exact test were used for comparison of the qualitative data. Pearson correlation test was used to study the relation between plasma paraoxonase, salt-stimulated activities, HDL, LDL, and triglyceride. A p value < 0.05 was considered significant.

RESULTS

Demographic and clinical data of the subjects are shown in Table 1. In the MetS group, body mass index was higher, triglyceride levels were higher and HDL levels were lower than in the control group, as expected. There were no significant differences between MetS subjects and the controls in terms of age, serum total cholesterol, LDL, prevalence of smoking or family history (Table 1). The PON1 activity and salt-stimulated PON1 activity were similar in women with MetS and controls (p = 0.902 and p = 0.877, respectively) (Table 1).

Table 1. Demographic profile, clinical and laboratory parameters of the study population

	Control subjects (n = 65)	MetS (n = 54)	P
Age [years]	51.1 ± 10.2	53.6 ± 9.9	0.175
Body mass index [kg/m^2]	29.1 ± 6.2	32.7 ± 5.2	< 0.001
Waist circumference [cm]	84.8 ± 10.1	102.7 ± 9.8	< 0.001
Smoking	2 (3.1%)	6 (11.1%)	0.083
Diabetes mellitus	0	18 (33.3%)	< 0.001
Impaired fasting glucose	18 (27.7%)	22 (40.7%)	0.135
Coronary artery disease	0	16 (29.6%)	< 0.001
Hypertension	13 (20%)	47 (87%)	< 0.001
Family history of CVD	11 (16.9%)	13 (24.1%)	0.232
Total cholesterol [mg/dL]	200.9 ± 37.5	204.3 ± 37.2	0.625
Triglycerides [mg/dL]	88.8 ± 51.8	165.4 ± 82.7	< 0.001
LDL cholesterol [mg/dL]	123.5 ± 32.6	126.3 ± 31.4	0.638
HDL cholesterol [mg/dL]	57.1 ± 14.1	48.2 ± 9.7	< 0.001
PON1 activity [U/L]	101.7 ± 48.1	98.4 ± 42.8	0.902
Salt-stimulated PON1 activity [U/L]	153.8 ± 88.9	143.8 ± 72.8	0.877

MetS — metabolic syndrome; PON1 — paraoxonase 1; CVD — cardiovascular disease

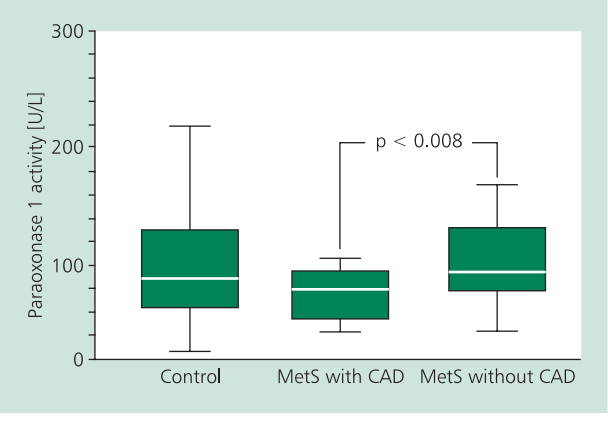

Figure 1. Serum paraoxonase 1 activitity in the metabolic syndrome (MetS) group and the control group; CAD — coronary artery disease

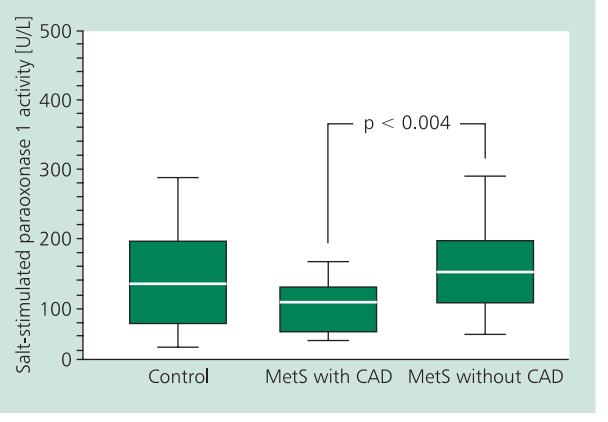

Figure 2. Serum salt-stimulated paraoxonase 1 activity in the metabolic syndrome (MetS) group and the control group; CAD — coronary artery disease

There was no significant difference in PON1 activity (p = 0.159), and salt-stimulated PON1 activity between diabetics (n = 18) and non-diabetics (n = 101; p = 0.139).

In the MetS group, patients with CAD had reduced PON1 and salt-stimulated PON1 activity compared to patients with MetS without CAD (75.6 ± 21.5 *vs* 108 ± 45.9 U/L, p < < 0.008 and 103.6 ± 37.2 *vs* 160.7 ± 77.7 U/L, p < 0.004, respectively; Figs. 1, 2). The mean age of these two subgroups was similar.

Serum PON1 and salt-stimulated activity did not correlate with HDL, LDL, and triglyceride (r = –0.045, p = 0.625, r = –0.003, p = 0.976; r = –0.023, p = 0.805, r = 0.006, p = 0.951; r = –0.129, p = 0.163, r = –0.196, p = 0.032, respectively). Serum PON1 and salt-stimulated activity negatively correlated with age (r = –0.194, p = 0.034, r = –0.187, p = 0.042, respectively).

DISCUSSION

MetS is a clustering of risk factors which predispose an individual to CV disease [1]. In several studies, MetS has been shown to be commoner in women than in men [11–13, 18], and it has been shown that the association between hypertension, CAD and early mortality is stronger in females [14].

Metabolic syndrome is associated with a higher fraction of oxidised LDL. Hyperinsulinaemia and impaired glycaemic control are related to increased *in vivo* LDL oxidation [19]. Many studies have suggested that PON1 activity is reduced in subjects with CAD [20], hypercholesterolaemia [7] and type 2 DM [7]. Decreased PON1 activity might contribute to an increased propensity for the development of CV disease in women with MetS in addition to known risk factors.

There is little data about PON1 activity in patients with MetS. One study showed that the activity of PON1 was not decreased in MetS compared with controls [21]. Recently, Tabur et al. [22] showed that non-diabetic MetS does not affect serum paraoxonase activity. We also did not find any significant difference in PON1 and salt-stimulated PON1 activity between the women with, and the women without, MetS. PON1 activity has been shown to be reduced in diabetic patients in several studies [8, 23]. By contrast, some investigators have not found any difference in paraoxonase activity between diabetic and control groups [24–26]. Kopprasch et al. [24] showed that PON1 activity was not significantly altered in impaired glucose tolerance and DM subjects when compared to normoglycaemic controls. In our study, there was also no significant difference in PON1 and salt-stimulated PON1 activity between diabetics and non-diabetics. However, there were only 18 patients with DM in our study. More marked glycaemia in overt diabetes, compared with MetS, may account for this difference [21]. Additionally, the lack of significant difference in PON1 activity between women with or without MetS may result from interethnic variability, something observed in other studies [3].

Some studies have found serum paraoxonase activity to be correlated with various lipid and lipoprotein parameters such as triglycerides and HDL [26, 27]. We did not find such a correlation. Also, serum paraoxonase activity was not correlated with the serum concentrations of lipids and lipoproteins in diabetic patients [8]. A recent report has indicated that paraoxonase genotype is a major determinant of serum lipid and lipoprotein concentrations. This report showed that genotypes of PON1 were significantly associated with a variation in plasma concentrations of total, HDL, non-HDL, and LDL cholesterol, and triglycerides [28]. We did not study genotypes of paraoxonase in our study.

The incidence of atherosclerosis increases with age. Cakatay et al. [29] showed in healthy subjects that PON1 activity of elderly and middle-aged individuals was significantly decreased compared to that in the young group, and they suggested that decreasing PON1 activity might predispose to atherosclerosis. Although paraoxonase activity showed negative correlation with age in our study in the MetS group, there was no significant difference in patients with or without CAD. This suggests that reduced PON1 and salt-stimulated PON1 activity levels could be related with CAD rather than with age in our study.

Cardiovascular diseases are the most important clinical consequences of MetS. Lopes et al. [30] showed that the presence of MetS conferred a 2.5-fold increase in the mortality risk in patients with CAD. Other studies have indicated a relationship between a low serum paraoxonase activity and the presence of atherosclerosis [31, 32]. Reduced serum PON1 activity has been found to be related to accelerated atherogenesis [33].

Limitations of the study

Some limitations should be underlined. Our study sample size was small. Additionally, the lack of patients with CAD in the control group was a possible limitation of this study.

CONCLUSIONS

In our study, we showed that in the MetS group, serum PON1 and salt-stimulated PON1 activity was reduced in patients with CAD as compared to those without CAD.

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Aktywność paraoksonazy 1 w surowicy kobiet z zespołem metabolicznym

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Streszczenie

Wstęp: Zespół metaboliczny (MetS) jest najważniejszym czynnikiem ryzyka choroby wieńcowej (CAD) u kobiet. Zmniejszenie aktywności paraoksonazy 1 (PON1) może wpływać na rozwój miażdżycy u osób z MetS poprzez zwiększenie podatności na peroksydację lipidów.

Cel: Celem badania było ustalenie, czy istnieje zależność między aktywnością PON1 w surowicy a występowaniem MetS u kobiet.

Metody: Badana grupa składała się z 54 kobiet z MetS. Zespół metaboliczny rozpoznawano zgodnie z kryteriami NCEP ATP III. Grupę kontrolną stanowiło 65 kobiet, u których nie stwierdzono ani MetS, ani CAD. U wszystkich pacjentek z grupy MetS wykonano angiografię wieńcową.

Wyniki: Aktywności PON1, zarówno podstawowa, jak i po stymulacji solą, nie różniły się istotnie między grupą kobiet z MetS i grupą kontrolną (odpowiednio $p = 0,902$; $p = 0,877$). Nie stwierdzono również istotnych różnic w podstawowej aktywności PON1 ($p = 0,159$) oraz aktywności PON1 po stymulacji solą ($p = 0,139$) między chorymi na cukrzycę i kobietami bez tego schorzenia. Wśród kobiet z MetS oraz z CAD ($n = 16$) stwierdzono istotnie zmniejszoną aktywność PON1 (podstawową i po stymulacji solą) w porównaniu z osobami bez CAD (odpowiednio $p = 0,008$; $p = 0,004$).

Wnioski: U kobiet z CAD i MetS aktywność PON1 w surowicy jest istotnie zmniejszona. Zespół metaboliczny *per se* nie powoduje zmiany aktywności PON1.

Słowa kluczowe: aktywność paraoksonazy, zespół metaboliczny, choroba wieńcowa

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