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ORIGINAL ARTICLE

SERUM PARAOXONASE ACTIVITY AND ITS RELATION TO SERUM LIPIDS IN CORONARY ARTERY DISEASE

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ABSTRACT: Human serum paraoxonase1 (PON1) is a calcium dependent esterase enzyme that hydrolyses lipid peroxides accumulating on low density lipoproteins. In the serum, PON1 enzyme is almost exclusively located on the high density lipoprotein (HDL). It hydrolyses oxidised lipids in low density lipoprotein (LDL) and could therefore retard the development of atherosclerosis. As PON1 activity has a role in preventing atherosclerosis and coronary heart disease is common in Kerala, this study was conducted to assess the activity of the enzyme and its corelation to serum lipids. **MATERIALS AND METHODS:** In this case-control study, one hundred patients with coronary artery disease and one hundred healthy controls were included. Serum paraoxonase activity was meausured using phenyl acetate as substrate and lipid profile was done in auto analyzer. **RESULTS:** The mean PON 1 activity {(70.21±27.62) in cases against (135.86±33.48) in controls (p value .000)} was significantly low in CAD group compared to the control group. The CHD group had a significantly lower mean total cholesterol level (202.82±57.77 against 219.45±46.08) and lower mean HDL level (40.88±10.08 against 56.79±17.24). Correlation of the activity of PON1 with other variables for the combined group (Cases and controls taken together) showed that age is negatively correlated and HDL is positively correlated with PON 1 activity. **CONCLUSIONS:** The activity of serum paraoxonase enzyme (PON1) is low in patients with coronary artery disease compared to healthy controls. Thus low serum PON1 enzyme activity is a risk factor for CAD. The activity of the serum PON1 enzyme increases with increase in HDL level and decreases with increase in age for the combined group.
KEYWORDS: paraoxonase, cardiovascular, atherosclerosis

INTRODUCTION: Coronary artery disease (CAD) is the most common cause of death in the developed world, and by the year 2020, it will be the chief cause of death worldwide. It is predicted that cardiovascular diseases will claim 25 million lives annually by 2020, and that coronary heart disease will surpass infectious diseases as the world's number one cause of death and disability.[1] India is experiencing an alarming increase in heart disease. Cardiovascular disease accounted for 32% of all deaths in 2000, and the World Health Organization (WHO) estimates that 60% of the world's cardiac patients will be Indian by 2020. Thus cardiovascular disease is a major cause of morbidity and mortality and a major public health concern.

Coronary artery disease is a complex trait caused by a number of genetic and environmental factors. The basis for most cardiovascular diseases is atherosclerosis. Atherosclerosis is the end product of a series of complex cascade of interactions among the cellular and non-cellular components of the arterial wall, blood constituents, mononuclear phagocytes and platelets, focal hemodynamic stresses and environmental and genetic factors.

Human serum paraoxonase/aryl esterase (PON 1),[2] is a calcium dependent enzyme that hydrolyses organophosphates such as paraoxon, diazoxon, sarin, and soman, and also arylesterases

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such as phenyl acetate. It has been implicated in the pathogenesis of atherosclerosis and is synthesised by the liver. In the serum, PON1 enzyme is almost exclusively located on the high density lipoprotein (HDL).[3] It hydrolyses oxidised lipids in low density lipoprotein (LDL) and could therefore retard the development of atherosclerosis. The capability of HDL to metabolise lipid peroxides and to protect against their accumulation on LDL has largely been attributed to the presence of PON 1 on it. As oxidation of LDL constitutes the principal atherogenic modification of serum LDLs, the ability of PON to limit oxidation represents a major antiatherogenic mechanism. Studies have pointed that serum PON1 activity and concentration are decreased in CAD.[4] Thus PON1 may be a determinant of resistance to the development of atherosclerosis by protecting lipoproteins against oxidative modification.

Genetic factors play an important role in predisposition to atherosclerotic coronary artery disease and its thrombotic complications. A large number of genes are likely to be involved in the pathogenesis of CAD. PON 1 activity is partly genetically determined. Paraoxonase gene family in humans has three members; PON1, PON2 and PON3. PON1 is the best studied of the family. Human paraoxonase 1 gene is located on the long arm of chromosome 7. Human serum paraoxonase 1 shows substrate activity polymorphism.[5] The enzymatic activity of paraoxonase varies among individuals by 10-40 folds.

In the present study we measured serum paraoxonase activity against phenyl acetate substrate (aryl esterase activity) in coronary artery disease patients and healthy controls. We also studied the relation of paraoxonase activity with serum lipids and correlation of paraoxonase activity with age, gender and BMI.

METHODS: Subjects: This case-control study included a total of 200 subjects. Informed consent was taken and hospital ethical committee approved the study. The case group consisted of 100 adult consecutive patients with angiographically proven CAD attending the Cardiology Department of Government Medical College, Thiruvananthapuram. Patients with hypertension, diabetes mellitus, hepatic disease and smokers were excluded from the study. Those with a history of myocardial infarction within 6 months before taking part in the study were also excluded.

One hundred healthy controls were selected from the general population and they included medical, paramedical staff of the institution and healthy attendants of the patients. Absence of coronary artery disease, diabetes mellitus and hypertension was assessed by the use of a standard proforma. Smokers were excluded from this group also.

Fasting venous blood was collected. 3ml of blood was transferred to a plain test tube and centrifuged at 3000r.p.m for 10 minutes to separate the serum using Eltek refrigerated centrifuge. Paraoxonase activity and lipid profile was assessed from serum.

All the chemicals used for reagent preparation were of analytical grade obtained from Sigma Aldrich. Double distilled deionised water was used for reagent preparation. Lipid profile was done in Erba XL 300 auto analyser by enzymatic methods using kit from Erba Manheim. LDL Cholesterol was calculated using Friedwald's formula. Serum paraoxonase activity was measured by using Jasco UV-VIS 560 spectrophotometer. An aliquot of serum is added to phenyl acetate (4mmol/L) in Tris–acetate buffer (50mmol/L) pH 7.5 containing calcium chloride (20mmol/L).The rate of formation of phenol is measured by monitoring the increase in absorbance at 270nm at 25°C. The normal range extends from 53- 186 k U/L.[6]

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RESULTS: Statistical analysis was performed using SPSS for windows version 15. The mean and standard deviation for quantitative variables and percentage for qualitative variables were calculated for both cases and controls .Difference in the group means of quantitative variable was compared by t test. Chi-square test or Fischer's exact test were used to compare the differences in the percentage of qualitative variables between groups. A p value of <0.05 was considered as significant. Pearson correlation coefficient was obtained to study correlation between PON1 activity and other continuous variables. Multiple regression analysis was done to study PON1 activity difference between the two study groups adjusting for other independent factors. Table 1 shows the demographic data, serum paraoxonase activity and lipid levels of the study group.

Cases & controls show significant difference in the distribution of age, sex, total cholesterol, HDL and PON1 activity. Out of the 100cases, 73 were males and 27 were females. In the control group, 51 were males and 49 were females. So when compared with control group, case group had a greater proportion of males (p value .002) and was significantly older with mean age of cases 54.78 and that of controls 49.41 (p value .000) [Table 1]. There was no significant difference in mean BMI [24.28±3.54 in cases against 24.13±3.05 in controls] between the groups.

The CHD group had a significantly lower mean total cholesterol level (202.82±57.77) against 219.45±46.08) (p value .027) and lower mean HDL level (40.88±10.08 against 56.79±17.24) (p value .000). No significant difference in the mean value of triglycerides and LDL cholesterol was noted between case and control groups. The mean PON 1 activity (70.21±27.62 in cases against 135.86±33.48 in controls) (p value .000) (Figure 1) was significantly low in CAD group compared to the control group. Multiple linear regression analysis was done to study PON 1 activity difference between cases and controls adjusting for age, sex, BMI, TC, HDL (Table 2). A p value of .000 was obtained which shows that there is a significant difference in the activity of PON1 between cases and controls when the confounding effect of other variables was adjusted. Serum PON1 activity show positive correlation with HDL and negative correlation with age when the case and control groups are combined (Table 3).

DISCUSSION: The activity of serum PON1 enzyme in CAD patients was significantly low in our study. The mean activity is approximately half when compared to controls. This is comparable to studies reported by Mackness et al [7]. The results of the Caerphilly study, the first prospective epidemiological study of PON1 and CHD, also showed that PON1 activity predicted coronary events independent of all other coronary risk factors, including HDL. The study suggested that PON1 activity might be developed as a biomarker of HDL function and cardiovascular risk independent of HDL concentrations.[8]

Phenyl acetate (aryl esterase activity) and paraoxon (paraoxonase activity) are the two substrates most commonly used to monitor PON1 activity**.** The present study focused on phenyl acetate hydrolytic activity as it was recently shown to be a more accurate marker for the antioxidant activity of PON1 than is paraoxon.[9] The exact physiological substrate of PON1 has not been identified to date. The role that PON1 play in retarding atherosclerosis is that it protects lipoproteins against oxidative modification, perhaps by hydrolysing phospholipid and cholesteryl-ester hydroperoxides. Experiments with inhibitors of PON1 suggest that it is responsible for the antioxidant effect of HDL.[10]

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The total cholesterol and LDL was found to be lower in the patients compared to controls in this study. Though the mean levels of total cholesterol showed statistically significant difference (p value .027) between cases and controls, the difference in mean LDL level was not statistically significant (p value .364, Table 1). This can be explained by the fact that the patient group was taking cholesterol lowering therapies while they took part in the study. The HDL cholesterol level was lower and triglycerides were higher in the case group. The difference in mean HDL level between cases and control was statistically significant (p value .000).

Correlation of the activity of PON1 with other variables for the combined group (Cases and controls taken together) showed that age is negatively correlated and HDL is positively correlated with PON 1 activity. Within the control group no correlation of the enzyme activity with other variables was noted. In the case group, TC level is negatively correlated with PON1activity. No correlation with other variables is noted.

The difference in the mean age between cases and controls were significant in this study and age is a conventional risk factor for CAD. But this much of age difference between case and control was not uncommon as it was recently reported in a similar study in Japanese subjects.[11] Multiple regression analysis after adjusting for other variables showed that PON1 activity is significantly low in case group. Low serum paraoxonase activity has been reported with diseases like diabetes mellitus, hypercholesterolemia, myocardial infarction and renal failure also.[12] Smoking reduces serum paraoxonase activity whereas alcohol shows no association.

In this study, the concentration of the enzyme was not measured and also gene polymorphisms which has been shown to modulate the activity of the enzyme was not taken into account. Moreover the activity of the enzyme towards paraoxon was not studied. Natalia Ferre et al., [13] suggest that the differential substrate activity of PON1 is more critical than the enzyme concentration for its protective effect against atherosclerosis. Sample size was also small. These are the major limitations of this study.

In conclusion, the activity of serum paraoxonase enzyme (PON1) is low in patients with coronary artery disease compared to healthy controls. The low serum PON1 enzyme activity is an independent risk factor for CAD. Confirmation that PON1 influences the risk of atherosclerosis must come from clinical trials in which PON1 activity is raised nutritionally or pharmacologically. Also the physiologic substrate of this enzyme should be discovered so that modifiers that can enhance the activity of PON1 can be developed.

Variable	Control	CHD	p value
Subjects(male/female)	100(51/49)	100(73/27)	.002*
Age (years)	49.41(8.42)	54.78(9.06)	.000*
BMI	24.13(3.05)	24.28(3.54)	.744
TC(mg/dl)	219.45(46.08)	202.82(57.77)	.027*
HDL(mg/dl)	56.79(17.24)	40.88(10.08)	.000*
TG(mg/dl)	138.55(66.42)	154.88(79.21)	.120
LDL(mg/dl)	135.11(36.37)	129.30(51.24)	.364
PON activity(kU/L)	135.86(33.48)	70.21(27.62)	.000*

Table 1: The demographic data, serum paraoxonase activity and lipid levels of the study group

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Values are shown as mean (Standard deviation). p value <.05 (Shown by asterisk) are significant.

Model		Unstandardized Coefficients		Standardized Coefficients	T	p value
		B	Std. Error	Beta		
1	(Constant)	133.405	25.181		5.298	0
	CC	-63.035	5.5	-0.7	-11.46	.000*
	Age	-0.029	0.266	-0.006	-0.11	0.913
	Sex	-5.519	4.971	-0.06	-1.11	0.268
	BMI	0.11	0.691	0.008	0.159	0.874
	TC	-0.03	0.049	-0.035	-0.626	0.532
	HDL	0.278	0.175	0.1	1.587	0.114

Table 2: Multiple linear regression analysis to study PON1 activity difference between two groups after adjusting for age, sex, BMI, TC, HDL

Dependent variable: PON 1 activity, p value .000.

		PON 1 activity
Age	Pearson Correlation(r value)	-.229(**)
	p value	0.001
	N	199
BMI	Pearson Correlation(r value)	-0.033
	p value	0.645
	N	199
TC	Pearson Correlation(r value)	0.108
	p value	0.133
	N	194
LDL	Pearson Correlation(r value)	0.014
	p value	0.852
	N	192
HDL	Pearson Correlation	.412(**)
	p value	0
	N	194
TG	Pearson Correlation(r value)	-0.071
	p value	0.327
	N	194

Table 3: Correlation of PON 1 activity with other study variables for combined group

** Correlation is significant at the 0.01 level (2-tailed).

* Correlation is significant at the 0.05 level (2-tailed).

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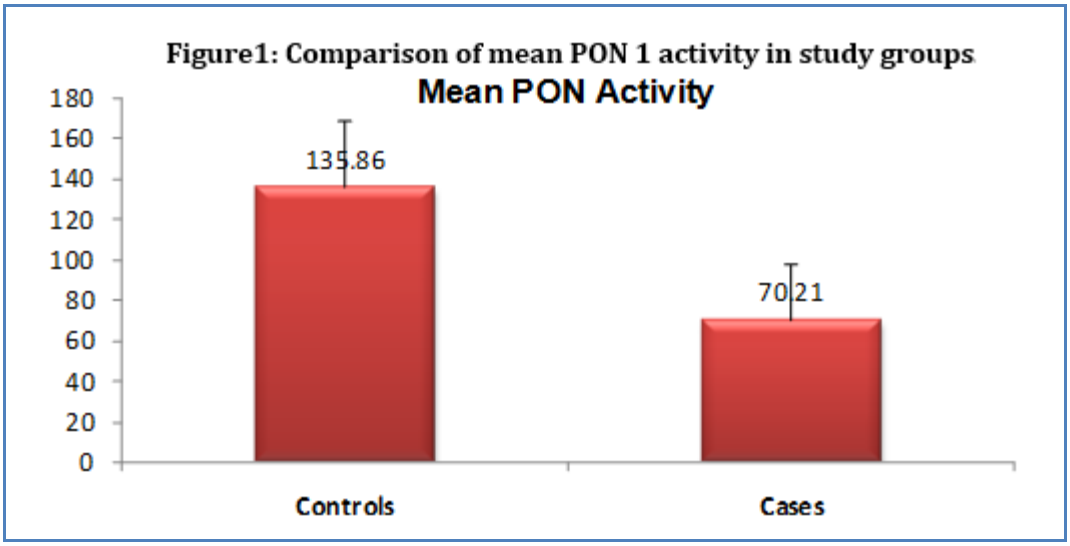

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