

Association of serum paraoxonase activity and coronary artery calcification

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Abstract. – **AIM:** It has been known that there was a relation between the activities of serum paraoxonase (PON) and the severity of the coronary artery disease. However, little is known about association of coronary artery calcification (CAC) and serum PON activities. The aim of this study is to evaluate the relationship between CAC and serum PON activities.

PATIENTS AND METHODS: We measure serum PON activities from 122 patients (42 female, mean age = 62±10 years) with angiographically documented CAC (Group I), and 138 patients (54 female, mean age = 60±10 years) without CAC (Group II). Coronary calcification was detected with fluoroscopy before coronary angiography. Serum PON activities were measured by spectrophotometrically method. Patient characteristics and baseline data were recorded from patient's files.

RESULTS: The triglyceride levels is lower in group I than group II ($p = 0.040$). Diastolic blood pressure and frequency of diabetes mellitus was higher in the group I than group II (respectively $p = 0.012$ and $p = 0.022$). The other clinic and laboratory parameters were similar in two groups (all $p > 0.05$). The only statistically significant differences between with CAC and without CAC groups in respect to serum PON activities were present (170.6 ± 59.6 vs. 209.6 ± 69.8 U/ml; respectively, $p < 0.001$). There was a negative correlation between serum PON activities and presence of CAC ($p < 0.001$).

CONCLUSIONS: These data indicate that the serum PON activities are decreased in patients with CAC. The serum PON activities may play a role in development of the CAC and reduced serum PON activity might represent a biochemical marker of CAC.

Key Words:

Coronary artery calcification, Serum paraoxonase activity.

Introduction

Paraoxonase (PON) is a well-known antioxidant molecule which inhibits atherosclerosis by protecting low-density lipoprotein (LDL) and high-density lipoprotein (HDL) from oxidation by hydrolyzing activated phospholipids¹ and lipid peroxide products²). Earlier studies have shown that serum PON1 activity is associated with the presence and extent of coronary artery disease (CAD)³, coronary collateral development⁴, modulation of endothelial functions⁵, and regulation of coronary vasomotor tone⁶.

Coronary artery calcification (CAC) is a definitive indicator for coronary atherosclerosis and it takes place in all vascular tissue^{7,8}. The amount of coronary calcium correlates with the amount of atherosclerosis in patients with CAD⁸. Vascular calcification reduces aortic and arterial elasticity, which impairs cardiovascular hemodynamics, resulting in substantial morbidity and mortality in cardiovascular diseases⁹. It was reported that there is a strong correlation between CAC and cardiovascular events in asymptomatic and symptomatic individuals^{10,11}.

Atherosclerotic calcification is the most common form of calcific vasculopathy. It is a multifactorial process and its pathophysiology is incompletely understood⁹. Coronary calcification is caused by the interaction of several risk factors such as hyperlipidemia, high glucose levels, chronic kidney disease, inflammatory factors, oxidative stress, osteopontin, fibroblast growth factor (FGF)-23, matrix metalloproteinases, transforming growth factor- β 1 (TGF- β 1)^{9,12} and is commonly detected by electron beam computed tomography (EBCT) and fluoroscopy^{8,13}.

In their previous study Thyagarajan et al¹⁴ reported no association of CAC on CT and serum paraoxonase activity (SPA) in their follow up study at the 15th and 20th years of the subjects. However their study population was far younger than the typical age of both CAD and CAC and no additional study was reported on the association of CAC and SPA. Accordingly we hypothesized that decreased SPA might be associated with increased CAC. Therefore, the current study was undertaken to assess whether SPA is associated with the angiographically visible CAC.

Patients and Methods

Study Population

Two-hundred sixty patients (mean age = 61±10 years, 96 female) whom underwent coronary angiography were included in this study after giving informed consent for participation to the study and study protocol conforms to the principles of Helsinki Declaration. Institutional Ethics Review Board approved the study protocol. All cases grouped into two: Group I included 122 cases with the presence of CAC (42 female, mean age = 62±10 years) and Group II included 138 subjects without presence of CAC (54 female, mean age = 60±10 years).

We have evaluated CAC and laboratory parameters besides medical records regarding details of past medical history and medications and details of physical examination. All patients were selected from individuals who underwent elective coronary angiography in our institution with a suspicion of CAD based on a typical chest pain or abnormal results on noninvasive tests.

Exclusion criteria included atrial fibrillation, valvular, myocardial or pericardial disease, hepatic or renal dysfunction (serum creatinine >1.5 g/dl). The patients admitted with acute coronary syndromes and who had suffered a myocardial infarction or major surgical procedure within the last 3 months, patients who had undergone revascularization procedures or patients with concomitant inflammatory diseases such as infections and autoimmune disorders, neoplastic diseases were excluded from this study. Patients taking alcohol, statins, antioxidant vitamins and drugs, such as carvedilol and zofenopril, were also excluded from the study.

Baseline Definitions and Measurements

Hypertension was defined as diastolic blood pressure more than or equal to 90 mmHg or sys-

tolic blood pressure more than or equal to 140 mmHg or self reported use of antihypertensive drug(s). Diabetes mellitus was diagnosed if the fasting plasma glucose concentration was more than or equal to 126 mg/dl on two separate occasions, or if the patient was on treatment with insulin or oral hypoglycemic agent(s). Current cigarette smokers were included in the cigarette smoking group. Height and weight were measured according to a standardized protocol. Body mass index (BMI) was calculated by dividing weight in kilograms by height in meters squared (kg/m²).

Angiographic Evaluation and Detection of CAC Using Fluoroscopic Technique

All patients were examined with a Toshiba intensifier and monitor (Toshiba Infinix: 2C308-011E*S[®], Otawara-Shi, Tochigi-Ken, Japan). The mA varied between 1.5 and 3.5 and the kVp between 70 and 110 depending upon the size of the patient. The x-ray unit was also equipped with an automatic brightness control which maintained the optimal kVp with the aid of a photoelectric cell at the level of the output phosphor. All patients were examined in at least four views (PA, left lateral, shallow RAO, and LAO), with the patient erect and holding his breath in full inspiration. All studies were performed by one fluoroscopist without knowledge of the patient's clinical or angiographic status. Careful fluoroscopic examination was done prior to the coronary angiography. The presence or absence of calcifications was recorded in each of the four major coronary arteries-left main, left anterior descending, left circumflex and right coronary artery and its branches. Angiographic Procedure and Criteria Selective coronary cineangiograms were obtained¹⁵. CAC was considered to be present when linear radio-opaque densities are encountered consistent with the trace of main coronary arteries and their major branches on non-contrast enhanced angiography frames taken just before intracoronary contrast agent injection.

Assessment of Biochemical Markers

Blood samples were obtained after an overnight fasting state just before angiography after insertion of the femoral catheter sheath and the serum was immediately separated from the cells by centrifugation at 3000 g for 10 min, stored at -70°C, and then analyzed. The levels of triglyceride (TG), total cholesterol, HDL cholesterol, LDL cholesterol, creatinine, calcium and

fasting blood glucose were determined using commercially available assay kits (Abbott®, Abbott Park, North Chicago, IL, USA) with Abbott Aeroset auto-analyzer (Abbott).

Measurement of Serum Paraoxonase Activity

Measurement of SPA was performed in the absence of NaCl (basal activity). The rate of paraoxon hydrolysis (diethyl-p-nitrophenylphosphate) was measured by monitoring the increase of absorbency at 412 nm at 37°C with auto-analyzer (Abbott®, Abbott Park, North Chicago, IL, USA). The amount of generated p-nitrophenol was calculated from the molar absorptivity coefficient at pH 8, which was 17000/mol/l/cm¹⁶. PON activity was expressed as U/l serum. Enzymatic activity was calculated from the molar absorptivity coefficient of the produced phenol; 1310/mol/l/cm. Coefficient of variation for measurement of serum PON activity was 2%.

Statistical Analysis

All analyses were conducted using SPSS 11.5 (SPSS Inc., Chicago, IL, USA). The Kolmogorov-Smirnov test was used to verify the hypothesis of normal distribution, followed by the independent Student t test in case of normal distribution. Systolic and diastolic blood pressure, heart rate, creatinine and fasting blood sugar were evaluated using Mann Whitney U test. Chi-

square test was used for categorical variables of groups. Continuous variables were expressed as mean ± SD or median (range) and categorical variables were expressed as percentages. The correlation between SPA and variables were evaluated using Pearson's rank correlation analysis. Two-sided *p* value of less than 0.05 was considered statistically significant.

Results

The clinical characteristics and laboratory parameters of groups were presented on Table I. Age, hypertension, cigarette smoking, BMI, systolic blood pressure, levels of fasting glucose and urea, total cholesterol (TC), LDL-C, HDL-C, and calcium levels were similar in patients with and without CAC (*p* > 0.05 for all). Frequency of cigarette smoking, sex, hypertension dyslipidemia were also similar between the two groups (*p* > 0.05 for all). The triglyceride levels were lower in group I than in group II (*p* = 0.040). Diastolic blood pressure and frequency of diabetes mellitus was higher in the group I than group II (respectively *p* = 0.012 and *p* = 0.022)

Paraoxonase activity was significantly decreased in group I than in group II (*p* < 0.001) (Figure 1). In bivariate correlation analysis SPA was only correlated with presence of CAC (*p* < 0.001) (Table II).

Table I. Demographic and clinical characteristics of patients with and without coronary artery calcification.

Variables	Patients with CAC (group I, n = 122)	Patients without CAC (group II, n = 138)	<i>p</i> value
Age, (years)	62±5	60 ± 10	0.062
Male/female, (%)	34/66	39/61	0.488
Body mass index, (kg/m ²)	27 ± 6	27 ± 5	0.593
Hypertension, (%)	40	36	0.515
Diabetes mellitus, (%)	40	27	0.022
Smoking, (%)	29	31	0.679
Hyperlipidemia, (%)	30	38	0.172
Systolic blood pressure, (mmHg)	136.7 ± 25.4	130.6 ± 18.5	0.155
Diastolic blood pressure, (mmHg)	83.4 ± 17.5	79.5 ± 14.8	0.016
Heart rate, (beat/minute)	78 ± 10	79 ± 18	0.577
Fasting blood sugar, (mg/dL)	125.4 ± 24.5	147.3 ± 17.2	0.083
Total cholesterol, (mg/dL)	194.2 ± 39.3	201.7± 45.4	0.403
LDL cholesterol, (mg/dL)	129.4 ± 39.7	128 ± 37.6	0.874
HDL cholesterol, (mg/dL)	39.2 ± 10.4	39.4 ± 15.3	0.918
Triglyceride, (mg/dL)	154.7 ± 76.6	193.4 ± 106.5	0.040
Creatinine, (mg/dL)	0.91 ± 0.4	0.89 ± 0.3	0.969
Calcium, (mg/dL)	9.0 ± 0.6	9.1 ± 0.5	0.519
Paraoxonase, (U/L)	170.61 ± 59.56	209.61 ± 69.82	< 0.001

CAC; coronary artery calcification, HDL: high-density lipoprotein; LDL: low-density lipoprotein; Data are presented as mean ± SD or %. Systolic and diastolic blood pressure, heart rate, creatinine and fasting blood sugar were presented as median (rang).

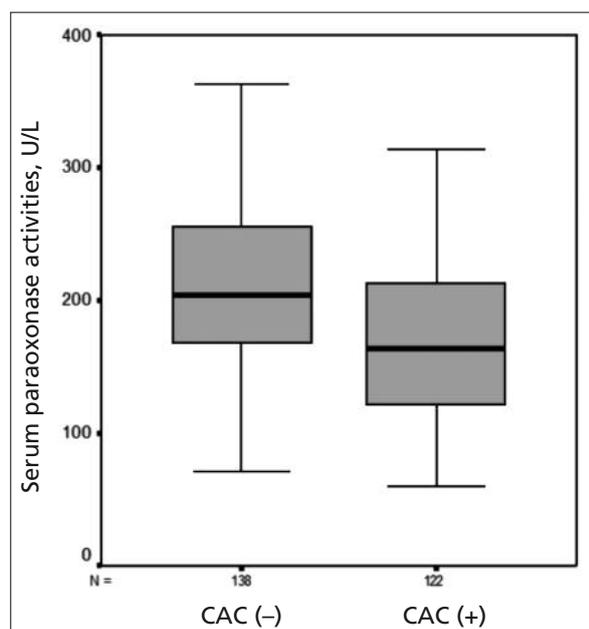


Figure 1. Serum paraoxonase activities in patients with and without coronary artery calcification (CAC: coronary artery calcification).

Discussion

In the present study, we used the fluoroscopic assay to assess the presence of CAC in patients undergoing coronary angiography. We found that SP activity was significantly decreased in patients with CAC.

Previous investigations have shown correlation of CAC and the total plaque burden in the coro-

Table II. Bivariate correlation and multiple linear regression analysis of serum paraoxonase activity and variables.

Variables	Bivariate analysis	
	r value	p value
Age	-0.049	0.431
Body mass index	-0.050	0.529
Diastolic blood pressure	-0.049	0.452
Systolic blood pressure	-0.057	0.387
Heart rate	0.029	0.657
Fasting blood sugar	-0.051	0.601
Total cholesterol	0.140	0.168
LDL cholesterol	0.052	0.609
Triglyceride	0.072	0.476
Creatinine	0.072	0.458
Calcium	0.071	0.644
Presence of CAC	-0.287	<0.001

CAC; coronary artery calcification, HDL: high-density lipoprotein; LDL: low-density lipoprotein.

nary arteries^{3,17-19}. Thyagarajan et al¹⁴ have shown that there was no association between paraoxonase enzyme activity and coronary CAC in young subjects both at 15 and 20 years old. Except for that study¹⁴), there is no published data in literature on the relationship between the serum PON activity and CAC. This is the first study revealing significant association between lower PON activity and CAC detected on coronary angiography.

First step of atherosclerosis is oxidation of LDL-C. Then, Ox LDL-C migrates to subintimal layer of arteries. Macrophages phagocyte Ox LDL-C and foam cells are formed. Foam cells are fragmented and atherosclerotic progression and calcification occurs in the plaque. CAC can be detected with microscopic methods^{20,21}. The evaluation of specimens of CAC has revealed small aggregates of crystalline calcium among the lipid particles of lipid cores in the atherosclerotic plaque²⁰. Calcification development in the atherosclerotic plaque is an organized, regulated process similar to bone formation. Non-hepatic gamma-carboxyglutamate (Gla)-containing proteins like osteocalcin and osteopontin have been reported to have roles in the pathogenesis of CAC⁸. Calcified human atherosclerotic plaque also contains cells capable of osteoblastic differentiation. Besides rapidly growing literature above-mentioned investigations indicate that calcification is an active process and not simply a passive precipitation of calcium phosphate crystals, as once thought^{22,23}.

Minimally oxLDL was previously shown to induce differentiation of calcifying vascular cells²⁴. Previous evidences have suggested that oxidative stress enhances differentiation of calcifying vascular cells and inhibits differentiation of bone cells²⁵. Another report showed that HDL regulates the osteoblastic differentiation and calcification of vascular cells and that vascular calcification is another target of HDL action in the artery wall²⁶. Our results suggest that low SPA is associated with increased CAC and PON may also be protective against vascular calcification and decreased PON might lead to increased atherosclerotic plaque calcification by increasing oxLDL-C¹⁸.

In current practice, fluoroscopy and EBCT are most commonly used noninvasive diagnostic modalities to detect CAC^{8,12}. Fluoroscopic method, we have used, has the advantages of no additional radiation, no extra cost. Contrarily fluoroscopic method is operator dependent, varies with fluoroscopic equipment, body habitus, over-

lying anatomic structures, and overlying calcifications in structures such as vertebrae and valve annuli and presence of calcium is not possible quantified on fluoroscopy.

Cross sectional study design is the main limitation of this investigation. Study population continued taking previously prescribed medications before entry into this research. Two groups did not, however, differ with regard to the baseline medications, and no association was observed between these drugs and serum PON activity. Presence and severity (Gensini score) of CAD was not different among two groups (data not shown) although we could not have opportunity to perform advanced diagnostic utilities (such as intravascular ultrasound) which would have provided extended information on lesion characteristics.

Conclusions

SPA is decreased in patients with CAC compared in patients without CAC and paraoxonase might be protective enzyme against CAC. Further comprehensive prospective studies are needed to verify or exclude our findings and to better elucidate the mechanisms of the negative association of SPA and CAC.

Conflict of Interest

None declared.

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