Relationship Between Paraoxonase-1 Activity and Lipid Peroxidation Marker Levels in Coronary Artery Disease Patients with and without Diabetes

Koroner Arter Hastalığı Olan Diyabetik ve Nondiyabetik Hastalarda Paraoksonaz 1 Aktivitesi ve Lipid Peroksidsasyonu Belirtç Düzeyleri Arasındaki İlişki

**ABSTRACT**

**Objective**: Increase in blood glucose concentration can inactivate paraoxonase-1 (PON1) and increase lipid peroxidation. The lack of protective effect of PON1 on lipid peroxidation may be a factor in acceleration of coronary artery disease (CAD) in diabetic patients. We therefore aimed to investigate the changes in PON1 activity and lipid peroxidation markers in diabetic and non-diabetic CAD patients.

**Material and Methods**: We investigated the relationship between serum lipids, lipoproteins, thiobarbituric acid reactive-substances (TBARS), conjugated dienes (CD) and lipoprotein(a) (Lp(a)) levels and PON1 activity in CAD patients with and without diabetes.

**Results**: Elevated levels of Lp(a), CD and TBARS and decreased level of high-density lipoprotein cholesterol (HDL-C) and the activity of PON1 were observed in CAD patients with and without diabetes by comparison to controls. Total cholesterol and HDL-C have increased, TBARS and CD have decreased in CAD patients without diabetes compared to those with diabetes. On multiple logistic regression analysis, risk factors associated with CAD were CD, TBARS and PON1/HDL-C ratio in non diabetic CAD patients whereas only glucose levels were associated with CAD in diabetic CAD patients.

**Conclusion**: PON1 activity and lipid peroxidation markers have associated with glucose levels in diabetic patients and were also found to be the major risk factor for CAD in diabetes. We suggest that maintaining the optimum glucose levels can be vital to regulate the oxidative balance in diabetic patients. Further studies with a large number of individuals might be performed for enhance our knowledge.

**Key Words**: Diabetes mellitus; arylalkylphosphatase; lipid peroxides; lipids

**ÖZET Amaç**: Kan glukoz konsantrasyonundaki artış, paraoksonaz-1 (PON1) inaktivasyonuna ve lipid peroksidsasyonunda artışa neden olabilir. Lipid peroksidsasyonu üzerine PON1 koryuyucu etkisinin azalması diyabetik hastalarda, koroner arter hastalığı (KAH) hızlandırıcısı bir etki yaratabilir. Bu nedenle bu çalışmada, PON1 ve lipid peroksidsasyonu belirteçlerinin diyabetik ve non-diyabetik KAH olanlardaki değişimini gözlemlemeyi amaçladık.

**Gereç ve Yöntemler**: Bu çalışmada, diyabetik ve non-diyabetik KAH olan bireylerde, serum lipitlerini, lipoproteinleri, tiyobarbiturik asit reaktift bileşikleri (TBARS), konju gel diener (KD), lipoprotein(a) (Lp(a)) düzeyleri ve PON1 aktivitesi arasındaki ilişkiyi araştırdık.

**Bulgular**: Hem diyabetik hem de non-diabetik KAH hastalarda, Lp(a), KD ve TBARS düzeylerinde artış gözlenmiş, kontrollere kıyasla yüksek dansiti lipoprotein kolesterol (HDL-K) düzeyi ve PON1 aktivitesinde azalma gözlenmiş. Diyabetiklerde kısasıyla, non-diyabetik KAH hastalarda total kolesterol ve HDL-K düzeyleri daha yüksek, IKB, TBARS ve KD düzeyleri daha dişikktir. Çoklu regresyon analizi yapıldığında, non-diyabetiklerdeki KAH risk faktörleri arasında KD, TBARS ve PON1/HDL-C oranı bulunmamış, diyabetik hastalarda KAH ile ilişkili risk faktörü olarak yalnız glukoz saptaılmıştır.

**Sonuç**: Diyabetik hastalarda, PON1 aktivitesi, lipid peroksidsasyon belirteçleri ile glukoz düzeyleri arasında ilişki vardır ve diyabette görülen KAH’da lipid peroksidsasyon belirteçleri major risk faktörüdür. Diyabetik hastalarda, optimum glukoz seviyelerinin korunması, oksidatif dengeyi düzenlenmek için hayati önemlernen fikrindendir. Bilgimizin arttırılması için daha çok sayıdaki bireyler kapasitenin daha ileri çalışmalar yapabilir.

**Anahtar Kelimeler**: Diabetes mellitus; arildialkilosfataz; lipid peroksitler; lipidler

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Diabetes is described as "one of the main threats to human health in the twenty-first century". Diabetes is associated with hyperglycaemia and autooxidation of glucose may lead to increased free radical synthesis, which in turn may lead to DNA mutations, changes in structure and function of proteins and peroxidation of lipids in combination with certain lipid abnormalities. Lipid peroxides are high toxic molecules. They might disturb the balance between vasoconstriction and vasodilatation by decreasing the synthesis of prostoglandins and increasing the vessel wall sensitivity to angiotensin II. All of these events might indicate elevated risks for cardiovascular and other macrovascular morbidity and mortality in type 2 diabetic patients.

High levels of glucose can produce permanent chemical alterations in high density lipoprotein (HDL) and increase lipid peroxidation while glycated HDL is less capable of binding the antioxidant enzyme paraoxonase-1 (PON1), a HDL-associated esterase/lactonase that metabolizes lipid hydroperoxides. Serum PON1 activity was found to be decreased in Diabetes Mellitus (DM), and could therefore be one of the reasons to increased mortality due to coronary artery disease (CAD).

Epidemiological data shows that the prevalence of diabetes and CAD are influenced by environmental factors. According to the results of the Turkish Diabetes Epidemiology (TURDEP) study, the prevalence of diabetes is 7.2 % in Turkey. We previously reported an association between PON1 activity and lipid peroxidation levels in CAD patients. In this study we concerned the relation between PON1 activity and lipid peroxidation indicators in CAD patients with diabetes. We matched the subjects for age and sex to eliminate the influence of age and gender and determine the relationship between lipids, lipoproteins, lipid peroxidation markers [thiobarbituric acid reactive substances (TBARS) and conjugated dienes (CD)] and PON1 activity. On the other hand, we wanted to compare all of the above parameters with diabetic and non-diabetic CAD patients and healthy controls.

MATERIAL AND METHODS

SUBJECTS
28 patients were with type-2 diabetes (16 male, 66.06 ± 2.62 old years (mean ± SEM) and 12 females, 64.25 ± 2.66 old years), 28 non-diabetic CAD patients (16 male, 65.38 ± 2.30 old years and 12 females, 62.67 ± 2.40 old years) and 28 healthy controls (16 male, 62.00 ± 2.27 old years and 12 females, 62.08 ± 2.89 old years) who underwent coronary angiography for diagnostic purposes. Diabetes was defined according to WHO criteria. Standard Judkin technique was used in coronary arteriographic examination and images were interpreted by a panel of experienced cardiologists blinded to data on biochemical parameters. The presence of CAD was characterized from irregularity of coronary wall to significant coronary lesions in at least one major coronary artery and non-diabetic subjects with no detectable CAD were classified as controls. All the subjects in 3 groups were age and sex matched. The study was approved by the University Ethics Committee of the Faculty of Medicine.

SERUM SAMPLES
After a 12 hour fasting, venous blood samples were obtained in the morning by the biochemists performing laboratory analyses that were blinded to the clinical status of patients. Serum was separated immediately by low-speed centrifugation (600 g for 10 min at 4°C). Serum samples were used freshly for analysis.

LIPID ASSAYS
Total cholesterol (TC), HDL-C and triglycerides (TG) were measured enzymatically using commercial kits (Abbott Aeroset system, Laboratories, Abbott Park, Illinois). The low density lipoprotein-cholesterol (LDL-C) fraction was calculated indirectly using the Friedewald equation. The factor [triglyceride] / 5 was used to estimate very low density lipoprotein cholesterol (VLDL-C) concentration. Lp(a) concentrations were measured by automated immunoturbidimetric method on a Roche/Hitachi 911 analyzer (Tina-quant; Roche Diagnostics, Mannheim, Germany) using specific antisera.
Levels of glucose and Hb\textsubscript{A1c} were determined on a Hitachi 911 automatic analyzer (Boehringer Mannheim, Mannheim, Germany).

**PARAOXONASE-1 ACTIVITY ASSAY**

Serum PON1 activity was assayed spectrophotometrically as described previously\(^{11}\). Briefly, the assay mixture consisted of 1500 µL of 6 mmol/L paraaxon substrate solution in 0.1 mol/L Tris–HCl buffer, pH 8.0, containing 1 mmol/L CaCl\(_2\) and 60 µL of fresh serum specimen. The absorbance was monitored photometrically at 405 nm and at 37°C at 1 minute intervals on a spectrophotometer (photometer 4010, Boehringer Mannheim, GmbH, Germany). One unit of PON1 activity was defined as 1 µmol of p-nitrophenol formed per minute and the activity expressed as U/L of serum.

**CONJUGATED DIENE ASSAY**

Conjugated diene levels were measured by the method of Recknagel and Glende\(^{12}\). Lipids were extracted with 2:1 (v/v) chloroform–methanol; the extract was evaporated to dryness under a stream of nitrogen and then redissolved in cyclohexane. The cyclohexane solution was assayed at 234 nm. The results were expressed as µmol/L using \(\epsilon_{\text{max}} = 25.200 \text{ M}^{-1} \text{ cm}^{-1}\).

**THIOBARBITURIC ACID REACTIVE-SUBSTANCES ASSAY**

Thiobarbituric acid reactive-substances levels were measured by a fluorometric method described by Gumuslu et al.\(^{13}\) with minimal modifications described by Wasowicz et al.\(^{14}\) using 1,1,3,3-tetramethoxypropane as a standard. In brief, 50 µL of serum or plasma or an adequate volume of MDA working standard solution was introduced into 10-mL glass tubes containing 1 mL of distilled water. After addition of 1 mL of the solution containing TBA in acetic acid (pH of the reaction mixture, 2.5). After mixing, the samples were placed in a water bath and heated for 1 h at 95-100°C. After the samples were cooled, 25 µL HCl was added and the reaction mixture was extracted by 5 mm with 3.5 mL of n-butanol. We separated the butanol phase by centrifugation at 1500 x g for 10 min, and measured the fluorescence of the butanol extract with the fluorescence of the butanol extract was measured in a spectrofluorometer (Shimadzu RF-5000; Shimadzu Corp, Japan) using wavelengths of 525 nm for excitation and 547 nm for emission. The results were presented in µmol MDA/L.

**Statistical Analysis**

The data were expressed as means ± SEM. Comparisons of parameters between groups were performed using One-way ANOVA test. The correlations between the variables were evaluated by linear regression analysis. Calculations were performed by SPSS version 13.0 (SPSS Inc., Chicago, IL, USA). P-values less than 0.05 were defined as statistically significant.

**RESULTS**

Table 1 shows the data related to the glucose, lipids, lipoprotein (a), paraoxonase and lipid peroxidation markers observed in controls and both group of patients.

Table 2 shows the correlation coefficients among various parameters including HDL-C, PON1, TBARS, CD, glucose and Hb\textsubscript{A1c} non diabetic and diabetic CAD patients and controls.

We chose PON1 activity as a dependent variable and found that CD, HDL-C, LDL-C levels and we found that TC/HDL-C and LDL-C/HDL-C ratios correlated (R=0.96, p<0.001) with PON1 activity. These parameters were explaining the 92% change in PON1 activity in this study population (Durbin Watson coefficient = 2.11).

On multiple logistic regression analysis, risk factors associated with CAD included TBARS (OR = 1394530, p< 0.001), PON1/HDL-C (OR = 0.234, p< 0.05) and LDL-C (OR = 2,542, p< 0.05) when diabetic and non diabetic patients were assayed against controls. When non diabetic CAD patients were compared against controls, lipid peroxidation markers (CD (OR = 1125, p< 0.001) and TBARS (OR = 164274, p< 0.05)) and PON1/HDL-C (OR = 0,229, p< 0.05) ratio whereas when diabetic CAD patients were assayed against controls only glucose (OR = 1.247, p< 0.05) levels were found to be associated with CAD risk.
TABLE 1: Glucose, lipids, lipid peroxidation markers (CD and TBARS) and Lp(a) levels and PON1 activity of non-diabetic and diabetic CAD patients and controls.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Controls (n=28)</th>
<th>Non-diabetic CAD patients (n=28)</th>
<th>Diabetic CAD patients (n=28)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose mg/dl</td>
<td>87.82 ± 1.36</td>
<td>88.43 ± 2.48</td>
<td>167.61 ± 6.79**, ***</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>4.51 ± 0.06</td>
<td>4.75 ± 0.06</td>
<td>7.61 ± 0.19**, ***</td>
</tr>
<tr>
<td>TC (mg/dl)</td>
<td>178.27 ± 20.50</td>
<td>213.46 ± 7.35*</td>
<td>194.12 ± 5.41***</td>
</tr>
<tr>
<td>LDL-C (mg/dl)</td>
<td>47.18 ± 1.93</td>
<td>62.26 ± 3.09 *</td>
<td>55.68 ± 2.32**</td>
</tr>
<tr>
<td>TG (mg/dl)</td>
<td>300.25 ± 18.60</td>
<td>380.85 ± 31.89 *</td>
<td>325.05 ± 23.91</td>
</tr>
<tr>
<td>VLDL-C (mg/dl)</td>
<td>59.34 ± 3.54</td>
<td>76.17 ± 6.20 *</td>
<td>65.54 ± 4.43</td>
</tr>
<tr>
<td>HDL-C (mg/dl)</td>
<td>42.54 ± 1.55</td>
<td>37.12 ± 1.93 *</td>
<td>32.10 ± 1.55**, ***</td>
</tr>
<tr>
<td>CD (µmol/L)</td>
<td>1.23 ± 0.06</td>
<td>2.13 ± 0.08 *</td>
<td>2.38 ± 0.08**, ***</td>
</tr>
<tr>
<td>TBARS (µmol MDA/L)</td>
<td>1.15 ± 0.05</td>
<td>1.81 ± 0.051 *</td>
<td>2.00 ± 0.04**, ***</td>
</tr>
<tr>
<td>Lp(a) (mg/dl)</td>
<td>12.41 ± 1.90</td>
<td>36.79 ± 3.78 *</td>
<td>30.90 ± 4.31**</td>
</tr>
<tr>
<td>PON1 (U/L)</td>
<td>399.40 ± 21.50</td>
<td>212.34 ± 13.54 *</td>
<td>220.98 ± 15.05**</td>
</tr>
<tr>
<td>PON1/HDLC (U/mmol)</td>
<td>361.14 ± 12.95</td>
<td>222.39 ± 8.01*</td>
<td>220.98 ± 15.05**</td>
</tr>
<tr>
<td>Age (years)</td>
<td>62.04 ± 1.76</td>
<td>64.21 ± 1.66</td>
<td>65.29 ± 1.87</td>
</tr>
</tbody>
</table>

Values are given as mean ± SEM.

TC, total cholesterol; LDL-C, low density lipoprotein-cholesterol; TG, triglycerides; VLDL-C, very low density lipoprotein-cholesterol; HDL-C, high density lipoprotein-cholesterol; CD, conjugated dienes; TBARS, thiobarbituric acid-reactive substances; Lp(a), lipoprotein (a); PON1, paraoxonase-1; CAD, coronary artery disease; n denotes the number of subjects.

*P < 0.05, non-diabetic CAD patients vs. controls.

** P < 0.05, diabetic CAD patients vs. controls.

***P < 0.05, diabetic CAD patients vs. non-diabetic CAD patients.

TABLE 2: Correlation coefficients between HDL-C, PON1, TBARS, CD, glucose and HbA1c levels.

<table>
<thead>
<tr>
<th>HDL-C</th>
<th>PON1</th>
<th>TBARS</th>
<th>CD</th>
<th>Glucose</th>
<th>HbA1c</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>1.000</td>
<td>0.778 **</td>
<td>-</td>
<td>-0.466 *</td>
<td>-</td>
</tr>
<tr>
<td>PON1</td>
<td>0.778 **</td>
<td>1.000</td>
<td>-</td>
<td>-0.554 *</td>
<td>-</td>
</tr>
<tr>
<td>Non-diabetic CAD patients</td>
<td>1.000</td>
<td>0.652 **</td>
<td>-</td>
<td>-0.297 **</td>
<td>-</td>
</tr>
<tr>
<td>Diabetic CAD patients</td>
<td>-0.467 *</td>
<td>-0.550 **</td>
<td>1.000</td>
<td>0.339 **</td>
<td>0.395 *</td>
</tr>
</tbody>
</table>

HDL-C, high density lipoprotein-cholesterol; PON1, paraoxonase-1; TBARS, thiobarbituric acid-reactive substances; CD, conjugated dienes; CAD, coronary artery disease.

DISCUSSION

This study demonstrates the levels of lipids, Lp(a), lipid peroxidation markers and the activity of PON1 in serum of CAD patients with and without diabetes, matched for age and gender.

The levels of TC were significantly higher in non-diabetic CAD patients than in controls and diabetic CAD patients in agreement with the report of Gazzaruso et al.\(^15\) that the greatest risk factor for coronary artery disease is hypercholesterolemia.

Coronary artery disease patients in both groups had elevated levels of LDL-C in respect to control group. Lahdenpera et al.\(^16\) reported that LDL-C was lower in type 2 diabetic patients than in non-diabetic subjects. However, we could not find any significant difference between CAD patients with and without diabetes.

The role of plasma TG as CAD risk factor has been controversial.\(^17\) The present study indicated that fasting TG and VLDL-C levels were significantly increased in non-diabetic CAD patients compared to controls supporting Acarturk et al.\(^17\) who reported that serum TG and VLDL-C were increased in patients with CAD as compared to subjects without the disease. This result is in
In accordance with the study of Turner et al., possibly because of the greater biological variability of triglyceride than cholesterol measurements and because patients were receiving dietary advice, lipid lowering drugs and had a more uniform dietary intake than in the general population. Postprandial triglyceride values may have an additional atherogenic role to the fasting values that were measured.\textsuperscript{18}

Earlier prospective studies have demonstrated that HDL cholesterol\textsuperscript{18,19} is a significant predictor of cardiovascular events among patients with diabetes. In the present study, we showed that the presence of diabetes had substantially lowered HDL-C levels. The mechanism by which HDL-C level is reduced in diabetes is poorly understood, but may be associated with an increase in blood glucose concentration. Glycation can increase lipid peroxidation in HDL. Glycated HDL also has a reduced ability to protect against oxidation.\textsuperscript{20} However, even normal levels of high-density lipoprotein cholesterol may have a diminished protective power in diabetes because of structural and functional alterations in HDL.\textsuperscript{21}

We have demonstrated that Lp(a) levels were increased in diabetic and non-diabetic CAD patients in respect to control group. Our finding does not support the study of Gazzaruso et al.\textsuperscript{15} who reported that the difference between controls and diabetic CAD patients was not significant. On the other hand, they have also reported that non-diabetic CAD patients had significantly higher Lp(a) levels than diabetic CAD patients whereas in our study, the increase in non-diabetic CAD patients was not statistically significant. Our results are in accordance with Pedreno et al.\textsuperscript{22} who reported that Lp(a) is not elevated with the presence of diabetes and in angiographically defined CAD, elevated Lp(a) levels are associated specifically with CAD but not with type 2 diabetes mellitus.

We observed increased oxidative stress markers (TBARS and CD) in patients, as were previously shown for diabetes\textsuperscript{23} and CAD.\textsuperscript{11} Diabetic CAD patients have higher CD and TBARS levels than non-diabetic CAD patients. The increased serum oxidative stress markers in diabetic patients could be the result of glycation and glycoxidation of LDL by glucose, or/and the decreased capability of the patients’ HDL to protect LDL against oxidation.\textsuperscript{24}

These data confirm our previous observations of low serum PON1 activity in CAD patients,\textsuperscript{8,11} and this fact may account for the increased serum oxidative stress. Indeed, we have demonstrated that PON1 activity is negatively correlated with CD levels in non-diabetic and with CD and TBARS levels in diabetic CAD patients. In diabetics, a significant amount of serum PON1 is dissociated from HDL to the lipoprotein deficient serum fraction and unlike PON1 in HDL, unbound PON1 is not able to protect against lipids peroxidation. Our data showed that in diabetic CAD patients, PON1 activity did not correlate with glucose levels or Hb\textsubscript{A1c} but on the other hand CD, HDL-C and LDL-C levels were explaining the 92% changes in PON1 activity in this study population. The present study clearly demonstrated the reduced PON1 activity in the patients’ serum might be due to decreased HDL-C levels, altered HDL composition and direct PON1 inactivation by oxidized lipids.\textsuperscript{25}

As glucose increases oxidative stress, increased oxidative stress was an expected result for diabetic CAD patients. In diabetic CAD patients, a positive correlation was found between glucose and TBARS levels which may account for the increased oxidative stress associated with hyperglycemia. Our results agree the hypothesis that hyperglycemia might contribute to both macrovascular and microvascular complications via increased oxidative stress.\textsuperscript{26}

In patients with CAD, association between elevated TBARS levels in serum and cardiovascular risk was demonstrated by a previous longitudinal study.\textsuperscript{27} In this study, when diabetic and non-diabetic CAD patients were tested against controls, risk factors associated with CAD included TBARS, PON1/HDL-C and LDL-C. An increased concentration of LDL-C was found to be the major risk factor for coronary artery disease.\textsuperscript{18,28} Our finding suggest that TBARS level is the major risk factor for
CAD in non diabetic patients while decreased HDL-C associated PON1 activity and increased LDL-C levels are risk factors that are not associated with diabetes whereas increased hyperglycaemia is a risk factor for CAD in diabetes.

This study made a comparison in age and gender matched diabetic and non-diabetic CAD patients and controls. PON1 activity, lipid peroxidation markers were correlated with glucose levels in diabetic patients and were also found to be the major risk factor for CAD.

**Conclusion**

We therefore suggest that maintaining the optimum glucose levels is vital to regulate the oxidative balance in diabetic patients. However, this study had a limited number of subjects and further studies with a large number of individuals should be performed to further enhance our knowledge.

**Acknowledgement**

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**REFERENCES**


