

PARAXONASE (PON) ACTIVITY IN LESS THAN 40 YEARS OLD NON-DIABETIC PATIENTS WITH AND WITHOUT SIGNIFICANT CORONARY ARTERY DISEASE

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Abstract

INTRODUCTION: Regarding the increasing incidence of cardiovascular diseases (CAD) in people younger than 40 years old, without any major risk factors, this study aimed to find if atherosclerosis of serum Paraxonase (PON) activity is a probable risk factor.

METHOD: This was a case-control study on 80 non diabetic persons younger than 40 years old, with chest pain. Patients were divided in two groups based on their results of coronary angiography test: patients with and without CAD (angio-positive and angio-negative). We also divided patients based on major coronary artery disease risk factors in two groups: with and without risk factors. We measured and compared PON activity, body mass index (BMI), serum triglyceride (TG), fasting blood sugar (FBS), C- reactive protein (CRP), apolipoprotein A1 and B (APO A1, and APO B), total cholesterol and low and high density lipoproteins (LDL and HDL) together in these groups.

RESULTS: In angio-negative and angio-positive groups, the difference between PON activity (121.44 vs. 89.58), HDL (44.58 vs. 37.11), TG (149.31 vs. 199.7), APO B (87.48 vs. 99.50), CRP (4.38 vs. 7.32) was significant ($P < 0.05$). There was not seen any significant difference between two groups regarding LDL, total cholesterol, APOA, and BMI ($P > 0.05$). We didn't find any relationship between PON activity and HDL levels.

CONCLUSION: This study suggests that low PON activity level might be considered as a risk factor for coronary artery disease, especially in patients who don't have any other major risk factors. Further studies are needed to evaluate the effect of these risk factors on each other.

Keywords: Paraoxonase activity, CRP, BMI, triglyceride, APO A1, APO B, HDL, LDL and total cholesterol, coronary artery disease.

ARYA Atherosclerosis Journal 2008, 4(2): 53-58

Date of submission: 20 Jan 2008, *Date of acceptance:* 20 Mar 2008

Introduction

Coronary artery disease (CAD) is the most important cause of death in Iran and in the industrial countries.^{1,2} There are multiple risk factors have the major role in the development of coronary atherosclerosis such as smoking, dyslipidemia, diabetes mellitus and hypertension,^{3,4} but in some patients suffer from CAD and specially in young patients with acute myocardial infarction, we can not find any risk factors for atherosclerosis. Such cases indicate that there are other unknown risk factors have important role in atherosclerosis. One of these risk factors that lately attract scientific attention is paraoxonase (PON) activity.

Paraoxonases are a group of hydrolyze organophosphates compounds which are widely used as insecticide and nerve gases.⁵ Human serum paraoxonase (PON1) is an enzyme synthesized in the liver and placed on HDL.⁵ The serum concentration of HDL has long been known to have an inverse correlation with the development of atherosclerosis.⁵ Several studies have shown that HDL protects LDL against oxidative processes.^{5,6} Oxidation of LDL plays major role in initiation and progression of atherosclerosis.⁵ Studies show that the antioxidant activity of HDL may be related to the enzyme associated with HDL,⁵ and PON1 can prevent lipid peroxidase accumulation on LDL.⁵

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Regarding the increasing incidence of CAD in individuals younger than 40 years of age without any major risk factors for atherosclerosis, study of serum PON activity as a probable risk factor is very important.

In this study we measured and compared PON activity in two groups of less than 40 years non diabetic persons with CAD and without CAD.

Also, we compared other probable risk factors such as body mass index (BMI), serum triglyceride (TG), fasting blood sugar (FBS), C-reactive protein (CRP), apolipoprotein A₁ and B (APO A₁, and APO B), total cholesterol and low and high density lipoproteins (LDL and HDL) together in these two groups.

Materials and Methods

Subjects and sample collection

This was a quantitative cross-sectional and case-control study. Study population included non diabetic patients referred to the outpatient heart clinic of Shahid Chamran and Noor hospitals in Isfahan with chest pain complain and were candidate for angiography from October 2007 to April 2008.

Patients were examined by taking their history and accurate physical examination. Risk factors were determined and inclusion and exclusion criteria were evaluated for the study. Exclusion criteria included age above 40 years and diabetes mellitus (either type 1 or type 2, for its effect on serum level and function of HDL). Inclusion criteria were chest pain suspected to be ischemic heart disease, and being a candidate for coronary angiography. Blood samples were collected for laboratory studies after at least 12 hours fasting. Then blood samples were centrifuged on 2200 round/min for 30 minutes, in 4°C. The resulted serum froze in -20°C and was carried to laboratory of the Seddiqeh Tahereh Heart Research Center in Isfahan with cold box. The serums were tested in this laboratory for FBS, HDL, LDL, CRP, TG, APOA₁, APOB, total cholesterol and PON activity.

After blood sampling, patients had coronary angiography. The results of the angiography were evaluated by a cardiologist that was unaware of the study groups. Patients were divided to two groups based on their results of coronary angiography:

- 1- Case group with significant CAD (angiopositive group): was explained by 50% or more stenosis in at least one of the coronary arteries
- 2- Control group without significant CAD (angioegative group): was explained by normal angiography or less than 50% stenosis in coronary arteries.

In case group, we had 35 patients with significant CAD and in control group we had 45 patients without significant CAD.

Also, we divided patients in two groups based on CAD major risk factors: with and without CAD major risk factors. CAD major risk factors were defined as smoking, total cholesterol > 200 mg/dl, systolic blood pressure > 140 mmHg, diastolic blood pressure > 90 mmHg. Diabetes mellitus, another CAD major risk factor, was one of the exclusion criteria.

Analytical methods

All blood samples were analyzed in laboratory of the Isfahan Heart Research Center by a biochemist unaware of the results of patients' coronary angiography. Two sets of serum were analysed: one set for biochemistry and one for PON activity.

Analysis of PON 1 activity

PON₁ activity was measured by adding serum to Trips buffer (100 mmol/l, pH = 8.0) containing 2 mmol/l calcium chloride and 5.5 mmol/l paraoxone (O, O- diethyl - o - p- nitro phenyl phosphate, Sigma Chemical co). The rate of generation of p-nitro phenol was determined at 405nm and 25c using a continuously recording spectrophotometer (Bechman-Du- 68).

Serum lipids

Patients' serums were analyzed for triglyceride, FBS, CRP, APO A₁, and APO B, total cholesterol and LDL and HDL with Hitachi II, 902 autoanalyzer.

FBS was analyzed by Biosystem Kit with photometric method. Total cholesterol and triglyceride were analyzed by Pars Azmun Kit with enzymatic method and CRP, APO A₁, and APO B, LDL and HDL were analyzed by Pars Azmun Kit with photometric method.

Statistical analysis

All data were collected by statistical software EP15 and were analysed by soft ware SPSS 15 and statistical quantitative t-test (independent). Sample size was 80 (35 sample with CAD positive and 45 sample without CAD positive) calculated by this formula: $N = (Z_{1-\alpha/2})^2 \times p(1-p) / (d \times d) = 80$.

Results

First, we divided patients into two groups: patients with significant CAD and patients without CAD. There was not any significant difference between two groups about sex distribution and age.

Considering the fact that CAD major risk factors are interverer variables; we divided patients into two other groups: patients with CAD major risk factors and patients without CAD major risk factors.

We compared CRP/APO B/triglyceride/HDL /PON activity and CAD in these two groups by t-test to eliminate the effects of interverer variables (CAD major risk factors).

Table 1. Patients' demographic data

variables	CAD positive mean \pm SD	CAD negative mean \pm SD
Age (year: mean \pm SD)	35.52 \pm 4.45	36.04 \pm 5.26
male	35.38 \pm 4.6	35.15 \pm 5.96
female	36.75 \pm 2.36	38.14 \pm 1.91
Sex	35	45
male	31	32
female	4	13

BMI. Difference between angio-negative and positive was not significant (85.34 vs 92.03) ($P > 0.05$) (SD = 17.2 vs 30.71).

CRP. Difference between angio-negative and positive was significant (4.38 vs 7.32) (t-test, $P < 0.05$) (SD = 3.00 vs 8.07).

Total Cholesterol. Difference between angio-negative and positive was not significant (175.1 vs 187.35 mg/dl) (t-test, $P > 0.05$) (SD = 28.66 vs 43.32).

LDL. Difference between angio-negative and positive was not significant (108.37 vs 107.30 mg/dl) (t-test, $P > 0.05$) (SD = 36.89 vs 6.94).

Apo A. Difference between angio-negative and positive was not significant (113.41 vs 115.67 mg/dl) (t-test, $P > 0.05$) (SD = 19.45 vs 22.40).

Apo B. Difference between angio-negative and positive is significant (87.48 vs 99.50 mg/dl) (t-test, $P < 0.05$) (SD = 16.36 vs 25.93).

Triglyceride (TG). Difference between angio-negative and positive was significant (149.31 vs. 199.70 mg/dl) (t-test, $P < 0.05$) (SD = 71.76 vs 109.44).

PON activity

Mean of PON activity in angio-negative group was 121.44 \pm 51.10mg/dl and in angio-positive group was 89.58 \pm 50.25 mg/dl. Difference between these two numbers is significant ($P < 0.05$).

The t-test for the group with no major risk factor showed the mean PON activity of 123.96 \pm 55.06 mg/dl in the angio-negative group and 72.04 \pm 22.03 mg/dl in the angio-positive group. Difference between these two numbers was significant ($P < 0.0001$).

But in the group with major risk factor, mean of PON activity was 118.32 \pm 46.88 mg/dl for the angio-negative patients and 96.72 \pm 56.77 mg/dl for angio-positive patients. Difference between these two numbers was not significant. ($P = 0.16$) (Table 2).

Table 2. Comparison of PON activity in patients with CAD history with and without risk factors

	CAD positive Mean \pm SD	CAD negative Mean \pm SD	P value
With Risk Factors*	96.72 \pm 56.77	118.32 \pm 46.88	0.16
Without Risk Factors	72.04 \pm 22.03	123.96 \pm 55.06	< 0.001

*With risk factors: col-t $>$ 200 or SBP $>$ 140 or DBP $>$ 90 or FBS $>$ 126 or smoking = yes.

HDL- cholesterol

Mean of HDL in angio-negative group was 44.58 \pm 12.33 mg/dl and in angio-positive patients was 37.11 \pm 8.35 mg/dl. Difference between these two numbers was significant ($P < 0.05$) (t-test). T-test in the group with no major risk factor mean of HDL was 43.23 \pm 11.29 mg/dl in the angio-negative group and 34.80 \pm 8.59 mg/dl in the angio-positive group. Difference between these two numbers was significant ($P = 0.01$). But in the group with major risk factor, mean of HDL was 46 \pm 13.49 mg/dl for angio-negative group and 37.96 \pm 8.26 mg/dl for the angio-positive group. Difference between these two numbers was significant ($P = 0.04$) (Table 3).

Table 3. Comparison of HDL- cholesterol in patients with CAD history with and without risk factors

	CAD positive Mean \pm SD	CAD negative Mean \pm SD	P value
*With Risk Factors	37.96 \pm 8.26	46 \pm 13.49	0.01
Without Risk Factors	34.80 \pm 8.59	43.23 \pm 11.29	0.04

* risk factors: col-t $>$ 200 or SBP $>$ 140 or DBP $>$ 90 or FBS $>$ 126 or smoking = yes.

Comparing HDL and PON activity

In the angio-negative group, Pearson correlation between HDL and PON activity was 0.025 ($P = 0.875$). In the angio-positive group, Pearson correlation between HDL and PON activity was -0.002 ($P = 0.98$).

This means that there is no correlation between HDL and PON activity neither in the angio-negative group nor in the angio-positive group.

Discussion

Many studies show that decreased paraoxonase activity is a risk factor for atherosclerosis,⁶⁻¹² but some studies reported low paraoxonase activity as a risk factor for CAD.¹³⁻¹⁵

We should consider that although PON₁ can hydrolyze a wide range of substrates, recent studies have demonstrated that PON₁ is a lactonase catalyzing

both the hydrolysis and the formation of a number of lactones.¹⁶⁻¹⁸

Also the paraoxonase *in vivo* role remains largely unclear.¹⁶ Some investigators believe that the PON₁ activity is a poor reflection of PON₁ native activity and Arylsterase activity on the other hand, is responsible for PON₁'s native lactones activity,^{16,19} and it is thought to be a better reflection of PON₁ antioxidative potential.^{16,19} These investigators don't know PON activity as a good indicator for anti-atherosclerosis activity.¹⁶ Also, some scientists believe that results of studies in Europe and USA about relationship between PON activity and CAD can not be generalized in Iran. These investigators believe that PON activity is not a good indicator of atherosclerosis in Iran.²⁰ They theorize that there is racial difference in genetically polymorphic distribution of paraoxonase in different populations. In Europe and USA this distribution is bimodal but in non European populations distribution of PON activity is unimodal. In this study, without elimination of major risk factors for atherosclerosis by statistical methods, mean paraoxonase activity in the atherosclerotic population (the case group) was significantly lower than non atherosclerotic population (the control group).

But with elimination of major risk factors for atherosclerosis, this difference was statistically significant only in patients without major risk factors. It seems that paraoxonase activity can only inhibit atherosclerosis if there are no CAD major risk factors. In other words, CAD major risk factors neutralize PON activity. For this reason, we advise measurement of PON activity if patient has no CAD major risk factors.

High serum HDL level is a modulator of CAD major risk factors.⁵ It decreases incidence of coronary atherosclerosis.⁵ In this study, average of serum HDL in the angio-negative group was higher than the angio-positive group, with and without elimination of CAD major risk factors effect. These findings are compatible with findings of other investigations.

In this study, we didn't find any significant correlation between PON activity and HDL serum level. Studies have shown positive associations of serum PON₁ mass and activity with HDL cholesterol and apoA₁.^{6,21-23} Based on the highly significant correlation between paraoxonase mRNA and HDL levels in the recombinant mice, it has been suggested that either the synthesis of paraoxonase and HDL in the liver are coordinately regulated or that PON expression influences HDL level.^{6,24} But we should consider that PON₁ present in serum is located on HDL, being tightly bound to a HDL sub fraction containing Apo AI and clustering^{5,16,25} low concentrations of HDL increase susceptibility to atherosclerosis and conse-

quently CAD. The PON₁-containing HDL particles constitute a very small fraction of the total plasma HDL.²⁶ Low serum PON₁ levels occur when HDL concentrations are profoundly low, for example in fish eye and Tangier diseases. However, when serum HDL levels are only moderately decreased (as in samples of this study), the decrease in PON₁ is independent of changes in HDL.^{5,27}

T-test analysis showed a significant difference between angio-negative and angio-positive groups regarding APO B, CRP and TG that are also three risk factors of CAD.^{6,12,28-31} These findings are compatible with findings of most other investigations.

BMI, APO A₁, LDL and total cholesterol are other risk factors of CAD.³² In this study, we didn't find any significant difference between BMI and mean of total cholesterol, APO A₁, LDL serum levels in the angio-negative and angio-positive groups. These results can be due to small volume sample, interverner variables effect or the age group of the patients we studied.

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