DOI: NJLM/2013/6309:1981

Original Article

Biochemistry Section

Study of Paraoxonase 1 (PON1) Activity as an Independent Risk Factor in Coronary Artery Disease

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ABSTRACT

Background: Coronary Artery Disease is the major cause of mortality and morbidity worldwide. Human serum paraoxonase-1 is a high density lipoprotein bound enzyme exhibiting antiatherogenic properties.

Aim: The aim of present study was planned to evaluate the serum paraoxonase-1 activity and lipid profile with coronary artery disease in addition to investigate the relationship between serum HDL-C and PON1 levels in patients with Coronary Artery Disease.

Material and Methods: In the present case-control study 142 with coronary artery disease (age range 26 to 72) and 115 age and sex matched healthy controls were recruited. Serum paraoxonase activities were measured spectrophotometrically by using phenyl acetate as substrate by kinetic assay while lipid profile was analysed by enzymatic method by cholesterol oxidase peroxidase (CHOD-PAP) method of total cholesterol and high density lipoprotein cholesterol and glycerol 3- phosphate oxidase (GPO-PAP) method of triglyceride. Values were expressed as mean ± standard deviation and data from patients and controls were compared by using student t-test.

Results: Significantly lower serum paraoxonase 1 activity ($p<0.001$) along with the lower high density lipoprotein cholesterol ($p<0.001$) and higher low density lipoprotein cholesterol, triglycerides and very low density lipoprotein cholesterol were observed in coronary artery disease patients as compared to healthy controls. The linear correlation in between serum paraoxonase-1 activity and HDL-C levels was found in the coronary artery disease like Myocardial infarction ($r=0.208$), stable angina ($r=-0.051$) and unstable angina ($r=-0.103$) and in the controls ($r=0.102$).

Conclusion: The low serum paraoxonase 1 activity may be an independent risk factor for coronary artery disease furthermore it can be used as primitive marker of progression of atherosclerosis and coronary artery disease.

Key words: Coronary artery disease, Lipid profile, Paraoxonase-1

INTRODUCTION

Coronary Artery Disease (CAD) is defined as acute or chronic cardiac disability arising from imbalance between the myocardial supply and demand for oxygenated blood. It is multifactorial in etiology and has spectrum of presentations ranging from stable angina, acute coronary syndrome to completely asymptomatic disease [1].

Cardiovascular disorders (CVD) which include CAD, heart failure and stroke are the leading cause of morbidity and mortality both in developed and developing countries and by 2020 CAD is expected to become the number one cause of death worldwide. CAD is the single most important contributor to increasing burden of CVD. It leads to more deaths than any other disease including cancer [2].

The oxidative modification of low density lipoprotein cholesterol (LDL- C) in the arterial wall is believed to be the major pathogenetic mechanism behind the initiation and acceleration of atherosclerosis and thus the coronary artery disease [3].

Serum paraoxonase (PON1) EC 3.1.8.1 an arylesterase synthesised in the liver. This is High Density Lipoprotein Cholesterol (HDL-C) associated enzyme which is responsible for the antioxidant properties of HDL. This enzyme plays an important role in preventing LDL –C oxidation, it is considered to protect against the development of coronary heart disease [4]. Knowledge about the link between paraoxonase activity and atherosclerosis comes largely from the biological rather than epidemiological studies as there is evidence that peroxidation of LDL-C is an important factor for atherosclerosis [5].

The lipoprotein profile has been investigated extensively in recent years which are found to be deranged in large proportion of CAD patients especially, Asians showing a

mixed picture of dyslipidaemia [6]. Numerous cohort studies and clinical trials have confirmed the association between the HDL-C concentration and increased risk of coronary heart disease. Low HDL-C is a marker for the presence of a small, dense, cholesterol depleted LDL in the circulation which itself increases risk of atherosclerosis probably because of its susceptibility to oxidation. Number of factors may play a role in its pathogenesis. Low PON-1 activity could be an independent risk factor [7]. Further studies have indicated that PON-1 can prevent lipid peroxide accumulation on LDL in-vitro and in-vivo. Lower PON-1 activity and concentration may be more important in determining the presence of CAD than paraoxonase genetic polymorphism [8].

Acquaintance of Paraoxonase status in CAD may help in planning proper strategies in clinical management of the disease. Thus aim of our study was to find PON-1 activity in patients with different types of CAD like stable angina (SA), unstable angina (USA), and myocardial infarction (MI) and compared it with that in healthy controls and also tried to find its correlation with lipid variables.

MATERIAL AND METHOD

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

Patients: The study included total 142 patients between age group 26 to 72 years of CAD. Of these 102 patients of Myocardial Infarction (MI) and Unstable Angina (UA) who admitted in the Intensive Cardiac Care Unit (ICCU) chest pain were taken for the study.

Remaining 40 patients of stable angina had taken from outpatients attending the cardiology department of same hospitals. The patients were diagnosed by physicians. Data included history, physical examination, serial 12-lead electrocardiogram and cardiac markers measurement.

Control subjects: A sum of 115 healthy age and sex matched individuals who didn't have any evidence of CAD as per clinical examination were taken as control subjects.

Exclusion criteria: Patients with diabetes mellitus, renal insufficiency, hypertension, current smokers, hepatic disease, and heart disease like congenital heart disease, diseases of heart valves & myocardium or taking lipid lowering drugs and antioxidant vitamin supplements were excluded.

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

Estimation of lipid profile:

Serum total cholesterol and HDL-C were determined by CHOD-PAP method. Serum triglyceride (TG) was measured enzymatic GPO-PAP method end point assay (using kit manufactured by span diagnostic Ltd) using semi-autoanalyser. LDL-C calculated by using friedewald formula (LDL-C= total cholesterol-TG/5- HDL-C) [9-12].

Estimation of PON1 activity:

The assay was based on the principle that PON1 catalyses the cleavage of phenyl acetate resulting in phenol. The rate of formation in phenol is measured by monitoring the increase in absorbance at 270nm. One unit of arylesterase activity is equal to 1μM of phenol formed per minute. The activity is expressed in KU/L based on the extinction coefficient of phenol of 1310 M-1 cm-1 at 270 nm at pH 8.0 and 250C. Blank sample without serum were used to correct for non-enzymatic hydrolysis [13].

STATISTICAL ANALYSIS

The statistical analysis was carried out by using the SYSTAT software version 12. The results were expressed in Mean± Standard Deviation (Mean±SD). To test the significance between the study group and the control groups, data was were analysed by student's t-test. p value $p<0.001$ was considered to be statistically highly significant. The relationships between PON1 activity and HDL-C were assessed using Pearson's correlations.

RESULTS

The demographic and clinical characteristics of the three groups of CAD and control group are listed in [Table/Fig-1] showed. There were no significant differences between the groups in age, gender and BMI.

As shown in [Table/Fig-2] serum PON1 activity and HDL-C levels were ($p<0.001$) significantly decreased in patients with SA, USA and MI as compared to controls. While total cholesterol, TG, LDL-C levels were significantly ($p<0.001$) higher in all types of CAD subjects as compared to healthy controls.

[Table/Fig-4] Illustrated PON1 activity and lipid profile in male and female patients. Serum PON1 activity and HDL-C levels were ($p<0.001$) significantly decreased while total cholesterol, TG, LDL-C levels were significantly ($p<0.001$) higher in both genders of all types of CAD subjects as compared to healthy controls.

Variables	Controls (n=115)	CAD		
		Stable angina (n=40)	Unstable Angina (n=43)	Myocardial Infarction (n=59)
Age in years	40.1± 12.34	41.3±14.06	43.1± 13.01	48.8±14.56
Gender (Men/Women)	67/48	29/11	31/12	43/16
Body Mass Index	20.23±1.88	23.04±2.38	23.53±2.99	22.9±2.89
Systolic blood pressure (mmHg)	110 ± 15.03	119.15± 30.11	120.07 ± 35.16	129.32 ± 22.58
Diastolic blood Pressure (mmHg)	75.62 ± 5.04	81.42± 14.28	79.53 ± 16.02	84.84 ± 23.36
Cigarette Smokers (n)	------	07	15	16
Tobacco Chewing(n)	------	26	19	28

[Table/Fig-1]: Demographical and clinical characteristic of Controls and CAD
Values were expressed in mean with Standard Deviation (Mean±SD), n = numbers

Variables	Controls (n=115)	CAD (n=142)		
		Stable Angina (n=40)	Unstable Angina (n=43)	Myocardial Infarction (n=59)
Total Cholesterol (mg/dl)	168.54±27.09	190.98±32.89	242.95±55.53*	264.76±64.24*
HDL-Cholesterol (mg/dl)	43.806±3.59	40.13±6.63*	37.68±3.70*	35.47±4.89*
LDL Cholesterol (mg/dl)	102.67±27.27	119.03 ±31.69	174.82±52.93*	195.52±62.49*
Triglyceride (mg/dl)	110.31±27.32	157.04±38.05*	155.01±44.49*	168.87±43.63*
Paraoxonase (KU/L)	103.67±20.52	44.65±8.7*	47.70±12.18*	50.43±10.21*

[Table/Fig-2]: Paraoxonase-1 activity and lipid profile in subjects with CAD and controls
Values were expressed in mean with Standard Deviation (Mean±SD), * p<0.001-- considered as highly significant

Group	Gender	Total Cholesterol (mg/dl)	HDL-Cholesterol (mg/dl)	LDL Cholesterol (mg/dl)	Triglyceride (mg/dl)	Paraoxonase (KU/L)
Control	Male (n=67)	166.46±22.97	43.71±3.62	101.45±20.85	106.43±24.10	107.37±17.63
	Female (n=48)	168.28±24.27	43.39±3.68	103.42±22.42	107.31±25.26	105.66±20.48
Stable Angina	Male (n=29)	191.17±33.71*	39.75±6.99*	119.12±39.92*	161.49±34.23*	41.26±11.06*
	Female (n=11)	207.08±46.94*	39.47±6.77*	133.58±48.42*	170.13±52.11*	42.95±9.12*
Unstable Angina	Male (n=31)	230.51±40.82*	38.37±3.32*	157.03±39.39*	177.35±59.04*	49.53±12.84*
	Female (n=12)	272.15±78.64*	36.63±4.29*	188.32±66.30*	194.31±73.39*	43.24±8.18*
Myocardial Infarction	Male (n=43)	260.86±59.27*	35.60±5.11*	191.22±59.92*	170.14±34.19*	49.59±9.45*
	Female (n=16)	273.72±66.71*	35.49±4.25*	201.67±58.72*	188.93±61.36*	50.99±10.77*

[Table/Fig-3]: PON1 activity and lipid profile in male and female CAD patients and controls
Values were expressed in mean with Standard Deviation (Mean±SD),* p<0.001-- considered as highly significant

Groups	Parameters	'r' Values
Controls	PON1 Vs HDL-C	0.102
Stable Angina	PON1 Vs HDL-C	-0.051
Unstable Angina	PON1 Vs HDL-C	-0.103
MI	PON1 Vs HDL-C	0.208

[Table/Fig-4]: Correlations between the PON1 and HDL-C in CAD

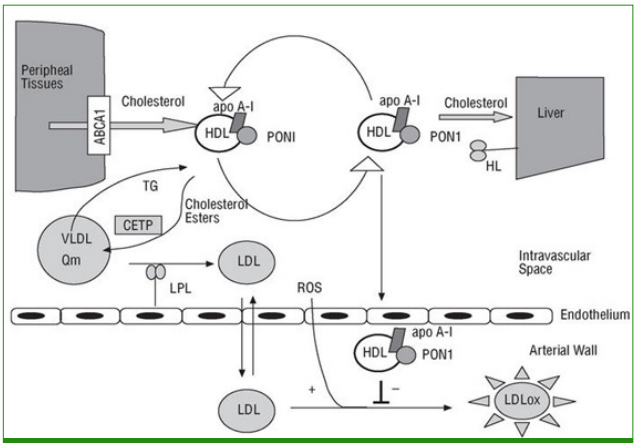
[Table/Fig-4] Indicates the correlation between PON1 and HDL-C in CAD and controls groups. 'r' values for serum PON1 verses HDL-C were -0.051,-0.103, and 0.208 in SA,USA and MI respectively while in the normal controls, it is 0.102.

DISCUSSION

Atherosclerosis is primary cause of CAD.CAD occurs due to narrowing and subsequent occlusion of the coronary arteries. [3]. Hypercholesterolemia is universally accepted as a major risk factor for atherosclerosis but at any given concentration of plasma cholesterol, there is variability in the occurrence of cardiovascular events as it has been shown that oxidative modification of LDL might be a crucially important step in development of atherosclerotic plaque [4].

HDL-C which plays an anti atherogenic role apart from inverse cholesterol transport protects LDL-C against oxidative modification which is attributed to PON1 enzyme located in a subfraction of HDL-C that contain apoA-1 and clusetrin [14]. The physiological function of PON1 seems to be a degrade specific oxidised cholesteryl ester and oxidised phospholipids in lipoproteins and cell membranes [Table/Fig-5] [15].

The paraoxonase gene family has at least three members PON1, PON2 and PON3 of which the PON1 plays an important



[Table/Fig-5]: Role of paraoxonase-1 in prevention of arteriosclerotic plaque formation. ABCA1 indicates ATP-binding cassette A1; apo A-I, apolipoprotein A-I; CETP, cholesterol ester transfer protein; HDL, high-density lipoprotein; LDL, low-density lipoprotein; LDLox, oxidized LDL; HL, hepatic lipase; LPL, lipoprotein lipase; PON1, paraoxonase-1; Cm, chylomicron; ROS, reactive oxygen species; TG, triglycerides; VLDL, very-low-density\ lipoprotein

role as it product paraoxonase is exclusively bound to HDL [5]. The last decade has seen that accumulation of evidence suggesting a role of PON1 in atherogenesis and CAD.

In the present study we have demonstrated that serum PON1 activity was significantly decreased in CAD like stable angina, unstable angina and myocardial infarction as compared to healthy controls. This finding is an agreement with the reports that have found diminution of PON1 activities in CAD [14-16].

PON1 activity measured using phenyl acetate as substrate has been through to be more closely reflecting PON1 concentration and it minimizes the risk of genotype variation [16]. Its activity can be decreased in subjects either due to diminish synthesis or inactivation under oxidative stress by lipid peroxidation [4,6] several studies have shown that reduced PON1 activity and increased malondialehyde level may contribute to increased susceptibility for development of acute myocardial infarction. [17]. Lipid peroxide which are substrate for PON1 and which have been shown to be raised in people with coronary heart disease 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 coronary heart disease [18].

The lower activity of PON1 can depress the ability of circulating HDL particle to protect LDL from oxidation, to participate in reverse cholesterol transport pathway and to inhibit monocytes-endothelial cell interaction. All these appear to be important in the inflammatory response in artery that promotes atherogenesis [14]. Serum PON1 activity is reduced in diabetes mellitus and metabolic syndrome which are associated with accelerated atherogenesis [5,14].

Gur et. al. have suggested that decline antioxidant capacity of PON1 activity and free sulphyhydryl groups occurred in CAD patients with three vessel disease. Our study supports this observation with finding of lower PON1 activity in CAD [19]. Many case controls studies have invented that measurement of PON1 activity was a better predictor than genotype in terms of CAD [7,20].

The Lipid abnormalities are one of the important risk factor for ischemic heart disease. There are a number of risk factors which influence the formation of plaques due to excess cholesterol. The plaques that are deposited on the walls of the blood vessels reduce blood flow to the heart muscle and cause ischemia.

Fasting levels of triglycerides, LDL-C and total cholesterol in patients of CAD were significantly higher as compared to those in controls ($p<0.001$) where as the levels of serum HDL-cholesterol was significantly ($p<0.001$) lower as compared to that in controls.

Our results are strongly supported to other studies [21-23]. In a prospective cardiovascular munster study, elevated TG has been found to be significant and independent risk factor for major coronary events even after adjustment for LDL-C and HDL-C levels and other risk factors [21]. Assessing the lipid ratio in a normal individual as it is one of the atherogenic factors for development of myocardial infarction and other coronary complications.

Lehto et al., have demonstrated that there was a direct correlation between the incidence of acute myocardial infarction and plasma lipid abnormalities. M.R. Abdullah has found that significant increase in lipid and lipoprotein, total cholesterol and LDL-C in the sera which showed severity of clinical symptoms of endothelial symptoms. According to him abnormal lipid profile along with other crucial factors in the cascade leading to ischemic damage in the cardium [24].

K Kusuma and Asna Vrooj have studied the nutritional status and lipid profile in selected Ischemic Heart Disease patients. They have found that waist to hip ratio and body fat % were higher along with low HDL- C and High TG levels in serum. These trends observed with respect to the diet type in Indian population [25].

In present study, univariate correlation analysis has performed in the CAD group and controls. Linear correlation between the PON-1 activity and HDL-C was found in CAD like MI, SA and USA and in controls. Very few investigators premeditated the correlation between PON1 and HDL-C in CAD. Andan Akey et al., have found that, no significant correlation in between PON1 activity and other metabolic parameter like HDL-C, TG, Insulin in the CAD and metabolic syndrome [14]. Amur Ayab et al., have established that sustained myocardial infarction did not show markedly decreased HDL-C concentration

but PON1 activity and PON concentration were profoundly decreased [26]. Kumar et al., have studied serum PON1 activity in normalipidimic patients with acute myocardial infarction. According to them no correlation was observed between PON1 activity and HDL-C in acute myocardial infarction which suggested that decreased PON 1 activity could be oxidative stress in acute myocardial infarction [21].

CONCLUSION

Thus higher TG and LDL-C and lower HDL-C levels are better indicators of CAD and their catabolism rate may play crucial role in the development and progression of atherosclerosis. But the study also confirmed no association between PON1-activity and HDL-C in severity of CAD. Hence the low serum PON1 activity may be an independent risk factor for CAD. Similar studies involving larger samples in different ethnic groups in India need to be done to find out the role of PON1 activity in pathogenesis of CAD.

ACKNOWLEDGEMENTS

The author deeply acknowledges all the doctors and technical staffs in intensive cardiac care unit of Vikhe Patil Hospital & Swasthya Hospital & Research Centre Ahmednagar for their cooperation in collection of blood within time.

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FINANCIAL OR OTHER COMPETING INTERESTS: None.

Date of Publishing: **Aug 30, 2013**