

How to Cite:

Varadhan, S., Venkatachalam, R., Ramasamy, J., & Ayyamkulamkara, S. S. (2022). Oxidized low density lipoprotein and paraoxonase activity in coronary artery disease. *International Journal of Health Sciences*, 6(S1), 3405–3413. <https://doi.org/10.53730/ijhs.v6nS1.5493>

Oxidized Low Density Lipoprotein and Paraoxonase Activity in Coronary Artery Disease

Sowmya Varadhan

Research Scholar, Department of Biochemistry, Bharath Institute Of Higher Education and Research, Selayur, Chennai, Tamil Nadu -600073, India

Ramesh Venkatachalam

Associate Professor, Department of Biochemistry, Sreelekshmi Narayana Institute Of Medical Science, Bharath Institute of Higher Education and Research, Chennai, Tamilnadu-600073, India

Jawahar Ramasamy

Professor and HOD, Department of Pathology, Aarupadaiveedu Medical College Hospital, Vinayaka Mission's Research Foundation (Deemed to be University), Kirumampakkam, Puducherry- 607402, India

Supriya Simon Ayyamkulamkara

Biochemist, Department of Biochemistry, Government Medical College, Trissur, Kerala-680596, India

Abstract---Background: High-density lipoprotein (HDL) has a reverse relation with incidence of coronary artery disease (CAD). Paraoxonase (PON) is an HDL associated enzyme responsible for antioxidant capacity of HDL. Oxidative modification of LDL results in formation of oxLDL. This can be reduced by Paraoxonase, which has anti-inflammatory and antioxidant function. Aim: To evaluate and compare the level of HDL, oxLDL and Paraoxonase (PON) activity in Coronary Artery Disease patients with controls. Effort was also made to correlate HDL with PON, also to correlate OxLDL with PON and HDL. Materials and Method: 60 clinically proven CAD patients within the age group of 35 to 70 were selected from OPD of Dr. Somervell Memorial CSI Medical College, Kerala. 60 controls within the same age group without any history of CAD was selected from subjects coming for Health Checkup. Results: HDL and PON were significantly lowered in CAD patients than in controls ($P < 0.05$). OxLDL, Total Cholesterol, Triglyceride, LDL and VLDL were significantly higher in CAD patients than in controls ($P < 0.05$). Correlation Analysis showed a positive correlation of HDL and PON. Statistically significant negative

correlation was observed in case of oxLDL with PON and HDL. Conclusion: Increased oxLDL and decreased PON, forms additional risk factors together with the conventional ones for development and progression of CAD. Hence these two parameters can be targeted for future therapeutic purposes. Methods can be introduced to increase paraoxonase activity, thereby increasing antioxidant capacity of HDL, which might help in reducing formation of oxLDL.

Keywords---Coronary Artery Disease, oxidized Low Density Lipoprotein, Paraoxonase, High Density Lipoprotein.

Introduction

Among the major lipoprotein classess, High-density lipoprotein (HDL) has an inverse relation with coronary artery disease (CAD) (1). HDL without any doubt is an anti-atherogenic molecule. Due to this action, together with its role in reverse cholesterol transport, HDL is referred to as good cholesterol. Many studies have considered reducing CAD risk by increasing the HDL quantitatively, but the expected clinical outcome in terms of atheroprotection was not attained (2). Lipid and protein constituents associated with functional HDL molecule have been extensively studied in the past, which throws more light into the antioxidant and anti-inflammatory roles of HDL particle. (3). This explains the fact that together with level of HDL, its functions or properties also needs to be studied extensively to reduce the impendent cardiac risk.

Paraoxonase (PON1) is an HDL associated enzyme assumed to be accountable for majority of the antioxidant capacity of HDL. The antioxidant property of PON was explained by the inverse correlation between PON activity and coronary heart disease and also by the increased levels of lipid peroxidation products (4).

Extensive oxidative modification of LDL results in formation of oxLDL, if the lipid peroxidation is not kept under check by the antioxidant mechanisms available in the body. Formation of oxLDL can be reduced or even inhibited by Paraoxonase enzyme, which has an anti-inflammatory and antioxidant function. This HDL associated enzyme has the capacity to hydrolyze substrates like lactones, aryl esters, organophosphates etc (5). Predictive role of PON in CAD has been analyzed and it is seen that PON contributes considerably to the materialization of dysfunctional HDL. PON is considered to be significantly associated with multi-vessel disease and is a suitable therapeutic target for preventing CAD (6).

Previous Studies have shown that oxLDL is a risk factor for CAD and the level of ox-LDL, ratio of oxLDL to Total cholesterol and other lipoproteins are better markers than individual lipoproteins to discriminate CAD from control subjects (7).The parameters discussed so far are having an opposing effect on posing a risk for CAD. Oxidised low density lipoprotein (oxLDL) promotes occurrence of CAD, whereas high density lipoprotein or the associated Paraoxonase is atheroprotective, mainly their role in removing oxidised lipids from oxLDL (8).

On the basis of these findings, decrease in activity of PON can be further studied as a causative factor for advancement of Coronary Artery Disease. Although there are studies that show the ability of PON in preventing atheroma, much more reports are required for the pharmacological and therapeutic effects of this HDL associated enzyme. More light can be thrown into the protective effect of Paraoxonase by studying in detail its role in preventing lipid peroxidation and formation of oxLDL. The specific objective of the present study was to assess and compare Paraoxonase and oxLDL level in CAD patients and controls. An attempt was also made to correlate HDL with PON and also to correlate OxLDL with PON and HDL.

Materials and Methods

Study Centre: Dr. SMCSI Medical College, Kerala.

Sample size: With 90 % power and 5 %, significance, assuming a difference of 100 U/ml of PON (standard deviation 150) sample size required was calculated to be 49 per group.

Study population: 60 CAD patients were selected from those attending Cardiology OPD in our institute. 60 age and sex matched subjects without CAD was taken as controls from subjects attending Medical OPD.

Blood samples were collected from patients after getting written informed consent as per the criteria laid down by the institute scientific and ethics committee. Venipuncture was done to collect sample from patients when admitted to CCU. Serum samples were separated by centrifugation at 3000 rpm for 10 minutes and were stored at -80 °C till they were taken for analysis.

Detailed demographic and other relevant information were recorded using Proforma. 4 ml of peripheral blood was collected from each patient and the following parameters were analyzed - Total Cholesterol (TC), High Density Lipoprotein Cholesterol (HDL), Low Density Lipoprotein Cholesterol (LDL), Triglyceride (TG), oxidized LDL (oxLDL), Paraoxonase (PON).

PON activity was estimated spectrophotometrically, using the method of Beltowski et al 1985. Plasma Lipid Profile was estimated in fully automated chemistry analyzers using commercial assay kits. VLDL value was calculated using Friedwalds equation.

Statistical analysis

Data represented as mean $\pm$ SD in normal distribution variables. t test used to test difference in baseline characteristic between two groups. Pearson Correlation Coefficient was calculated to evaluate correlation among parameters. *P* value of <0.05 was taken as statistically significant. SPSS software used for statistical analysis.

Results

The study was planned to assess the extent of oxidation in CAD patients by determining the level of oxLDL. Level of oxLDL was correlated to activity of HDL

associated Paraoxonase. Oxidation product, oxLDL, anti-atherogenic PON, and serum lipid profile were estimated in CAD patients. These values were compared with that of age and sex matched controls. The test and control group comprised of 60 subjects. Baseline demographic characteristics of patients and controls, with risk factors of CAD are shown in Table 1.

Table 1: Baseline demographics and risk factors of CAD and control group

		CAD (n=60)	Control (n=60)
Age		39-70 (54.5)	34-65 (49.5)
Gender	M	69 %	33 %
	F	31 %	27 %
Body Mass Index (BMI)		29.41 $\pm$ 3.44	24.03 $\pm$ 3.16
Waist Circumference (WC)		101.5 $\pm$ 11.05	78.5 $\pm$ 12.5
Diabetes Mellitus		65 %	19 %
Family History of CAD		21 %	11 %
Smokers		79 %	24 %
Hypertension		64 %	18 %
Dyslipidemia		65 %	14 %

Biochemical parameters

Lipid profile in Coronary Artery Disease patients and controls were compared. Mean value of TC, HDL, LDL, TG and VLDL of these two groups are given in table 2. TC, TG, LDL, VLDL showed significant increase in CAD patients compared to that of controls .HDL value was significantly high in controls.

Table 2: Lipid profile in CAD and control

PARAMETERS	CAD	Control	P-Value
Total Cholesterol (mg/dl)	223.99 $\pm$ 60.46	171.37 $\pm$ 30.33	.04
Triglycerides (mg/dl)	163.84 $\pm$ 60.89	114.14 $\pm$ 54.45	.000
High Density Lipoprotein (mg/dl)	44.95 $\pm$ 12.25	57.88 $\pm$ 10.64	.000
Low Density Lipoprotein (mg/dl)	151.93 $\pm$ 41.48	133.33 $\pm$ 25.70	.000
Very Low Density Lipoprotein (mg/dl)	32.76 $\pm$ 12.17	22.82 $\pm$ 10.89	.000

Results are expressed in mean $\pm$ SD

Oxidized LDL and antioxidant enzyme PON1 was evaluated and compared in Coronary Artery Disease patients and controls. The mean value of oxLDL and PON1 in CAD patients and control subject were compared and given in table 3. oxLDL of CAD patients were significantly high compared to control subjects. PON1 was significantly lowered in patients group as compared to controls.

Table 3: Level of oxLDL and PON1 in CAD and control

Parameters(U/ml)	CAD	Control	P-Value
PON1	315.17 ± 80.05	410.05 ± 86.53	.000
oxLDL	40.94 ± 49.32	20.75 ± 19.05	.000

Results expressed as mean ± SD

Correlation analysis was done between HDL and PON given in Table 4. A statistically significant positive correlation was observed between HDL and PON. Correlation analysis was done between HDL and oxLDL given in Table 5. Negative correlation with statistical significance was observed between oxLDL and HDL. Correlation analysis was done between oxLDL and PON given in table 5. OxLDL and PON also exhibited a statistically significant negative correlation.

Table 4. Pearson correlation analysis of HDL with PON

			PON
HDL	CAD	Pearson Correlation	0.316
		Sig. (2-tailed)	0.006
	CONTROL	Pearson Correlation	0.091
		Sig. (2-tailed)	0.322

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

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

Table 5. Pearson correlation analysis of oxLDL with HDL and Pearson correlation analysis of oxLDL with PON

			HDL	PON
OxLDL	CAD	Pearson Correlation	-0.21	-0.309
		Sig. (2-tailed)	0.05	0.006
	CONTROL	Pearson Correlation	0.168	0.018
		Sig. (2-tailed)	0.196	0.463

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

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

Discussion

There are many cellular and molecular processes involved in development and progression of CAD in patients. If studied in depth these may serve as future therapeutic targets. It is undisputed that older patients are at a greater risk for adverse events and complications from nearly all pharmacological and procedural therapies (9).

Males and females reporting for suspected angina exhibits noticeable variation in severity of CAD. Females probably have less disease severity than age matched males, particularly if they do not present with myocardial infarction (10). In this study, waist circumference and BMI was increased in CAD patients. Findings of study conducted by Janssen et al revealed that BMI and WC can be used to predict CAD risk factors among children and adolescents. (11)

If we look at the risk factors included in our study, it shows that diabetes, family history of CAD, Smoking, Hypertension all are more in patients with CAD. Based on a study in 2017, the risk of developing Coronary artery disease increases with age in individuals, mostly age more than 45 years in men and more than 55 years in women. This study shows that family history of early heart disease is risk factor for CAD (12). Study by Hajar suggests that cardiac complications of diabetes makes it an important risk factor in the causation of Coronary Heart Disease. They also suggests that Diabetes is high risk for cardiovascular disease development. Cholesterol-lowering therapy and hypoglycemic drugs should be used in diabetic patients for lowering this risk (13). Study by Nowbar et al has shown that there is a high prevalence of hypertension and smoking in a few countries, which correlates with increased mortality rate from Ischemic Heart Disease (14).

It is an already established fact that Coronary artery Disease exhibits an abnormal lipid profile. In our study we observed similar findings. CAD group showed increased TC, LDL-C, TG and VLDL. HDL fraction in this group were significantly lower. Study by Rao et al shows that Total cholesterol, LDL, Triglycerides was high in cases than controls. In this study also HDL cholesterol was significantly lower in cases than controls (15). Study by Borzyszkowska et al also shows high total cholesterol, high LDL cholesterol and low HDL cholesterol (16) and these have been marked as risk factors for development of CAD.

Our study showed statistically significant reduction in paraoxonase activity. Similar findings were obtained by other studies also. Study by Zhou et al suggests that PON has a significant role in arresting atherogenesis. Reduced PON activity go together with increased inflammatory status and is strongly linked to development of atherosclerosis. This decreased enzyme activity can therefore be an essential inflammatory marker for severity of CAD. Efforts to boost PON activity may prove to be an assuring approach to reduce CAD risk and for averting enhancement of cardiovascular atherosclerosis. Study by Shen et al shows that decreased HDL-associated PON activities are associated with the presence and severity of stable CAD in patients (17, 18).

We assessed the level of oxLDL in CAD and control group. Our results showed increased amount of oxLDL in CAD as compared to controls. Study by Khalili et al suggests that assessing the level of oxLDL is important in establishing Coronary Heart Disease. They also obtained a significant correlation between severity of CAD and oxLDL level (19). It is known fact that PON is an HDL allied enzyme. In CAD patients we had observed a significant decrease in HDL and associated PON. Correlation analysis of PON against HDL showed a positive correlation, suggesting that as functional HDL decreases, PON activity also decreases.

According to Singh et al suggests that the low level of HDL and PON together with their correlation in CAD group may explain the process by which CAD develops. If we take HDL as a dependent variable, PON becomes the parameter responsible for change of HDL in CAD (20). Correlation analysis was done between oxLDL and PON enzyme activity. As per our study it was seen that as PON activity decreases oxLDL value increased. Similar result was obtained in case of Hackenhaar et al who showed that PON activity and HDL levels are decreased. They propose that decreased PON and increased oxLDL can forecast CAD (21).

Conclusion

HDL quantity is seen to be low in Coronary Artery Disease. Activity of Paraoxonase, an HDL related enzyme with antioxidant property is also found to be low in CAD patients. Enhanced lipid peroxidation increases oxLDL level in CAD patients. Lack of shielding effect of HDL associated PON enzyme on lipid peroxidation may be a causative factor in oxLDL surge and hastening of CAD. Reduced HDL and PON together with rise in oxLDL and their significant correlation in CAD may explain the rationale of occurrence of this disease. Available medical proof indicates that the lowering of PON activity is associated with increase of inflammatory status and is interrelated with advancement of atherosclerosis. Results of our study suggests that lower PON activity and increased oxLDL can used as an inflammatory markers for CAD. Together with the established therapeutic target HDL, attention should also be given to enhancing the activity of PON, which can help in reducing cardiovascular risk.

Acknowledgments: The authors would like to express their sincere gratitude to all staff of Department of Biochemistry and Cardiology, Dr. Somervell Memorial CSI Medical College, Trivandrum, Kerala in any capacity for the support in the publication of this manuscript.

Conflict of interests: Authors have declared that no conflict of interests exist.

Consent: All authors declare that written consent to participate in the study was obtained from the participants before the start of the study.

Ethical approval: Ethical approval was obtained from the institute where research was carried out, SMCSIMCH/EC(PHARM)02/02/12.

Funding: This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Authors' contributions: This work was carried out in collaboration among all authors.

1. SV: Acquisition of Data, Conception and design.
2. RV: Methodology, Supervision, Final Approval
3. JR: Software, Validation, Reviewing

4. SSA: Analysis and interpretation of data, and Editing.

Reference

1. Pérez-Méndez Ó, Pacheco HG, Martínez-Sánchez C, Franco M. HDL-cholesterol in coronary artery disease risk: function or structure?. *Clinica Chimica Acta*. 2014 Feb 15;429:111-22.
2. Ali KM, Wonnerth A, Huber K, Wojta J. Cardiovascular disease risk reduction by raising HDL cholesterol—current therapies and future opportunities. *British journal of pharmacology*. 2012 Nov;167(6):1177-94.
3. Arora S, Patra SK, Saini R. HDL—a molecule with a multi-faceted role in coronary artery disease. *Clinica chimica acta*. 2016 Jan 15;452:66-81.
4. Soran H, Schofield JD, Durrington PN. Antioxidant properties of HDL. *Frontiers in pharmacology*. 2015 Oct 16;6:222.
5. Razavi AE, Ani M, Pourfarzam M, Naderi GA. Associations between high density lipoprotein mean particle size and serum paraoxonase-1 activity. *Journal of research in medical sciences: the official journal of Isfahan University of Medical Sciences*. 2012 Nov;17(11):1020.
6. Variji A, Shokri Y, Fallahpour S, Zargari M, Bagheri B, Abediankenari S, Alizadeh A, Mahrooz A. The combined utility of myeloperoxidase (MPO) and paraoxonase 1 (PON1) as two important HDL-associated enzymes in coronary artery disease: Which has a stronger predictive role?. *Atherosclerosis*. 2019 Jan 1;280:7-13.
7. Huang H, Mai W, Liu D, Hao Y, Tao J, Dong Y. The oxidation ratio of LDL: a predictor for coronary artery disease. *Disease markers*. 2008 Jan 1;24(6):341-9.
8. Rasmiena AA, Barlow CK, Ng TW, Tull D, Meikle PJ. High density lipoprotein efficiently accepts surface but not internal oxidised lipids from oxidised low density lipoprotein. *Biochimica et Biophysica Acta (BBA)-Molecular and Cell Biology of Lipids*. 2016 Feb 1;1861(2):69-77.
9. Madhavan MV, Gersh BJ, Alexander KP, Granger CB, Stone GW. Coronary artery disease in patients ≥ 80 years of age. *Journal of the American college of Cardiology*. 2018 May 8;71(18):2015-40.
10. Chiha J, Mitchell P, Gopinath B, Plant AJ, Kovoov P, Thiagalingam A. Gender differences in the severity and extent of coronary artery disease. *IJC Heart & Vasculature*. 2015 Sep 1;8:161-6. <https://doi.org/10.1016/j.ijcha.2015.07.009>.
11. Janssen I, Katzmarzyk PT, Srinivasan SR, Chen W, Malina RM, Bouchard C, Berenson GS. Combined influence of body mass index and waist circumference on coronary artery disease risk factors among children and adolescents. *Pediatrics*. 2005 Jun 1;115(6):1623-30.
12. Hajar R. Risk factors for coronary artery disease: historical perspectives. *Heart views: the official journal of the Gulf Heart Association*. 2017 Jul; 18(3):109.
13. Hajar R. Diabetes as “coronary artery disease risk equivalent”: A historical perspective. *Heart views: the official journal of the Gulf Heart Association*. 2017 Jan;18(1):34.
14. Nowbar AN, Gitto M, Howard JP, Francis DP, Al-Lamee R. Mortality from ischemic heart disease: Analysis of data from the World Health Organization

- and coronary artery disease risk factors From NCD Risk Factor Collaboration. *Circulation: Cardiovascular Quality and Outcomes*. 2019 Jun;12(6):e005375.
15. Rao V, Kiran R. Evaluation of correlation between oxidative stress and abnormal lipid profile in coronary artery disease. *Journal of cardiovascular disease research*. 2011 Jan 1;2(1):57-60.
 16. Borzyszkowska J, Stanislawska-Sachadyn A, Wirtwein M, Sobiczewski W, Ciecwierz D, Targonski R, Gruchala M, Rynkiewicz A, Limon J. Angiotensin converting enzyme gene polymorphism is associated with severity of coronary artery disease in men with high total cholesterol levels. *Journal of applied genetics*. 2012 May;53(2):175-82.
 17. Zhou C, Cao J, Shang L, Tong C, Hu H, Wang H, Fan D, Yu H. Reduced paraoxonase 1 activity as a marker for severe coronary artery disease. *Disease markers*. 2013 Jul 28;35(2):97-103.
 18. Shen Y, Ding FH, Sun JT, Pu LJ, Zhang RY, Zhang Q, Chen QJ, Shen WF, Lu L. Association of elevated apoA-I glycation and reduced HDL-associated paraoxonase1, 3 activity, and their interaction with angiographic severity of coronary artery disease in patients with type 2 diabetes mellitus. *Cardiovascular diabetology*. 2015 Dec;14(1):1-0.
 19. Khalili A, Ghorbanihaghjo A, Rashtchizadeh N, Gaffari S. Association between serum ferritin and circulating oxidized low-density lipoprotein levels in patients with coronary artery disease. *Journal of cardiovascular and thoracic research*. 2012;4(1):1.
 20. Singh K, Singh R, Chandra S, Tyagi S. Paraoxonase-1 is a better indicator than HDL of atherosclerosis-A pilot study in North Indian population. *Diabetes & Metabolic Syndrome: Clinical Research & Reviews*. 2018 May 1;12(3):275-8.
 21. Hackenhaar FS, Martinez D, Medeiros TM, Klein C, Alabarse PV, Wainstein MV, Goncalves SC, Benfato MS. Oxidized-LDL and paraoxonase-1 as biomarkers of coronary artery disease in patients with sleep-disordered breathing. *Current medicinal chemistry*. 2012 Sep 1;19(25):4359-66.