Plasma Osteopontin as a Predictor of Coronary Artery Disease: Association With Echocardiographic Characteristics of Atherosclerosis

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Osteopontin (OPN), a bone-related protein, is present within the atherosclerotic plaques, most strikingly in calcified plaques. Valvular calcifications are accepted as a part of the spectrum of atherosclerosis and are associated with atherosclerotic calcification in the coronary arteries. The study aimed to evaluate the association of plasma OPN with the presence and extent of coronary stenosis, mitral annular calcification (MAC), and aortic valve sclerosis in stable angina patients. We studied 120 subjects who underwent coronary angiography because of ischemic chest pain. Coronary artery disease (CAD) was defined as $\geq 50\%$ stenosis in ≥ 1 coronary artery. MAC and aortic valve sclerosis were detected by echocardiography. Lipid profile, high sensitive C-reactive

protein (hsCRP), and OPN were measured in all studied subjects. Patients with CAD had increased plasma OPN when compared with those without CAD ($P < 0.001$). Plasma OPN levels were significantly positively correlated with atherogenic lipid profile, hsCRP, MAC grading, aortic valve sclerosis grading, and the number of stenosed coronary vessels in CAD patients. In multivariate analysis, OPN was an independent predictor of CAD ($P = 0.01$), MAC ($P = 0.01$), and aortic valve sclerosis ($P = 0.04$). In conclusion, OPN is an independent predictor of MAC and aortic valve sclerosis. Plasma OPN levels reflect the extent of coronary stenosis and can be used as a biomarker to identify patients with coronary atherosclerosis. *J. Clin. Lab. Anal.* 24:201–206, 2010. © 2010 Wiley-Liss, Inc.

Key words: osteopontin; coronary artery disease; aortic valve sclerosis; mitral annular calcification; coronary calcification.

INTRODUCTION

Atherosclerosis is a complex multifactorial process resulting from an excessive inflammatory response to various forms of injurious stimuli to the arterial wall (1). The transition of a stable coronary atherosclerotic lesion into a ruptured and/or eroded plaque results in the clinical manifestation of an acute coronary syndrome. Plaque instability is the consequence of a complex inflammatory response of the vessel wall ignited by activated macrophages and T-cells, leading to proteolytic degradation of connective tissue matrix, excessive proinflammatory cytokine production, and apoptosis of vascular wall cells (2).

Various calcification processes (e.g., aortic valve calcification, mitral annular calcification, and coronary artery calcification, and aortic atheroma) may all be a part of the spectrum of atherosclerosis. Vascular

calcification, with its reduced compliance and altered mechanical properties, is a predisposing factor for plaque rupture and a predictor of cardiovascular (CV) mortality (3). Calcium deposition typically occurs in areas of atherosclerotic lipid accumulation and is a highly organized and regulated process that is very similar to cortical bone formation (4). Accordingly, several bone-related proteins have now been identified as being capable of inducing or inhibiting CV diseases.

Osteopontin (OPN), a calcium-binding phosphorylated glycoprotein, was originally identified as a mediator

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involved in bone remodeling (5). OPN has been implicated in numerous physiological and pathological events including cell-mediated immunity, inflammation, tumor progression, and cell survival (6). In recent years, it has become apparent that OPN exerts important CV effects (7). OPN is a component of human atherosclerotic lesions, where it is secreted by cells of the monocyte/macrophage lineage and, to a lesser extent, by endothelial and vascular smooth muscle cells (8). OPN mRNA expression in human atherosclerotic plaques was reported to be closely associated with the severity of atherosclerosis and calcification (8,9). These findings suggest that OPN may play a role in the development of vascular disease and could also be a marker of atherosclerosis lesions.

In stable angina patient, in contrast to acute coronary syndromes, there is a paucity of biomarkers that can be used in clinical practice to characterize patients (7). This study aimed to evaluate the association of plasma OPN with the presence and extent of coronary artery stenosis, some echocardiographic characteristics of atherosclerosis (mitral annular calcification, aortic valve sclerosis), and other CV risk factors in stable angina patients.

MATERIAL AND METHODS

Study Groups

This study was performed on 120 subjects with the main complaint of ischemic chest pain, who underwent coronary angiography at the cardiology department of Zagazig University Hospitals. All patients fulfilled the criteria of ischemic chest pain and/or signs of myocardial ischemia on exercise electrocardiography for finding subjective evidence for clinical indication of coronary angiography.

Coronary angiography was recorded by a femoral approach with judkin technique to assess the degree of coronary artery stenosis. According to coronary angiography findings, subjects were divided into two groups: coronary artery disease (CAD) group with ≥ 1 coronary artery having $\geq 50\%$ luminal diameter stenosis (10) ($n = 74$), and control group with $< 50\%$ stenosis ($n = 46$). The extent of coronary artery stenosis was determined by the number of coronary vessels with $\geq 50\%$ stenosis.

Patients with acute coronary syndromes or those with a history of revascularization, valvular heart disease, heart failure, cardiomyopathies, infectious, inflammatory, autoimmune, bone, renal (creatinine levels > 2.0 mg/dl), liver, or neoplastic diseases, and patients receiving systemic glucocorticoids or immunosuppressants were excluded from the study.

Complete medication profiles were obtained from all subjects. Diabetes was considered present if a patient had a fasting glucose level ≥ 126 mg/dl or was treated

with insulin or oral agents. Hypertension was defined by systolic blood pressure ≥ 140 mm Hg, diastolic blood pressure ≥ 90 mm Hg, and/or the current use of anti-hypertensive treatment. The study protocol was approved by the institutional ethics committee and written informed consent was obtained from all participants.

Echocardiographic Assessment

Transthoracic 2-dimensional echocardiography was performed by Hewlett Packard Sonos 5500 machine (Soma Technology, Inc., Bloomfield, CT) for detection of mitral annular calcification (MAC) and aortic valve sclerosis. MAC was defined as dense high reflective area at the base of the posterior mitral leaflet in the parasternal short axis view. Mild MAC involvement affected one-third or less of the annulus, moderate involvement was between one-third and two-third of the annulus, and severe involvement exceeded two-third of the annulus (11). Aortic valve sclerosis was diagnosed by two-dimensional short and long axis view. Aortic sclerosis relied on visualization of bright echoes at the base of the aortic valve leaflets. Mild aortic valve calcification (AVC) was scored as small isolated spots, moderately calcified included multiple larger spots, and heavily calcified involved extensive thickening and calcification of all cusps (12).

Laboratory Assessment

Fasting blood samples were collected in plain and EDTA containing tubes. Serum samples were used for determination of lipid profile, High sensitive C-reactive protein (hsCRP). EDTA samples were centrifuged at 1,000xg for 15 min within 30 min of collection and the plasma was stored at -80°C for analysis of OPN. Total cholesterol, triglycerides were determined on ADVIA 1650 autoanalyzer (Siemens Medical Solutions Diagnostic, Newark, DE). HDL-C was determined using Eli-tech kit (France). LDL-C was calculated according to Friedewald's Equation (13). hsCRP was measured by particle enhanced immunonephelometry using BN Prospec (Siemens). OPN was measured with a sandwich enzyme-linked immunosorbent assay using a commercially available kit (Quantikine®, R&D systems Inc., Minneapolis, MN) according to the manufacturer's protocol. The intra-assay precision is 2.6–4.0%, inter-assay precision is 5.4–6.6%, and lower detection limit is 0.011 ng/ml.

Statistical Analysis

The sample size was calculated with 80% statistical power and 95% confidence interval. Kolmogorov–Smirnov test was used to verify the normality of distribution of continuous variables. Data are presented as

mean $\pm$ standard deviation (SD) and range for continuous variables, frequency, and percentage for categorical ones. Differences between the studied two groups were evaluated by independent sample *t* test and χ^2 respectively. Differences between more than two groups were evaluated by one-way ANOVA. Bivariate correlations between OPN levels and other studied parameters were performed using the Pearson and Spearman rank correlation, when appropriate. Multiple logistic regression analysis were used to elucidate independent predictors of CAD, MAC, and aortic valve sclerosis. The variables included in the model are all the demographic, clinical, and biochemical characteristics of the study subjects. Two-tailed *P*-value of <0.05 was considered to be statistically significant. Statistical analysis was performed with SPSS software (Statistical Package for the Social Science; SPSS Inc., Chicago, IL) version 15 for the Microsoft Windows.

RESULTS

Coronary stenosis was present in 74 patients, in whom 28 had one vessel disease, 32 had two, and 14 had three vessel disease. There was a significant increase in the prevalence of MAC and aortic valve sclerosis in patients with CAD when compared with those with no CAD ($P = 0.003$, $P < 0.001$, respectively). No significant differences were found in demographic and other clinical characteristics between the two groups ($P > 0.05$) (Table 1).

Patients with CAD had higher LDL-C ($P = 0.01$), hsCRP ($P < 0.001$) and OPN ($P < 0.001$), and lower

HDL-C ($P = 0.02$) than those with no CAD (Table 2). Notably, there was no significant difference in mean OPN levels between patients receiving statins and those who did not take these drugs (80.5 ± 14.2 vs. 93.4 ± 33.5 ng/ml, respectively, $P = 0.07$).

On studying the correlation between plasma OPN and other studied parameters in CAD patients, OPN levels were significantly positively correlated with atherogenic lipid profile, hsCRP ($r = 0.47$), the number of stenosed vessels ($r = 0.54$), MAC ($r = 0.32$) and AVC grading ($r = 0.60$) (Table 3). In multivariate analysis, OPN was the only independent predictor of MAC (β coefficient $\pm$ SE = 0.04 ± 0.02 , $P = 0.01$) and aortic valve sclerosis (β coefficient $\pm$ SE = 0.03 ± 0.03 , $P = 0.04$). A stepwise

TABLE 2. Laboratory Findings of the Studied Groups

Variables	No CAD <i>n</i> = 46	CAD <i>n</i> = 74	<i>P</i> -value
Cholesterol (mg/dl)	197.3 $\pm$ 19.8 (170–243)	204.7 $\pm$ 30.9 (161–298)	0.15
Triglycerides (mg/dl)	148.8 $\pm$ 30.6 (81–190)	141.2 $\pm$ 46.7 (86–265)	0.28
HDL-C (mg/dl)	46.2 $\pm$ 4.3 (38–53)	44.6 $\pm$ 5.4 (35–54)	0.02*
LDL-C (mg/dl)	120.9 $\pm$ 21.5 (88–164)	133.5 $\pm$ 28.0 (98–198)	0.01*
hsCRP (mg/l)	2.95 $\pm$ 1.2 (0.7–5.0)	6.3 $\pm$ 2.3 (2.8–14.1)	<0.001 *
OPN (ng/ml)	56.3 $\pm$ 11.5 (34–74)	85.0 $\pm$ 23.3 (40–153)	<0.001 *

Results are represented as mean $\pm$ SD (range). *Significant. *n*, number of subjects; HDL-C, high density lipoprotein-cholesterol; LDL-C, low density lipoprotein-cholesterol; hsCRP, high sensitive C-reactive protein; OPN, Osteopontin.

TABLE 1. Demographic, Clinical and Therapeutic Characteristics of the Studied Groups

Variables	No CAD <i>n</i> = 46	CAD <i>n</i> = 74	<i>P</i> -value
Age (years)	52.7 $\pm$ 7.8 (41–69)	55.0 $\pm$ 6.5 (42–69)	0.07
Male gender ^a	28 (60.9)	46 (62.2)	0.09
BMI (kg/m ²)	27.0 $\pm$ 2.6 (23–32)	27.8 $\pm$ 3.0 (22–33)	0.16
Smoking ^a	14 (30.4)	30 (40.5)	0.26
Family history ^a	10 (21.7)	20 (27.0)	0.52
Diabetes ^a	12 (26.7)	16 (21.6)	0.57
Hypertension ^a	24 (52.2)	44 (59.5)	0.43
MAC ^a	6 (13)	28 (37.8)	0.003*
Aortic valve sclerosis ^a	14 (30.4)	68 (91.9)	<0.001 *
Medications ^a			
Nitrate	26 (56.5)	48 (64.9)	0.36
β Blockers	10 (21.7)	18 (24.3)	0.74
Aspirin	32 (69.6)	60 (81.1)	0.14
Statins	20 (43.5)	36 (64.9)	0.02*

^aResults are represented as mean $\pm$ SD (range) or *n*(%). *Significant; *n*, number of subjects; BMI, body mass index; MAC, mitral annular calcification.

TABLE 3. Correlation of OPN With Other Studied Parameters in CAD Patients

Variables	OPN	
	<i>r</i>	<i>P</i> -value
Age	0.25*	0.03
BMI	0.31**	0.008
Cholesterol	0.39**	0.001
Triglycerides	0.26*	0.03
HDL-C	−0.41**	<0.001
LDL-C	0.40**	<0.001
hsCRP	0.47**	<0.001
Stenosed vessels number	0.54**	<0.001
MAC grading	0.32**	0.006
AVC grading	0.60**	<0.001

*Correlation is significant at 0.05 level. **Correlation is significant at 0.01 level. OPN, Osteopontin; BMI, body mass index; HDL-C, high density lipoprotein-cholesterol; LDL-C, low density lipoprotein-cholesterol; hsCRP, high sensitive C-reactive protein; MAC, mitral annular calcification; AVC, aortic valve calcification.

Fig. 1. Scatter plot showing plasma OPN levels in subjects with no, 1-, 2-, 3-vessel disease (VD).

TABLE 4. Multiple Logistic Regression Analysis Showing Independent Predictors of CAD

Variables	β coefficient $\pm$ SE	P-value
AVC grading	1.93 ± 0.88	0.03*
MAC grading	0.14 ± 0.06	0.02*
hsCRP	1.96 ± 0.87	0.02*
OPN	0.15 ± 0.06	0.01*

*Significant. SE, standard error; AVC, aortic valve calcification; MAC, mitral annular calcification; hsCRP, high sensitive C-reactive protein; OPN, Osteopontin.

increase in plasma OPN levels were found depending on the number of diseased vessels ($P < 0.001$) (Fig. 1).

Multiple logistic regression analysis revealed that OPN ($P = 0.01$), hsCRP ($P = 0.02$), MAC grading ($P = 0.02$) and aortic valve sclerosis grading ($P = 0.03$) were independent predictors of CAD (Table 4).

DISCUSSION

This study reported that plasma OPN levels were significantly associated with the presence and extent of coronary stenosis. Plasma OPN levels were significantly positively correlated with atherogenic lipid profile, hsCRP levels, and the number of diseased coronary vessels, MAC grading, and AVC grading in CAD patients. However, it is important to note that at multivariate analysis, the OPN level was found to be an independent predictor of CAD, and the only independent predictor of MAC and aortic valve sclerosis.

The results of this study extend earlier evidence suggesting an association between elevated circulating OPN levels and severity of coronary atherosclerosis in stable angina patients (7,14), unstable angina and non-ST elevation myocardial infarction (15). Close correlations were also reported earlier between OPN and several major CV risk factors (7). Elevated baseline OPN levels were independently associated with an increased risk of future adverse CV events in chronic stable angina patients (7) and in patients with history of percutaneous coronary intervention (16). OPN may play a role in plaque formation (14), and high plasma levels of OPN may reflect plaque instability or may be involved in plaque destabilization (16). The present findings, combined with previous observations, suggest that OPN may have clinical utility as a biomarker to identify the presence of atherosclerotic disease.

A potential explanation for the association between elevated level of OPN and the extent of coronary atherosclerosis is that OPN is known to induce chemotactic movement of several cells involved in the atherosclerotic process such as vascular smooth muscle cells and macrophages (6). In addition, data from experimental studies indicate that OPN has the capacity to induce the proliferation of vascular smooth muscle cells (17) and may inhibit nitric oxide synthase expression in macrophages and endothelial cells (18). Anti-OPN antibody treatment inhibits neointimal formation in rat arteries (19). Interestingly, plasma OPN levels were positively correlated with hsCRP in our study and others (7,14), suggesting the possible involvement of OPN in the atherosclerotic inflammatory process. Taken together, these findings suggest that OPN may be critically involved in the inflammatory processes that take place within the arterial wall in atherogenesis and atheromatous plaque disruption.

Alternatively, another explanation may involve coronary calcifications. Coronary calcification occurs in the majority of patients with CAD, that its presence is related to lesion vulnerability, and that its extent is directly related to the overall burden of atherosclerotic disease (20). Plasma OPN was found to be positively correlated with coronary artery calcification in stable angina patients (14), and in diabetic patients (21).

Valvular calcifications are accepted as a manifestation of atherosclerosis because they are associated with high prevalence of CV risk factors and display similar histopathological findings as atherosclerotic lesions (22,23). Valvular calcifications are associated with atherosclerotic calcifications in the coronary arteries and aorta (24). Our findings, coupled with earlier reports (11,23), demonstrated that MAC and aortic valve sclerosis grading were independent predictors of the presence of CAD.

Our results reported that plasma OPN was the only independent predictor of MAC and aortic valve sclerosis. OPN was significantly positively correlated with MAC and AVC grading. To our knowledge, this is the first study investigating the relationship between plasma OPN, MAC, and aortic valve sclerosis in CAD patients. OPN was earlier found to be expressed in early calcific aortic valve lesion (25), whereas in a community-based sample, plasma OPN was not associated with the presence of MAC (26). Although OPN mRNA expression was reported to be high in calcified plaques (8,9), OPN was reported to inhibit the calcification of smooth muscle cell cultures in vitro (27). No calcification was found in the aorta of transgenic mice overexpressing the OPN gene, in spite of markedly increased medial thickness (28). OPN-deficient mice were reported to have enhanced calcification (29). Hence, the high levels of OPN expression in calcified plaques may represent an adaptive mechanism aimed at preventing calcification (14). Chronic inflammation is a unifying feature of all dystrophic soft tissue calcification including the response to injury (5). Thereby, it is not surprising that OPN, secreted from infiltrating macrophages that are intimately associated with calcified atherosclerotic plaque, influences the inflammatory processes that take place within the arterial wall (30).

Some limitations of our study merit consideration. First, the relatively small number of patients analyzed; thus, these results should be confirmed in a larger population. Second, the cross-sectional relationship observed between plasma OPN and CAD did not allow the cause inferences. Third, there was significant increase in the number of CAD patients receiving statins compared with those with no CAD. The use of statins was reported to be associated with lower plasma OPN (7). However, in our study, plasma OPN levels were significantly higher in CAD patients, and no significant difference was observed in OPN levels between patients receiving statins and those who did not take the drug. Therefore, this limitation could not have affected the results.

CONCLUSION

Our results revealed for the first time that plasma OPN is an independent predictor of MAC and aortic valve sclerosis. The positive association between OPN and the severity of CAD highlight the potential use of OPN as a biomarker to identify patients with or at risk of coronary atherosclerosis. A blood assay that could quantify the severity of coronary atherosclerosis would be less expensive, avoid exposure to radiation and be more accessible than the present imaging-based methods. Future studies in populations with subclinical disease

(e.g., <50% angiographic stenosis or plaque by other imaging modalities) will be crucial to assess the prognostic value of OPN in primary disease prevention.

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