

Research Article

Paraoxonase and vitamin D status in subjects with elevated LDLMohammed Muiz¹, Ashok Prabhu K.², Durga Rao Y.³, Pooja³, Nandini M.³¹Kasturba Medical College, Mangalore, 575001, Manipal Academy of Higher Education, Manipal, Karnataka, India²Department of Clinical Biochemistry, Kasturba Medical College Hospital, Ambedkar Circle, Mangalore, 575001, Karnataka, India³Department of Biochemistry, Kasturba Medical College, Mangalore, 575004, Manipal Academy of Higher Education, Manipal, Karnataka, India

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Corresponding author: **Nandini M.** Email: nandini.m@manipal.edu**ABSTRACT**

Introduction and Aim: Of the various causes for atherosclerosis vitamin D deficiency/insufficiency is well-known. Low levels of vitamin D are linked to alterations in certain markers of cardiovascular disease risk. Paraoxonase (PON-1) protects against CVD by preventing the oxidation of LDL as well as HDL. Reports on the correlation of PON-1 and vitamin-D status are very few. The present study assessed the correlation of PON1 and vitamin-D status in subjects with elevated LDL levels.

Materials and Methods: Serum samples of subjects with elevated LDL were assessed for lipid profile, vitamin-D and Paraoxonase. Vitamin D and lipid profile were estimated using COBAS auto-analyzer. Paraoxonase activity was assessed by the spectrophotometric method. 60 subjects with elevated LDL were taken as test and 30 with normal LDL were taken as the control group.

Results: Reduction in PON1 activity coupled with low HDL cholesterol and vitamin-D levels was seen in subjects with elevated LDL. Negative correlation of basal PON-1 with LDL was seen in the test group. The control group showed a negative association of Paraoxonase with HDL and a positive association with Vitamin D.

Conclusion: Elevated LDL along with vitamin-D deficiency enhances the risk of atherosclerosis. Future interventional studies (dietary supplements and drugs) leading to the enhancement of the PON-1 activity and lower LDL may give more insights on its anti-oxidant role.

Keywords: Atherosclerosis; BPON; HDL; LDL; SPON; Vitamin D

INTRODUCTION

Vitamin D deficiency/insufficiency, is a problem encountered in several parts of the world. Atherosclerosis is characterized by chronic inflammation and lipid accumulation. It is a commonly known fact that LDL which is atherogenic undergoes oxidative modification and is crucial in the onset and the chain of events occurring in atherosclerosis. On the other hand, HDL plays an anti-atherogenic role.

Paraoxonase (PON-1), functions as an inhibitor of LDL oxidation *in vitro* thus preventing CVD (1). More than 95% of PON1 resides in the HDL particles in the circulation and protects against oxidative damage of cells and lipoproteins. Susceptibility of HDL and LDL to undergo atherogenic modifications (2) is modulated by PON1. The protective effect of HDL against lipid peroxidation is attributed to PON-1.

Low vitamin D levels have been found to be associated with several markers of cardiovascular disease risk, including metabolic syndrome (3). The anti-atherogenic role of vitamin D causes hindrance in

the genesis of foam cells, cholesterol uptake by the macrophages, and enhances HDL transport (4). Decreased levels of 25-hydroxyvitamin D in the serum were associated with low HDL cholesterol concentration leading to metabolic syndrome (5).

Several studies have found inverse relationships with vitamin D and the incidence of various chronic conditions, especially cardiovascular disease, as analyzed by the circulating concentration of 25-hydroxyvitamin D (6, 7). A study done in Turkey, further highlighted the increased incidence of reduced coronary flow in patients with low vitamin-D levels (8).

A cross-sectional study conducted in PIMS, Islamabad (July 2016 to January 2017), showed a significant inverse relationship between the mean level of LDL-C and the vitamin-D values. The patients with lowest vitamin-D values were recorded to have higher LDL-C values. The same study also found similar relation between TC (Total cholesterol) and triglycerides also. In a study done among the population of rural China, positive relation was found between 25(OH) D3 levels and HDL-C also,

signifying the possible role of vitamin-D in preventing dyslipidaemia (9).

The functions of PON-1, an ester hydrolase are linked to HDL in circulation. PON-1 catalyses the hydrolysis of organophosphate insecticides and oxidized phospholipids. The growing evidence as seen in various studies underline the importance of reduced activity of HDL associated PON-1 as a strong marker of cardiovascular diseases in humans (10, 11).

Diabetic patients with micro vascular complications showed significant reduction in PON-1 activity (12). Study of Jayadip *et al.*, have shown that decreased levels of 25 hydroxy vitamin D was independently associated with dyslipidemia (13). Present study aimed to determine the PON-1 and vitamin D in subjects with elevated LDL and assess the correlation of PON-1 with vitamin D status.

MATERIALS AND METHODS

Study design

This was a hospital based cross-sectional study. Blood samples of 90 subjects attending the OPD at KMC Hospital Ambedkar Circle (KMCHAC) were analysed (60 subjects with elevated LDL and 30 subjects with LDL in the normal range).

Data collection

Patient data was obtained from Clinical Biochemistry section - KMC Laboratory Services KMCHAC. Blood samples of subjects in the age group of 35-75 whose serum lipid profile and vitamin D was assessed were selected for the study. Remaining serum was then stored at -20 degree celsius for two weeks for the paraoxonase assay.

Estimation of lipid parameters, HDL and LDL was done in the COBAS C-6000 auto-analyzer by using the ROCHE Diagnostic kits. Assay of paraoxonase was carried out in the department of Biochemistry by the spectrophotometric method (14). 4-nitrophenyl phosphate, in 20 mM Tris-HCl buffer, pH 8.0 was used as the substrate. The increase in absorbance due to the formation of the yellow 4-nitrophenol by the action of paraoxonase was monitored at 412 nm for 3 minutes. Both basal and salt stimulated paraoxonase (BPON and SPON) activity was estimated.

Statistical analysis

The mean difference in HDL and LDL between the groups was compared by student's independent t test. Mann Whitney U test was used for PON-1 and vitamin D. Correlation of paraoxonase with vitamin D was analysed using Pearson's correlation coefficient. $p < 0.05$ was considered statistically significant.

RESULTS

Table 1 shows the gender and age distribution of the subjects. The mean age is comparable between the groups. Majority of the subjects with normal LDL levels were females and those with elevated LDL were males.

Table1: General characteristics of the subjects

Parameters	LDL >100 (n = 60)	LDL < 100 (n = 30)	p-value
Age (Years)	45.56 ± 16.3	50 ± 15	0.25
Sex (Male / Female)	28/32	9/21	0.19

Results are given as Mean ± SD, Student's independent t-test n= number of subjects in each group, * $p < 0.05$ – significant

The mean values of LDL and HDL of the subjects is presented in Table 2. The decrease in HDL levels observed in subjects with elevated LDL is statistically significant. A significant decrease in the basal PON-1 and vitamin D values are observed in subjects with elevated levels of LDL (Table 3). However there was no significant change in the salt stimulated paraoxonase (SPON) activity in the two groups.

Table 2: Levels of lipid parameters

Parameter	LDL >100 (n=60)	LDL <100 (n = 30)	p-value
LDL (mg/dl)	137 ± 26	78 ± 16.6	-
HDL (mg/dl)	47 ± 14.60	61 ± 32	< 0.01*

Results are given as Mean ± SD, Student's independent t-test * $p < 0.05$ - Significant

Table 3: Levels of paraoxonase and vitamin D

Parameter	LDL >100 (n=60)	LDL < 100 (n = 30)	p-value
BPON	57 ± 25.70	89 ± 36.5	<0.001*
SPON	3.42 (2.41, 4.53)	4 (2.44, 8)	0.30
Vitamn-D ^s	14 (7.09,28.30)	29 (17.44,37.10)	< 0.01*

Results expressed as Mean±SD for Basal PON-1, Median with interquartile range for SPON-1, Student's independent t-test, # = Chi-square test, * $p < 0.05$ = Significant. \$- variables showed skewness.

Correlation of PON-1 with various study parameters are given in Table 4. Negative correlation of basal PON-1 with LDL is observed in both the groups. However it is significant only in the group with elevated LDL. Correlation of basal PON-1 with HDL shows a negative association only in subjects whose

LDL is within the normal range ($p < 0.01$). A positive association of basal PON-1 with Vitamin D is also observed in this group. Correlation of BPON with SPON shows significant positive association in both the groups. Fig. 1 depicts the percentage of subjects in the vitamin D deficient, insufficient and sufficient groups.

Table 4: Correlation of PON-1 with study parameters

Parameter	Correlation	LDL > 100 (n=60)	LDL < 100 (n = 30)
LDL	r	-0.51	0.30
	p	<0.001	0.2
HDL	r	0.10	- 0.60
	p	0.40	< 0.01*
Vitamin D	r	- 0.08	0.50
	p	0.52	0.03*
SPON	r	0.27	0.54
	p	0.03	0.01*

r: Correlation coefficient, p: p value, * $p < 0.05$ – significant

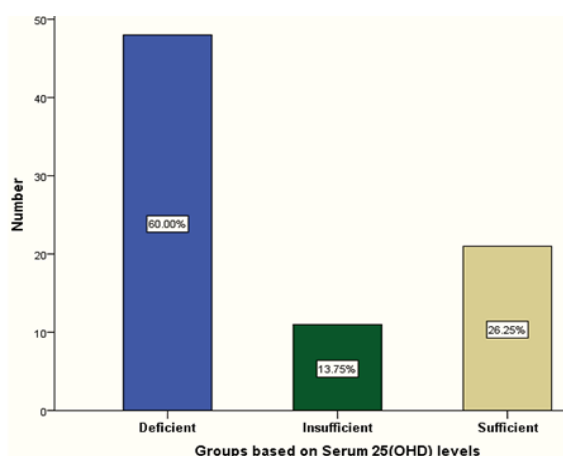


Fig 1: Vitamin D status in subjects

DISCUSSION

It is a well-known fact that dyslipidemia is one of the main causative factors that contribute to coronary artery disease. Various studies have firmly established the association of high plasma concentration of LDL and decreased levels of HDL with atherogenesis (15,16). The present study showed a decrease in HDL and basal PON-1 activity in subjects with elevated LDL levels. Significant reductions in the vitamin D levels were also observed.

Correlation of PON-1 with vitamin D showed a positive association in the control group. Negative association of BPON-1 with LDL seen in subjects with elevated LDL is an important finding. Likewise negative association of BPON-1 with HDL observed in the control group is of relevance.

The primary determinant of the antioxidant potential of HDL is considered to be PON-1. Boemi *et al.*, have

reported reduction in serum paraoxonase in type 1 diabetes individuals (17). Marie Claude *et al.*, (18) and Narayani *et al.*, have reported decreased PON-1 levels in patients with CAD (19). Studies have demonstrated that serum PON-1 is decreased in patient with cardiovascular diseases and other pathologies which increase the cardiovascular disease risk (20, 11). Decreased levels of PON-1 observed in subjects with elevated LDL are similar to the above studies. Kopparasch *et al.*, did not find any association between PON-1 and increased oxidation of LDL (21).

There exists a positive association between HDL and PON1 (22). One study conducted by Narayani *et al.*, suggested that there was no positive correlation (23). However in our study a negative correlation was seen, in subjects with normal LDL values which suggest that there are several other factors that modulate the relationship with HDL and the enzyme.

In a study conducted by Rosenblat *et al.*, the authors suggest that PON-1 can augment the capacity of HDL to remove cholesterol from macrophages(24). This could be justified by the finding of a positive correlation of HDL and PON-1 only in the group with LDL cholesterol within the normal range.

A study done by Martyn *et al.*, attests that serum vitamin D is autonomously associated with HDL and the metabolic syndrome in both genders, suggesting the role of different factors in the pathogenesis of metabolic syndrome (25).

Activity of PON-1 may be affected by acquired risk factors that include a change in diet, life style and other metabolic diseases. Negative association of BPON-1 with LDL suggests the potential risk of CVD in subjects with elevated LDL.

CONCLUSION

The present study proved that PON1 activity, HDL cholesterol and vitamin D are lower in subjects with elevated LDL. A negative correlation of paraoxonase with LDL is seen in subjects with elevated LDL. Low levels of PON-1 and vitamin D enhances the propensity to develop atherosclerosis due to dyslipidaemia. Future studies which lead to the enhancement of the PON-1 activity by modification of diet and specific medications may throw more insights on its anti-oxidant role with respect to lowering of LDL. Optimal level of vitamin-D in the body via PON-1 activity may also play a role in decreasing LDL, which may be suggestive of the importance of vitamin D in prevention of atherosclerosis.

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CONFLICT OF INTEREST

There is no conflict of interest.

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