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Clinical Investigations

Association between Paraoxonase-1 Activity and Lipid Peroxidation Indicator Levels in People Living in the Antalya Region with Angiographically Documented Coronary Artery Disease

AYŞE YEŞIM GÖÇMEN, SAADET GÜMÜŞLÜ, PH.D., ENDER SEMIZ, M.D.*

Department of Biochemistry and *Department of Cardiology, Faculty of Medicine, Akdeniz University, Antalya, Turkey

Summary

Background: Paraoxonase-1 (PON1) is a high-density lipoprotein (HDL)-associated enzyme capable of hydrolyzing lipid peroxides. Thus, PON1 plays a preventing role in atherosclerosis by protecting against lipid peroxidation.

Hypothesis: The incidence of coronary artery disease (CAD) is high in the Turkish population, and many risk factors have been studied as determinants of CAD. In Turkish people living in the Antalya region, we aimed to determine serum PON1 activity and its relation to lipoproteins and lipid peroxidation markers.

Methods: We measured the activity of serum PON1 together with concentrations of a variety of lipid constituents—total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), very low-density lipoprotein cholesterol (VLDL-C), HDL cholesterol (HDL-C), triglycerides (TG), apolipoprotein (apo) A-I, apoB, and lipid peroxidation indicators (conjugated diene [CD] and thiobarbituric acid-reactive substances [TBARS])—in 108 patients with CAD and 64 healthy subjects (controls).

Results: We found that the PON1 activity was significantly reduced in patients with CAD (222.37 ± 11.31 IU/l) compared with controls (331.75 ± 20.98 IU/l). These patients had significantly lower HDL-C, PON1/HDL-C, apoA-I, PON1/ApoA-I, and ApoA-I/ApoB, and higher LDL-C, TC/HDL-C, LDL-C/HDL-C, apoB, CD and TBARS than did controls. Total cholesterol and apoA-I concentrations were significantly higher in women than in men in both groups. After multiple logistic regression analysis, TBARS (odds ratio [OR] 568.87; p = 0.000), age (OR 1.10; p = 0.000), gender (OR 4.58; p = 0.008), apoA-I/apoB (OR 0.046; p = 0.003), and PON1/apoA-I (OR 0.58; p = 0.007) were independently indicative of the presence of CAD.

Conclusions: This is the first report of decreased serum PON1 activity and increased lipid peroxidation indicators (CD and TBARS) of patients with CAD living in Antalya, Turkey. Our results indicate that TBARS levels, age, gender, apoA-1/ApoB, and PON1/apoA-I ratios are important markers of CAD.

Key words: coronary artery disease, paraoxonase, lipids, lipoproteins, lipid peroxidation, conjugated diene, thiobarbituric acid-reactive substances

Introduction

Coronary artery disease (CAD) is a complex trait caused by a number of genetic and environmental factors.[1] Atherosclerotic cardiovascular disease is the principal cause of mortality and morbidity in developed countries.[2,3] The hallmark of atherosclerosis is the accumulation of cells containing excessive lipids within the arterial wall. Plasma lipoproteins are a major source of the lipid that accumulates in atherosclerotic lesions.[3] High-density lipoprotein (HDL) can protect low-density lipoprotein (LDL) from oxidative damage.[4] The mechanism of this protective effect may involve paraoxonase-1 (PON1), an HDL-associated enzyme capable of hydrolyzing lipid peroxides.[5] Human serum PON1 is a calcium-dependent esterase that hydrolyzes organophosphates such as paraoxon, and its activity is reduced in hypercholesterolemia and vascular cardiac diseases.[6]

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Address for reprints:

Saadet Gümüşlü, Ph.D.
Department of Biochemistry
Faculty of Medicine
Akdeniz University
07070 Antalya, Turkey
e-mail: sgumuslu@akdeniz.edu.tr

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Turkey has a high incidence of CAD, and its various geographic regions differ with regard to environment, diet, and genetics. The people living in the Antalya region have Mediterranean climate, diet, and life style. The status of PON1 activity, lipids, lipoproteins, apolipoproteins, and lipid peroxidation indicator (conjugated diene [CD] and thiobarbituric acid-reactive substance [TBARS]) levels has not been previously studied in the serum of patients with CAD and controls living in Antalya. Therefore, the present study aimed to investigate the activity of PON1 and the levels of lipids, lipoproteins, apolipoproteins (apo), and lipid peroxidation indicators in patients with CAD and healthy subjects living in Antalya, Turkey. The predictive power of parameters as markers of CAD was also investigated.

Materials and Methods

Subjects

Between May 2000 and March 2001, individuals who underwent coronary angiography at the Cardiology Department of the Medical Faculty of Akdeniz University participated in this study. They were inhabitants of the south of Turkey (Antalya region) and were unrelated. Exclusion criterion for subjects was the presence of diabetes mellitus.

The standard Judkin's technique was used for coronary arteriographic examination, and images were interpreted by a panel of experienced cardiologists blinded to data on all biochemical analyses. A stenosis estimated as >25% in at least one major coronary artery was considered to indicate CAD. Participants with no detectable coronary artery anomaly were classified as controls.

The CAD patient group included 108 individuals aged 60.00 ± 10.31 years (32–79) and comprised 20 women, aged 62.40 ± 9.07 years (49–79), and 88 men aged 59.47 ± 10.69 years (32–79). Controls were 64 individuals, aged 52.81 ± 10.72 years (29–79) and comprised 33 women, aged 54.70 ± 9.76 years (33–79) and 31 men, aged 50.81 ± 11.31 years (29–79). Fasting (12-h) blood samples were obtained from all subjects. Serum was separated by centrifugation of the blood samples at 1500 × g for 10 min at 4 °C.

Lipid Assays

Total cholesterol, HDL cholesterol (HDL-C), and TG were assayed on an Abbott Aeroset autoanalyzer (Abbott Aeroset System Laboratories, Abbott Park, Ill., USA), using enzymatic colorimetric methods. The LDL-C fraction was calculated indirectly, using the Friedewald equation.[7] The factor (triglyceride)/5 was used to estimate very low-density lipoprotein cholesterol (VLDL-C) concentration.[7]

Apolipoprotein A-I and apoB concentrations were measured by immunoturbidimetric method on a Roche/Hitachi 911 analyzer using specific antisera (Tina-quant, Roche Diagnostics GmbH, Mannheim, Germany).

Paraoxonase-1 Activity Assay

Serum PON1 activity was assayed by the methods described by Furlong *et al.*[8] and Mackness *et al.*[9] with minimal modifications. Briefly, the assay mixture consisted of 1,500 μl of 6 mM, paraoxon substrate solution in 0.1 M Tris-HCl buffer, pH 8.0, containing 1 mmol/l $CaCl_2$ and 60 μl of fresh serum. The generation of p-nitrophenol was measured at 405 nm and at 37° C. A molar extinction coefficient for p-nitrophenol was calculated as 3,030 $M^{-1}cm^{-1}$ to determine the enzymatic activity. One unit (IU) was defined as 1 μmol of p-nitrophenol formed per min and the activity was expressed as IU/l of serum.

Conjugated Diene and Thiobarbituric Acid-Reactive Substance Assays

Serum conjugated diene levels were measured by the method of Recknagel and Glende.[10] The TBARS levels were measured using the fluorometric method adapted to our laboratory by Gumuslu *et al.*,[11] which is a modification of the method described by Wasowicz *et al.*,[12] and the results were expressed as nmol malondialdehyde (MDA)/l.

Statistics

Data are expressed as mean ± standard deviation, and values of $p < 0.05$ were considered significant. Student's *t*-test was used for comparison of means. Pearson's correlation coefficient was used to test the strength of any associations between different variables. Multiple logistic regression analysis was used to identify associations between CAD and the independent parameters.

Results

The data obtained from patients with CAD and controls are shown in Tables I and II.

In the CAD patient group, PON1 activity correlated positively with TC ($r = 0.213$, $p < 0.05$) and HDL-C levels ($r = 0.207$, $p < 0.05$), and correlated negatively with CD levels ($r = -0.202$, $p < 0.05$). The HDL-C levels correlated positively with TC ($r = 0.360$, $p < 0.0001$), LDL-C ($r = 0.224$, $p < 0.05$), and apoA-I levels ($r = 0.402$, $p < 0.0001$), and correlated negatively with apoB/LDL-C ratio ($r = -0.313$, $p < 0.005$). The PON1/HDL-C ratio correlated positively with PON1 activity ($r = 0.845$, $p < 0.0001$), TG ($r = 0.223$, $p < 0.05$), VLDL-C ($r = 0.223$, $p < 0.05$), TC/HDL-C ($r = 0.325$, $p < 0.005$), LDL-C/HDL-C ratio ($r = 0.272$, $p < 0.005$), and apoB levels ($r = 0.198$, $p < 0.05$), and correlated negatively with apoA-I/apoB ($r = -0.294$, $p < 0.005$) and CD levels ($r = -0.200$, $p < 0.05$).

The TBARS levels (odds ratio [OR] 568.87; 95% confidence interval [CI] 57.40–5638.09; $p = 0.000$), age (OR 1.10; 95% CI 1.05–1.16; $p = 0.000$), gender (OR 4.58; 95% CI 1.49–14.15; $p = 0.008$), apoA-I/apoB (OR 0.046;

TABLE I Paraoxonase-1 (PON 1), lipids, lipoproteins, apolipoproteins, conjugated diene (CD) and thiobarbituric acid-reactive substances (TBARS) in controls and patients with coronary artery disease (CAD)

Parameters	Controls (n = 64)	Patients with CAD (n = 108)
PON1(IU/l)	331.75 ± 167.80	222.37 ± 117.57[d]
TC (mg/dl)	194.39 ± 39.55	202.67 ± 41.41
HDL-C (mg/dl)	42.97 ± 9.25	38.89 ± 9.88[b]
LDL-C (mg/dl)	123.46 ± 33.27	134.96 ± 34.98[a]
VLDL-C (mg/dl)	27.72 ± 12.12	28.65 ± 13.58
TG (mg/dl)	138.63 ± 60.65	143.13 ± 68.08
PON1/HDL-C (IU/mg)	0.80 ± 0.41	0.60 ± 0.31[d]
TC/HDL-C	4.68 ± 1.16	5.52 ± 1.78[d]
LDL-C/HDL-C	2.99 ± 0.96	3.70 ± 1.49[c]
ApoA-I (mg/dl)	113.41 ± 22.89	105.22 ± 21.78[a]
ApoB (mg/dl)	96.22 ± 23.83	108.56 ± 27.16[c]
PON1/ApoA-I (IU/mg)	0.31 ± 0.18	0.22 ± 0.12[c]
ApoA-I/HDL-C	2.72 ± 0.62	2.84 ± 0.78
ApoB/LDL-C	0.81 ± 0.19	0.83 ± 0.21
ApoA-I/ApoB	1.23 ± 0.31	1.01 ± 0.26[d]
CD (μmol/l)	1.35 ± 0.50	2.03 ± 0.58[d]
TBARS (nmolMDA/l)	1.23 ± 0.36	1.75 ± 0.26[d]

Values are means ± SD.
[a] $p < 0.05$, vs. controls.
[b] $p < 0.01$, vs. controls.
[c] $p < 0.005$, vs. controls.
[d] $p < 0.001$, all vs. controls.
Abbreviations: TC = total cholesterol, HDL-C = high-density lipoprotein cholesterol, LDL-C = low-density lipoprotein-cholesterol, VLDL-C = very low-density lipoprotein cholesterol, TG = triglicerides, ApoA-I = apolipoprotein A-I, ApoB = apolipoprotein B.

95% CI 0.06–0.36; $p = 0.003$), and PON1/apoA-I (OR 0.58; 95% CI 0.39–0.86; $p = 0.007$) were independently indicative of CAD after multiple logistic regression analysis.

Discussion

In this study, only subjects without evidence of coronary atherosclerosis (<25 stenosis) were taken as control population, and diabetic subjects were not considered, as the increased risk of cardiovascular disease in diabetic patients is well documented.[9] Many contradictory results have been obtained in the alteration of PON activity in patients with CAD. Paraoxonase activity has been reported to be decreased[13,14] or unchanged.[15] We observed that PON1 activity was decreased in patients with CAD.

High-density lipoprotein cholesterol and apoA-I concentrations were decreased in patients with CAD. This result agrees with previously published data in patients with CAD in Taiwan.[16] In angiographically defined Indian[17] and Chinese[18] patients with CAD, the apoA-I levels were found to be significantly lower than in the controls. A low plasma HDL-C concentration is an important risk factor for coronary heart disease.[19] Hernandez *et al.*[20] reported that PON activity in pericardial fluid correlated with the levels of HDL-C. Paraoxonase-1 activity correlated with HDL-C in patients with CAD. This indicated that PON1 can decrease in activity dependent on HDL-C concentration. Women have generally higher HDL-C concentration;[19] this result agrees with our findings. Our findings are also in accordance with the concept that women have lower CAD incidence and that the prevalence of risk factors is greater in men. Thus, the lower incidence of CAD in women has been attributed to their higher HDL-C levels.

Since PON1 activity, HDL-C, and apoA-I concentrations were decreased in patients with CAD, we were interested in determining whether the observed reduction in PON1 activity was due to decreased HDL-C and apoA-I concentrations. Therefore, we standardized the PON1 activity for HDL-C (PON1/HDL-C ratio) and apoA-I (PON1/apoA-I ratio) concentrations. We found that the PON1/HDL-C and PON1/apoA-I ratios were lower in patients with CAD and that the decrement in PON1/HDL-C ratio may be due to reduced PON1 activity in such patients. The decrement in PON1 activity may be due to the inactivation of the enzyme by lipid peroxidation products, decreased synthesis, or enhanced catabolism of PON1. The PON1/HDL-C and PON1/apoA-I ratios contribute to the HDL-associated antioxidant activity of PON1. Patients with CAD had higher concentrations of LDL-C and apoB, a result that is consistent with those in some studies.[21,22] In our study, female patients with CAD had higher levels of LDL-C than male patients. Increased apoB and LDL-C are both associated with increased incidence of CAD. In the control group, apoB concentrations were found to be higher in women than in men, but the significance of difference between patients with CAD and male controls was stronger than the difference between those patients and female controls. Our results contradict those of Mansur *et al.*,[23] who reported that elevated levels of apoB are an independent risk factor for CAD in men.

Women in the CAD patient and control groups had higher levels of TC than did men. Kannel[24] reported that the average TC levels at which CAD events occurred were substantially higher in women.

In the present study, we demonstrated that the apoA-I/apoB ratio was lower in patients with CAD. Bahl *et al.*[17] and Azizi *et al.*[15] suggested that apoA-I/apoB was the variable with the strongest predictive power for CAD and we agree with this finding.

Apolipoprotein-B levels correlated positively with TC, LDL-C, TG, VLDL-C and apoA-I levels, as well as with the PON1/HDL-C ratio. This leads us to conclude that HDL-related PON1 activity might interact with apoB-carrying lipoproteins.

Several lines of evidence support a role for oxidatively modified LDL in atherogenesis and its in vivo existence. Oxidation of LDL is a free radical-mediated process involving the peroxidative modification of polyunsaturated fatty acids.[25] The various LDL oxidation pathways produce sev-

TABLE II Paraoxonase-1 (PON1), lipids, lipoproteins, apolipoproteins, conjugated diene (CD), and thiobarbituric acid-reactive substances (TBARS) in female and male controls and patients with coronary artery disease (CAD)

Parameters	Sex	Controls	p_1	CAD patients	p_2	p_3
PON1 (IU/l)	Female	341.60 ± 188.56		233.51 ± 121.16		<0.05
	Male	321.26 ± 144.86		219.83 ± 117.30		<0.001
TC (mg/dl)	Female	205.58 ± 37.36		225.10 ± 34.54		
	Male	182.48 ± 38.89	<0.05	197.57 ± 41.31	<0.01	
HDL-C (mg/dl)	Female	45.91 ± 7.67		41.05 ± 10.50		
	Male	39.84 ± 9.87	<0.01	38.40 ± 9.73		
LDL-C (mg/dl)	Female	130.97 ± 34.69		155.12 ± 27.99		<0.05
	Male	115.47 ± 30.22		130.38 ± 34.91	<0.005	<0.05
VLDL-C (mg/dl)	Female	28.69 ± 11.72		28.94 ± 13.91		
	Male	26.69 ± 12.64		28.58 ± 13.58		
TG (mg/dl)	Female	143.58 ± 58.65		145.00 ± 69.76		
	Male	133.35 ± 63.24		142.71 ± 68.09		
PON1/HDL-C (IU/mg)	Female	0.76 ± 0.44		0.61 ± 0.34		
	Male	0.84 ± 0.37		0.59 ± 0.31		<0.005
TC/HDL-C	Female	4.58 ± 1.00		6.00 ± 2.56		<0.05
	Male	4.78 ± 1.32		5.41 ± 1.55		<0.05
LDL-C/HDL-C	Female	2.93 ± 0.88		4.21 ± 2.20		<0.01
	Male	3.05 ± 1.05		3.58 ± 1.25		<0.05
ApoA-I (mg/dl)	Female	121.18 ± 21.03		119.30 ± 24.69		
	Male	105.16 ± 22.18	<0.005	102.03 ± 19.86	<0.005	
ApoB (mg/dl)	Female	102.03 ± 23.48		116.00 ± 25.51		<0.05
	Male	90.03 ± 22.98	<0.05	106.88 ± 27.38		<0.005
PON1/ApoA-I (IU/mg)	Female	0.30 ± 0.21		0.20 ± 0.11		<0.05
	Male	0.31 ± 0.13		0.22 ± 0.13		<0.005
ApoA-I/HDL-C	Female	2.70 ± 0.60		3.02 ± 0.68		
	Male	2.73 ± 0.65		2.80 ± 0.80		
ApoB/LDL-C	Female	0.81 ± 0.19		0.75 ± 0.14		
	Male	0.80 ± 0.19		0.85 ± 0.22		
ApoA-I/ApoB	Female	1.24 ± 0.33		1.06 ± 0.25		<0.05
	Male	1.21 ± 0.30		1.00 ± 0.26		<0.001
CD (μmol/l)	Female	1.29 ± 0.38		1.85 ± 0.80		<0.001
	Male	1.41 ± 0.60		2.07 ± 0.51		<0.001
TBARS (nmolMDA/l)	Female	1.21 ± 0.35		1.68 ± 0.21		<0.001
	Male	1.26 ± 0.38		1.76 ± 0.26		<0.001

p_1 = p values for comparison of control females vs. control males.
p_2 = p values for comparison of female vs. male patients with CAD.
p_3 = p values for comparison of female patients with CAD vs. female controls and male patients with CAD vs. male controls.
Abbreviations as in Table I.

eral lipid peroxidation products such as CD and MDA.[26] It is more accurate to use the term TBARS instead of MDA for the products of the reaction with thiobarbituric acid.[21] One single assay of lipid peroxidation is probably not sufficient to serve as a marker for cardiovascular risk, and there is a need for the measuring of several markers.[26] In this study, CD and TBARS were measured as an indicator of lipid peroxidation. Increases in CD and TBARS levels in the serum of patients with CAD suggested increased production of lipid peroxidation. The results obtained for TBARS levels are consistent

with recently published observations, which showed increased TBARS levels in patients with CAD.[27] No comparable report is available in the literature on the serum levels of CD in patients with CAD. The present study demonstrates for the first time that CAD is associated with increased CD levels. Our study supports the earlier hypothesis that lipid peroxidation is involved in CAD. In the CAD group, the TBARS level correlated with the CD level, which, in turn, correlated negatively with PON1 activity, suggesting a possible mechanism by which overproduction of lipid peroxidation product (CD) inactivated PON1.

Conclusions

This is the first report of decreased serum PON1 activity and increased lipid peroxidation indicators (CD and TBARS) of patients with CAD living in Antalya, Turkey. Conjugated diene levels that correlated negatively with PON1 activity suggested a possible mechanism by which overproduction of lipid peroxidation product (CD) inactivated PON1. Our results indicate that TBARS levels, age, gender, apoA-1/ApoB, and PON1/apoA-I ratios are important markers of CAD.

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