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Original Research Article

Association of Myeloperoxidase and Paraoxonase 1 Activity in Dyslipidemic and Non-Dyslipidemic Individuals: A Cross Sectional Analytical Study in a Tertiary Care Hospital

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Abstract:

Background: The lipid profile consisting of lipoprotein and triglyceride (TG) concentration reflects lipid metabolism that is modulated by genetic and environmental factors. The value of lipid profile parameters like TC, TG, LDL, HDL alters in dyslipidemia which can be due to some intrinsic & extrinsic factors, physical inactivity, genetic predisposition or the combination of aforesaid factors. Myeloperoxidase (MPO), a product of systemic inflammation plays important role both in the process of oxidative stress and inflammation and promotes oxidation of lipoproteins. 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.

Objectives: This study was done to find out the myeloperoxidase and paraoxonase1 activity and the association between athrogenic index with Paraoxonase1 activity in dyslipidemic individuals in a population attending tertiary care hospital in eastern India

Methods: 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. The sample size for the study was calculated using Open Epi (Version-3) software and was found to be 280 (140dyslipidemics&140 non dyslipidemics) Serum HDL, LDL were measured by automated analyser using ERBA XL 640 system pack reagents. The calculation of atherogenic index was performed by the formula $\log_{10}$ (Triglyceride/HDL) for each dyslipidemic individuals (atherogenic index1) Estimation of serum myeloperoxidase activity was done by using standardized kit (Elabscience) Myeloperoxidase activity assay kit.

Results: The aim of the present study was to compare the Paraoxonase 1 (PON1) and Myeloperoxidase (MPO) activity in dyslipidemic and non dyslipidemic individuals and to find the ratio of MPO and PON1 activities in serum of both the study groups and the correlation of MPO/PON1 and the atherogenic index of plasma (AIP). Statistically significant differences ($p<0.05$) had been found between dyslipidemics and non dyslipidemics in LDL, HDL, MPO, PON1, MPO/PON1 ratio and the atherogenic index of plasma in our study In our study there was significantly decreased PON1(Z value +10.6, p value <0.05) level and increased level of AIP (Z value -0.06, p value <0.001)in dyslipidemics in comparison to non dyslipidemics.

Conclusions: Therefore, it may be safely concluded that low levels of PON1 activity and high level of MPO activity may be associated with high cardiovascular risks. In future, the cardiovascular risk may be predicted by estimation of these parameters in the serum.

Keywords: Myeloperoxidase, Paraoxonase 1, atherogenic index, cardiovascular risk.

Introduction

Dyslipidemia, characterised by increased total cholesterol, triglycerides(TG), low density cholesterol(LDL) and decreased high density cholesterol (HDL) has been firmly established as a

major risk factor for atherosclerosis and cardiovascular diseases (CVD).[1] Numerous clinical and epidemiological studies have strongly advocated the reduction of LDL cholesterol as the cornerstone of management of atherosclerosis and coronary artery disease.[2] But, in clinical settings it is a common experience that residual atherogenic risks remains even after reduction of LDL below the recommended targets. That is why the focus has now gradually shifted to the usage of a combination of lipid indices for a more comprehensive assessment of the lipid status.

One such lipid index is the Atherogenic Index of Plasma (AIP) which is is defined as the base 10 logarithm of the ratio of the concentration of triglyceride (TG) to high density lipoprotein cholesterol (HDL-C), where each concentration is expressed in mmol/L.[3] This is particularly relevant in the Indian context because the typical Indian lipid profile shows high triglyceride with low HDL.[4]AIP is believed to be a potent indicator of cardiovascular disease, surpassing the importance of the traditional parameters of lipid profile. It acts as an indicator of the concentration of small low density lipoprotein levels, which is the main incriminating factor for atherosclerosis and coronary artery disease.Myeloperoxidase (MPO), a product of systemic inflammation plays important role both in the process of oxidative stress and inflammation and promotes oxidation of lipoproteins. [5] MPO is a leukocyte-derived enzyme that catalyzes the formation of oxidative reactants and participates in innate immunity against infections. It is a potential participant in promotion and propagation of atherosclerosis [6] and impairing endothelial function, it can participate in destabilization of atherosclerotic plaques. [7]

Paraoxonase 1 is an enzyme which is located on the membrane of high density lipoprotein (HDL) particle and it protects both high and low-density lipoproteins (LDL) against oxidative free radical damage. Decreased PON1 activity has been found to be associated in several diseases such as coronary artery diseases (CAD),[8] type I diabetes,[9] obesity,[10] and renal failure.[11] PON1, MPO and bind to HDL, forming a ternary complex, reciprocally modulate each other's activity. The ratio, if more than 1.5, has been shown to be a useful marker for secondary prevention of coronary artery disease through modulation of HDL function in several studies.

In this study, we intended to explore the association between the AIP and the MPO/PON1 ratio in dyslipidemic individuals. The aim of the study was done to estimate the myeloperoxidase and paraoxonase1 activity and the association between atherogenic index with MPO/PON1ratio in dyslipidemic individuals in a population attending tertiary care hospital in eastern India.

Methodology:

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. Accordingly dyslipidemia is defined as low-density lipoprotein cholesterol (LDL-C) ≥140 mg/dL, high-density lipoprotein cholesterol (HDL-C) ≤40 mg/dL, and triglycerides ≥150 mg/dL. [12,13] All the available literature showed 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 280 (140 dyslipidemics & 140 non dyslipidemics).

For estimation of paraoxonase activity, paranitrohenylacetate (PNPP) of strength 5.5 mmol/l, activator solution (Calcium chloride 20 mmol/L and sodium chloride 155 mmol/L) mixed in Tris HCL buffer (0.1 mmol/L, pH 8) were taken. After adding diluted serum sample, absorbances were taken at 0,1 and 3 minutes at 412 nm in a UV-Vis spectrophotometer T60 manufactured by LABINDIA, Thane, India. The calculated delta absorbances were finally multiplied with molar extinction coefficient 17000/mol/cm to quantify the enzymatic activity. Serum HDL, LDL were measured by automated analyser using ERBA XL 640 system pack reagents. The calculation of atherogenic index was performed by the formula, AIP=log10 (TG/HDL-C) (mmoL). So, for the calculation of AIP, triglyceride, and HDL values were first converted from mg/dL to mmoL/L and then AIP was calculated.

Estimation of serum myeloperoxidase activity was done by using standardized kit (Elabscience Myeloperoxidase activity assay kit; Lot no. F6CS2MQVQK). The principle of the test is based on the reduction of hydrogen peroxide by MPO to form a complex with HOCl which again reacts with o- dianisidine to finally produce a yellow coloured compound measured at 460 nm. The absorbance from the patient's sample obtained finally deducted from absorbance of standard and the final enzyme activity was quantified (in U/L) by multiplying molar extinction coefficient 175/mol^{-1}/cm^{-1}.

Results

Continuous data were expressed as Mean ± SD and the distribution of the data was tested for normality by the Shapiro-wilk test. Unpaired t-test (for con-

tinuous variables) and chi-square test (for categorical variables) were used for group comparison. Differences in mean and median of serum lipoproteins (namely LDL & HDL), myeloperoxidase and Paraoxonase 1 level between dyslipidemic and non dyslipidemic individuals were analyzed using Mann Whitney U test and the Spearman's Rank correlation and coefficient test as the distributions were non-parametric. P value<0.05 was considered as statistically significant. In Table1, the descriptive statistics of various parameters and the results of the Man Whitney's U test in dyslipidemics and non dyslipidemics have been shown. Results of the Spearmann's rank correlation between the lipoprotein and MPO, lipoproteins and PON1, MOP/PON1ratio and lipoproteins and the MPO/Pon1 ratio and AIP have been depicted in Table 2. The correlation curve between HDL and Myeloperoxidase has been shown in Figure 1. Last but not the least, the correlation curve among HDL and PON1 has been shown in Figure 2. Statistically significant differences ($p<0.05$) had been found between dyslipidemics and non dyslipidemics in LDL, HDL, MPO, PON1, MPO/PON1 ratio and the atherogenic index of plasma in our study. The ratio of MPO/PON1 has been calculated and it showed positive correlation with LDL (+0.39) and negative correlation with HDL(-0.327). MPO/PON1 ratio was higher in dyslipidemics (3.39±1.27) compared to nondyslipidemics (1.3±3.39).

Table 1: Shows the descriptive statistics of various parameters and the results of the Man Whitney's U test in dyslipidemics and non dyslipidemics

Parameters	Dyslipidemics (Mean H±SD)	Dyslipidemics (median)	Non dyslipidemics Mean SD)	Non Dyslipidemics (median)	Z value	P value
HDL (mg/dl)	41.24 5.79	39.8	57.28 + 0.78	52.8	-11.2	<0.001
LDL (mg/dl)	107.56+29.9	109.4	74.85+20.08	56.1	+9.47	<0.05
MPO (U/L)	507.26=99.35	503	248.08+64.7	252.45	-11.8	<0.05
PON1 (U/L)	153.1+2.02	155	193.2+3.6	197	+10.6	<0.05
MPO:PON1	3.39+1.27	3.16	1.3+3.39	1.29	+14.39	<0.001
Atherogenic index	0.294 - 0.143	0.311	-0.09 + 0.13	-0.06	+13.5	<0.001

Table 2: Results of the Spermann Rank correlation tests

Spearman correlation	Rho value	P value
HDL & MPO	-0.3646	<0.05
HDL & PON1	+0.208	<0.05
LDL & PON1	-0.112	0.184
LDL & MPO	+0.456	<0.001
MPO/PON1 & LDL	+0.39	<0.05
MPO/PON1 & HDL	-0.396	<0.05
MPO/PON1 & AIP	0.686	<0.001

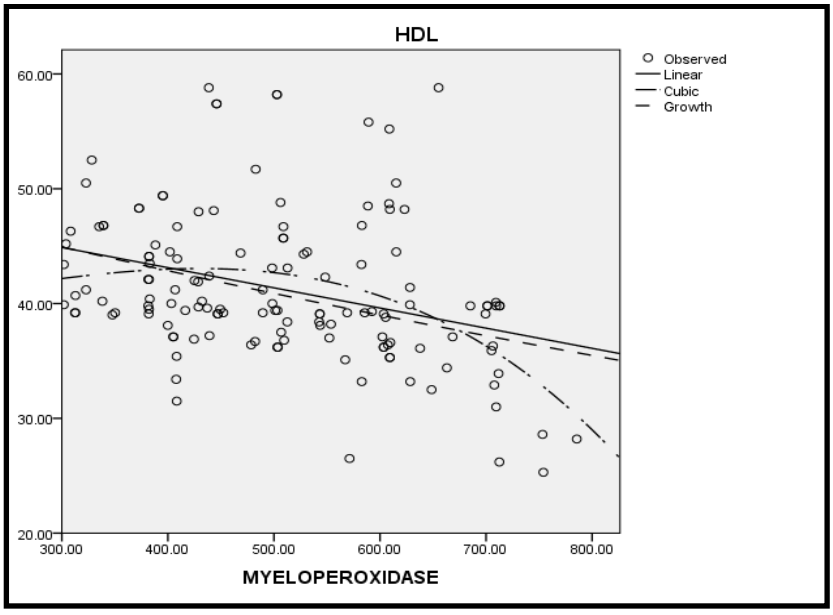

Figure 1: Shows the correlation curve between HDL and Myeloperoxidase

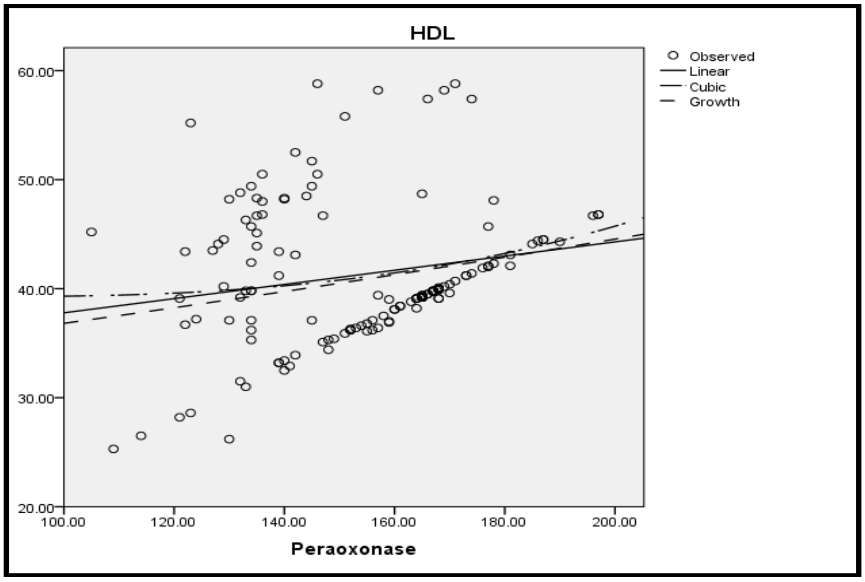

Figure 2: Shows the correlation curve between HDL and Paraoxonase1

Discussion

A cursory glance through the results throws up some interesting points. In Table 1 which shows the descriptive statistics and the results of the Mann Whitney test between the dyslipidemic and non dyslipidemic study groups, we find statistically significant differences ($p<0.05$) in LDL, HDL, MPO, PON1, MPO/PON1 ratio and the AIP. It is evident that, LDL is raised and HDL is decreased in dyslipidemics compared to nondyslipidemics, both being statistically significant.

These findings are well known and expected and well bourne out by several other studies .Further, there was significantly decreased PON1 (p value <0.05) and increased level of AIP (p value <0.001) in dyslipidemics in comparison to non dyslipidemics (vide table1). The MPO/PON1 ratio in our study was significantly increased (Z value +14.39, $p<0.001$) in dyslipidemic group which indicates the risk of atherosclerosis and CAD.

Table 2 shows the results of the Spearmann Rank correlation tests (theoretically lies between − 1 and + 1) performed in order to assess the correlation between different parameters. Our study also showed significant positive correlation between PON1 and HDL, negative correlation between LDL and PON1, a negative correlation between HDL and MPO and positive correlation in between LDL and MPO. In order to have a better understanding of the oxidative state, the ratio of MPO/PON1 has been calculated and the ratio showed positive correlation with LDL (+0.39) and negative correlation with HDL (-0.396). The MPO/PON1 ratio was also found to be positively correlated with the atherogenic index of plasma (0.686)

Previous studies have explained that there is inactivation of PON1 by oxidized LDL, which leads to reduction of PON1 activity. [14,15] Oxidized-LDL inactivates the PON1 by developing communications among the enzyme free sulfhydryl group and oxidized lipids. [16] It might be due to the dislodgment of calcium ions, necessary for PON1 activity [17].

Similar findings were bourne out by other studies wherein a population at increased CHD risk such as hyperlipemia had 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.18

In another study done by Variji A et al, PON1, MPO and the MPO/PON1 ratio were done to also evaluate which one has a stronger predictive role in coronary artery disease (CAD). They observed that the diagnostic performance of PON1 alone was comparable to that of the MPO/PON1 ratio for CAD risk assessment; however, MPO may increase the true positive rate. [19]

The atherogenic index of the plasma, the predictor of cardiovascular disease was significantly higher in dyslipidemics which also positively correlated with the MPO/PON1 ratio and this indicates that there is a chance of oxidative stress which increases the oxidized LDL concentration and hence the chance of atherosclerosis and coronary artery diseases increase in dyslipidemics. This finding is corroborated in many other previous studies. [20,21,22]

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. [23] The Indian lipid profile is characterized by high triglycerides and low

HDL (together called atherogenic dyslipidemia) and this picture is found in both urban and rural population and even in migrant Indians settled abroad.

The high AIP could be due to high carbohydrate diets of the Indian subcontinent where rice and/or wheat is the staple diet. [24] High AIP is believed to be one of the reasons for a higher relative risk burden, an earlier age of onset, higher case fatality and higher premature deaths due to cardiovascular diseases in the Indian population. Interestingly, AIP positively correlated with BMI, waist circumference, triglycerides, systolic blood pressure and even fasting blood sugar in South East Asia. AIP is sensitive measure and can be easily calculated especially when other lipid values are within normal range. [25]

The most important limitation of the study is the lack of established reference ranges of PON1and MPO in the population and the fact that there are no gold standard method for estimation of these parameters activity so far.

Conclusion

Therefore, it may be safely concluded that low levels of PON1 activity and high level of MPO activity may be associated with high cardiovascular risks.It may be safely concluded that the MPO:PON1 ratio positively correlates with the Atherogenic index of plasma (AIP) which has been established to be a sensitive marker of the cardiovascular disease. In future, the cardiovascular risk may be predicted by estimation of AIP in the serum.

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