Original Article

Association of Serum Pentraxin 3 and High-Sensitivity C-Reactive Protein with Severity of Coronary Stenosis

Sujatha Mahadevarao Premnath, Sunil Kumar Nanda, Lopamudra Ray[1], Mark Christopher Arokiaraj[2], Kandasamy Ravichandran[3]

Departments of Biochemistry, [2]Cardiology and [3]Biostatistics, Pondicherry Institute of Medical Science, Pondicherry University, Puducherry, [1]Department of Biochemistry, Shri Balaji Institute of Medical Sciences, Raipur, Chhattisgarh, India

Abstract

Background: Atherosclerosis being the keystone in the pathology of coronary artery disease (CAD) is a chronic inflammation of arterial intima mediated by various inflammatory markers. Pentraxin 3 (PTX3) and high-sensitivity C-reactive protein (hs-CRP) are the two important biomarkers of chronic inflammation that causes atherosclerosis. **Aims:** This study aims to investigate the association of serum PTX3 and hs-CRP with the severity of coronary stenosis in patients undergoing coronary angiogram. **Subjects and Methods:** A total of 80 patients who underwent elective coronary angiogram were included. Their blood sample was collected for PTX3 and hs-CRP estimation prior to angiogram. Based on the angiogram, the participants were divided into four groups based on the number of arteries affected. PTX3 was estimated using enzyme-linked immunosorbent assay and hs-CRP was assayed using latex-enhanced immunosorbent assay. **Statistical Analysis Used:** Kruskal–Wallis test was used to find the association of PTX3 and hs-CRP in each group and Pearson's correlation was used to correlate PTX3 and hs-CRP with the extent of stenosis. **Results:** The mean PTX3 and hs-CRP levels in patients with some lesions in the coronary artery were 231.5 ± 129.9 pg/mL and 2.4 ± 0.4 mg/mL, respectively. The PTX3 levels elevate gradually with the severity of stenosis with $P = 0.000$ which is highly significant. A strong positive correlation was observed ($R = 0.7929$, $P < 0.00001$) with PTX3 and severity of stenosis. Whereas, for hs-CRP, the correlation was weaker ($R = 0.3011$, $P = 0.006$). **Conclusions:** PTX3 and hs-CRP can not only predict the number of arteries affected but also can differentiate between normal coronaries and CAD which can minimize the use of angiography.

Keywords: *Atherosclerosis, coronary stenosis, high-sensitivity C-reactive protein, Pentraxin 3*

Introduction

Cardiovascular disease (CVD) remains the paramount cause of death worldwide and has contributed to nearly 32% of the total deaths globally.[1] The prevalence of cases has doubled from 271 million in 1990 to 523 million in 2019.[2] India has been ranked second with the highest number of cases and contributes to one in every five deaths due to CVD.[3] This created an exigency in health sector for an effective and early diagnosis to reduce morbidity and mortality. Although there are many conventional risk prediction algorithms, 20% of the individuals with CVD have no risk factors and nearly 40% of the individuals have only one risk factor.[4] Cardiac-specific bioassay diagnoses the disease only after the insult and accurate biomarkers are still lacking to adumbrate any event during ongoing pathogenesis.[5]

Atherosclerosis being the keystone in the pathology of coronary artery disease (CAD) is a chronic inflammation of arterial intima mediated by various cytokines right from onset, progression, plaque formation, thrombus dislodgement, and calcification.[6-8] Many recent works have focused on how these inflammatory biomarkers can help in the noninvasive diagnosis of atherosclerotic disease in patients who are at risk and to prognosticate the disease.[9-11]

C-reactive protein (CRP) is a well-known acute phase reactant of the pentraxin family and is secreted from the liver in response to inflammation. It is well known that CRP is a nonspecific marker widely used in many assessing the risk for future CVD.[11,12] High levels of CRP are associated with high risk for future CVD.[13] However, its role in predicting the severity of atherosclerosis is not much explored.

Pentraxin 3 (PTX 3) is a recently discovered inflammatory marker belonging

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Address for correspondence:
Dr. Sujatha Mahadevarao Premnath,
C5 Staff Quarters, PIMS Campus, Ganapathychettykulam, Kalapet - 605 014, Puducherry, India.
E-mail: drsuj85@gmail.com

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to the pentraxin superfamily, similar to CRP. PTX 3 is synthesized from the inflammatory cell of the plaque.[11,14,15] Hence, it is considered a specific and propitious marker for assessing the extent of inflammation in coronary arteries.[16]

In the present study, we aim to investigate the association of serum PTX3 and CRP with the severity of coronary stenosis in patients undergoing coronary angiogram. Expecting PTX3 and CRP levels may correlate with the extent of blockade in coronary arteries, and this could be a promising biomarker in predicting the disease severity before any invasive procedure.

Subjects and Methods

The present study was an observational cross-sectional study, conducted by the department of biochemistry and cardiology in a tertiary care hospital. Sample size was calculated by iterative procedure using the formula for analysis of variance $N = \lambda/f^2$: λ is noncentrality parameter and f is effect size using Cohen's f (0.4498) calculated from $f = \sigma_k/\sigma$, where $\sigma_k = \sqrt{\sum_{i=1}^{k} (\mu_i - \mu)^2 / k}$. Assuming type I error 0.05, power $(1 - \beta)$ is 80. Considering the larger difference between mean of the groups μ as 50.04 and expected standard deviation (SD) σ_k as 55.63, the required sample size was 76 for 4 groups together. Consecutive sampling was the sampling technique used.

A total of 80 participants who underwent coronary angiogram for the diagnosis of CAD were included. Inclusion criteria: patients of age group 30–50 years posted for elective coronary angiography either to find the extent of lesion or for therapeutic angioplasty were included. Exclusion criteria: patients who were known cases of valvular heart disease, acute or chronic kidney disease, peripheral vascular disease, and stroke were excluded.

Since this was a cross-sectional study, single venous blood samples were collected from the participants for PTX3 and high-sensitivity CRP (hs-CRP) assay prior to angiogram. PTX3 was estimated using sandwich enzyme-linked immunosorbent assay and hs-CRP was estimated using latex-enhanced immunoturbidimetry. There are two different tests to measure CRP levels, and each test measures a different range of CRP in the blood for different purposes. The standard CRP test measures markedly high levels of the protein to detect diseases that cause significant inflammation. It measures CRP in the range from 10 to 1000 mg/L. The hs-CRP test accurately detects lower levels of the protein than the standard CRP test. It measures CRP in the range from 0.5 to 10 mg/L. This test is used to evaluate individuals for risk of CVD which, in turn, detects low-lying inflammation.

Few study participants who had elevated cardiac markers such as troponin and creatinine kinase and with typical electrocardiogram (ECG) finding underwent diagnostic coronary angiogram to find the extent of blockage with angioplasty. Some of the study participants were posted for diagnostic angiogram for atypical chest pain, ECG findings, and strong risk factors. Based on the angiogram, the participants were divided into four groups: normal coronaries (NCs), single-vessel disease (SVD) with stenosis >50% in single coronary artery, double-vessel disease (DVD) with stenosis >50% in two of the coronary arteries, and triple-vessel disease (TVD) with stenosis >50% in all the three main coronary arteries. The PTX3 and hs-CRP values which were estimated prior were compared between the four groups. The study was approved by the institutional research and ethics committee (Ref No. RC/15/03). Informed consent was taken from all the participants.

Statistics

Data were entered in MS Excel 2007 and analyzed by SPSS for Windows (version 20.0, Armonk, New York, USA, IBM Corporation). Descriptive statistics with numbers, mean, and SD were used for continuous variables. Chi-square test was employed for the comparison of categorical variables. Kruskal–Wallis test was used to find the association of PTX3 and CRP in each of the groups since they were nonnormally distributed. Pearson's correlation was used to correlate PTX3 and CRP with the extent of stenosis. $P < 0.05$ was considered significant and value <0.01 highly significant.

Results

The study sample had four groups, namely NC, SVD, DVD, and TVD with 20 participants in each group. Table 1 represents the baseline characteristics of the study participants. A total of 64 males and 16 females were studied. Each of the groups had higher number of males compared to females. The mean age and standard deviation of the males was 57.1 ± 9.5 years and for females 61.8 ± 10.8 years. There was a significant difference in age not only among the gender but also between the four groups, and a progressive increase in severity of the stenosis with age was noted which was expected.

Table 2 compares the mean and SD of PTX3 and hs-CRP levels between the four groups. Both the PTX3 and the hs-CRP values were higher in the patients with some lesions in the coronary stenosis compared with NCs. The mean PTX3 and hs-CRP levels in patients with NCs were

Table 1: Baseline characteristics of the study participants

Variables	NC	SVD	DVD	TVD	*P*
Age, mean±SD	53.3±8.9	58.3±9.8	58.6±10.2	62.1±9.0	
Males	13	16	19	15	0.040
Females	7	4	1	5	
Total	20	20	20	20	

SD: Standard deviation, NC: Normal coronary, SVD: Single-vessel disease, DVD: Double-vessel disease, TVD: Triple-vessel disease

Table 2: Comparison of mean and standard deviation pentraxin 3 and C-reactive protein levels in the four groups

Parameters (mean±SD)	NC	SVD	DVD	TVD	*P*
PTX3 (pg/mL)	91.9±30.7	119.4±31.2	201.4±79.1	373.9±113.9	0.000
hs-CRP (mg/L)	1.2±1.3	2.0±1.2	2.6±1.7	2.8±2.1	0.034

P<0.05 significant, <0.01 highly significant. SD: Standard deviation, NC: Normal coronary, SVD: Single-vessel disease, DVD: Double-vessel disease, TVD: Triple-vessel disease, PTX3: Pentraxin 3, hs-CRP: High-sensitivity C reactive protein

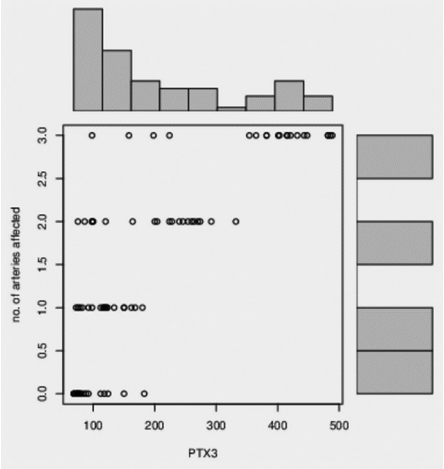

Figure 1: Correlation of PTX3 with severity of coronary stenosis. PTX3: Pentraxin 3

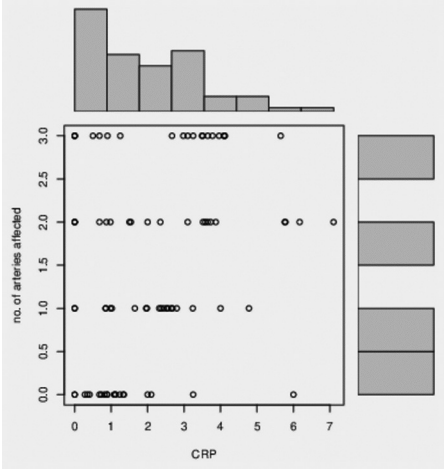

Figure 2: Correlation of CRP with severity of coronary stenosis. CRP: C-reactive protein

91.9 ± 30.7 pg/mL and 1.2 ± 1.3 mg/L, respectively. The mean PTX3 and hs-CRP levels in patients with some lesions in the coronary artery were 231.5 ± 129.9 pg/mL and 2.4 ± 0.4 mg/L, respectively. The PTX3 levels elevate gradually with the severity of stenosis with $P = 0.000$ which is highly significant. *Post hoc* test is also indicative of significant difference between all of the groups. Similarly, there is a significant difference in hs-CRP levels between the groups, $P = 0.034$. CRP values also showed an increase with increase in severity of coronary stenosis. A strong positive correlation was observed ($R = 0.7929$, $P < 0.00001$) with the serum levels of PTX3 and severity of stenosis shown in Figure 1. Whereas, for hs-CRP, although a positive correlation is obtained, it is weaker compared to PTX3 ($R = 0.3011$, $P = 0.006$) as shown in [Figure 2].

Discussion

It is well known that chronic inflammation mediated by various circulating and local inflammatory markers arbitrates the process of atherosclerosis in vessels. This study appraises the predictive potential of two of the inflammatory markers in finding the extent of the lesion in CVD.

CRP belonging to the pentraxin family is a nonspecific inflammatory marker synthesized in the liver as an acute phase reactant and also at the site of the inflammation. It is one of the significant inflammatory marker levels which increases during atherosclerosis.[12] High levels of hs-CRP is an independent risk factor for prediction of CVD.[17,18]

PTX3 belonging to the same family as CRP is specifically produced by the smooth muscles, endothelial cells, and fibroblast of the atheroma but not in the liver. Rolph *et al*. first found PTX3 in atherosclerotic lesions through immunohistochemistry, thus concluding that PTX3 is specific to the atherosclerotic plaques.[19] Many studies have proved that PTX3 is a valid and potential biomarker for CAD.[14,16,20]

In this study, we have found higher PTX3 and hs-CRP levels in patients with CAD compared to NC. There is a significant rise in the serum values of the same with the severity of the coronary stenosis. One of the significant findings in our study is that the more the number of vessels affected, the more is the inflammation resulting in raised serum levels.

Implications of CRP specifically on the pathogenesis of atherothrombosis and angiogenesis have been extensively studied. Many studies support that high levels of CRP correspond with the high intensity of atherosclerosis plaque build up in arteries. A study conducted by Habib and Al Masri confirmed that patients with high GENSINI score after angiogram showed high levels of hs-CRP.[21] Studies done over the world have found that hs-CRP levels increase with the severity of stenosis.[22,23] However, in a study done by Razban *et al*. in 102 subjects, they have found that no correlation exists between serum hs-CRP levels and the number of vessels.[24]

Results of many similar studies using PTX3 have been consistent with the same findings as our study. A study

done by Yahia *et al*. also showed a significant correlation between PTX3 levels and SYNTAX score which was used to access the severity of CAD.[25] Another study conducted by Liu *et al*. using GENSINI score to assess the extent of lesion in coronary arteries also showed similar outcomes.[26] In a similar study done in Serbia, the results showed an increasing trend of the PTX3 and hs-CRP levels with the increasing number of vessels affected in patients with CAD.[27]

In future, PTX3 can be used as a therapeutic target and levels can be used to evaluate the patient whether invasive angiographic procedure is needed or not. Further studies need to be done to identify the levels in stable angina and also the functionality of this marker in prognosis after any intervention.

Studies are needed to find out whether PTX3 could perform better when compared to CRP, since PTX3 is a more specific inflammatory protein pertaining to coronary atherosclerosis. Establishing a cutoff for both PTX3 and hs-CRP to differentiate between CAD and healthy individuals will be of immense help. Large studies on the level of PTX3 and hs-CRP after the medical or surgical management and its correlation with various cardiovascular risk factors have many future implications.

Limitation

The only limitation of the study was small sample size, and we included participants with only more than 50% lesions in each vessel. Similar studies with large sample size and in coronary plaques size less than 50% should be done. Levels of PTX3 and CRP after angioplasty or coronary artery bypass grafting need to be evaluated to analyze the specificity of the above markers.

Conclusions

The main observation in this study is that PTX3 and hs-CRP levels are higher in CVD and elevate significantly with the severity of the atherosclerotic lesion in the coronary arteries. Their levels can not only predict the number of arteries affected but also can differentiate between NCs and CAD. Thus, PTX3 should also be included in many risk prediction algorithms to evaluate the future risk for predicting CVD.

Ethical statement

The study involved minimal risk. Ethical clearance was given by the Institution Ethics Committee, Pondicherry Institute of Medical Sciences (Ref No RC/15/03). The study procedure and objectives was explained to the participants and informed consent was taken.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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