e-ISSN: 0976-822X, p-ISSN:2961-6042

Available online on http://www.ijcpr.com/

International Journal of Current Pharmaceutical Review and Research 2024; 16(5); 17-23

Original Research Article

A Study on Association of Serum Paraoxonase -1 Status with Atherogenic Index in Dyslipidemic Individuals in a Government Medical College in Kolkata

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Received: 01-02-2024 Revised: 15-03-2024 / Accepted: 21-04-2024
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Conflict of interest: Nil

Abstract

Introduction: Dyslipidaemia is due to altered lipid metabolism, often characterised by elevation of LDL & depletion of HDL concentration. Paraoxonase 1 (PON1) is an enzyme associated with HDLc and its atheroprotective and antioxidant role. Atherogenic index (AIP) is a predictor of atherosclerosis which is dependent on both serum triglyceride and HDLc concentration

This study was done to find out the association between atherogenic index and paraoxonase 1 activity in dyslipidemic individuals.

Methodology: 63 dyslipidemic patients (diagnosed by lipid profile parameters attending OPD were compared with 63 normolipidemic individuals. Serum paraoxonase1 activity of both dyslipidemic & no dyslipidemic individuals were measured using paranitrophenylacetate substrate. Serum atherogenic index along with other cardiac indices (cardiac risk ratio I and II and atherogenic coefficient) were calculated from lipid profile parameters.

Results: Significantly increased parameters of lipid profile including total cholesterol, Triglyceride and LDLc and significantly decreased HDLc and PON1 activity has been found among the dyslipidemics in comparison to normolipidemics ($p<0.001$). AIP and other cardiac indices are also significantly increased in dyslipidemic group ($p<0.001$). Statistically significant negative correlation has been found between PON1 & AIP ($r= - 0.425$ and $p<0.001$).

Conclusion: Low levels of PON1 is associated with high AIP and hence there a higher risk of cardiovascular disease in dyslipidemics.

Keywords: Paraoxonase 1, Atherogenic index, Dyslipidemia.

Introduction

Dyslipidemia is an increasing burden of society of not only in the developed countries but also in a developing country like ours. Dyslipidemia can be defined according to the National Cholesterol Education Programme (NCEP) as hyper cholesterolemia, hypertriglyceridemia & hyper lipoprotenemia and combination of these. [1] There is an elevation of serum low density lipoprotein (LDL) in dyslipidemia, but decreased serum concentration of high density lipoprotein (HDL) is a more frequent finding.

HDL is called the scavenger lipoprotein because it is required for transfer of cholesterol to the liver from throughout the body for breakdown & synthesis. [2] Human serum paraoxonase 1 (PON1) is an enzyme which has paraoxonase as well as arylesterase activity and is physically bound to high-density lipoproteins (HDL). It plays a key role in the biological action of HDL of protecting the lipoprotein, by preventing the oxidative damage of the HDL biological membrane. Serum PON1 is almost exclusively found in association with HDL particles. The lipid composition of HDL can influence its size and structure and is determined by a combination of various factors including dietary intake, metabolism, and storage. [3,4] So, dietary fats may affect PON1 activity by changing the phospholipids composition of HDL. On the other hand, it seems that any variation in HDL fatty acid composition could alter the function of HDL and the activity of its related enzyme, PON1.

Studies have shown that PON1 contributes to the atheroprotective function of HDL by decreasing lipid peroxidation along with an anti –inflammatory

component. Though the exact mechanism is not known, it is widely believed that PON1 inhibits the oxidative inactivation of Lecithin Cholesterol Acyl Transferase (LCAT), well known for the reverse cholesterol transport enzyme associated with HDL. Recent studies have demonstrated that HDL was extremely potent in protecting LDL against oxidative modification. [5] PON1 was the first enzyme shown to prevent /retard LDL oxidation in vitro by Mackness. [6] The enzyme was shown to reduce both the oxidative stress of macrophages as well as their ability to oxidise LDL. Further, PON1 present in human tissue fluid may protect the LDL even after it has crossed the vascular endothelium.

Atherogenic index of plasma (AIP) is defined as the logarithm of the ratio of plasma triglyceride concentration to the HDL concentration. (AI= log TG/HD). It is based on TG and HDL concentrations, both of which are independent risk factors for CAD. [7] Recently, AIP is used as a predictor of atherosclerosis. Individuals with AIP values of -0.3 to 0.1 are categorised as with low risk, those with 0.1 to 0.24 are categorised as medium and individuals with an AIP more than 0.24 are categorised as patients with high Cardiovascular risk. [8,9]

In order to predict the cardiovascular risk in susceptible individuals, some atherogenic indices or lipoprotein ratios have been defined. They are Castelli Risk Index I and II (CRI), atherogenic coefficient (AC), and non-high-density lipoprotein (HDL-c) (NHC). Castelli Risk Index I is calculated as Serum total cholesterol/Serum HDLc and Castelli Risk Index II (CRII) is calculated as Serum low-density lipoprotein (LDL)-cholesterol/Serum HDLc. It has been seen previously that indices calculated from several lipid profile parameters together had a better predictive capacity than an isolated lipoprotein. [10,11,12]

Objective

The aim of this study was to find out the association between atherogenic index and paraoxonase 1 activity in dyslipidemic individuals in the population attending a tertiary care hospital in Eastern India.

Materials & Methods

This observational analytical study was conducted in the Department of Biochemistry in College of Medicine & Sagore Dutta Hospital, Kolkata from June 2020 to May 2021 after obtaining the necessary Institutional Ethical Clearance.

Dyslipidemic patients attending the Department of Biochemistry for measurement of Serum lipid profile & belonging to the working age group of 20 - 60 years were included in the study. Patients were identified to be dyslipidemics based on National Cholesterol Education Programme (NCEP) guidelines. According to the NCEP guidelines, dyslipidemia was defined as low-density lipoprotein cholesterol (LDL-C) ≥140 mg/dL, high-density lipoprotein cholesterol (HDL-C) ≤40 mg/dL, triglycerides ≥150 mg/dL. [13] All the available literature show that all the studies on PON1 were performed in the age group of 20-60 years. Patients having diabetes mellitus or on anti-diabetic medications or hypolipidemic drugs and patients belonging to the age group of below 20 years or above 60 years were excluded from the study. Diabetic patients were excluded from the study as diabetes mellitus itself is a predisposing factor of dyslipidemia. [14]

The sample size for the study was calculated using Open Epi (Version-3) software and was found to be 130 (65 dyslipidemics & 65 non dyslipidemics).

Blood samples were collected for serum lipid profile after 12 hours of fasting & were stored at -20°C. All the serum samples were analyzed for serum paraoxonase-1 activity. Paraoxonase activity was analysed by using paranitrophenylacetate as substrate.

For estimation of paraoxonase activity, paranitrohenylacetate (PNPP) of strength 10 mmol/l, 4 Aminoantipyrine (8.6 mmol/L) and activator solution (Calcium chloride 20 mmol/L and sodium chloride 155 mmol/L) mixed in Tris acetate buffer (100 mmol/L, pH 7.5) were taken. This substrate was mixed with serum samples. After 20 minutes of incubation, Potassium ferricyanide (213 mmol/L) were added & mixed & absorbances were taken at 5 and 120 min at a wavelength of 492nm in a UV-Vis spectrophotometer T60 manufactured by LABINDIA. The calculation was done against the absorbances of blank. [15]

Serum HDL, LDL was measured by automated analyser using ERBA XL 640 system pack reagent. The HDL assay was based on a modified polyvinyl sulfonic acid (PVS) and polyethelene -glycol -methyl ether (PEGME) coupled precipitation method with the improvements in using optimized quantities of PVS/PEGME and selected detergents. While the LDL, VLDL, chylomicrons reacted with PVS/PEGME, the enzymes selectively reacted with HDL to produce hydrogen peroxide which was detected by the Trinder reaction. [16]

The assay of LDL was based on a modified polyvinyl sulfonic acid (PVS) and poly Ethelene glycol methyl ether (PEGME) coupled precipitation method with the improvements in using optimized quantities of PVS/PEGME and selected detergents. LDL, VLDL, CM reacted with PVS/PEGME and got precipitated. The supernatant contained HDL reacted with cholesterol esterase &

cholesterol oxidase. Addition of TODB N, N-Bis(4-ssulfobutyl)-3-methylalanine in detergent & MES buffer (pH 6.5) reagent containing a specific detergent released LDL from the PVS/PEGME complex.

The released LDL reacted with the enzyme cholesterol esterase & oxidase, to produce hydrogen peroxide which reacted with 4 amino antipyrine by the enzyme peroxidase and produce quinone. The intensity of colour formed was proportional to the LDL concentration in the sample. [17] The calculation of atherogenic index was performed by the formula log10 (Triglyceride/ HDL) for each dyslipidemic individuals (atherogenic index1) Data were tabulated in excel sheet & analysed by standard statistical method SPSS 16. The $p<0.05$ were considered significant at 95% confidence interval. The distribution of all the variables was determined & was found to be parametric. So Pearson's correlation tests were carried out between the PON 1 activity & HDL, between PON 1 & LDL and atherogenic index & PON1 Categorical variables were tested by chi square ($\chi 2$) test and continuous variables were tested by t- test.

Result:

Table 1: Demographic characteristics of the study population according to dyslipidemia and non-dyslipidemia (n=136)

Characteristics (sex)	Dyslipidemia	Non dyslipidemia	P value
Male (%)	57.6	42.4	0.110
Female (%)	44.2	55.8	

Table 2: independent t test of the parameters of lipid profile:

	Non dyslipidemia N=68	Dyslipidemia N=68	t value	P value
Total cholesterol (mmol/l) (Mean ± SD)	4.14± 0.68	6.02± 0.81	19.16	<0.001
Triglyceride (mmol/l) (Mean ± SD)	2.58±0.68	5.41±2.09	15.5	<0.001
HDL (mmol/l) (Mean ± SD)	1.32 ± 0.25	1.19 ± 0.21	5.45	<0.001
LDL (mmol/l) (Mean ± SD)	2.45±0.48	4.11± 0.63	17.44	<0.001
Paraoxonase (U/L) (Mean ± SD)	170.50 ± 26.19	137.45 ± 32.31	6.37	<0.001
Atherogenic index (Mean ± SD)	0.23±0.09	2.11±0.11	147.4	<0.001
Atherogenic Coefficient (Mean ± SD)	2.29±0.92	4.32±1.87	8.26	<0.001
Cardiac risk ratio1 (Mean±SD)	3.29±0.92	5.32±1.87	0.83	<0.0001
Cardiac risk ratio 2 (Mean ± SD)	1.95±0.59	3.61±1.3	9.86	<0.001

$p<0.05$ were considered significant at 95% confidence interval.

The mean value total cholesterol among the dyslipidemics was 235 mg/dl, their mean LDL was also 157.74 mg/dl, and the HDL was 46.20.

We have enrolled these patients in the dyslipidemics group because the LDL and total cholesterol values have been given due weightage as per ATP III guidelines, in spite of the fact that HDL is greater than 40mg/dl in these patients. [13] The number of dysdlipidemic patients is 68 based on LDL & total cholesterol level. Among this population, 36 cases have HDL values >40mg/dl giving rise to an apparent increase in the level of HDL in the dyslipidemic group.

Table 3: Pearson's correlation coefficient between Paraoxonase1 with HDL & LDL given in table 3:

Parameters	r value	p value
HDL & PON1	r = 0.152	0.02
LDL & PON1	r = -0.427	<0.001 #

r = Karl Pearson's correlation co-efficient. There was positive correlation between HDL and Paraoxonase activity whereas LDH was negatively correlated. # $p<0.05$ were considered significant at 95% confidence interval.

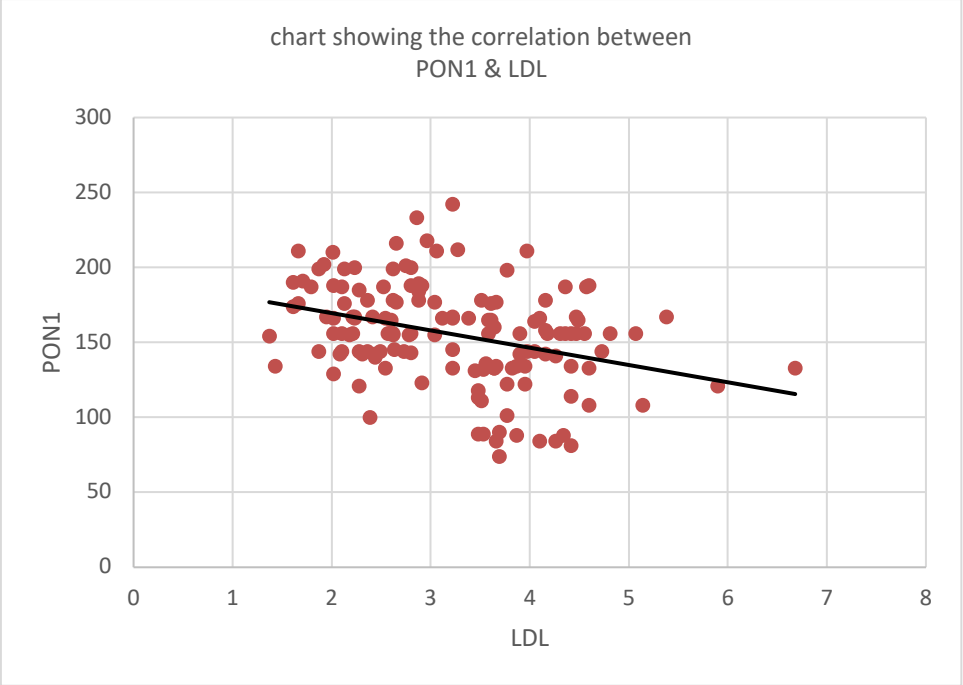

Figure 1: Shows correlation between LDL & Paraoxonase1 (using panitrophenylacetate substrate)

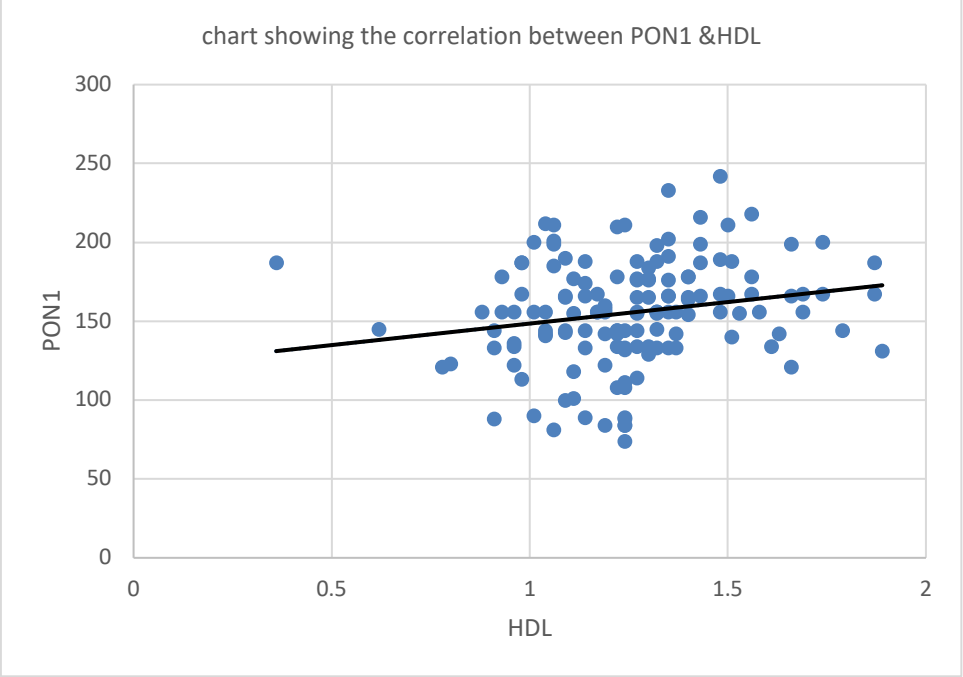

Figure 2: Shows correlation between HDL & Paraoxonase1 (using paranitrophenylacetate substrate)

Table 4: Pearson's correlation coefficient between atherogenic index & Paraoxonase1 given in table 4:

	r value	**P value**
Atherogenic index & PON1	-0.425	<0.00001

r = Karl Pearson's correlation co-efficient. # $p<0.05$ were considered significant at 95% confidence interval. Statistically significant negative correlation has been found between atherogenic index & Paraoxonase activity

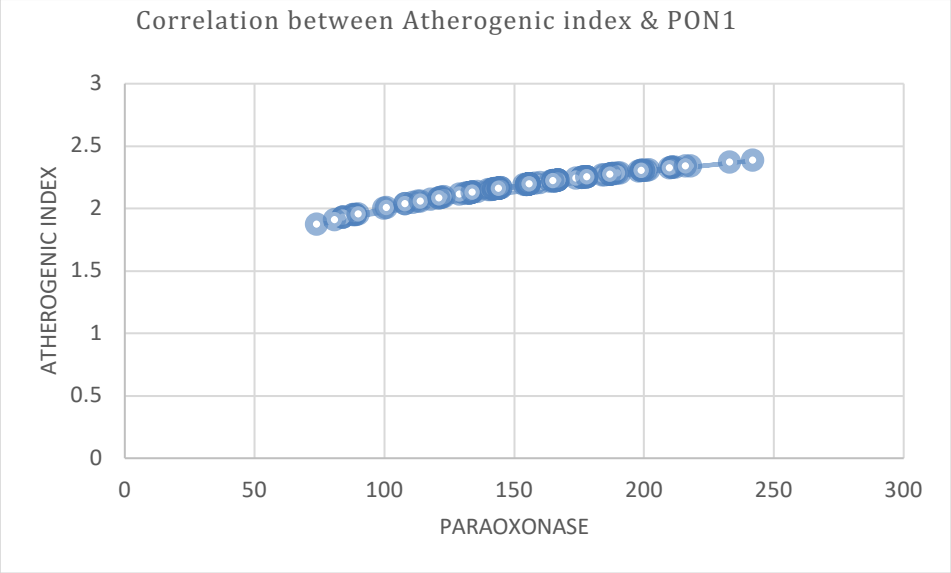

Figure 3: Correlation between atherogenic index & PON1

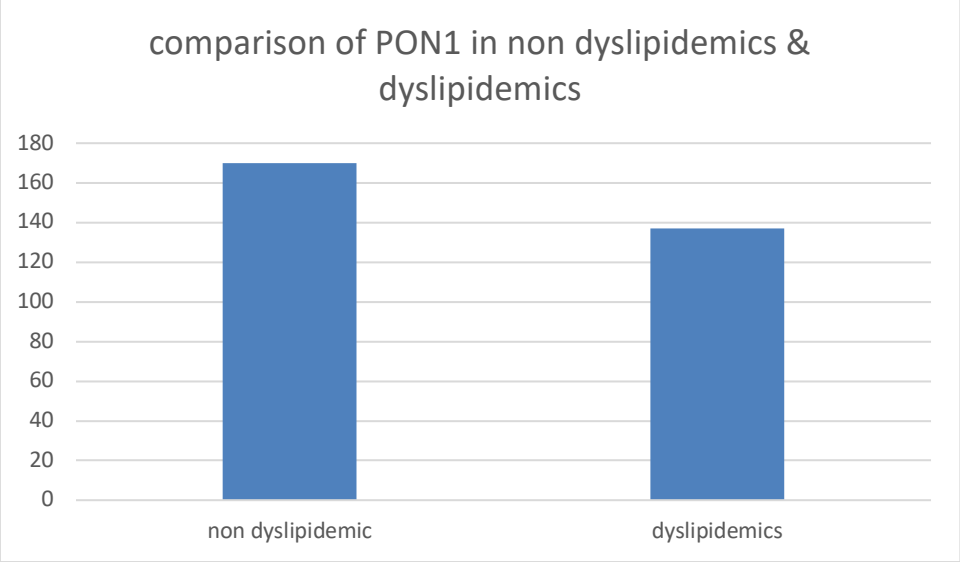

Figure 4: comparison of PON1 in dyslipidemics & non dyslipidemics

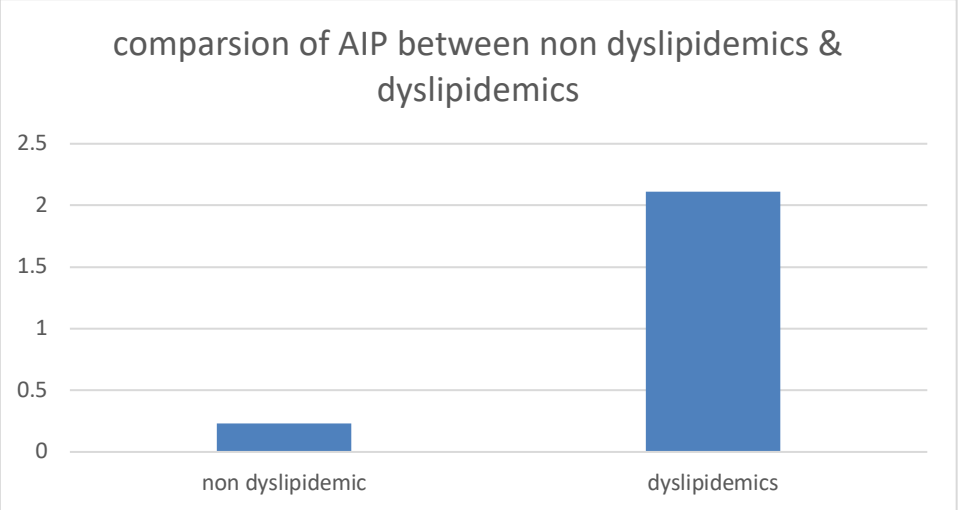

Figure 5: comparison of AIP between non dyslipidemics & non duslipidemics

Discussion

Serum PON1 is a glycoprotein synthesized mainly by the liver. It is found with the high-density lipoprotein & circulates along with it. But without binding with HDL, PON 1 is catalytically inactive. [14]. The enzyme also plays an important role in decreasing oxidative stress by free radical scavenging in the human body. [13] It plays a significant role in inhibiting the oxidation of both low-density lipoprotein (LDL) and HDL particles [15,16] Thus the PON1 has a protective role against atherosclerosis and cardiovascular disease. As per table1 57.6% of dyslipidemic individuals were male & 44.2% were female (Table 1).

In the dyslipidemic group the mean fasting LDL was 4.11±0.63 mmol/l, the mean HDL was 1.19±0.21mmol/l, PON1 was 137.45±32.31 U/L and AIP was 2.11±0.11. In individuals of non dyslipidemic group the mean fasting LDL was 2.45±0.48mmol/l, HDL was 1.32±0.25mmol/l, PON1 was 170.5±26.19 U/L % AIP was 0.23±0.09. Individuals in the dyslipidemic group have statistically significant higher fasting LDL-c ($p<0.001$), and significantly lower fasting HDL level ($p<0.001$), significantly higher AIP ($p<0.001$) and significantly lower PON1($p<0.001$) values. [Table 2]

A positive correlation between HDL & PON1 ($r=0.152$ & $p=0.02$) and a negative correlation between PON1 & LDL ($r=-0.427$ & $p<0.001$) has been seen. This is an expected finding since PON1 activity is associated with HDL (table 3, Figure1 &2) [18,19,20]

Statistically significant negative correlation has been found between AIP & PON1 ($r=-0.425$ & $p<0.001$) among all individuals (table 4 & Figure3)

Comparing the last 2 figures (fig 4 &5) we see that the AIP was higher and PON1 was lower in dyslipidemics. Many studies have shown that AIP is a measure of cardiovascular disease. In dyslipidemics it is therefore evident that there is a high risk of cardiovascular disease in dyslipidemics as corroborated by many other similar studies. [21,22,23,24]

Here, it may be pointed out that the levels of PON1 activity were also very low in dyslipidemics. At the same time, the Cardiac risk factor I and II and the atherogenic coefficient are all higher ($p <0.0001$) in dyslipidemics compared to normolipidemics (vide table-2). Therefore, it may be safely concluded that low levels of PON1 activity may be associated with high cardiovascular risks.

In future, the cardiovascular risk may be predicted by estimation of PON1 activity in the serum.

Limitations: The most important limitation of the study is the lack of established reference ranges of PON1 in the population and the fact that there is no gold standard method for estimation of PON1 activity so far.

Conclusion

This study was performed in the department of in a tertiary care hospital. The aim was to evaluate the association between AIP and PON1 in dyslipidemics. A negative correlation was found between AIP and PON1 activity. It may be concluded that low levels of PON1 is associated with high AIP and hence a higher risks of cardiovascular disease in dyslipidemics.

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