Int J Clin Exp Pathol 2016;9(2):2625-2630
www.ijcep.com /ISSN:1936-2625/IJCEP0018963

Original Article

High pentraxin 3 expression in aorta of patients with atherosclerosis and its correlation with other risk factors

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Received November 1, 2015; Accepted January 5, 2016; Epub February 1, 2016; Published February 15, 2016

Abstract: Objectives: Pentraxin 3 plays an important role in the process of atherosclerosis. We aimed to investigate the correlation between pentraxin 3 expression in the aorta of patients with coronary atherosclerosis and other risk factors for atherosclerosis. Patients and methods: Aortic wall tissues from 43 patients with atherosclerosis who underwent coronary artery bypass graft surgery were studied and compared with control renal arterial tissues obtained from 12 healthy kidney donors without atherosclerosis. We measured serum biochemical parameters and calculated Gensini scoring, and aortic pentraxin 3 expression levels were evaluated by immunohistochemistry in both groups. Results: Levels of aortic pentraxin 3 expression were significantly higher in the experimental group and were also elevated in diabetic patients and smokers. Aortic pentraxin 3 expression was positively correlated with serum pentraxin 3, high-sensitivity C-reactive protein, triglycerides, total cholesterol, low density lipoprotein cholesterol, and lipoprotein(a) levels ($P<0.05$) but negatively correlated with high density lipoprotein cholesterol levels ($P<0.05$). We also found that aortic pentraxin 3 expression correlated significantly with Gensini coronary disease severity scores ($P<0.01$). Conclusions: Pentraxin 3 expression is elevated in the aorta of patients with coronary atherosclerosis, which correlates with the severity of coronary artery disease and other risk factors for atherosclerosis.

Keywords: Atherosclerosis, pentraxin 3, risk factors, coronary artery bypass graft, inflammation

Introduction

The inflammatory hypothesis is one of the most important mechanisms behind the pathogenesis of atherosclerosis. Pentraxin-3 (PTX-3), a newly discovered reactive protein, involved in inflammatory responses, belongs to the family of high-sensitivity C-reactive proteins (hs-CRP).

PTX-3 is released mainly by vascular endothelial cells, smooth muscle cells and mononuclear macrophages in response to inflammatory factors such as interleukin 1 (IL-1) and tumor necrosis factor (TNF). PTX-3 levels reflect local cardiovascular inflammation more closely than serum hs-CRP, which is secreted by liver cells. Therefore the correlation between PTX-3 levels and the severity of coronary artery disease and plaque instability may be stronger than correlations with other inflammatory markers [1].

By studying patients with ST-segment elevation myocardial infarction, Latini et al. [2] identified high PTX-3 levels as a predictor of mortality risk for these patients, independent of hs-CRP and troponin T levels. Although previous studies validated that the use of serum PTX-3 levels as a risk stratification parameter for coronary heart diseases (CHD), the existence of a correlation between arterial PTX-3 expression levels in patients with atherosclerosis and severity of coronary artery diseases was not reported.

Here, we investigate the correlation between local aortic PTX-3 expression levels and the severity of coronary artery disease and other atherosclerotic risk factors using human aortic tissue samples obtained during aorta-coronary artery bypass graft (CABG) procedures in patients with atherosclerosis.

Materials and methods

Study population

Forty-three patients, 28 males and 15 females aged 63.2 ± 9.3, who underwent CABG surgery

at Qianfoshan Hospital between 2010 and 2013 were included in the experimental group. In the present study, indications for CABG surgery included (1) left main coronary disease; (2) unstable angina with medication failure; (3) disease of the three coronary vessels; (4) disease of two coronary vessels, with high-grade stenosis of proximal left anterior descending artery (LAD); and (5) failed percutaneous coronary intervention. The exclusion criteria were acute infection, acute state of a chronic inflammatory disease, stroke, pulmonary edema, acute or chronic liver or kidney disease and trauma. As control, renal arterial tissues without atherosclerotic lesions were obtained from 12 healthy kidney donors (7 males and 5 females aged 53.6 ± 5.82). All subjects signed an informed consent form before the test, and the study was approved by the ethics committee of Qianfoshan Hospital.

Serum biochemical measurements

Venous blood samples from the patients in experimental and control groups were obtained after an overnight fast. Serum PTX-3 levels were measured by enzyme-linked immunosorbent assay, and hs-CRP levels were measured by scattering turbidimetry. Triglyceride (TG) and total cholesterol (TC) levels were measured by terminal colorimetric analysis. High density lipoprotein cholesterol (HDL-C) and low density lipoprotein cholesterol (LDL-C) levels were assessed by a chemically modified enzyme method. Lipoprotein(a) (Lp(a)) levels were measured by immunoturbidimetry. All serum biochemical measurements were analyzed using the MODULAR Biochemical Analyzer (Roche Diagnostics Ltd, Basel, Switzerland).

Coronary arteries stenosis scoring

Patients in the experimental group were subjected to coronary angiography using the Judkins technique. Angiograms evaluations and quantitative assessment of atherosclerotic lesions were performed by experienced cardiologists. Gensini scoring defined 1% to 25% narrowing of a coronary artery as 1, 26% to 50% narrowing as 2, 51% to 75% narrowing as 4, 76% to 90% narrowing as 8, 91% to 99% narrowing as 16, and 100% occlusion as 32 [3]. Coefficients were calculated according to lesion site: 5 for the left main coronary artery, 2.5 for the LAD or proximal left circumflex artery (LCX), 1.5 for the mid-region of the LAD, and 1 for the distal LAD or mid-distal region of the LCX. The above scores were added up to obtain the final Gensini score for each patient.

Tissue collection, section preparation and immunohistochemical staining

Full-thickness aortic tissues were collected during CABG surgeries from patients included in the experimental group. Control renal arterial tissues were obtained from healthy kidney donors. All tissue samples were fixed in 10% formal in and embedded in paraffin. Then, tissue blocks were sectioned and 3 μm sections were mounted on glass slides. After baking, the slides were then placed in the BenchMark immunohistochemical staining system (Ventana, Tucson, AZ). After deparaffinization and antigen retrieval, a monoclonal mouse anti-PTX-3 primary antibody (Abcam, Cambridge, MA) was used at a dilution of 1:100 followed by incubation with a goat anti-mouse IgG secondary antibody. The slides were washed in PBS between staining steps throughout the process. Immunoreactive products were detected using a DAB chromogenic reagent kit. The sections were counterstained with hematoxylin (Ventana) and then rinsed and dehydrated before microscopic examination.

The Image-Pro plus 6.0 software was used to analyze specific PTX-3 staining digital images at high magnification. We randomly selected6 high-power magnification fields of view per section and then measured the mean optical density (OD) of positive PTX-3 signal from each field. An average OD value of the 6 fields was used as the PTX-3 expression level calculated for each section.

Statistical analysis

SPSS 18.0 was used for statistical analyses. All results were expressed as the mean ± SD. Student's *t*-test and the χ^2 test were used to analyze continuous normally distributed variables and categorical variables, respectively. Correlation analysis was performed with linear correlation. $P<0.05$ was considered statistically significant.

Results

Serum PTX-3, hs-CRP and lipid levels in the experimental group patients

Among the 43 experimental group patients who underwent CABG, 27 patients suffered from

Table 1. Serum PTX-3, hs-CRP and lipid levels in patients in the experimental group

	n	Mean ± SD	Reference values	*t*	*P*
PTX-3 (ug/L)	43	5.98 ± 0.67	2.70 ± 0.32	5.159	0.000
hs-CRP (mg/L)	43	10.40 ± 2.89	2.15 ± 2.10	7.259	0.000
TG (mmol/L)	43	2.43 ± 1.17	1.13 ± 0.29	4.745	0.004
TC (mmol/L)	43	5.25 ± 1.53	4.42 ± 0.79	2.126	0.031
LDL-C (mmol/L)	43	3.09 ± 1.15	2.59 ± 0.26	2.659	0.006
HDL-C (mmol/L)	43	1.15 ± 0.23	1.46 ± 0.24	-4.257	0.000
Lp(a) (mg/dl)	43	27.18 ± 15.38	14.40 ± 6.64	3.457	0.001

Abbreviations: HDL-C: high density lipoprotein cholesterol; hs-CRP: high-sensitivity C-reactive protein; LDL-C: low density lipoprotein cholesterol; Lp(a): lipoprotein(a); PTX-3: Pentraxin 3; TG: triglycerinate; TC: total cholesterol.

unstable angina and 12 had a history of myocardial infarction. In addition, 15 patients had a history of diabetes, and 19 had hypertension. Serum PTX-3, hs-CRP and lipid levels from the patients included in the experimental group are shown in **Table 1**. In comparison with reference values, serum PTX-3, hs-CRP, TC, TG, LDL-C, and Lp(a) values were elevated in these patients, whereas their serum HDL-C values were decreased.

Endogenous expression levels of PTX-3 in aortic tissue

As shown in **Figure 1**, PTX-3 protein expression was elevated dramatically in the aortic tissue samples from all 43 patients constituting the experimental group, with a mean PTX-3 expression level of 0.27 ± 0.07 OD units, ranging from 0.10 to 0.48 OD units. Positive PTX-3 expression by immunohistochemistry was detected as uneven diffuse dark brown products, which were granular, sliced, or clustered.

In the control group, the renal artery sections of the 12 kidney donors were normal, as determined by macroscopic observation and routine histopathological examination, and showed low immunohistological staining intensity for PTX-3 expression indicated by the presence of light blue and light brown products.

Relationship between aortic PTX-3 expression and coronary disease severity

As shown in **Figure 2**, aortic PTX-3 expression was highly correlated with Gensini coronary disease severity scores in the experimental group (r = 0.702, P<0.01), with a mean Gensini score of 78.12 ± 23.67.

Correlation of PTX-3 protein expression with clinical risk factors for atherosclerosis

We also analyzed the relationship between PTX-3 expression and clinical risk factors such as gender, smoking and diabetes. As shown in **Figure 3**, PTX-3 expression was significantly elevated in diabetic patients and smokers with values of 0.28 ± 0.07 vs. 0.21 ± 0.04 OD for diabetic vs. non-diabetics patients (P<0.05), and values of 0.27 ± 0.05 vs. 0.20 ± 0.04 OD for smokers vs. non-smokers (P< 0.05), respectively. However, no correlation between gender and PTX-3 expression was observed, with values of 0.24 ± 0.05 vs. 0.23 ± 0.08 OD for males vs. females respectively (P>0.05).

Positive correlation between aortic PTX-3 expression levels and serum PTX-3, hs-CRP and serum lipid levels

As shown in **Table 2**, the linear correlation analysis revealed that aortic PTX-3 expression was positively correlated with serum PTX-3 (r = 0.702, P<0.01), hs-CRP (r = 0.690, P<0.05), TG (r = 0.400, P<0.05), TC (r = 0.469, P<0.01), LDL-C (r = 0.435, P<0.01), and Lp (a) (r = 0.506, P<0.01). By contrast, aortic PTX-3 expression was negatively correlated with serum HDL-C levels (r = -0.453, P<0.05).

Discussion

Atherosclerosis is the result of multi-factor synergism [4], and the inflammation hypothesis is now recognized as one of the most important mechanisms of the pathogenesis of atherosclerosis [5]. As a member of the hs-CRP family, PTX-3 plays a key role in the development of atherosclerosis [6] PTX-3 is released mainly by vascular endothelial cells, smooth muscle cells and mononuclear macrophages following induction by inflammatory factors (such as IL-1 and TNF) [7]. It was previously suggested that the correlation between PTX-3 levels and vascular inflammation would be closer than the correlation between PTX-3 and hs-CRP and could better reflect the degree of coronary atherosclerosis, independent of hs-CRP levels [8]. Rolph et al. [9] also found elevated PTX-3

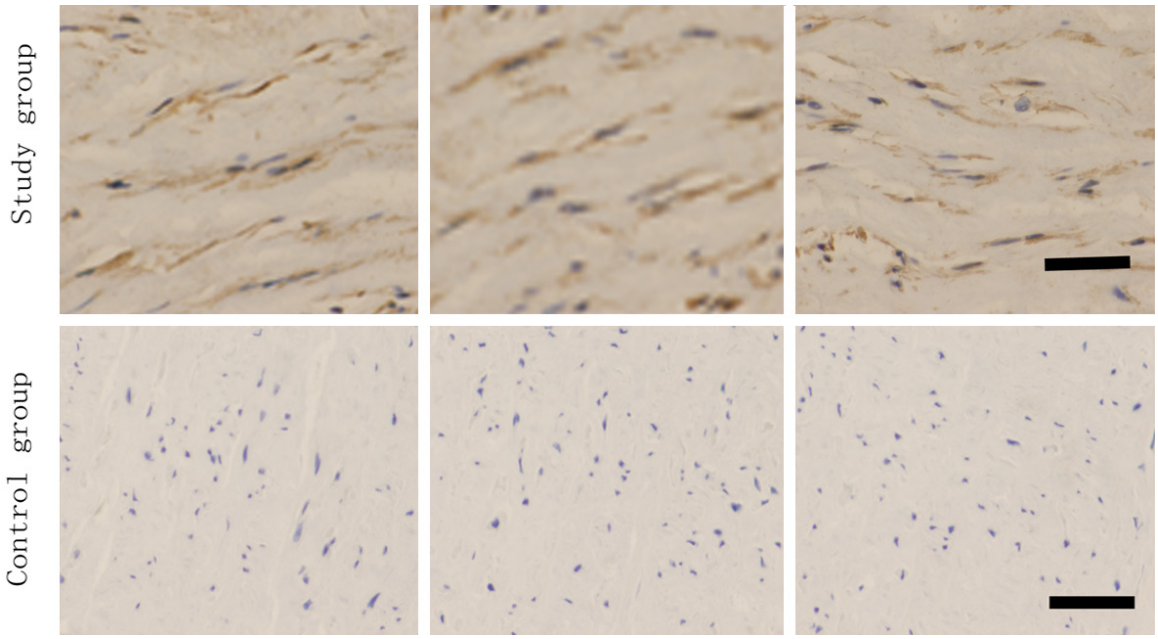

Figure 1. Top: Low levels of PTX-3 expression in normal renal artery tissues from kidney donors (×400). Bottom: High level PTX-3 protein expression in aortic tissues from the experimental group patients who underwent CABG surgery (×400). (Brown staining is considered positive staining; scale bar, 50 μm). CABG: Coronary artery bypass graft; PTX-3: Pentraxin 3.

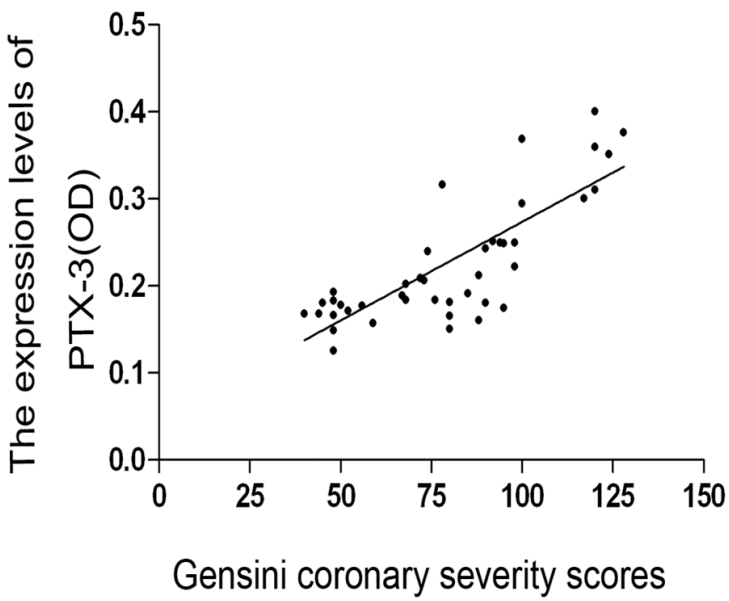

Figure 2. Correlation between aortic PTX-3 expression and Gensini coronary severity scores. PTX-3: Pentraxin 3.

expression levels in plaques from patients with atherosclerosis compared with lower PTX-3 levels found in artery biopsies of patients without atherosclerosis. Consistent with this, we found that serum and aortic PTX-3 levels were significantly increased in the experimental group. We also found that the aortic PTX-3 expression level was highly correlated with Gensini coronary disease severity scores, which suggests a critical role of PTX-3 inflammasome in atherosclerosis.

Our study also identified a positive correlation between aortic PTX-3 expression levels and serum PTX-3, hs-CRP, TC, TG, LDL-C and Lp(a) levels. However, aortic PTX-3 expression was found to be negatively correlated with serum HDL-C levels. Previous research showed that ox-LDL can increase PTX-3 expression levels in endothelial cells and macrophages by activating NF-kB [9, 10]. Klouchel et al. [11] also found that ox-LDLs promote PTX-3 mRNA expression in vascular endothelial cells, while PTX-3 promotes the formation of free oxygen radicals which accelerate the oxidation of LDLs. Low levels of HDL-C reduce the cellular resistance to oxidative stress in arterial

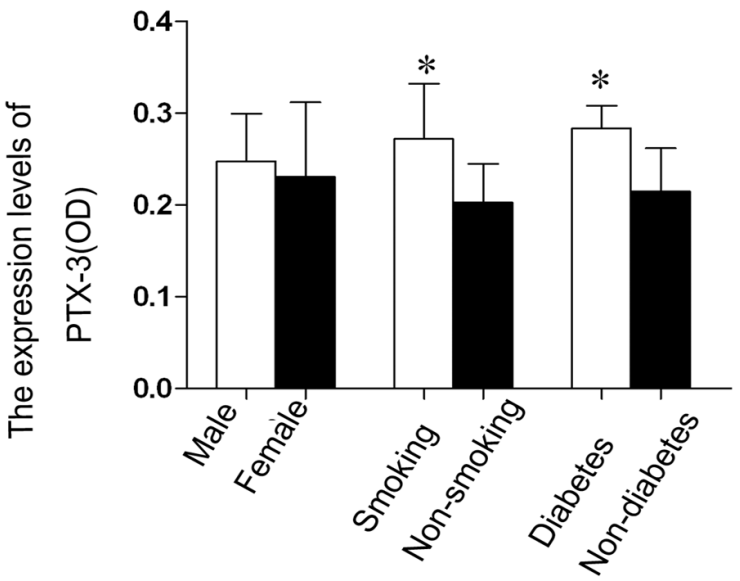

Figure 3. Aortic PTX-3 expression in patients from the research group clustered by gender, smoking profile or diabetic status. *P<0.05. PTX-3: Pentraxin 3.

Table 2. Association between aortic PTX-3 expression levels and serum PTX-3, hs-CRP and lipid levels

	PTX-3	hs-CRP	TG	TC	LDL-C	HDL-C	Lp(a)
r	0.702	0.690	0.400	0.469	0.435	-0.453	0.506
P	0.002	0.012	0.016	0.004	0.008	0.014	0.002

Abbreviations: HDL-C: high density lipoprotein cholesterol; hs-CRP: high-sensitivity C-reactive protein; LDL-C: low density lipoprotein cholesterol; Lp(a): lipoprotein(a); PTX-3: Pentraxin 3; TG: triglycerinate; TC: total cholesterol.

areas affected by atherosclerosis, resulting in a larger amount of lipid s accumulating on the endarterium which favors the accumulation of blood lipid deposits, leading to atherosclerosis progression [12, 13]. However, it is unclear why aortic PTX-3 expression levels were negatively correlated with serum HDL-C levels, although this may be explained by an adjustment of PTX-3 mRNA expression by HDL-C via the PI3K signaling pathway [14]. Additionally, PTX-3 can cause a release of membrane coagulation phospholipids, enhancing platelet reactivity and aggravating the inflammatory response [15], as well as promoting the progression of atherosclerosis.

This study also found that aortic PTX-3 expression levels were higher in smokers than non-smokers, which indicates that smoking promotes PTX-3 expression. As CHD risk equivalents, diabetes and atherosclerosis have the same pathological basis. Under these similar contexts, inflammatory responses promote PTX-3 secretion by macrophages. Our study found that aortic PTX-3 expression was higher in diabetic vs. non-diabetic patients, indicating that diabetes plays a role in the progression of atherosclerosis via PTX-3.

The coronary artery Gensini score is an effective method to evaluate the degree of coronary artery lesions and increases in patients with serious coronary artery disease. Our results showed that aortic tissue PTX-3 expression increased with the Gensini score, suggesting that higher PTX-3 expression can accelerate the development and progression of atherosclerosis, increase the instability of vulnerable plaques, and ultimately lead to the formation of complex atherosclerotic lesions.

Most studies on atherosclerosis are performedon animal models, while others are conducted using human tissue samples obtained during autopsy. In animal models, atherosclerosis develops rapidly, whereas in humans, the disease develops progressively over decades. These differences in etiology and pathophysiology hinder the translation of results obtained in animals models directly to humans. In the case of human samples collected during autopsy, autolytic destruction of antigens cannot be avoided and impairs tissue quality. Therefore, surgical material appears to be the optimal choice. In this study, we collected button-sized full-thickness aortic tissues discarded during CABG surgeries, which have the unparalleled advantageof a native aortic tissue architecture consisting of intima, media, and adventitia. We can thus improve the reliability and scientific credibility of the findings based on these unique samples, which provide a new and reliable approach to studying the pathogenesis of atherosclerosis [16, 17].

In summary, the present study shows that aortic PTX-3 expression levels are significantly elevated in patients with atherosclerosis, and that elevated PTX-3 expression is highly associated with coronary Gensini scores. The significant correlations between aortic PTX-3 expressionand other cardiovascular risk factors support the hypothesis that PTX-3 contributes to atherogenesis induced by numerous clinical risk factors, and may serve as an inflammatory marker associated with atherosclerosis.

Acknowledgements

This work was supported by the Shandong Provincial Natural Science Foundation of the People's Republic of China [No. Y2007C066].

Disclosure of conflict of interest

None.

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