The Evaluation of Serum Paraoxanase Activity and Malondialdehyde Levels in Type 2 Diabetic Patients with Retinopathy

Retinopatisi Olan Tip 2 Diabetes Mellitus Olgularında Serum Paraksonaz Aktivitesinin ve Malondialdehit Seviyelerinin Belirlenmesi

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Abstract
Purpose: In the present study, we aimed to evaluate paraoxonase1 (PON1) activities and malondialdehyde (MDA) levels, one of the end products of lipid peroxidation induced by reactive oxygen species in diabetic patients with retinopathy.

Material and Methods: Serum MDA levels and PON1 levels were measured spectrophotometrically in 57 diabetic patients with retinopathy and in 24 healthy subjects which constituted the control group.

Results: In the DM group, mean serum basal PON1, stimulated PON 1 and MDA levels are detected as 151.72 ± 76.26 U/L, 329.86 ± 234.05 U/L and 3.79 ± 2.11 nmol/mL, respectively. In the control group, mean serum basal PON1, stimulated PON 1 and MDA levels are detected as 272.69 ± 104.44 U/L, 554.29 ± 214.58 U/L and 1.12 ± 0.29 nmol/mL, respectively. When compared to healthy controls, there was a statistically significant decrease in serum basal PON1, stimulated PON 1 levels (p <0.001) and a significant increase in serum MDA levels (p <0.001) in diabetic patients.

Conclusion: Increased oxidative stress caused by hyperglycemia results in a decrease in PON activities, therefore, PON might be used as a marker in the management of glycemic control and the development of complications.

Keywords: Diabetes Mellitus; Malondialdehyde; PON1 protein, human.

Özet
Amaç: Bu çalışmada Tip 2 diabetes mellitusulu (DM) oglularında lipit peroksidasyonunun son ürünlerinden olan ve reaktif oksijen parçaları tarafından indüklenen, paraksonaz 1 (PON1) ve malondialdehit (MDA) seviyelerinin belirlenmesi amaçlanmıştır.

Materiyal ve Metod: Eliyedi diabetik oğul ve kontrol grubunun oluşturduğu 24 sağlıklı oğulda serum MDA ve PON1 seviyeleri spektrofotometrik olarak ölçüldü.

Bulgular: DM grubunda, ortalamal serum bazal PON1 düzeyi 151.72 ± 76.26 U/L, ortalamal stimüle PON 1 düzeyi 329.86 ± 234.05 U/L ve ortalamal MDA düzeyi 3.79 ± 2.11 nmol/mL olarak bulundu. Kontrol grubunda ise ortalamal serum bazal PON1 düzeyi 272.69 ± 104.44 U/L, ortalamal stimüle PON1 düzeyi 554.29 ± 214.58 U/L ve ortalamal MDA düzeyi 1.12 ± 0.29 nmol/mL olarak bulundu. Diabetik oğularda serum bazal PON1 ve stimüle PON 1 düzeyleri sağlıklı kontrolleri göre anlamı olmayan düzük seviyelerdir (p <0.001), serum MDA düzeyleri anlamı olarak yüksekti (p <0.001).

Sonuç: Hiperglisemi nedeniyle artış oksidatif stres PON aktivitesinde azalma neden olmaktadır. Bu nedenle PON glikemik kontrolünün ve komplikasyon gelişiminin bir göstergesi olarak kullanılabilir.

Anahtar Kelimeler: Diabetes Mellitus; Malondialdehit; İnsan PON1 proteini.

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Introduction
To date, many investigations have focused on the antioxidant status and oxidative stress in diabetes mellitus (DM) (1–3). It has been shown that an increase in free radical production in type 2 diabetes mellitus occurs due to nonenzymatic glycosylation of proteins, auto-oxidation of glucose, or increased metabolism of glucose by the sorbitol pathway. Poor glycemic control in type 2 DM also has been associated with the depletion of protective serum antioxidant activity in these cases (4).

Paraoxonase (PON1) is a calcium-dependent esterase that is known to catalyze hydrolysis of organophosphates. PON1 is widely distributed among tissues such as liver, kidney, intestine, and also plasma (5, 6). PON1, which is exclusively bound to high-density lipoprotein (HDL), is recognized as an antioxidant enzyme, because it hydrolyses lipid peroxides in oxidized lipoproteins (7, 8). PON1 activity was suggested to be inversely associated with oxidative stress in serum and macrophages (9). Reduced PON1 activities have been reported in several groups of patients with diabetes, hypercholesterolemia and cardiovascular disease who are under increased oxidative stress (10, 11).

In the present study, we aimed at evaluating the basal-stimulated PON1 activities and malondialdehyde (MDA) levels, one of the end products of lipid peroxidation induced by reactive oxygen species in patients with diabetic retinopathy.

Patients and Methods
In this study we measured serum basal-stimulated PON1 and MDA levels in patients with type 2 DM with retinopathy who were followed up by our Retina clinic of the Ophthalmology Department and Internal Medicine Department.

The study included 57 patients with type 2 DM (33 male and 24 female) with a mean age of 57.94 ± 9.09 years (range 46-75 years) and a mean diabetes duration of 14.02 ± 4.41 years (range 12-25 years). All of the patients were treated with oral antidiabetic drugs. Total cholesterol, HDL-cholesterol, LDL cholesterol and triglycerides were measured by conventional enzymatic methods. HbA1c was determined on venous blood by routine laboratory methods.

Retinopathy was assessed ophthalmoscopically and confirmed by fluorescein angiography. Patients had adult onset form of DM with no history of ketoacidosis. Chronic renal insufficiency, uncontrolled primary and secondary hypertension, previously documented myocardial infarction, angina and cardiovascular disease or other life threatening diseases such as cancer were the exclusion criteria from the study.

The control group consisted of 24 age-matched healthy volunteers (14 male, 10 female). The study was approved by the Ethical Committee of Human Studies of Erciyes University and all subjects gave informed consent.

All reagents were purchased from Sigma and Merck. After blood samples were obtained serum samples were immediately separated and stored at –70 °C until analysis.

Serum MDA levels were measured according to a method described elsewhere (12). The principle of the method was based on the spectrophotometric measurement of the color occurred during the reaction to thiobarbituric acid with MDA. Concentration of thiobarbituric acid reactive substances (TBARS) was calculated by the absorbance coefficient of malondialdehyde-thiobarbituric acid complex and expressed as nmol/mL. As a standard MDA bis (dimethyl acetal)-TBA (thiobarbituricacid) complex was used.

Serum PON1 activity was measured according to a method described elsewhere (13). We measured the rate of hydrolysis of paraoxon by monitoring the increase of absorbance at 405 nm and at 25 °C. The basal assay mixture included 1.0 mM paraoxon and 1.0 mM CaCl; in 0.05 M glycine buffer pH 10.5 and salt-stimulated PON included 1.0 M NaCl in addition to this mixture. One unit (IU) of paraoxonase activity is defined as 1 mol of p-nitrophenol formed per min, and activity was expressed as U/L of serum. Paraoxonase assay were made either without any added NaCl (basal activity) or with 1 M NaCl included (salt-stimulated activity).

Statistical evaluation was carried out with the SPSS® 10.0 (Statistical Packages for Social Sciences; SPSS Inc, Chicago, Illinois, USA). Data obtained from the study groups were compared by the student t-test; p value less than 0.05 was considered as statistically significant.

Results
The demographic and clinical characteristics of control subjects and diabetic patients are presented in Table 1. HDL levels were lower and total cholesterol, fasting
glucose, HbA1c levels were higher in type 2 DM patients compared to controls and these comparisons were statistically significant (p<0.05). Triglyceride and LDL-cholesterol levels were higher not different in type 2 DM patients compared to controls (p=0.05).

In the DM group, mean serum basal PON1, stimulated PON 1 and MDA levels are determined as 272.69 ± 104.44 U/L, 554.29 ± 214.58 U/L and 1.12 ± 0.29 nmol/mL, respectively. In the control group, mean serum basal PON1, stimulated PON 1 and MDA levels are shown on Table II. When compared to healthy controls, there was a statistically significant decrease in serum basal PON1, stimulated PON 1 levels (p<0.05) and a significant increase in serum MDA levels (p<0.05) in diabetic patients.

Table I. Clinical Characteristics of Patients and Controls

<table>
<thead>
<tr>
<th></th>
<th>Controls (n=24)</th>
<th>Type 2 DM (n=57)</th>
<th>p  value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender (male/female)</td>
<td>14/10</td>
<td>33/24</td>
<td></td>
</tr>
<tr>
<td>Age (yr)</td>
<td>52.31±11.28</td>
<td>57.94±9.09</td>
<td>p&gt;0.05</td>
</tr>
<tr>
<td>Fasting glucose (mg/dL)</td>
<td>86.5±11.27</td>
<td>212.56±84.27</td>
<td>p&lt;0.05</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>3.11±0.76</td>
<td>9.19±1.84</td>
<td>p&lt;0.05</td>
</tr>
<tr>
<td>T-cholesterol (mg/dL)</td>
<td>145.90±32.57</td>
<td>218.08±65.54</td>
<td>p&lt;0.05</td>
</tr>
<tr>
<td>LDL-cholesterol (mg/dL)</td>
<td>121.77±32.79</td>
<td>138.96±47.98</td>
<td>p&gt;0.05</td>
</tr>
<tr>
<td>HDL-cholesterol (mg/dL)</td>
<td>58.04±15.44</td>
<td>42.64±13.86</td>
<td>p&lt;0.05</td>
</tr>
<tr>
<td>Triglyceride (mg/dL)</td>
<td>174.77±44.61</td>
<td>196.78±95.23</td>
<td>p&gt;0.05</td>
</tr>
<tr>
<td>Duration of diabetes (yr)</td>
<td>-</td>
<td>14.02±4.41</td>
<td></td>
</tr>
</tbody>
</table>

There was a statistically significant difference between patients with DM and controls, student t-test (p< 0.05). Data given as mean ± SD.

Table II. Serum PON and MDA levels in patients with DM and controls

<table>
<thead>
<tr>
<th></th>
<th>DM</th>
<th>Control</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>PON1 (U/L) (basal)</td>
<td>151.72±76.26 U/L</td>
<td>272.69±104.44 U/L</td>
<td>p&lt;0.05</td>
</tr>
<tr>
<td>PON1 (U/L) (stimulated)</td>
<td>329.86±234.05 U/L</td>
<td>554.29±214.58 U/L</td>
<td>p&lt;0.05</td>
</tr>
<tr>
<td>MDA (nmol/mL)</td>
<td>3.7±2.11</td>
<td>1.12±0.29</td>
<td>p&lt;0.05</td>
</tr>
</tbody>
</table>

There was a statistically significant difference between patients with DM (n=57) and controls (n=24). Values are given as mean:SD. Student t test (p< 0.05).

Discussion

Diabetes mellitus is associated with increased oxidant stress and the changes in antioxidant enzyme activities. The increased oxidative stress in type 2 DM may result from the changes in energy metabolism, alterations in sorbitol pathway activity, changes in the level of inflammatory mediators and the status of the antioxidant defence systems, or localized tissue damage following hypoxia and ischemic reperfusion injury (3). Maxwell co-workers (4) also suggest that persistent hyperglycemia promotes nonenzymatic glycosylation of proteins and local free radical production.

Previously reported studies indicated that there is a strong correlation between antioxidant enzyme activities and poor glycemic control in diabetes, which probably accounts for the diabetic complications (3, 14, 15).

In diabetes mellitus, there is an increased production of free radicals, which in turn promotes lipid peroxidation. MDA is formed as an end product of lipid peroxidation. When compared to healthy controls, we found a statistically significant increase in serum MDA levels in patients with diabetic retinopathy (p<0.001). This is in agreement with many previously reported studies (16-18).
In our study, the finding that basal and stimulated PON 1 activities were significantly different in DM patient group compared with control is similar to the report of Mackness co-workers, who showed a reduction in PON activity in patients with insulin-dependent diabetes mellitus (IDDM) (19). Previous data on PON activity in DM show conflicting results. Although some recent investigations demonstrate no significant association between the PON activity and complications of DM (14), other reports show that reduction in PON activity is associated with vascular complications of DM (19-22).

In a recent study by Unur and co-workers (23) the relationship of oral disturbances of diabetes mellitus patients with paraoxonase gene polymorphisms was evaluated. It was found that PON1 55 M allele carriers had greater risk for general periodontal and/or gingival problems.

In conclusion increased oxidative stress caused by hyperglycemia results in a decrease in PON activities, therefore, PON might be used as a marker in the management of glycemic control and the development of complications. Since it is yet unclear whether low PON activity by itself and/or presence of PON phenotypes constitute any risk factors for complications, further prospective and molecular biology studies are necessary to determine the role of PON phenotypes on the development of diabetic complication.
References


