Article published online: 2023-04-18

Effect of Statins on the Inflammatory Markers in Patients with Coronary Artery Disease

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J Lab Physicians 2023;15:498–502.

Abstract

Introduction Atherosclerosis mediated by inflammatory markers is the corner stone in the pathology of coronary artery disease (CAD). Hyperlipidemia, one of the risk factors is treated with statins. Statins also have a pleotropic role in reducing inflammation. Effect of statins on two inflammatory markers pentraxin 3(PTX 3) and high sensitivity C-reactive protein (hs-CRP) is explored in this study.

Objective This article estimates the levels of serum PTX 3 and hs-CRP in CAD patients with and without statin therapy and correlates the levels with low-density lipoprotein cholesterol (LDL-c) and high-density lipoprotein cholesterol (HDL-c) in CAD patients without statin therapy.

Material and Methods This was a cross-sectional study conducted on 62 patients with CAD diagnosed by coronary angiogram. They were divided into two groups. Group I were the CAD patients on statin therapy and group II were CAD patients who never had any lipid lowering drugs irrespective of their lipid values. Serum PTX3, hs-CRP, and lipid profile were estimated in these groups. Comparison between the groups was done using Student's *t*-test and correlation analyzed using Pearson's correlation.

Results Serum PTX 3 and hs-CRP levels were higher than the reference range in both the groups. But group I showed significantly low PTX 3 levels (p-value = 0.032) compared with group II. There was a significant positive relationship between PTX 3 and LDL-c ($p = 0.003$) in group II.

Conclusion CAD patients on statin therapy have lower vessel wall inflammation compared with patients without statin therapy.

Keywords
- C-reactive protein
- coronary artery disease
- pentraxin 3
- statins

Introduction

Coronary artery disease (CAD) is one of the notable causes of death worldwide. According to the Global Burden of Disease Study 2019 nearly 9.14 million people died due to ischemic heart disease which accounts to 1.76% of world population.[1] India currently has the highest burden of acute coronary syndrome and ST-segment elevation myocardial infarction

received
December 1, 2022
accepted after revision
February 6, 2023
article published online
April 18, 2023

DOI https://doi.org/10.1055/s-0043-1768167.
ISSN 0974-2727.

Thieme Medical and Scientific Publishers Pvt. Ltd., A-12, 2nd Floor, Sector 2, Noida-201301 UP, India

in the world.[2] The Global Burden of Disease Study estimate of death rate for cardiovascular disease (CVD) in India is higher than the global average. Ischemic heart disease (IHD) and stroke constitute the majority of CVD mortality in India (83%), with IHD being predominant.[3]

Atherosclerosis being the primary pathology of CAD, is a chronic inflammation characterized by lipid accumulation and clot formation triggered by multiple risk factors. Many circulating inflammatory markers of both systemic and vascular origin like interleukins-1 (IL-1), IL-6, and IL-10, adhesion molecules such as intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule, serum amyloid A, C-reactive protein (CRP), fibrinogen, and pentraxin 3 (PTX 3) play different roles right from initiation of fatty streak, progression, and clot rupture.[4,5] CRP is a well-known acute phase reactant of the pentraxin super family and is secreted from the liver in response to inflammation. It is well known that modestly elevated levels of CRP above the baseline are associated with future risk of CVDs. Even though CRP is a nonspecific marker, it is robustly used in many risk prediction algorithms.[4,6] PTX 3 also belonging to the pentraxin super family is a vascular inflammatory marker secreted from the clot itself.[7] Epidemiological studies have proved that it is a valid biomarker for atherosclerosis and evidences suggest that it is a sensitive and specific marker for CAD compared with CRP.[8,9]

Hyperlipidemia is one of the traditional risk factors for CAD and treating it with hydroxymethylglutaryl-coenzyme A reductase inhibitors or statins has proven to reduce the future risk and regression of atherosclerotic plaques.[10]

Apart from the lipid lowering role of statins, they also have a pleiotropic role by modulating above-mentioned inflammation in vessel wall.[10] Long-term statin therapy significantly reduces the CRP levels[11,12] But studies on the effect of the statin on the more specific vascular marker PTX 3 are limited. So the effect of statins on two inflammatory markers PTX 3 and high sensitivity CRP (hs-CRP) and its correlation with the lipid profile parameters is explored in this study.

Objective

1. To compare the levels of PTX 3 and hs-CRP levels in CAD patients with and without statin therapy.
2. To correlate the PTX 3 and CRP levels with low-density lipoprotein cholesterol (LDL-c) and high-density lipoprotein cholesterol (HDL-c) levels in CAD patients without statin therapy.

Materials and Methods

Study Setting

This study was conducted in a tertiary care hospital in Pondicherry which caters around 500 patients per day. The study was conducted in the department of biochemistry and cardiology during 2016 to 2017. The laboratory is fully automated and the department of cardiology has a dedicated cardiac catheterization laboratory and state of art critical care unit for managing CAD patients. Approximately 50 patients with CAD are managed every day in the department of cardiology.

Ethical Considerations

Since minimum risk was involved in the study, clearance from the Institutional Ethical committee was obtained (Ref No RC/15/03). The study procedure and objectives were explained to all participants and informed consent was taken.

Study Design

This is an observational cross-sectional study.

Study Participants and Study Group

Patients of above the age of 25 years with evidence of CAD in coronary angiogram were taken up for the study. Furthermore, they were divided into two groups based on whether they are on statin therapy for hyperlipidemia or not. Group I consists of patients on statin therapy for hyperlipidemia. Group II consists of participants who never had any lipid lowering drugs irrespective of their lipid levels.

Inclusion Criteria

Adult, stable patients above 25 years with evidence of more than 50% blockade in one or more coronary arteries in coronary angiogram.

Exclusion Criteria

Patients with normal coronaries in coronary angiogram, less than 50% blockage in one or more artery, history of percutaneous transluminal coronary angioplasty done earlier, patients with chronic renal disease, liver diseases, chronic inflammatory disease, peripheral vascular disease, and known case of malignancy were all excluded from the study.

Sample Size Calculation and Sampling

Sample size for Student's *t*-test calculated using the formula $n = (t_{n\text{-}1,\ \alpha/2} + t_{n\text{-}1,\ \beta})^2/d^2$, where d is the mean standard deviation, α is the probability of detecting false effect, and $\beta = 1$–power. Assuming a pooled standard deviation of 1.05 units the study would require a sample size of 26 for the test group and 34 for reference groups (ratio of two groups assuming to be 1.3) and total sample size 60 to achieve a power of 80% and a level of significance of 5%, for detecting a true difference in means.[12] Consecutive sampling was done to achieve the sample size.

Study Tools and Study Variables

Using a structured case report form demographic details, personal history, history of statin therapy, and duration were recorded from the participants. Study variables were serum PTX 3, hs-CRP, and lipid profile parameters like total cholesterol (TC), triglycerides (TGL), LDL-c, and HDL-c.

Study Procedure

Sixty-two adult patients with evidence of CAD on coronary angiogram were included after taking formal consent for the study. Based on the history of statin therapy they were divided into two groups. Thirty-six patients were included in group I (patients on statin therapy for hyperlipidemia) and 26 patients were included in group II (patients who never had any lipid lowering drug irrespective of their lipid profile).

Biochemical Measurements

Note that 3 mL of venous blood was collected, serum was separated. A part of the serum was subjected to TC, TGL, LDL-c, and HDL-c assay and other part of the serum was stored for PTX 3 and hs-CRP assay. Lipid profile parameters were estimated using colorimetric enzymatic assay using COBAS Integra 400 plus automated analyzer by Roche Diagnostics.

Serum PTX 3 assay was done using standard sandwich enzyme-linked immunosorbent assay (ELISA). Human PTX 3 96-well ELISA kit, from Life Span BioSciences, was used for this purpose. Seven standards with serial dilutions for standard curve and a negative control were used. The kit had a detection range of 0.313 to 20 ng/mL and sensitivity typically less than 0.313 ng/mL. Average serum levels of PTX 3 in healthy individuals were reported as 2.28 ± 1.33 ng/mL.[13]

Estimation of CRP was done using latex-enhanced hs-CRP immunoturbidimetry assay. The principle of the assay is that the CRP reacts with specific antibody producing immune complexes. The turbidity caused by these insoluble immune complexes is proportional to the CRP concentration which is measured spectrophotometrically. The hs-CRP test accurately detects lower levels (range 0.5–10 mg/L) compared with the conventional CRP (range 10–1000 mg/L). It is the hs-CRP which is used to evaluate individuals for risk of CVD whereas the conventional CRP is used to detect systemic inflammation or infection. The reference range for hs-CRP is less than 0.3 mg/dL for normal healthy individuals.[14]

Statistical Analysis

Data was entered in MS Excel 2007 and analyzed by Statistical Package for Social Sciences for Windows (version 20.0, IBM Corporation, Armonk, New York, United States). The quantitative data are expressed as mean ± standard error of the mean. Student's *t*-test was employed to compare the mean of the biochemical parameters between the two groups. The correlation of the lipid profile parameters especially LDL-c and HDL-c with PTX 3 and hs-CRP was done using Pearson's correlation coefficient. *p*-Values < 0.05 was considered statistically significant.

Table 2 Comparison of the biochemical parameters between two groups

Parameters	Group I ($n = 36$)	Group II ($n = 26$)	*p*-Value
TC (mg/dL)	191 ± 3.03	248 ± 4.12	0.0001
TGL (mg/dL)	155.40 ± 7.12	172.60 ± 7.68	0.1107
LDL-c (mg/dL)	120.50 ± 3.9	160.38 ± 4.17	0.0013
HDL-c (mg/dL)	49.20 ± 1.88	35.50 ± 3.54	0.0004
PTX 3 (ng/mL)	4.06 ± 0.13	6.25 ± 1.10	0.032
hs-CRP(mg/L)	6.32 ± 0.52	6.54 ± 0.83	0.8144

Abbreviations: HDL-c, high-density lipoprotein cholesterol; hs-CRP, high sensitivity C-reactive protein; LDL-c, low-density lipoprotein cholesterol; PTX 3, pentraxin 3; TC, total cholesterol; TGL, triglycerides.
Note: All parameters expressed as mean ± standard error of the mean. Group I include coronary artery disease (CAD) patients on statins and group II includes CAD patients without any lipid lowering drugs.

Results

With 62 participants with CAD recruited, 36 patients belonged to group I (patients on statin therapy for hyperlipidemia) and 26 patients for group II (patient who never had any lipid lowering drugs irrespective of their lipid profile).

►**Table 1** shows the baseline characteristics of the two groups. There was a significant difference in the mean age between the two groups (*p*-value 0.032). The proportion of males was higher in both the groups and the groups maintained the same gender proportions. There was a significant difference in the prevalence of diabetes in both the groups (*p*-value 0.029). No significant difference in the prevalence of other risk factors like smoking and hypertension or body mass index was noted.

►**Table 2** shows the mean lipid profile parameters, PTX 3, and hs-CRP levels between the groups. In group I nearly 33.33% had hyperlipidemia (TC greater than 200 mg/dL) despite statin therapy, whereas in group II nearly 56.2% had hyperlipidemia. All the lipid profile parameters were high and statistically significant in group II. The mean serum PTX 3 in group II showed statistically higher levels

Table 1 Baseline characteristics of the groups

Variables	Group I	Group II	*p*-Value
n	36	26	
Age in years (mean ± SE)	64.77 ± 1.34	53.30 ± 8.9	0.032
Males	25 (69%)	18 (69%)	
Females	11 (31%)	8 (31%)	
BMI (mean ± SE)	30.35 ± 0.87	32.26 ± 2.1	0.449
Participants with history of smoking	5 (14%)	7 (27%)	0.199
Participants with history of diabetes	13 (36%)	3 (11%)	0.029
Participants with history of hypertension	12 (33%)	4 (15%)	0.118
Mean years of statin therapy in years	4.5	NA	

Abbreviations: BMI, body mass index; SE, standard error.
Note: Group I include coronary artery disease (CAD) patients on statins and group II includes CAD patients without any lipid lowering drugs.

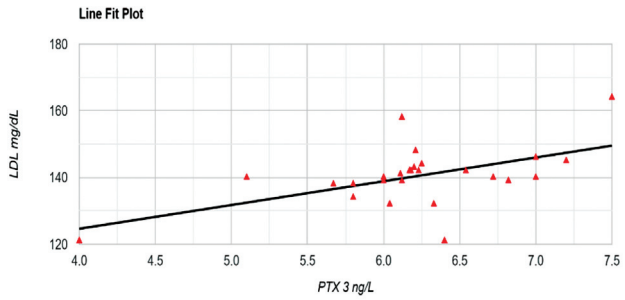

Fig. 1 Correlation between pentraxin 3 (PTX 3) and low-density lipoprotein cholesterol (LDL-c) in group II.

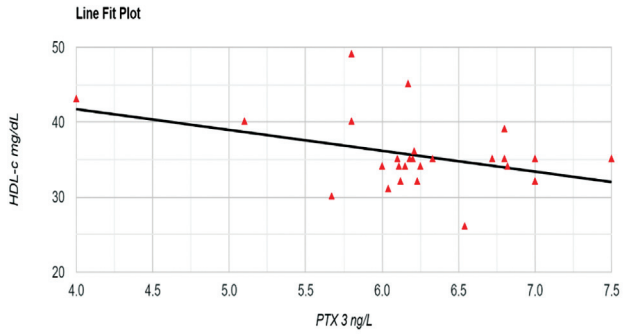

Fig. 2 Correlation between pentraxin 3 (PTX 3) and high-density lipoprotein cholesterol (HDL-c) in group II.

(6.25 ± 1.10 ng/mL) compared with group I (4.06 ± 0.13 ng/mL). There was no much difference noted in the hs-CRP levels between the groups. Both the groups showed high PTX 3 and hs-CRP levels above the reference range.

►**Fig. 1** shows the Pearson's correlation between PTX 3 and LDL-c. A strong positive correlation ($r = 0.662$, p-value 0.002) was obtained. ►**Fig. 2** shows moderate negative correlation between PTX 3 and HDL-c ($r = -0.312$, p-value 0.04). Similarly, insignificant minimal positive correlation between hs-CRP and LDL-c ($r = 0.281$ and p-value 0.256) and no significant correlation between hs-CRP and HDL-c ($r = -0.224$, $p = 0.061$) were observed.

Discussion

The two groups though had a common feature of CAD but they were different in many aspects. Foremost, the mean age of group I patients who were on statins was significantly higher compared with group II who never had any lipid lowering drugs. This indicates that patients not on any treatment for hyperlipidemia endure adverse events earlier. In many widely conducted studies about statins it was proved that statins not only reduce the risk but also postpone any adverse events.[15] Relatively high prevalence of diabetes was also noted in group I which indicates that this group inadvertently had high risk factors than group II.

In this study, we found that both PTX 3 and hs-CRP levels are above the reference range for healthy individual. Both CRP and PTX 3 are inflammatory proteins which belong to the pentraxin superfamily. PTX 3 is specifically produced by the smooth muscles, endothelial cells, and fibroblast of the atheroma but not in the liver. Rolph et al first found PTX 3 in atherosclerotic lesions through immunohistochemistry thus concluding plaques produce long-chain PTX 3 molecules.[16] Many studies have proved that PTX 3 levels are high in CAD compared with normal controls.[17,18] The high hs-CRP above the reference range is consistent with any adverse event which is CAD here. This could be the reason for lesser difference of hs-CRP levels in both groups.

The most important finding of this study is that significantly low PTX 3 in patients on statins compared with other group who were not on any lipid lowering drugs and no much difference in the hs-CRP levels. This is attributed to the anti-inflammatory action of statin which was found in many studies.[10,15] Many studies have proved that there is a significant reduction in CRP levels on taking statins in many other conditions like obesity, diabetes, etc.[19,20] Similar results were obtained in studies done with PTX 3 also.[21,22] But our study has not shown any significant difference in the hs-CRP levels because the cohort had a recent adverse event, such as acute coronary syndrome.

Though the exact mechanism on how the statins reduce the levels of inflammation is still unclear, it has been speculated that it inhibits the action of ICAM-1, IL-6, and IL-8. It also reduces the expression of integrins and actin polymerization in vasculature.[10]

PTX 3 levels correlated well with LDL-c levels and found a minimal negative correlation with HDL-c. There are studies which explain that PTX 3 has both cardioprotective and tissue damaging characteristics. In the Dong-gu study there was a positive correlation of PTX 3 with HDL and other CVD risk factors explaining a cardioprotective role.[23] In another study done by Bosutti et al there was an increased expression of PTX 3 gene in adipose tissues in individuals with high LDL levels.[24] Thus, there is a incertitude on whether statins or LDL has an effect on the PTX 3 levels which needs to be explored.

With all the above-mentioned evidence it is proved that statins definitely have a role in reducing vessel wall inflammation. So it would be beneficial to check the PTX 3 and hs-CRP levels in all high-risk patients and if at all if these markers are above the reference prophylactic statins may reduce the state of inflammation and future risk of CVD. Large-scale studies might be required to check the level of inflammatory markers in individuals who are at risk for CVD and the effect of statins on their levels.

The only limitation of the study was the sample size due to which many subgroup comparisons like markers levels with different types of statins and dosage were not feasible. This could also be one of the reasons for small correlation. Moreover, the study group consisted of only CAD patients and comparison of the same with controls also should have been done.

Conclusion

The main observation in this study is that statin therapy reduces the levels of inflammatory markers especially PTX 3 which is a more specific marker compared with hs-CRP levels

and the levels of PTX 3 is lower in patients with low LDL-c. Studies are required to find whether statins can be used to reduce vessel wall inflammation in high-risk patients with normal lipid levels and it also opens doors for the management of many inflammatory diseases too.

Conflict of Interest
None declared.

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