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Serum Osteopontin Concentrations in Relation to Coronary Artery Disease

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Background and Aims. Coronary artery disease (CAD) is a common form of vascular disease and is associated with high mortality and morbidity globally. It has been suggested that serum osteopontin (OPN) may be a useful biomarker of atherosclerosis and vascular calcification. The aim of this study was to assess the association between serum OPN levels and severity of CAD.

Methods. Three hundred and four subjects were studied, 111 with clinically significant angiographically defined CAD (CAD+) (>50% stenosis), 96 with negative angiography (CAD-) (<50% stenosis) and 97 healthy controls. Fasting blood samples were collected from all patients before coronary angiography and serum OPN levels were determined using ELISA.

Results. Serum concentrations of OPN were significantly higher in both CAD+ (72.99 [51.05−103.64]) and CAD- (11.11 [8.11−18.23]) ($p = 0.001$) groups compared with the control group (5.99 [4.26−7.91]) ($p = 0.001$). CAD+ subjects also had higher serum OPN levels compared with CAD-subjects ($p = 0.001$). However, OPN levels were comparable between subgroups of CAD+ subjects stratified according to the number of narrowed vessels in angiography.

Conclusions. The present results suggest a positive association between circulating OPN concentrations and the presence but not the extent of CAD. © 2015 IMSS. Published by Elsevier Inc.

Key Words: Coronary artery disease, Atherosclerosis, Osteopontin.

Introduction

According to updated statistics, cardiovascular disease is the first cause of death both in the U.S. (source: American Heart Association, 2013) and worldwide (data from the World Health Organization, 2013). Cardiovascular disorders represent the foremost cause of preventable death worldwide (1). Coronary artery disease (CAD) is the most prevalent form of cardiovascular disease with high mortality and morbidity rates. Calcification of the arterial wall has been shown to be associated with an elevated risk of cardiovascular events, although the causality of this association remains elusive (2,3). Osteopontin (OPN) is a glycoprotein secreted by macrophages, vascular smooth muscle cells, and endothelial cells and has been demonstrated to promote macrophage chemotaxis (4,5). Expression of OPN has been shown in the neointima of injured vessels, calcified atheromatous plaque (6,7), and macrophages at the site of inflammation where it is thought to mediate monocyte adhesion (8), migration (9) differentiation (10), and phagocytosis (11). Serum OPN has also been reported to be increased in patients with atherosclerosis, valvular stenosis and myocardial infarction (12−14). Circulating OPN levels are associated with increased aortic pulse

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wave velocity in patients with rheumatoid arthritis (15) and with increased intima-media thickness and mean systolic and diastolic flow velocities in patients with essential hypertension (16). It is known that oxidative stress plays an important role in the pathogenesis of atherosclerosis and that an association exists between OPN and atherosclerosis. Previous studies have shown an association between OPN and malondialdehyde (MDA) levels in patients with CAD, suggesting potential involvement of oxidative stress in the regulation of OPN expression (17). In light of the aforementioned observations, the present study aimed to assess the association between serum OPN concentrations and severity of CAD in a group of patients undergoing coronary angiography.

Methods

Study Population

The study was performed on a total of 304 patients. Of these (111 + 96), 207 underwent angiography. Of the 207 patients, 111 were CAD+ and 96 were CAD-. The remaining enrolled persons (n = 97) were healthy controls who did not undergo coronary angiography. The study subjects were selected from those subjects who underwent coronary angiography in the Ghaem Hospital (Mashhad, Iran). Written informed consent was obtained from all participants using protocols approved by the Ethics Committee of the Mashhad University of Medical Science and a standardized questionnaire was used to collect demographic information.

Coronary Angiography

Angiography was indicated principally for stable angina in patients who were positive for at least one objective test of myocardial ischemia including exercise stress test, dobutamine stress echocardiography, and thallium SPECT (single photon emission computed tomography). Exclusion criteria were as follows: oral contraceptives or hormone replacement therapy, pregnancy, prior history of coronary angioplasty or coronary artery bypass graft, and having overt clinical features of infection or chronic inflammatory disease. All subjects were negative for viral markers of hepatitis and anti-HIV antibody. Moreover, patients with myocardial infarction within the previous 3 months or with renal, hepatic or malignant diseases were excluded. Subjects who were candidates for emergency percutaneous coronary intervention were also excluded from the study. Coronary angiograms were performed using routine procedures. Analysis of the angiograms was performed offline by a specialist cardiologist. The presence of one or more stenoses ≥50% in diameter of at least one major coronary artery (left main, right coronary artery, left anterior descending, circumflex) was considered evidence of significant CAD (18). Patients with significant CAD were further stratified according to the number of narrowed vessels into those with one (SVD), two (2VD), or three-vessel disease (3VD) depending on the number of coronary arteries involved. A ≥50% narrowing of the left main coronary artery was considered as two-vessel disease. Eighty-three age- and sex-matched healthy volunteers were also recruited as a normal control group. These individuals had no personal or family history of cardiovascular disease or diabetes. Information on smoking, drug use and family history of CAD was obtained via a questionnaire. The study protocol was approved by the ethics committee of Mashhad University of Medical Sciences and written informed consent was obtained from each participant.

Laboratory Evaluation

Blood samples were collected from all patients before coronary angiography and after an overnight fast. Serum OPN levels were determined using a commercially available ELISA kit (D0ST00; R&D Systems, Italy) according to the manufacturer's instructions. Sensitivity of the assay method was 3.33 ng/mL with an intra- and inter-assay CV of <5 and $<10\%$, respectively. OPN was measured with a sandwich enzyme-linked immunosorbent assay using a commercially available kit (D0ST00; R&D Systems). In brief, 1:2 diluted testing samples were incubated in the N-terminal OPN antibody pre-coated wells at 37°C for 1 h. Following washing, 100 μL of labeled OPN antibody solution was added to each well and incubated for 30 min at 4°C. After washing, tetramethylbenzidine was added and the absorbance at 450 nm was measured with an automatic ELISA reader (Bio-Rad, Segrate, Italy).

Statistical Analysis

Statistical analyses were performed using the SPSS software v.16.0 (Chicago, IL). Data were expressed as mean ± SD (for normally distributed variables) or median (interquartile range) (for non-normally distributed variables). Comparison of serum OPN levels among study groups was made using one-way ANOVA (for normally distributed data) or Kruskal-Wallis (for non-normally distributed data) tests. Pearson or Spearman correlation coefficients were used to determine the association between OPN levels and clinical and biochemical factors. The impact of confounding parameters including age, gender, smoking status, diabetes mellitus and BMI on the association between serum OPN levels and CAD was assessed using binary logistic regression. In all analyses, a two-sided p value of ≤ 0.05 was considered as statistically significant.

Results

There were a total of 304 participants, of which 111 were CAD+, 96 CAD-, and 97 apparently healthy control