Study of Correlation between the Paraoxonase 1 (PON1) Activity and Lipid Profile in Various Types of Coronary Heart Disease

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ABSTRACT

Aim and background: Coronary heart disease (CHD) is the major cause of mortality and morbidity worldwide. Human serum paraoxonase-1 is a high-density lipoprotein (HDL)-bound enzyme exhibiting anti-atherogenic properties.

Objective: The current study aimed to determine the serum paraoxonase-1 activity and lipid profile with CHD in addition to correlate the relationship between serum high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein (LDL) cholesterol, and paraoxonase-1 level in patients with CHD.

Materials and methods: In the present case-control study, 265 with coronary artery disease (age range 26–75) and 120 age- and sex-matched healthy controls were recruited. Serum paraoxonase activity was measured spectrophotometrically by using phenylacetate as substrate by kinetic assay while lipid profile was analyzed by an enzymatic method by cholesterol oxidase peroxidase (CHOD-PAP) method of total cholesterol and HDL-C and glycerol 3-phosphate oxidase (GPO-PAP) method of triglyceride. Values were expressed as mean $\pm$ standard deviation and data from patients and controls were compared by using the Student's "t" test.

Results: Serum paraoxonase-1 activity was significantly ($p < 0.01$) decreased in CHD when compared with healthy controls. Similarly, there was a significant difference between mean values of paraoxonase-1 when all CHD groups compared with each other ($p < 0.01$). Correlation between paraoxonase-1 vs HDL-C was positive in stable angina (Karl Pearson's correlation coefficient $r = 0.04671$), myocardial infarction (MI) ($r = 0.2643$), and controls ($r = 0.06384$) and it was negative in unstable angina (UA) ($r = -0.098$). There was negative correlation between paraoxonase-1 vs low-density lipoprotein (LDL)-cholesterol in stable angina ($r = -0.0291$), MI ($r = -0.2265$), and control group ($r = -0.1767$), and was positive in UA ($r = 0.6185$).

Conclusion: Low paraoxonase-1 may be reducing the capacity of HDL to prevent the oxidation of LDL cholesterol, therefore, lead to CHD. So interventional means of dietary antioxidants to conserve or even to raise paraoxonase-1 activity may contribute to attenuation of atherogenesis.

Keywords: Coronary heart disease, Lipid profile, Paraoxonase-1.

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INTRODUCTION

Coronary heart disease (CHD) is defined as acute or chronic cardiac disability arising from an imbalance between the myocardial supply and demand for oxygenated blood.¹ Cardiovascular disorders (CVD) are the foremost cause of morbidity and mortality both in developed and developing countries. Coronary heart disease is the single most important contributor to this increasing burden of CVD.² The burden of CHD is raising in India. According to WHO, 7.2 million deaths, i.e., (12.8% of total deaths) of CHD occurs in 2008.^{3,4}

Raised blood levels of low-density lipoprotein (LDL), total cholesterol (TC), and triglycerides (TG)^{5,6} were seen in CHD with hypertension. In contrast, a low level of high-density lipoprotein (HDL) is a risk factor for mortality from CHD.⁵ So any abnormalities of lipoproteins may cause a higher incidence of microvascular and macrovascular obstacles in CHD. Dyslipidemia (high TG, high TC, and low HDL) is an major complication in patients with CHD which is a risk factor for increased CHD incidences and the mortality rate.⁶

Human paraoxonase (PON1) E.C. 3.1.8.1. is an arylesterase synthesized in the liver and is an HDL-associated enzyme that is responsible for the antioxidant properties of high-density lipoprotein cholesterol (HDL-C). This enzyme plays an important

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role in preventing LDL oxidation and is considered to protect against the development of CHD.⁷ Serum levels of PON1 and HDL-C showed inversed association with the presence of CAD but are not related to the severity of disease in terms of several diseased vessels. Interventional means by diet and drugs to enhance PON1 activity may contribute to the attenuation of atherosclerosis.⁸ The status of PON1, i.e., its activity and concentration is more important and significantly increased in CHD.⁹

View of the above information and several worthwhile information, acquaintance of PON1 status in CHD may help in planning proper strategies in the clinical management of the disease. Hence, the present study aimed to find PON-1 activity in patients with different types of CHD like stable angina, unstable angina (UA), and myocardial infarction (MI) and compared it with that in healthy controls and also tried to find its correlation with lipid variables.

MATERIALS AND METHODS

The present case-control study was conducted at the Department of Biochemistry DVVPF's Medical College, Ahmednagar and Swasthya Hospital and Research Center, Ahmednagar (Maharashtra) in collaboration with the Department of Biochemistry, BJ Medical College and Sassoon General Hospital (S.G.H), Pune. The study was approved by the Ethics Committee of BJMC and SGH, Pune, with all participants providing informed consent and utmost care was taken during the experimental procedure according to the declaration of Helsinki 1975.

Study Duration

4 years and 6 months.

Study Design

Type: Analytical case-control study.

Population: A total of 385 subjects were enrolled in the present study. Of which stable angina = 55, UA-100, MI-110, and controls-120.

Sampling: Simple random sampling.

In the present study, study was carried on available individuals that were accessible population. Then, it was divided into a group according to the type of CHD and random samples of varying sizes were drawn for each group.

Sample Size Calculation

The present study was quantitative thus the sample size was calculated by using the following formula:

$$\text{Sample size } n = 4 \times \sigma^2 / E^2$$

n = sample size, σ = standard deviation in population E = Allowable error

Control Group

One hundred and twenty healthy age- and sex-matched individuals without any evidence of CHD as per clinical examinations were taken as control subjects.

Patients Group

The study included a total of 265 patients between the age-group 26 years and 75 years of CHD. Of these, patients with MI and UA had been taken from the intensive cardiac care unit (ICCU) having chest pain.

Patients of stable angina had taken from outpatients attending the cardiology department of the same hospitals. The patients were diagnosed by physicians, blinded to the results of markers; data included history, physical examination, serial 12-lead electrocardiogram, and cardiac markers measurement.

Inclusion Criteria

The diagnosis of all patients with CHD was made by physicians, and patients, who had typical symptoms of CHD like chest pain, sweating, breathlessness, etc., and specific abnormalities for CHD on electrocardiogram, elevated cardiac markers were included in the present study.

Exclusion Criteria

All patients having heart disease like congenital heart disease, diseases of heart valves, and myocardium. Confounding factors that could interfere in the biochemical analyses of study subjects and alter the results like diabetes mellitus, renal insufficiency, hypertension, hepatic disease, inflammatory disease, history of recent infection, and febrile disorders were excluded. Patients taking antioxidant vitamin supplements which could influence PON1 enzyme status were also excluded.

After taking informed consent, all subjects were screened for inclusion and exclusion criteria. All the subjects were categorized into different groups as follows.

Controls: Age- and sex-matched healthy subjects ($n = 120$)

Stable angina ($n = 55$)

Unstable angina ($n = 100$)

Myocardial infarction ($n = 110$)

Approximately 5 mL blood was collected by venipuncture from the antecubital vein of the forearm of each subject between 9.00 and 11.00 am after fasting from 10.00 pm the previous day in plain vacutainer (Yucca Diagnostics) under aseptic conditions and centrifuged for serum collection.

Estimation of a Lipid Profile

Serum total cholesterol and HDL-C were determined by the CHOD-PAP method. Serum triglyceride (TG) was measured enzymatic GPO-PAP method endpoint assay (using kit manufactured by Span Diagnostics Ltd) using semiautoanalyser. LDL-C calculated by using Friedewald formula ($\text{LDL-C} = \text{total cholesterol} - \text{TG}/5 - \text{HDL-C}$).¹⁰⁻¹²

Estimation of PON1 Activity

The assay was based on the principle that PON1 catalyzes the cleavage of phenylacetate resulting in phenol. The rate of formation in phenol is measured by monitoring the increase in absorbance at 270 nm. One unit of arylesterase activity is equal to 1 μM of phenol formed per minute. The activity is expressed in Kilo international Unit/L based on the extinction coefficient of phenol of 1,310 $\text{M}^{-1} \text{cm}^{-1}$ at 270 nm at pH 8.0 and 25°C. Blank samples without serum were used to correct for non-enzymatic hydrolysis.^{13,14}

Statistical Analysis

Statistical software SYSTAT version-12 (by Cranes software, Bengaluru) was used to analyze the data. The results were expressed in mean $\pm$ standard deviation (mean $\pm$ SD).

Data were analyzed by descriptive statistics as mean, SD, percentage, etc. Comparisons of study groups and study groups to control groups were done by applying the Z test of the difference between two sample means at 5% ($p, 0.05$) and 1% ($p, 0.01$) level of significance. Also, correlation analysis was done by determining Karl Pearson's correlation coefficient for a positive and negative correlation between various parameters in all groups under study. A correlation was tested by Student's "t" test at 5% ($p, 0.05$) and 1% ($p, 0.01$) level of significance.

Regression analysis was done to estimate or predict unknown variables when one variable is known and this was done by constructing two lines of regressions for each parameter in all groups under study.

RESULTS

As shown in Table 1, serum PON1 activity and HDL-C levels were $p < 0.01$ significantly lower in patients with SA, UA, and MI when compared with controls. While total cholesterol, TG, LDL-C levels were significantly ($p < 0.01$) increased in all types of CHD subjects as compared to healthy controls.

Correlation diagram No. 1, 3, 5, and 7 showed that correlation between PON1 vs HDL-C was positive in SA (Karl Pearson's

correlation coefficient $r = 0.04671$), MI ($r = 0.2643$), and controls ($r = 0.06384$) and it was negative in UA ($r = -0.098$).

Correlation diagram No. 2, 4, 6, and 8 presented that negative correlations were seen in-between PON1 vs LDL-C in SA ($r = -0.0291$), MI ($r = -0.2265$), and control group ($r = -0.1767$), and positive correlation was seen in UA ($r = 0.185$).

Table 2 indicates that the regression analysis. Lines of regression have been done in-between PON1 and HDL-C, LDL-C in all groups under study. With the help of the regression line, it may be possible to estimate PON1 when HDL-C, LDL-C are known in all groups and to estimate HDL-C, LDL-C when PON1 is known in all groups (Figs 1 to 8).

Table 1: Biochemical changes in CHD and controls

Variable	Controls (n = 120) Mean $\pm$ SD	CHD		
		Stable angina (n = 55) Mean $\pm$ SD	Unstable angina (n = 100) Mean $\pm$ SD	Myocardial infarction (n = 110) Mean $\pm$ SD
Total cholesterol (mg/dL)	169.76 $\pm$ 28.35	192.48 $\pm$ 31.65*	267.33 $\pm$ 63.02*	269.91 $\pm$ 65.01*
HDL-C (mg/dL)	43.53 $\pm$ 3.72	39.74 $\pm$ 6.39*	36.57 $\pm$ 4.23*	35.32 $\pm$ 4.88*
LDL-C (mg/dL)	103.82 $\pm$ 28.24	120.98 $\pm$ 31.39*	194.79 $\pm$ 56.90*	199.17 $\pm$ 62.37*
TG (mg/dL)	112.02 $\pm$ 28.59	158.80 $\pm$ 35.41*	180.52 $\pm$ 54.33*	117.08 $\pm$ 48.01*
PON1 (IU/L)	103.97 $\pm$ 20.46	47.66 $\pm$ 16.04*	50.18 $\pm$ 11.12*	49.21 $\pm$ 9.67*

Values were expressed in mean with standard deviation (mean $\pm$ SD)

* $p < 0.01$ —considered as highly significant

Table 2: Regression analysis: lines of regression between PON and HDL-C, LDL-C in all groups under study: $X = \text{PON1}$, and $Y = \text{HDL-C, LDL-C}$

Line of regressions	Regression analysis			
	Stable angina (n = 55)	Unstable angina (n = 100)	Myocardial infarction (n = 110)	Controls (n = 120)
*PON1 on HDL-C	PON1 = 0.117 HDL-C + 42.99	PON1 = -0.258 HDL-C + 59.60	PON1 = 0.524 HDL-C + 30.71	PON1 = 0.351 HDL-C + 119.25
**HDL-C on PON1	HDL-C = 0.186 PON1 + 30.87	HDL-C = -0.037 PON1 + 51.53	HDL-C = 0.133 PON1 + 28.77	HDL-C = 0.0116 PON1 + 44.74
*PON on LDL-C	PON1 = -0.015 LDL-C + 49.47	PON1 = 0.12 LDL-C + 26.63	PON1 = -0.035 LDL-C + 56.20	PON1 = 0.128 LDL-C + 117.26
**LDL-C on PON1	LDL-C = -0.037 PON1 + 51.53	LDL-C = 3.165 PON1 + 35.97	LDL-C = -1.46 PON1 + 271.06	LDL-C = -0.24 PON1 + 129.17

*This regression line can be used to estimate PON1 when HDL-C, LDL-C are known in all groups

**This regression line can be used to estimate HDL-C, LDL-C when PON1 is known in all groups

Fig. 1: Correlation between PON1 and HDL-C in stable angina

Fig. 2: Correlation between PON1 and LDL-C in stable angina

Fig. 3: Correlation between PON1 and HDL-C in unstable angina

Fig. 4: Correlation between PON1 and LDL-C in unstable angina

Fig. 5: Correlation between PON1 and HDL-C in myocardial infarction

Fig. 6: Correlation between PON1 and LDL-C in myocardial infarction

Fig. 7: Correlation between PON1 and HDL-C in healthy controls

Fig. 8: Correlation between PON1 and LDL-C in healthy controls

DISCUSSION

Hypercholesterolemia is universally accepted as a major risk factor for atherosclerosis variability regarding lipid profile in the occurrence of cardiovascular events. Oxidative modification of LDL might be a crucially important step in the development of atherosclerotic plaque.⁷

High-density lipoprotein cholesterol plays an anti-atherogenic role which protects LDL-C against oxidative modification which is attributed to the PON1 enzyme located in a subfraction of HDL-C that contains apoA-1 and clusterin.¹⁵ The physiological function of PON1 seems to be to degrade specifically oxidized

cholesteryl ester and oxidized phospholipids in lipoproteins and cell membranes.¹⁶

PON1 was termed because of its capability to hydrolyze the organophosphate substrate paraoxon which is a harmful metabolite of the insecticide parathion. PON1 could also hydrolyze aromatic ester such as phenylacetate so-called arylesterase activity.²

There are at least three members PON1, PON2, and PON3, out of which the PON1 plays a significant role as its product paraoxonase is entirely bound to HDL.⁷

In the present study, we have shown that serum PON1 activity was significantly ($p < 0.01$) diminished in all types of CHD like SA, UA, and MI when compared with healthy controls. Similarly, there

was a significant difference between mean values of PON1 when all CHD groups compared with each other ($p < 0.01$). This outcome is in agreement with the results that have found a diminution of PON1 activities in CHD.^{7,15,16} Its activity can be reduced either due to diminishing its synthesis or inactivation of the enzyme under oxidative stress by lipid peroxidation or also because of acute-phase response that inhibits hepatic synthesis of PON1.^{2,7,17} Lipid peroxide which are the substrate for PON1 and which are raised in people with CHD are inhibitors of PON1. Low PON1 has been shown to reduce the capacity of HDL to prevent the oxidation of LDL and may therefore lead to CHD.¹⁸

Aviram et al. have proved that inactivation of PON1 by oxidized LDL, consequently reduces PON1 activity and its ability to protect LDL from oxidation. The enzyme is time-dependently inactivated throughout the formation of oxidized LDL. Oxidized-LDL inactivates the PON1 by developing communications among the enzyme-free sulfhydryl group and oxidized lipids. It might be due to the dislodgment of calcium ions which are necessary for PON1 activity.¹⁹ At the time of infarction, declined PON1 activity could be due to the overwhelming production of oxygen-free radicals.²⁰

Mackness et al. have assumed that populations at increased CHD risk have diminished serum PON1 concentration. According to their study, the ability of PON1 to hydrolyze paraoxon is inversely related to its capacity to hydrolyze lipid peroxide and thus to its anti-atherogenic action.²¹

Durrington et al. have suggested that diminished PON1 activity acts as an etiologic factor in the development of CHD.²² Ayub et al. have concluded that low serum PON1 activity in patients with MI may be a consequence of the coronary event itself or could have been present before MI.²³

PON1 present in serum is located on HDL-C, being tightly bound to an HDL subfraction containing apoA1 and clusterin. So the lower activity of PON1 can depress the ability to circulate HDL particles to protect LDL from oxidation, to participate in reverse cholesterol transport pathway, and to inhibit monocytes-endothelial cell interaction.^{24–26} Hence, in the current study, an attempt has been made to correlate the PON1 vs HDL and PON1 vs LDL-C in all CHD groups and controls.

As shown in correlation diagram 1, 3, 5, and 7, correlation between PON1 vs HDL-C was positive in SA (Karl Pearson's correlation coefficient $r = 0.04671$), MI ($r = 0.2643$), and controls ($r = 0.06384$) and it was negative in UA ($r = -0.098$).

There was negative correlation (correlation diagram 2, 4, 6, and 8) between PON1 vs LDL-C in SA ($r = -0.0291$), MI ($r = -0.2265$), and control group ($r = -0.1767$), and was positive in UA ($r = 0.6185$).

The regression analysis was performed, lines of regression have done in-between PON1 and HDL-C, LDL-C in all groups under study. With the help of the regression line, it may be possible to estimate PON1 when HDL-C, LDL-C are known in all groups and to estimate HDL-C, LDL-C when PON1 is known in all groups. (Table 2) There are very few reports which premeditated the correlation between PON1 and HDL-C, LDL-C in CHD. Saha et al. have found a correlation between PON1 activity and several lipids and lipoproteins parameters like HDL-C and TG.²⁷ Robert et al. have reported that association of PON1 with HDL may be required for the appropriate physiological distribution of the enzyme, to have a significant biological impact.²⁸ Multiple linear regression analysis has been performed by Gupta et al., as per their study, the low PON1 activity is a predictive risk factor for CAD, independent of all other established risk factors such as age, sex, smoking, alcohol,

and HDL-C levels. This finding indicates that PON1 activity plays an important role in the pathogenesis of CAD.²⁹

Akçay et al. have found that there is no significant correlation between PON1 activity and other metabolic parameters like HDL-C, TG, insulin in the CAD and metabolic syndrome.¹⁵ Ayab et al. have established that sustained MI did not show markedly decreased HDL-C concentration but PON1 activity and PON concentration were profoundly decreased.²⁰ Kumar et al. have studied serum PON1 activity in normolipidemic patients with acute MI. According to this study, no correlation was observed between PON1 activity and HDL-C in acute MI which is contradictory to our results.³⁰ These contrary results may be due to the association of the PON1 enzyme with HDL-C.

Thus in the current study, the mean value serum PON1 activity was significantly lower in SA, UA, and MI as compared with healthy controls which might be decreased synthesis of PON1 in the liver or inactivation of it by oxidized LDL-C. This low PON1 may be reducing the capacity of HDL to prevent the oxidation of LDL-C, therefore, lead to CHD. So interventional means of dietary antioxidants to conserve or even to raise PON1 activity and maybe contribute to attenuation of atherogenesis.

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