Plasma Osteopontin Levels Are Elevated in Non-ST-Segment Elevation Acute Coronary Syndromes

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Background: The regions of ruptured atherosclerotic plaques have numerous macrophages. Osteopontin that modulates macrophage function has been shown in atherosclerotic plaques. We aimed to study the plasma levels of osteopontin in patients with unstable angina or non-ST-segment elevation myocardial infarction (NSTEMI) and the relationship between osteopontin and the extent of the coronary artery disease (CAD).

Methods: We studied 65 patients with unstable angina or NSTEMI, 25 patients with stable angina and 18 patients as the control group. The extent of coronary artery stenosis was determined by the number of vessels with >50% stenosis. Plasma osteopontin concentrations were measured from the blood samples that were drawn immediately after admission to the emergency department in unstable angina/NSTEMI patients and before the coronary angiography in the stable angina and control groups.

Results: The plasma osteopontin concentration was (495 ± 118 ng/ml) significantly higher in the patients with unstable angina/NSTEMI compared to the stable angina group (319 ± 106 ng/ml) and control group (125 ± 54 ng/ml) (p=0.0001). The plasma osteopontin levels were lower in the patients with stable angina pectoris who had one-vessel disease compared to those with two-vessel disease (p=0.01). However, in the unstable angina/NSTEMI group, the plasma osteopontin levels were statistically not different among the patients with one-vessel, and two-vessel and three-vessel disease (p=NS). There was no correlation between the plasma osteopontin levels and the extent of coronary stenosis.

Conclusions: The plasma osteopontin levels are elevated in patients with unstable angina/NSTEMI, but there appears to be no correlation with the extent of CAD. These results may suggest that osteopontin may have a role in the pathobiology of ACS.

Key words: osteopontin ■ unstable angina ■ non-ST-segment elevation myocardial infarction ■ acute coronary syndrome ■ coronary artery disease

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INTRODUCTION

The primary pathological event that underlies the thrombosis implicated in acute coronary events is physical disruption of atherosclerotic plaques.[1,2] Within the fibrous caps of these prone-to-rupture plaques, macrophages are found—particularly those associated with multiple proinflammatory processes, and the release of cytokines, evidenced by the rise in several serum inflammatory markers.[3,8]

Osteopontin, a phosphorylated glycoprotein that is normally found in bone and teeth, is thought to be involved in the regulation of bone mineralization and calcium metabolism.[9] In addition, osteopontin is a multifunctional protein, not only expressed by bone cells but also by inflammatory cells, including macrophages, endothelial cells, smooth-muscle cells and fibroblasts; and serves in inflammatory response to modulate the functions of the macrophages, such as adhesion, migration, generation of reactive oxygen species, and cytokine release.[10-11] Osteopontin is abundant at the sites of calcification in human atherosclerotic plaques.[12-16]

Previously, it has been demonstrated that the plasma osteopontin levels are elevated in patients with stable angina pectoris and ST-segment elevation myocardial infarction.[17-19] However, to the best of our knowledge, it has not yet been studied in patients with non-ST-segment elevation acute coronary syndrome. The aim of this study was to evaluate the plasma osteopontin levels in patients with unstable angina and non-ST-segment elevation myocardial infarction (NSTEMI) and to demonstrate its association with the extent of CAD.

METHODS

Patient Population

Sixty-five patients admitted to our emergency department between May 2004 and December 2004 with acute coronary syndrome and diagnosed to have unstable angina or NSTEMI were studied. Unstable angina was diagnosed in patients presenting with chest pain at rest consistent with myocardial ischemia within the preceding 12 hours, and transient ST segment depression or T-wave inversion—or both—in ≥2 contiguous electrocardiographic leads. A diagnosis of NSTEMI was made on the basis of a history consistent with unstable angina and a circulating cardiac troponin T concentration >0.01 ng/ml.[20]

Exclusion criteria were: ST-segment elevation or Q waves on the electrocardiogram, healed myocardial infarction, dilated cardiomyopathy, valvular heart disease, known or suspected thrombotic disorder, patients already treated with a drug that interfered with coagulation before blood sampling and statins or other lipid-lowering drugs, ongoing or recent major disorder, bone diseases, infection, thyroid dysfunction, surgery or trauma within one month, malignant, autoimmune or inflammatory disease, chronic renal or liver disease, and antimicrobial or corticosteroid treatment.

Stable Angina Group

Twenty-five patients (19 men and six women) who had typical exertional angina but no angina at rest, along with a positive exercise test and ≥75% luminal diameter stenosis of ≥1 coronary artery, were included in the stable angina group.

Control Group

Eighteen patients who underwent coronary angiography for an abnormal exercise treadmill testing and/or typical chest pain but were found to have minimal (<25% narrowing) or no lesions in their coronary angiogram were included as the control group.

Blood Sampling and Assays

In patients with acute coronary syndrome, the first blood samples were drawn immediately after admission to the emergency department. A second blood sample for serum cardiac markers was taken two hours later. Plasma osteopontin concentrations were measured from the blood samples that were drawn immediately after admission to the emergency department in unstable angina/NSTEMI patients and before the coronary angiography in stable angina and control groups. Routine commercial assays for creatine kinase, creatine kinase-MB subfraction, levels of troponin T and myoglobin were used for detection of myocardial injury. The biochemical parameters and routinely performed complete blood count were also noted.

Venous blood samples for both patients and control group were obtained by the venipuncture of the antecubital vein without stasis. The blood samples for determination of plasma levels of osteopontin were collected in EDTA (ethylenediaminetetraacetic acid)-containing tubes and then centrifuged. Derived plasma samples were preserved frozen at -70°C until thawed and analyzed on a single occasion. The plasma osteopontin levels were measured by using an enzyme-linked immunosorbent assay (ELISA), a

Table 1. Baseline characteristics and laboratory parameters of study groups

	Acute Coronary Syndrome (n=65)	Stable Angina (n=25)	Control (n=18)
Age (Years)	57 ± 11	51 ± 13	55 ± 5
Sex (Male)	45 (69%)	19 (79%)	13 (72%)
Diabetes Mellitus	8 (12%)	3 (12%)	2 (11%)
Hypertension	10 (15%)	4 (16%)	3 (16%)
Current Smoking	24 (36%)	10 (40%)	7 (39)
Total Cholesterol (mg/dl)	198 ± 42	202 ± 50	177 ± 35
LDL Cholesterol (mg/dl)	112 ± 34	117 ± 28	96.5 ± 39
HDL Cholesterol (mg/dl)	47 ± 13	42 ± 11	49.0 ± 7
Triglycerides (mg/dl)	183 ± 105	199 ± 96	195 ± 55
Hb (gr/dl)	14 ± 1	14 ± 2	13 ± 2
Platelets ($x10^3/\mu l$)	248.969 ± 55.748	274.854 ± 47.165	236.111 ± 54.147
WBC ($x10^3/\mu l$)	8132 ± 2824	7920 ± 2415	7166 ± 2088
Creatinin (mg/dl)	0.8 ± 0.2	0.7 ± 0.3	0.8 ± 0.1
ALT (mg/dl)	21 ± 9	18 ± 7	19 ± 7
BUN (mg/dl)	15 ± 5	14 ± 4	14 ± 2
Medications			
Nitrates	49 (75%)	16 (64)	13 (%72)
ACE inhibitors	13 (20%)	6 (24%)	4(22%)
Calcium antagonists	21 (32%)	9 (36%)	7 (38%)
Beta blockers	16 (23%)	6 (24%)	4 (22%)
Aspirin	65 (100%)	65 (100%)	18(100%)

LDL cholesterol: low-density lipoprotein cholesterol; HDL cholesterol: high-density cholesterol; Hb: hemoglobin; WBC: white blood count; ALT: alanine amino transferase; BUN: blood urea nitrogen; ACE: angiotensin converting enzyme; P value >0.05 for all

commercially available kit (human osteopontin assay kit, IBL, Japan). This ELISA kit was recently developed based on the method reported by Kon et al.,[21] and it measures total concentration of phosphorylated and nonphosphorylated forms of osteopontin in plasma.

Coronary Angiography

Coronary angiographies were performed via femoral approach with the Judkins technique and recorded using a cineangiogram system (Siemens, Germany). All the coronary angiograms were evaluated by two cardiologists, blinded to the clinical findings and osteopontin data. CAD was defined as ≥1 coronary artery having >50% luminar diameter stenosis. The extent of coronary artery stenosis was determined by the number of vessels with >50% stenosis. Normal coronary angiogram was defined as no or minimal lesion (<25%).

Study Design and Endpoints

The study was approved by the local ethics committee, and all patients granted their written inform consent to participate. After baseline blood sampling at the time of initial evaluation in the emergency department, all patients with acute coronary syndrome were treated with aspirin 325 mg, p.o. metoprolol 50 mg, p.o. nitroglycerin 5–10 μg/min IV infusion, and subcutaneous enoxaparin 100 IU/kg; all the coronary angiographies were performed within the 24 hours of presentation. Patients with stable angina pectoris and control group subjects received aspirin, beta blockers, calcium channel blockers and nitrates as needed. The endpoint of the study was the assessment of the plasma levels of osteopontin and its relationship with the extent of CAD in acute coronary syndrome patients.

Statistical Analysis

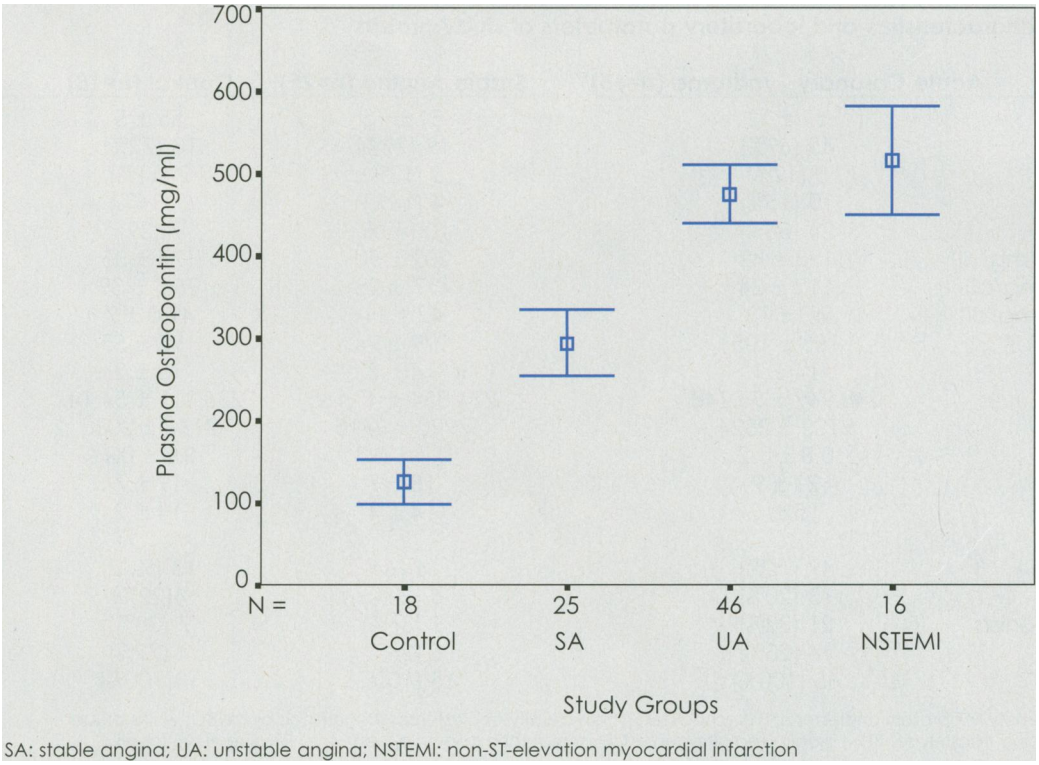
All results were expressed as mean ± SD. Kolmogorov-Smirnov test revealed that data distribution was normal. Any differences among the ≥3 groups were evaluated by ANOVA with Scheffe's test for parametric variables. Associations of plasma osteopontin levels with the extent of coronary stenosis and biochemical markers were evaluated by Spearman's correlation coefficient. A p value of <0.05 was considered to be statistically significant. All analyses were performed with SPSS® 11.0 software.

RESULTS

The clinical characteristics of the patients with acute coronary syndrome and stable angina, and the control subjects are shown in Table 1. There was no significant difference among the three groups with respect to age; sex; total cholesterol; LDL cholesterol; total leukocyte counts; platelet counts; and cardiovascular risk factors, including smoking, diabetes mellitus and hypertension.

Sixty-five acute patients with coronary syndrome were studied (45 male and 20 women), and the mean age was 57 ± 11 years. Sixteen patients were diagnosed

Figure 1. Plasma osteopontin levels of the control subjects, and patients with stable angina, unstable angina and non-ST-elevation myocardial infarction



SA: stable angina; UA: unstable angina; NSTEMI: non-ST-elevation myocardial infarction

as NSTEMI and 49 patients as unstable angina. Nineteen men and six women (mean age 50 ± 11 years) were included in the stable angina group. The age- and sex-matched control group involved 18 patients with chest pain and normal coronary angiograms.

Coronary angiography was performed in 45 of the 65 patients presenting with acute coronary syndrome. Fifteen patients had one-vessel disease (acute coronary syndrome group 1), 12 patients had two-vessel disease (acute coronary syndrome group 2), and 14 patients had three-vessel disease (acute coronary syndrome group 3). In the stable angina pectoris group, 13 patients had one-vessel disease and 12 patients had two-vessel disease.

The plasma osteopontin concentration of the acute coronary syndrome patients (495 ± 118 ng/ml) was significantly higher than the stable angina group (319 ± 106 ng/ml) and the control group (125 ± 54 ng/ml) (p=0.0001) (Figure 1). The patients in the stable angina group had higher mean plasma osteopontin concentrations than the control group (p=0.0001). The patients with unstable angina and NSTEMI had similar mean plasma osteopontin levels (487 ± 117 ng/ml, and 518 ± 173 ng/ml, respectively) (p=NS). The plasma osteopontin levels were lower in the patients with stable angina pectoris who had one-vessel disease (233 ± 86 ng/ml) compared to those with two-vessel disease (359 ± 54 ng/ml) (p=0.01) (Figure 2).

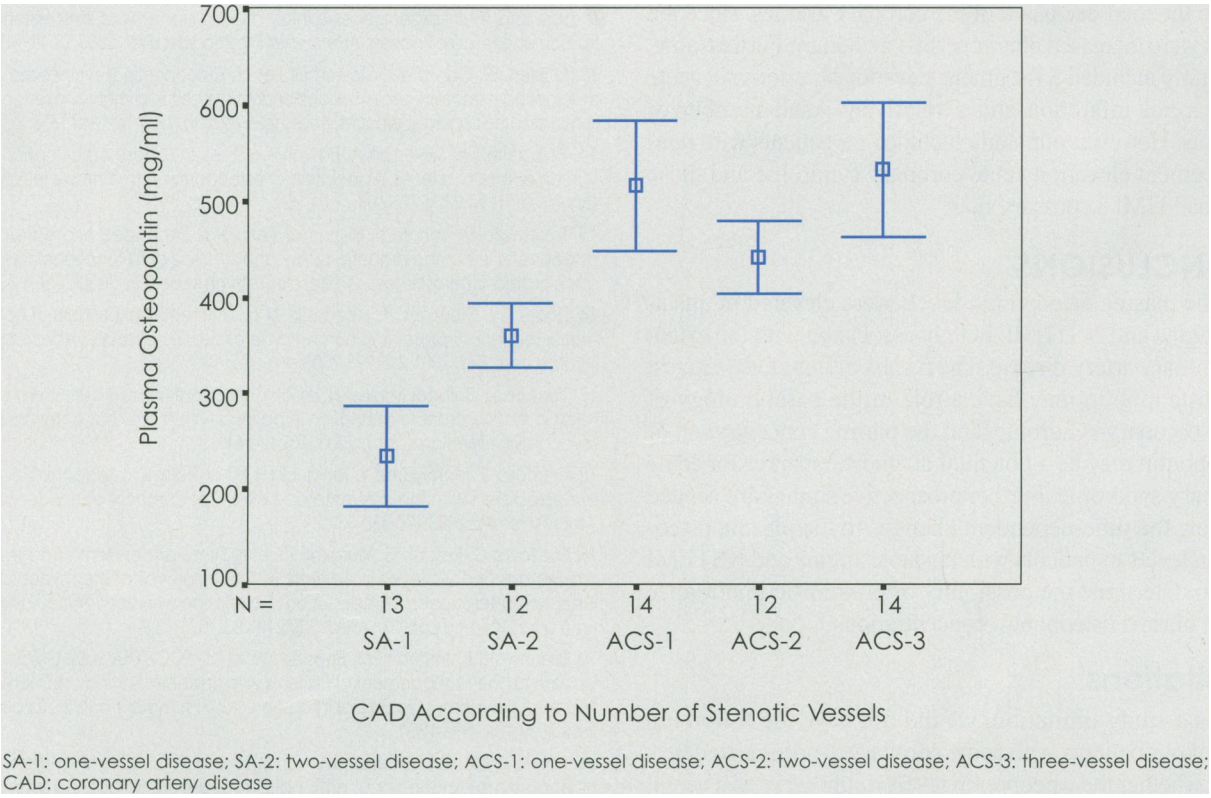
The plasma osteopontin levels in patients with acute coronary syndrome with one-, two- and three-vessel disease were similar (515 ± 116 ng/ml, 440 ± 59 ng/ml and 532 ± 122 ng/ml, respectively) (p=NS). Hence, we found no stepwise increase in the plasma osteopontin levels depending on the number of the vessels with >50% stenosis (Figure 2). The plasma osteopontin levels in patients with acute coronary syndrome did not correlate with the extent of coronary stenosis (r=-0.1, p=0.5) and, similarly, there was no correlation between the plasma osteopontin levels and the cardiac troponin-T levels as well (r=0.1, p=0.6).

DISCUSSION

This study demonstrates that the plasma osteopontin levels are significantly elevated in patients with unstable angina and NSTEMI. This study provides the first evidence that plasma osteopontin levels increase in the patients with non-ST-segment elevation acute coronary syndromes. However, we found no relationship between the plasma osteopontin levels and the extent of CAD extent in those patients

Coronary atherosclerosis is a chronic inflammatory process with acute exacerbations leading to unstable coronary syndromes. The pathophysiological mechanisms responsible for acute coronary syndromes are believed to involve an acute inflammatory stimulus that

Figure 2. Plasma osteopontin levels of patients with acute coronary syndrome and stable angina, according to the extent of coronary artery disease



SA-1: one-vessel disease; SA-2: two-vessel disease; ACS-1: one-vessel disease; ACS-2: two-vessel disease; ACS-3: three-vessel disease; CAD: coronary artery disease

contributes to coronary plaque disruption.[6,7] Pathological studies have demonstrated an increased inflammatory process at the site of plaque rupture with a dense inflammatory cell infiltrate, including macrophages.[3-5] The presence of osteopontin in atherosclerotic plaque is believed to exert its effect through upregulation within and in proximity to activated cells.[13-16] Elevated levels of osteopontin may be an important indicator of active inflammation and unstable coronary plaque.

We found that plasma osteopontin levels were high in the patients with stable angina pectoris who had two-vessel rather than one-vessel disease; however, in patients with acute coronary syndrome, osteopontin levels did not increase depending upon the number of vessels with >50% stenosis, nor did we find a correlation between osteopontin levels and CAD. This finding differs from Ohmoria et al., who found a correlation between osteopontin levels and CAD as well as a correlate between its extent in patients with stable coronary artery disease.[18]

A recent study evaluating the plasma osteopontin levels in 18 patients with acute myocardial infarction after successful reperfusion with primary coronary angioplasty showed that plasma osteopontin levels on the day of admission were similar to the control group; however, it increased in a time-dependent manner and reached to the maximal level on day three.[19] The normal plasma osteopontin levels on the day of admission might be due to the lack of a coronary angiographically documented healthy control group since those patients had a mean age 62 ± 8 years. Delayed elevation of plasma osteopontin levels observed in that study might be due to the total occlusion of the coronary arteries, since the levels were increased after reperfusion therapy. Furthermore, that study included ST-segment elevation anterior-wall acute myocardial infarction and a relatively small number of patients. However, our study included 65 patients with non-ST-segment elevation acute coronary syndrome and those who had TIMI 3 coronary flow.

CONCLUSIONS

The plasma osteopontin levels were elevated in unstable angina and NSTEMI, but not associated with the extent of coronary artery disease. The results of this study suggest that osteopontin may have a role in the pathobiology of acute coronary syndrome, and the plasma concentration of osteopontin may be a potential diagnostic marker for acute coronary syndrome. Further prospective studies are needed to show the time-dependent changes in the plasma osteopontin levels in patients with unstable angina and NSTEMI and to determine the possibility of prognostic importance of the plasma osteopontin concentrations.

Limitations

As a study limitation, we did not have the follow-up data of our patients with acute coronary syndrome to determine whether the osteopontin levels might serve as a useful marker for the prognostic purposes. In addition, we consider that the assessment of coronary calcification with computed tomography should be performed to show association between the plasma osteopontin levels and the coronary calcification. However, this seems to be time consuming, and not practical in the acute clinical settings.

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