Original Article

A Comparative Analysis of the Serum Paraoxonase (PON1) Activity and the Concentration of Parameters of Lipid Profile after 3 Months of Statin Therapy

Sharmistha Chatterjee[1], Biswajit Majumder[2], Prajwal Kumar Sinha[3], Indranil Chakraborty[4]

[1]Associate Professor, Department of Biochemistry, College of Medicine and Sagore Dutta Hospital Kamarhati, Kolkata, [2]Professor and Head, Department of Cardiology, R.G.Kar Medical College, Kolkata, [3]Senior resident, Cardiology, R.G.Kar Medical College, [4]Professor and Head, Department of Biochemistry, College of Medicine and Sagore Dutta Hospital, Kamarhati, Kolkata

How to cite this article: Sharmistha Chatterjee, Biswajit Majumder, Prajwal Kumar Sinha et.al. A Comparative Analysis of the Serum Paraoxonase (PON1) Activity and the Concentration of Parameters of Lipid Profile after 3 Months of Statin Therapy. Indian Journal of Public Health Research and Development/Volume 15 No. 2, April - June 2024.

Abstract

Background: Human serum paraoxonase (PON1) residing on HDL can prevent the oxidation of low density lipoprotein (LDL), the initiating factor in atherosclerosis. Statins are commonly used to treat dyslipidemia, aknown risk factor for coronary artery disease (CAD).The aim of the studyis to evaluate the alterations in the concentrationof PON1 along with that of other parameters of lipid profile in patients of CAD before and after 3 months of statin therapy.

Materials and Methods: The study included 30 new patients who were put on statin therapy following the diagnosis of acute coronary syndrome. The activity of PON1 (units-IU/L) and the concentration of lipid profile parameters (units-mg/dl) were estimated before starting statin therapy and again after three months. Patients with co-morbidities like diabetes, kidney disease, liver disease and other cardiac diseases of infectious aetiology were excluded.

Results and Analysis: As expected, both PON1 and HDL have increased after 3 months. There was a statistically significant increase in both PON1 ($p<0.05$)and HDL ($p<0.001$) and a decrease ($p <0.05$, also statistically significant) in LDL after 3 months of statin therapy.

Conclusion: This knowledge may be exploited in the follow up CAD patients. The increase in PONI and the similar increase in HDL after 3 months of statin therapy may be exploited in the follow-up of cardiac patients.

Keywords- Paraoxonase I, High density lipoprotein, statin therapy, cardiac patients, follow-up.

Introduction

Human serum paraoxonase (PON1) produced by the liver and almost exclusively residing on high density lipoproteins (HDL) has been demonstrated to prevent the oxidation of low density lipoprotein(LDL), which is the central initiating factor in the causation of atherosclerosis.[1,2] In fact, PON1is responsible

Corresponding Author: Sharmistha Chatterjee, Associate Professor, Department of Biochemistry, College of Medicine and Sagore Dutta Hospital Kamarhati, Kolkata.

E-mail: sharmisthacmajumder@yahoo.co.in

Phone: 9830477927

Submission date: July 20, 2023, **Revision date:** Aug 18, 2023, **Published date: Apr 4 2024**

for the antiatherogenic property of HDL and has been also been shown to prevent accumulation of lipid peroxides on LDL in vivo and in vitro.[3] Thus, PON1 alongwith HDL plays an important role in the pathophysiology of atherosclerosis and consequently coronary artery disease. Several studies have demonstrated a higher risk of cardiovascular mortality in patients with lower levels of PON1. [4] Dyslipidemia (high TG, high TC, and low HDL) is a known risk factor for coronary artery disease(CAD) and predisposes to the microvascular and macrovascular complications of CAD. Several studies have tried to correlate the activity of PON1 with HDL and other atherogenic indices.

Statins have been the cornerstone of management of dyslipidemia to prevent cardiovascular complications for quite some time now. Studies have shown the concentration of LDL decreased and that of HDL increased following administration of statins. Thus statins are known to have a favourable effect on the lipid profile.Thus,PON1 may be used to monitor the trends in dyslipidemia and cardiovascular complications during the management and follow up of these patients.[5.6.7] In this study, we have tried to evaluate to changes in the PON1 concentration along with the concentrationsof HDL and LDL in patients of CAD before and after 3 months of statin therapy in patients of CHD.

Aims and Objectives

The aim of the study is to evaluate the alterations in the activity of PON1 and the other parameters of the lipid profile, namely the HDL and LDL before and after three months of statin therapy in patients of CAD. The purpose is to explore whether PON1 can be used as a comparable marker for assessment of the atherosclerotic risk in individuals with that of LDL and HDL in the follow up of patients of CAD.

Materials and methods

The study was performed in the Biochemistry department in the College of Medicine and Sagore Dutta Hospital in association with the Cardiology department of R.G.Kar Medical College.30new patients who were put on statin therapy following acute coronary syndrome in the cardiology outdoor at the R.G.Kar Medical College were included in the study after taking proper informed consent from them. Patients with co-morbidities like diabetes, chronic kidney disease, chronic liver disease and other cardiac diseases of infectious etiology were excluded from the study. Before starting statin therapy, fasting venous blood samples were drawn from the selected patients in clot vials and analysed for PON1 and the parameters of lipid profile in the clinical laboratory in the Department of Biochemistry at College of Medicine and Sagore Dutta Hospital.

The activity of serum PON 1 was measured by a manual method using a spectrophotometer T60 in the research lab of the Department of Biochemistry. Blood samples (4ml) were drawn from the subjects prior to starting the statin therapy. The blood was allowed to clot, centrifuged and the serum was separated and analysed in the same day.Since calcium ions are required for the stability and activity of the enzyme, and the enzyme is inhibited by EDTA, the blood samples were drawn in plain clot vials,not in EDTA vials.[4]Sodium citrate cannot be used as an anticoagulant as it also chelates the calcium.[5] The activity of PON1 was evaluated by the help of its arylesterase activity when paranitrophenylacetate was used as a substrate.5.5mM of 4-nitrophenylacetate was used as a substrate in 20mM Tris –HCl buffer at a pH of 8.0.The increase in absorbance due to the formation of the yellow coloured 4-nitrophenol was measured by the spectrophotometer at 412 nm for 3mins.PON1 was taken as 1 U/L when the rate of formation of the 4-nitrophenol(substrate)was 1 micro mol /min under the given assay conditions.

After three months of the statin therapy, blood samples were again drawn from the subjects and once again analysed for PON1 and LDL and HDL in a similar manner.

To the best of the author's knowledge, there exists no available control serum for PON1 analysis. Frozen aliquots of pooled samples were used as controls. A single aliquot was analysed ten times to obtain the CV %.A retained aliquot was run on the next day to test the adequacy of the storage conditions. The serum samples were stored at -80 °C for further reference.

Results and Analysis

The data thus generated was tabulated in Excel sheets. The mean, median and standard deviation

of the PON1,LDL and HDL, both before and after starting statin therapy, were calculated and the distribution determined.

Table 1 showing the descriptive statistics of PON1, HDL and LDL.

	PON1(IU/L)		HDL(mg/dl)		LDL(mg/dl)	
	Before therapy	**After therapy**	**Before therapy**	**After therapy**	**Before therapy**	**After therapy**
Mean	36.69	50.358	39.36	44.7	85.4	72.3
Median	29.39	40.655	35.25	43.5	80.3	68
Standard deviation	31.76	38.36	4.91	4.30	17.79	14.5
P --value	<0.05		<0.001		<0.05	
Distribution	Non-normal	Non-normal	Non-normal	Non-normal	Non-normal	Non-normal

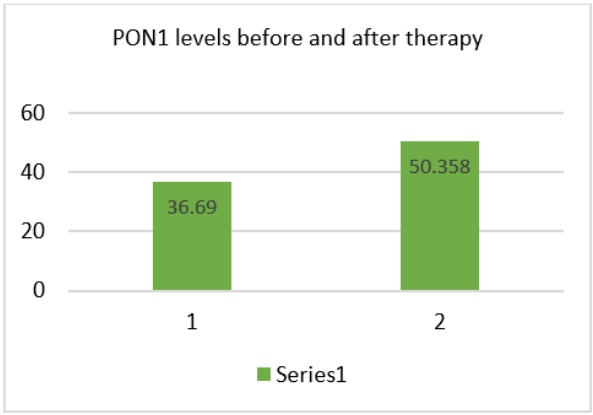

Figure 1: To show the levels of Paraoxonase I before and after 3 months of statin therapy.

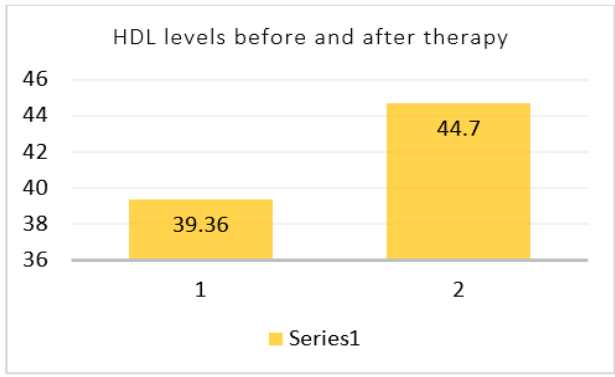

Figure 2: To show the levels of HDL before and after 3 months of statin therapy.

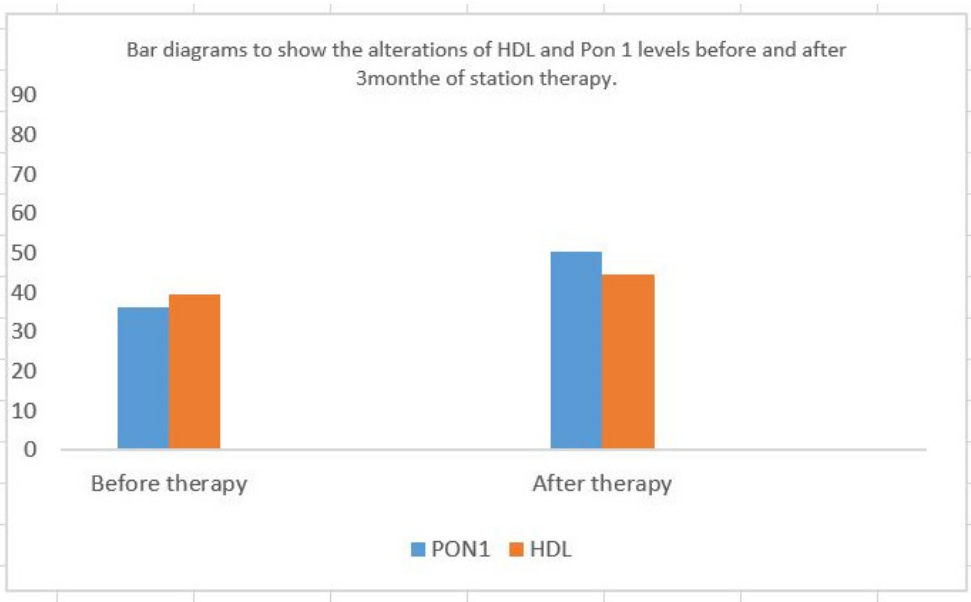

Figure 3: To compare the alterations of HDL and PON1 levels before and after 3 months of statin therapy.

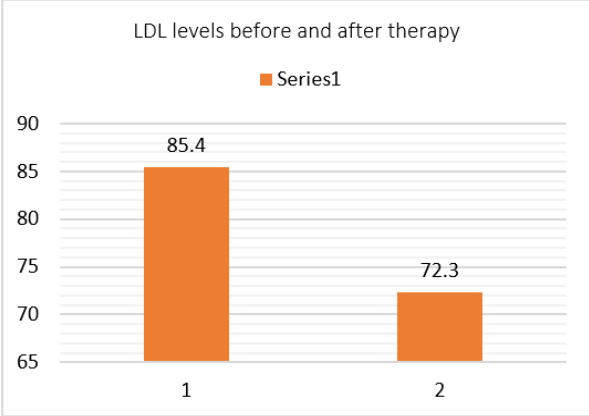

Figure 4: To show the alterations in the levels of LDL before and after 3 months of statin therapy.

Discussion

A quick glance through the above tables and figures throws up some interesting results.The mean value ofPON1 before statin therapy was 36.69 IU/l and after 3 months of statin therapy was found to be 50.35IU/L , an increases which is statistically significant (P<0.05) . On the other hand , the mean values of HDL increased from 35.25mg/dl in statin naïve patients to 44.7 mg/dl in those receiving statins for 3 months .This increase in HDL was also found to be statistically significant. (P<0.001). The mean value of LDL, which is the major incriminating factor for CAD decreased from 85.4 to 72.3 after 3 months of administration of statins. This decrease was also statistically significant (P<0.05).

PON1,nowclassifiedasanaryldialkylphosphatase (EC 3.1.8.1) by the Enzyme Commission of the International Union of Biochemistry and Molecular Biology is a Ca2+-dependent lactonase associated mostly with the small dense HDL3 subfraction.[8-10].A glycoprotein of approximately 354 amino acids, PON1 retains its hydrophobic signal sequence at the amino terminal (barring the methionine) to help it in its attachment with HDL.Of the three members of the paraoxonase (PON) multigene family, PON1 and PON2 are limited to HDL in their distribution , but PON3 is also found in other cells. But, all the three members, PON1,PON2 and PON3 are bestowed with the ability to prevent oxidation of LDL, so they all possess anti-atherogenic property.[11-14].

The antiatherosclerotic mechanisms of PON1 has been explained in several ways. PON1 was the first enzyme shown to prevent /retard LDL oxidation in vitro by Mackness.[15]The enzyme was shown to reduce both the oxidative stress of macrophages as well as their ability to oxidise LDL. The underlying mechanism is the hydrolysis of truncated fatty acids fromphospholipid, cholesteryl ester, and triglycerides whichactually produce substrates which can be naturally degraded by PON1.[16,17]The LCAT enzyme associated with HDL which is mainly responsible for the reverse cholesterol transport, is susceptible to oxidative stress.PON1 has been shown to prevent this oxidative inactivation of LCAT thus prolonging the duration for which that HDL can prevent LDL oxidation.[18]Other mechanisms include prevention of LDL glycation[18], normalization of endothelial function[20] and disposal of the apoptotic toxic products.[21,22] In fact, low PON1 concentrations were associated with major cardiovascular events (MACE; i.e., death, MI, stroke) in both primary and secondary prevention cohorts.[23,24]

It follows from the above discussion that PON1 activity is atheroprotective. Our subjects, when enrolled into our study presented with symptoms of CAD had low levels of PON1 alongwith dyslipidemia (i.e HDL was low and LDL quite high). These patients were prescribed statins and the serum PON1 and HDL and LDL were again estimated after 3 months of statin therapy. The results as presented above show that there was a statistically significant increase in HDL concentrations and a statistically significant decrease in LDL after 3 months. The alterations in lipid profile are predictable and supported by other studies. But, the serum PON 1 concentration also showed a statistically significant increase after three months paralleling the increase in HDL. This encouraging trend in PON1 may be explored as to its utility in being used as a marker in the follow up of patients of CAD. But our study was limited by a small sample size (only 40 samples—this being a pilot study.). The fact that there is a lacuna in our knowledge regarding the regulatory pathways of PON1 in vivo is also a major deterrent to the exploration of our hypothesis.

Limitations

There were a few limitations in our study. First and foremost, the sample size was very small, but this was intentionally maintained since the method

of estimation of PON1 was entirely manual and resources were poor. Secondly, to the best of author's knowledge no reference range of PON1 was available .This makes it very difficult to objectively compare the results across similar studies. It is therefore suggested that larger population studies be planned to determine the reference range of PON1.

Conclusion

It may be concluded from the above study that the activity of PON1 may be used to indicate the changes in the lipid profile in the follow up of patients of CAD being treated with statins. It is however necessary to conduct larger, well designed studies to explore whether it may be used as a marker better than HDL in the follow up of these patients and whether it is practically feasible in clinical practice.

Ethical clearance: Taken from The Institutional Ethics Committee at R.G.Kar Medical College

Source of funding: None

Conflict of Interest: nil

References

1. Parthasarathy S, Barnett J, Fong LG. High-density lipoprotein inhibits the oxidative modification of low-density lipoprotein. **Biochim Biophys Acta**. . 1990; *1044*: 275–283.
2. Steinberg D, Parthasarathy S, Carew TE, Khoo JC, Witztum JL. Beyond cholesterol modifications of low-density lipoprotein that increase its atherogenicity. **N Engl J Med**. . 1989; *320*: 915–924.
3. Wang F, Liu Y, Cong Y, Li P, Li Y, Yan Z, et al. Shift of the interconnection from the reaction system of paraoxonase 1 to the peroxidation reaction system of myeloperoxidase with HDL-C levels: A marker of atherosclerosis in patients with normal cholesterol levels. Clin Chim Acta.2015; 438: 370-5.
4. Ikeda Y, Suehiro T, Itahara T, Inui Y, Chikazawa H, Inoue M, et al. Human serum paraoxonase concentration predicts cardiovascular mortality in hemodialysis patients. Clin Nephrol 2007;67:358–65.
5. M.J. Chapman, Are the effects of statins on HDL-cholesterol clinically relevant?, *European Heart Journal Supplements*, Volume 6, Issue suppl_C, 1 July 2004, Pages C58–C63, https://doi.org/10.1016/j.ehjsup.2004.04.002
6. Stroes E. Statins and LDL-cholesterol lowering: an overview. Curr Med Res Opin. 2005;21 Suppl 6:S9-16. doi: 10.1185/030079905X59102. PMID: 16138936.
7. Feingold KR. Cholesterol Lowering Drugs. [Updated 2021 Mar 30]. In: Feingold KR, Anawalt B, Boyce A, et al., editors. Endotext [Internet]. South Dartmouth (MA): MDText.com, Inc.; 2000-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK395573/
8. Mackness B, Durrington PN, Mackness MI. Human serum paraoxonase. Gen Pharmac 1998;31:329–36.
9. Davidson WS, Silva RAGD, Chantepie S, Lagor WR, Chapman MJ, Kontush A. Proteomic analysis of defined HDL subpopulations reveals particle-specific protein clusters—Relevance to antioxidative function. Arteriioscler Thromb Vasc Biol 2009;29:870–6.
10. Rajkovic MG, Rumora L, Barisic K. The paraoxonase 1, 2 and 3 in humans. Biochemia Medica 2011;21:122–30.
11. Rodriguez-Sanabria F, Rull A, Beltran-Debon R, Aragones G, Camps J, Mackness B, et al. Tissue distribution and expression of paraoxonases and chemokines in the mouse: the ubiquitous and joint localisation suggest a systemic and coordinated role. J Mol Histol 2010;41:379–86.
12. Aviram M, Rosenblat M. Paraoxonases 1, 2, and 3, oxidative stress, and macrophage foam cell formation during atherosclerosis development. Free Radic Biol Med 2004;37:1304–16.
13. Reddy ST, Devarajan A, Bourquard N, Shih D, Fogelman AM. Is it just paraoxonase 1 or are other members of the paraoxonase gene family implicated in atherosclerosis? Curr Opin Lipidol 2008;19:405–8.
14. Deakin SP, James RW. Genetic and environmental factors modulating serum concentrations and activities of the antioxidant enzyme paraoxonase-1. Clin Sci 2004;107:435–47
15. Mackness MI, Arrol S, Durrington PN. Paraoxonase prevents accumulation of lipoperoxides in low-density lipoprotein. FEBS Letts 1991;286:152–4.
16. Mackness MI, Arrol S, Abbott CA, Durrington PN. Protection of low-density lipoprotein against oxidative modification by high-density lipoprotein associated paraoxonase. Atherosclerosis 1993;104:129–35.
17. Tavori H, Aviram M, Khatib S, Musa R, Mannheim D, Karmeli R, et al. Paraoxonase 1 protects macrophages from atherogenicity of a specific triglyceride isolated from human carotid lesion. Free Rad Biol Med 2011;51:234–42.
18. Hine D, Mackness B, Mackness M. Co-incubation of PON1, APO A1 and LCAT increases the time

HDL is able to prevent LDL oxidation. IUBMB Life 2012;64:157– 61.

19. Mackness B, Hine D, Liu Y, Mastorikou M, Mackness M. Paraoxonase 1 inhibits oxidised LDL-induced MCP-1 production by endothelial cells. BBRC 2004;318:680–3.
20. Mackness B, Quarck R, Verreth W, Mackness M, Holvoet P. Human paraoxonase-1 overexpression inhibits atherosclerosis in a mouse model of metabolic syndrome. Arterioscler Thromb Vasc Biol 2006;26:1545–50.
21. Jakubowski H. Homocysteine thiolactone: metabolic origin and protein homocysteinylation in humans. J Nutr 2000;130:377S–81S.
22. Jakubowski H. Calcium-dependent human serum homocysteine thiolactone hydrolase—a protective mechanism against protein s-homocysteinylation. J Biol Chem 2000;275:3957–62.
23. Tang WHW, Hartiala J, Fan Y, Wu Y, Stewart AFR, Erdmann J, et al. Clinical and genetic association of serum paraoxonase and arylesterase activities with cardiovascular risk. Arterioscler Thromb Vasc Biol 2012;32:2803–12.
24. Jarvik GP, Rozek LS, Brophy VH, Hatsukami TS, Richter RJ, Schellenberg GD. Furlong, C.E. Paraoxonase (PON1) phenotype is a better predictor of vascular disease than is PON1192 or PON155 genotype. Arterioscl Thromb Vasc Biol 2000;20:2441–7.