Study of Oxidative Stress Marker Serum Paraoxonase in Metabolic Syndrome

Metabolik Sendromda Oksidatif Stres Marker serum Paraoxonazın Çalışması

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Abstract

Purpose: The metabolic syndrome (MetS) is a constellation of interrelated risk factors of metabolic origin that appear to promote development of cardiovascular disease. It has become one of the most important topics for this decade because of marked increase in cardiovascular disease associated with risk factors. Paraoxonase (PON) is a family of three enzymes, PON1, PON2 and PON3, the gene which is located on chromosome 7. This is high-density lipoprotein (HDL)-associated enzyme with antioxidant activity. The objective of the present study aimed at measurement of PON1 activities in patients with MetS, to compare PON1 activities in patients with MetS with those in controls and assess its relationship with the lipoproteins in serum.

Material and Method: Cases were included as per the criteria put forth by the International Diabetes Federation. Serum PON1 activities were measured using spectrophotometer. Statistical analysis was done using SPSS 20.0.

Result: Serum PON1 arylesterase and lactonase activities were found to be reduced significantly in patients with MetS than in controls. PON1 activities showed a positive correlation with HDL, fasting blood glucose and diastolic blood pressure. Linear regression analysis showed a significant correlation between PON1 activities and body mass index.

Discussion: From the present study, it is clear that reduction in PON1 activities in MetS is mainly due either to abnormalities with synthesis or secretion of HDL cholesterol or oxidative stress as a consequence of excess production of the free radicals. This study also iterates that it is the quality and not the quantity of HDL cholesterol is important while studying the pathophysiology of MetS.

Keywords: Paraoxonase, arylesterase, lactonase, metabolic syndrome, oxidative stress

Öz


Gereç ve Yöntem: Çalışmaya Uluslararası Diyabet Federasyonu tarafından belirlenen kriterlere uygun olgular dahil edilmiştir. Serum PON1 aktivitesi spektrofotometre kullanılarak ölçülmüştür. Istatistiksel analiz SPSS 20.0 kullanarak yapılmıştır.

Bulgular: Serum PON1 arylesterase ve lactonase aktiviteleri MetS hastalarda kontrollere göre istatistiksel olarak azalmış bulunmuştur. PON1 aktivitesi HDL, açlık kan şeker ve diastolik kan basıncı ile pozitif korelasyon göstermiştir. Lineer regresyon analizi PON1 aktivitesi ile beden kitle indeksi arasında anlamlı korelasyon göstermiştir.

Tartışma: Bu çalışma açıkça göstermiştir ki MetS hastalarındaki PON1 aktivitelerinde azalma HDL kolestrol sentez ve sekresyonunun anormallikler ve serbest radikallerin fazla oluşumu sonucu oluşan oksidatif stres nedeniyledir. Bu çalışma aynı zamanda HDL kolestrolünün miktar değişikliğini de MetS patofizyolojisi çalışmalarında önemli olduğunu göstermektedir.

Anahtar kelimeler: Paraoksonaz, arilesteraz, lactonaz, metabolik sendrom, oksidatif stres

Introduction

Now-a-days, as a result of change in lifestyles and eating habits, the chemistry of human body is changing. Junk foods, lack of exercise, and psychosocial stress are important among the various reasons leading to dreadful metabolic disorders. Metabolic syndrome (MetS) is one of them. MetS is a constellation of interrelated risk factors of metabolic origin - metabolic risk factors - that appear to promote the development of atherosclerotic cardiovascular disease (CVD) (1). It consists of atherogenic dyslipidemia, elevations of blood pressure (BP) and glucose, prothrombotic and proinflammatory states (2). The

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A constellation of dyslipidemia (hypertriglyceridemia and low levels of high-density lipoprotein cholesterol (HDLc)), elevated BP, impaired glucose tolerance, and central obesity is now classified as MetS [3]. The definitions of MetS include those proposed by the World Health Organization, the European Group for the Study of Insulin Resistance, the US National Cholesterol Education Program (NCEP) and the International Diabetes Federation (IDF) [4,5,6,7]. Obesity and physical inactivity are the driving forces behind the syndrome while metabolic susceptibility is required for MetS to manifest [8]. This includes adipose tissue disorders, genetic and racial factors, aging, and endocrine disorders [9]. Two primary clinical outcomes of MetS are CVD and non-insulin-dependent diabetes mellitus, which both are leading causes of morbidity and mortality worldwide. In fact, all individual components of MetS are risk factors for CVD and MetS has been shown to be the most important factor responsible for myocardial infarction in the population younger than 45 years of age [10].

**Insulin Resistance and Obesity**

The most accepted hypothesis to describe the pathophysiology of the MetS is insulin resistance. Risk factors for the syndrome appear to be abdominal obesity and insulin resistance [11]. Insulin resistance is contributed by free fatty acids (FFAs) which reduce insulin sensitivity in muscle by inhibiting insulin-mediated glucose uptake. Increased circulating glucose results in hyperinsulinemia. In the liver, FFAs increase the production of glucose, triglycerides and very low-density lipoproteins leading to reduced glycogen formation and increased lipid accumulation in triglycerides (TG) [12]. Insulin resistance leads to increased lipolysis to produce more fatty acids, further inhibiting the antilipolytic effect of insulin, leading to lipolysis [13]. Abnormal production of inflammatory cytokines from adipose tissue may affect insulin resistance [14]. Obesity is related to control of BP and insulin resistance through oxidative stress [15,16]. Ultimately, oxidative stress has been shown to correlate with dyslipidemia and abdominal fat in patients with MetS and thus might be considered as a linking factor between various components of MetS.

**Paraoxonase**

In the history, paraoxanos (PONs) dates back long as Aldridge in 1953, classified esterases depending upon their interaction with organophosphate (OP) compounds. According to this, “A” esterases are those hydrolyzing OPs and “B” esterases are those inhibited by OPs. PONs belong to group “A” while cholinesterases belong to group “B” [17,18]. PON requires Ca++ for its stability and action which distinguishes it from other group “A” esterases which require Co++, Mn++, Mg++. They are widely distributed in the body including liver, kidney, small intestine, serum [19]. PON enzymes were originally discovered as enzyme-hydrolyzing exogenous OP compound, such as insecticide paraoxon. PON is a family of three enzymes, PON1, PON2 and PON3, the gene which is located on chromosome 7q21.3-22.1 [20].

**Paraoxonase 1**

The enzyme was first noticed as PON1 which is exclusively associated with HDLs and is a genetically polymorphic enzyme. This is a HDL-associated enzyme which plays a vital role in the prevention of micro-vascular complications due to oxidative stress and various toxic chemicals [21]. HDL oxidation is prevented by PON1-mediated hydrolysis of lipid peroxides and of cholesteryl linoleate hydroperoxides because peroxidase-like activity of PON1 protects against oxidation [22]. By far the most studied member of the family is serum PON1 (E.C 3.1.8.1), a 45 kDa protein, a calcium-dependent esterase/lactonase whose primary physiological role is to protect low-density lipoproteins (LDLs) from oxidative modifications [23]. Recent investigations have suggested that the hydrolytic activity towards lactones (cyclic esters) is the native activity of PON1: Structure-activity studies show that lactones are PON1’s preferred substrate for hydrolysis [24]. Certain studies have shown that molecules implicated in acute-phase inflammatory responses, cytokines and oxidized phospholipids reduce both PON1 expression and its PON activity [25]. Diabetes mellitus which is a component of MetS is associated with an elevated level of oxidative stress, increased susceptibility to coronary heart disease, and reductions in PON1 concentration and PON activity [26]. It is noted in obese diabetes patients that PON1 activities are reduced with elevated leptin and oxidative stress [27]. Human PON1 is predominantly synthesized in the liver from where it is secreted into the blood and associated with HDL. The objective of the present study was to estimate PON1 arylesterase (ARE), lactonase activity (LACT) in patients with MetS and to compare PON1 activities in patients with MetS and controls.

**Materials and Methods**

The study has been undertaken with due approval from the Institutional Ethics Committee. A total of 80 subjects were included in the study. Sample size is calculated using 2-sided confidence interval 95% and power 80%. Using the mean and standard deviation form in our pilot study, necessary sample size comes out to be 33. Therefore, a total of 40 subjects were enrolled each as cases and controls. Cases have been selected randomly from patients visiting the hospital to avoid the selection bias. Cases were included as per the criteria put forth by the IDF. Age- and sex-matched controls were selected from the population attending the regular medical checkup in the hospital. IDF criteria is: Central obesity plus 2 or more of the following - fasting glucose ≥100 mg/dL or previously diagnosed type 2 diabetes, TG ≥150 mg/dL or specific treatment for this lipid abnormality, HDLc <40 mg/dL in males and <50 mg/dL in females or specific treatment for this lipid abnormality and systolic BP ≥130 mm Hg diastolic BP ≥85 mm Hg or treatment of previously diagnosed hypertension. As per the Declaration of Helsinki, written informed consent were taken from all the participants. With all aseptic precautions, early morning fasting blood samples were collected by venipuncture from all patients. From control subjects, blood samples were collected through venipuncture at the time of their routine clinical visits. The samples were analyzed immediately after processing. Serum PON1 ARE activity was measured spectrophotometrically at 270 nm with 3 mL buffer-substrate solution containing 20 mmol Tris-hydroxymethyl (HCl) buffer and 4 mmol phenylacetate as substrate at pH 8.0 and 5 μl serum. Serum PON1 LACT was measured spectrophotometrically at 270 nm with 2 mL buffer-
substrate solution containing 50 mmol Tris-HCl buffer and 1 mmol dihydroxycoumarine as substrate at pH 8.0 and 10 μl serum. Normality of the distribution of the ARE and LACT was assessed by the Shapiro-Wilk test (28,29). Normality of the distribution of the ARE and LACT was assessed by the Shapiro-Wilk test. The two-sample t-test was applied for hypothesis testing. The results were expressed as mean ± standard deviation for all continuous variables. The statistical significance level was set at 0.05. There were no differences between the two groups with regard to age and sex. Linear regression was used to examine the relationship between one dependent and one independent variable. After performing an analysis, the regression statistics can be used to predict the dependent variable when the independent variable is known. Regression goes beyond correlation by adding prediction capabilities. Therefore, we also used the linear regression for the analysis. The result of linear regression is given in terms of R² which is a statistical measure of how close the data are to the fitted regression line. It is also known as the coefficient of determination. It indicates the proportion of the variance in the dependent variable that is predictable from the independent variable. In general, the higher the R², the better the model fits your data. The results obtained were analyzed by using SPSS 20.0.

**Results**

Serum PON1 ARE activity was found to be reduced significantly in patients with MetS than in controls. Similarly, serum PON1 LACT was found to be reduced significantly in patients with MetS. The results show that the ARE and lactonase singly can correlate well with body mass index (BMI) and their combination even more so. The study parameters are shown in the Table 1.

Figure 1 and 2 show the distribution of the PON1 ARE and LACT respectively in cases and controls. Figure 3 and 4 show correlation of PON1 ARE and lactonase respectively with BMI.

Correlation coefficient (r) for HDL with PON1 ARE comes out to be 0.150 (p<0.001) while for PON1 LACT was 0.071 (p=0.014). This positive correlation of HDL with PON activities shows that PON activities are well associated with HDL. Linear regression analysis shows R²=0.246 indicating significant correlation between ARE and BMI (p<0.001). Similarly, serum LACT of PON1 also gets reduced with increased BMI. Its linear regression analysis showed R²=0.417 suggesting a significant correlation between lactonase and BMI (p<0.001). Both these show a significant negative correlation with BMI. Along with other parameters, the difference between patients and controls for fasting blood glucose and diastolic BP was also statistically significant.

<table>
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<tr>
<th>Table 1. The study parameters</th>
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<tbody>
<tr>
<td>Parameter</td>
</tr>
<tr>
<td>Age (years)</td>
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<tr>
<td>BMI (kg/m²)</td>
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<tr>
<td>Diastolic BP</td>
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<tr>
<td>Fasting glucose (mg/dL)</td>
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<td>HDL cholesterol (mg/dL)</td>
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<tr>
<td>PON1 arylesterase (kU/L)</td>
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<td>PON1 lactonase (U/L)</td>
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BMI: Body mass index, PON: Paraoxonase, SD: Standard deviation, BP: Blood pressure, HDL: High-density lipoprotein

**Figure 1.** Serum paraoxonase 1 arylesterase activity in controls and cases

**Figure 2.** Paraoxonase 1 lactonase activity in cases and controls

**Figure 3.** Correlation of paraoxonase 1 arylesterase with body mass index

**Figure 4.** Correlation of paraoxonase 1 lactonase with body mass index
(r) for diastolic BP was 0.083 (p=0.036) with PON1 ARE and 0.079 (p=0.042) with PON1 LACT. Correlation coefficient (r) for fasting blood glucose was 0.134 (p=0.018) with PON1 ARE and 0.102 (p=0.029) with PON1 LACT.

Discussion

To the best of our knowledge, ours is the first study investigating the correlation of MetS with serum ARE and LACT in combination. Plasma PON1 activity was found to be altered, usually decreased in number of pathological conditions and diseases (30). In the present study, we found that there was a significant reduction in PON1 ARE and LACT in cases of MetS. It appears that ARE and LACT of PON1 are new and independent markers for MetS. Some of the researchers have found a significant negative correlation between oxidative stress and PON1 levels (31). It can be hypothesized that oxidative damage to the lipoproteins especially to HDL leads to reduced PON1 activity as oxidative stress levels are high in MetS due to deranged glycemic control and hyperinsulinemia (32). Biochemical findings of MetS have shown reduced HDLc. Decreased concentration of HDLc is correlated with reduced PON1 activities (33). Our results depict, though not very significant, a positive correlation of HDLc with ARE and lactonase.

Reduced PON1 activities are supposed to be a consequence of dysfunctional or modified HDLc which is responsible for reduced antioxidant capacity (34). As PON1 prevents oxidation of LDL and HDL, qualitative changes in lipoproteins coupled with reduced HDLc concentration leading to reduced PON1 activity, render them more susceptible to oxidative damage. There is a progressive decline in enzymatic capacity of PON1 with disease severity of MetS. Inactivation of PON1 (35) is likely to be the consequence of oxidative stress in, exceeding the antioxidant capacity of the enzyme. This can be the possible explanation of reduced PON1 activity.

The literature states that severity of MetS is directly proportional to oxidative stress which inactivates PON1 function (36). However, at the same time, the possibility that low PON1 function fails to provide an efficient protection against MetS-related oxidative damage can not be excluded. PON1 is capable of protecting lipoproteins from lipid peroxidation by degrading specific oxidized cholesteryl esters and phospholipids, and antioxidant properties of HDL have been attributed, at least partially to PON1 (36). This may be the possible explanation for reduced PON1 activity found in MetS. Some researchers revealed that a low PON1 concentration is characteristic of MetS, independently of low HDLc concentration. It has also been proposed that these results invite to take into account not only HDL quantity but also HDL quality, which could be reflected, at least in part by PON1 concentration or activity (37). This is because PON1 is a HDL-associated enzyme. This supports the findings that PON1 correlates well with MetS. The reductions in the activities of PON1 found in the present study and their relationship with HDL-cholesterol suggest that the decrease in these serum enzymes may participate in the pathogenesis of MetS.

The altered PON1 ARE and LACT in MetS could have two possible explanations. One is that serum PON1 activities may be lowered as a result of an altered synthesis or secretion of HDLc. The other one is the overproduction of circulating inhibitors like lipid peroxidation products and various free radicals. Free radicals are disproportionately formed in metabolic abnormalities, such as chronic hyperglycemia (38) dyslipidemia (39) by oxidation and subsequent oxidative degradation of proteins. Insulin resistance seems to stimulate endothelial superoxide anion production via nicotinamide adenine dinucleotide phosphate hydrolase (40) and hence, can worsen the degree of oxidative stress. Taken together, these observations strongly suggest that the activation of oxidative-stress pathways is a key component of MetS. From the present study, it is clear that reduction in PON1 activities in MetS are mainly due either to abnormalities with synthesis or secretion of HDLc or oxidative stress which is a consequence of excess production of free radicals. This study also iterates that it is the "quality" and not the quantity of HDLc which needs to be taken into account while studying the pathophysiology of MetS and PON1 effectively put forth the 'quality' of HDLc. It is also suggested from the present study that obesity is an important factor for enhanced oxidative stress and it is associated with lower antioxidant PON1 enzymatic activity. The combination of oxidative stress, obesity complication, dyslipidemia, deranged glycemic control and insulin resistance could contribute to the greater risk of MetS. Therefore, our study recommends PON1 as a tool to assess the severity of MetS.

Ethics

Ethics Committee Approval: Approved by Institutional Ethical Committee. Informed Consent: Written informed consent was taken as per Helsinki declaration. Peer-review: Externally peer-reviewed.

Authorship Contributions


Conflict of Interest: No conflict of interest was declared by the authors.

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