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The value of serum osteoprotegerin levels in patients with angina like chest pain undergoing diagnostic coronary angiography

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Abstract

Background: *Osteoprotegerin (OPG) is a member of the tumor necrosis factor superfamily. Recent evidence supports a relationship between serum OPG level and atherosclerosis. The aim of this study was to evaluate the possible association of OPG with the presence of coronary artery disease (CAD), its severity and prognosis in patients with chest pain and suspected coronary stenosis.*

Methods: *In this cross-sectional analytic study, 180 candidates of elective coronary artery angiography were recruited. Serum level of OPG was measured by ELISA method in all patients and its relation with presence and severity of CAD based on a coronary atherosclerosis score (CAS) was assessed. Patients were followed for a mean period of about 24 ± 3.2 months and the relationship between OPG levels and future cardiac events were evaluated.*

Results: *The mean serum level of OPG was 1637 ± 226 pg/mL in those with CAD and 1295 ± ± 185 pg/mL (nonparametric p = 0.001) in those without it. There was a significant direct correlation between the level of serum OPG and CAS (rho = 0.225, p = 0.002). The optimal cut-off point for predicting a significant coronary artery obstruction was a serum level of ≥ 1412 pg/mL with a sensitivity and specificity of 60% and 57.8%, respectively. Major adverse cardiac events (MACE) including cardiovascular death, admission with acute coronary syndrome, or heart failure, was significantly higher in those with higher OPG levels (22 [34.3%] vs. 15 [16%], p = 0.012).*

Conclusions: *There was a direct and significant correlation between the serum level of OPG and CAS. MACE occurred more commonly in those with higher baseline OPG levels.* (Cardiol J 2013; 20, 3: 261–267)

Key words: coronary artery disease, osteoprotegerin, atherosclerosis

Introduction

The receptor activator of nuclear factor-K_β ligand (RANKL) is recently known as an important factor in osteoclastogenesis. Osteoprotegerin (OPG), a member of the tumor necrotic factor receptor family and a decoy receptor for RANKL plays its role in bone remodeling by blocking RANKL-ligand [1]. Actually it is the indirect inhibitor of the osteoclasts.

Osteoprotegerin is expressed in vivo by endothelial cells, vascular smooth muscle cells, and osteoblasts [2]. High serum OPG levels may lead to vascular calcification, formation of atheroscle-

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rotic plaques, increasing blood pressure, and cardiovascular diseases [3]. Recent studies proposed a relationship between the serum OPG level with prevalence and severity of coronary artery disease (CAD) [4, 5], the degree of arterial calcification [6–8] and presence of unstable coronary plaques [9, 10]. However, the predictive value of OPG in determining CAD, compared with simple diagnostic tools like exercise tolerance test (ETT) was not studied before.

In this study we aimed to investigate the serum level of OPG in patients who referred to our hospital with chest pain and were candidates for coronary angiography. We tried to determine whether the serum OPG level could be used as a diagnostic tool for identifying the presence and severity of CAD or not. Almost all of the previous studies have used the number of stenotic coronary arteries as a marker of CAD severity, however, we used a coronary artery severity score [11] to evaluate more accurately the possible relationship between CAD severity and OPG levels. Also we evaluated the prognostic impact of OPG level in this cohort of our patients during midterm follow-up period.

Methods

This cross-sectional study was conducted in Shahid-Madani Heart Hospital of Tabriz with the approval of Scientific and Ethical Review Boards of Tabriz University of Medical Sciences, Tabriz, Iran. Written informed consent was obtained from all patients.

Participants

Between April 2008 and April 2009, 180 patients who fulfilled the criteria for suspected stable angina pectoris based on history and/or positive provocational tests (ETT, stress echocardiography or myocardial perfusion imaging) and were candidates for diagnostic coronary angiography were enrolled in the study. Patients with previously documented CAD, those with history of myocardial infarction, unstable angina, or coronary artery bypass graft, or those with any specific condition which could affect on the serum levels of OPG (including proved malignancy, serum creatinine > 2 mg/dL, treatment with corticosteroids or immunosuppressant drugs and being menopaused) were excluded from the study. Demographic data and distribution of risk factors were assessed through an interview preceding the coronary angiography. Diabetes was considered present if a patient was treated with insulin or oral agents or had a fasting glucose level ≥ 126 mg/dL. Hypertension was defined by current use of antihypertensive treatment, systolic blood pressure ≥ 140 mm Hg or diastolic blood pressure ≥ 90 mm Hg, or a combination of these. Hyperlipidemia was defined as total cholesterol level ≥ 240 mg/dL, the current use of lipid-lowering treatment, or both. Patients were followed for a mean period of 24 ± 3.2 months with telephone call or outpatient visit to determine the future cardiac events or mortality. All cause death, cardiovascular death, any admission with acute coronary syndrome (ACS) or heart failure (HF) were recorded.

Angiography and coronary atherosclerosis scoring

For angiography we used the usual method with 4 views for left coronary artery and 2 views for right coronary arteries. The severity of vascular obstruction was determined according to the Coronary Atherosclerosis Scoring (CAS) system described by Gensini [11].

In CAS system, the entire coronary tree was divided into 8 segments: main left coronary artery (MLCA), left anterior descending artery (LAD), proximal segment (first one-third) of the septal branch of LAD, proximal segment of the diagonal branch of the LAD, circumflex artery, proximal segment of obtuse marginal branch of circumflex artery, right coronary artery, and proximal segment of the posterior descending artery. The percentage of luminal obstruction in the coronary arteries' circulation was measured. The severity of obstruction was determined according to the following scores: Score 1 for plaques with < 50% luminal narrowing, score 2 for 50–74% reduction in luminal diameter, score 3 for 75–99% reduction in luminal diameter and score 4 for total occlusion.

The patients were categorized into 2 groups according to the findings of angiographic evaluation: a group of 135 patients with CAD, and a group consisted of 45 patients without any evidence of CAD in angiography.

Laboratory experiments

Blood samples were drawn from all participants at the morning of the day they scheduled to undergone angiography, after 12 hours of fasting. The blood samples were centrifuged and the sera were freezed at –70°C. The concentration of both total cholesterol and triglyceride were measured using Bio Systems spectrophotometric assay kits. High- and low-density lipoprotein was measured

Table 1. Clinical and paraclinical characteristics of patients in both groups with and without coronary artery disease (CAD).

Characteristics	CAD (n = 135)	Controls (n = 45)	P
Age: mean ± SD (median)	56.7 ± 10.5 (56)	51.3 ± 10.6 (51)	0.002
Male sex	121 (89.6%)	25 (55.6%)	< 0.001
Body mass index [kg/m^2]	26.6 ± 3.8 (26)	28.4 ± 5.9 (28)	0.016
Familial history of CAD	15 (11.1%)	4 (8.9%)	0.459
Hypertriglyceridemia	39 (28.9%)	8 (17.8%)	0.099
Hypertension	58 (43%)	25 (55.6%)	0.142
Diabetes mellitus	26 (19.3%)	4 (8.9%)	0.106
Current smoking	47 (34.8%)	8 (17.8%)	0.039
Recent therapy with statins	47 (34.8%)	16 (35.6%)	0.928
Serum hemoglobin [mg/dL]	14.1 ± 1.7 (14)	13.7 ± 1.7 (13.5)	0.205
Low-density lipoprotein [mg/dL]	194.6 ± 79.4 (187)	181.9 ± 81.8 (167)	0.363
High-density lipoprotein [mg/dL]	27.5 ± 15.4 (24)	35.6 ± 19.9 (29)	0.094
Total cholestrol [mg/dL]	209.9 ± 65.7 (197)	200.1 ± 66.6 (176)	0.389
Triglyceride [mg/dL]	257.3 ± 64.1 (234)	237.1 ± 74.4 (212)	0.080
Creatinine [mg/dL]	0.9 ± 0.2 (0.9)	0.9 ± 0.2 (0.9)	0.630
Serum osteoprotegerin [pg/mL]	1637 ± 226	1295 ± 185	0.001

for all sera. The serum OPG level was measured using the enzyme-linked immunosorbent assay (ELISA).

Statistical analysis

The patients' characteristics including age, sex, body mass index (BMI), familial history of CAD, hyperlipidemia, hypertension, diabetes mellitus, history of smoking, and recent therapy with statins were collected and compared among two groups with and without CAD.

We used the SPSS software version 16 for statistical analysis. The data were provided as mean ± SD for quantitative variables, and frequency (%) for the qualitative variables. Quantitative variables were compared among groups using Independent T-test, Mann-Whitney U-test, or one-way ANOVA and Tukey test. The quantitative (categorical) variables were compared using contingency tables, and by χ^2 test, and Fisher's exact test, when needed. Receiver operating characteristics (ROC) curve and coordinates of the curve were used in order to determine the cutoff point of serum OPG level in identifying patients with CAD. Logistic regression was used to determine the independent factors. $P \leq 0.05$ was considered statistically significant. The sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and accuracy of the serum OPG level was calculated separately.

Results

One hundred-eighty subjects were categorized according to the findings of angiographic study into two groups: a group of 135 (75%) patients with CAD, and a group consisted of 45 (25%) patients without CAD in angiography. Regarding the severity of CAD, 40 (22.2%) subjects had single vessel disease (SVD), 54 (30%) cases had 2 vessels disease (2VD), 41 (22.8%) patients had 3 vessels disease (37 cases; 20.6%) or narrowing of the MLCA with or without other branch stenosis (4 cases; 2.2%). The mean CAD-score was 46.7 ± ± 4.1 (median = 46). The clinical and laboratory characteristics of the patients in both groups were summarized and compared in Table 1. The mean age, frequency of male patients and the rate of smoking were higher in the CAD group, and the mean BMI was higher in the control group. There was not statistically significant difference among 2 groups for other variables.

There was no significant difference in cholesterol or triglyceride or creatinine levels between 2 groups. The mean serum level of OPG was significantly higher among CAD patients compared to the others (1637 ± 226 pg/mL in CAD group vs. 1295 ± 185 pg/mL in control group; $p < 0.001$).

In general, ETT was performed in 79 (43.9%) patients. ETT was positive in 48 (82.8%) subjects of the CAD group, and 13 (61.9%) subjects of the

Table 2. The diagnostic value of exercise tolerance test (ETT) vs. serum osteoprotegerin (OPG) level.

	ETT	OPG	ETT + OPG
True positive	48 (60.8%)	81 (45%)	57 (72.2%)
True negative	8 (10.1%)	26 (14.4%)	4 (5.1%)
False positive	13 (16.5%)	19 (10.6%)	17 (21.5%)
False negative	10 (12.7%)	54 (30%)	1 (1.3%)
Sensitivity	82.8%	60%	98.3%
Specificity	38.1%	57.8%	19.1%
Positive predictive value	79%	81%	77%
Negative predictive value	44.4%	32.5%	80%
Accuracy	70.9%	59.4%	77.2%

control group. The diagnostic value of ETT as compared to angiography was demonstrated in Table 2.

The area under the ROC curve for the serum OPG level in predicting CAD was calculated to be 0.609 ($p = 0.028$). The most appropriate cutoff point for both patient groups with and without CAD was serum OPG level of above 1412 pg/mL. The accuracy of serum OPG level (with above mentioned cutoff point) in predicting CAD was summarized in Table 2. The frequency of patients with serum OPG level ≥ 1412 pg/mL was higher in the case group compared to the control group ($p = 0.038$, OR = 2.1, 95% CI 1–4.1). This difference remained statistically significant, when adjusted for age, sex, BMI, and the history of smoking ($p = 0.042$, ExpB = 0.52). There was a significant correlation between serum level of OPG and CAD-score ($p = 0.002$, rho = 0.225). The mean serum OPG level was 1332.3 ± 135.6 pg/mL for patients with SVD, 1262.5 ± 121.2 pg/mL for patients with 2VD, and 1637.5 ± 163.5 pg/mL for patients with 3VD. The difference among 3 groups of patients (SVD, 2VD, 3VD) was not statistically significant ($p = 0.598$). During a mean period of about 24 ± 3.2 months and excluding 22 (12.2%) patients we lost to follow, major adverse cardiac events (MACE; cardiovascular death, admission with ACS or HF) occurred in 37 (23.4%) patients (15 [16%] in those with OPG level less than cut off point and 22 [34.3%] in those with OPG levels above cut off limit, $p = 0.013$). Figure 1 shows that most of this difference was due to higher frequency of admissions with ACS in patients with high OPG levels. Figure 2 shows Kaplan-Meier survival curves for patients with OPG levels higher or lower than 75th percentile. Multivariate Cox analysis showed OPG level and positive ETT result as independent predictors of time to occurrence of MACE (Table 3).

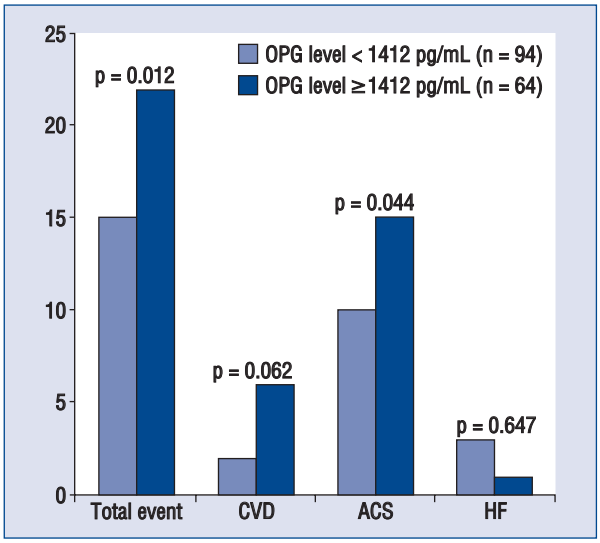

Figure 1. The distribution of major adverse cardiovascular events between groups with osteoprotegerin (OPG) levels higher and lower than cut off level; CVD — cardiovascular death; ACS — acute coronary syndrome; HF — heart failure.

Discussion

Our study showed that adding serum OPG measurement to ETT in evaluating patients with chest pain and suspected CAD increases its sensitivity and NPV. Also we showed that serum OPG level is correlated with the severity of atherosclerotic coronary involvement as reflected in CAS. Finally we showed that patients with higher OPG levels have higher MACE rates in midterm follow-up mainly due to higher occurrence of ACS related to plaques instability.

Jono et al. [5] in their study on 201 patients who underwent coronary angiography found

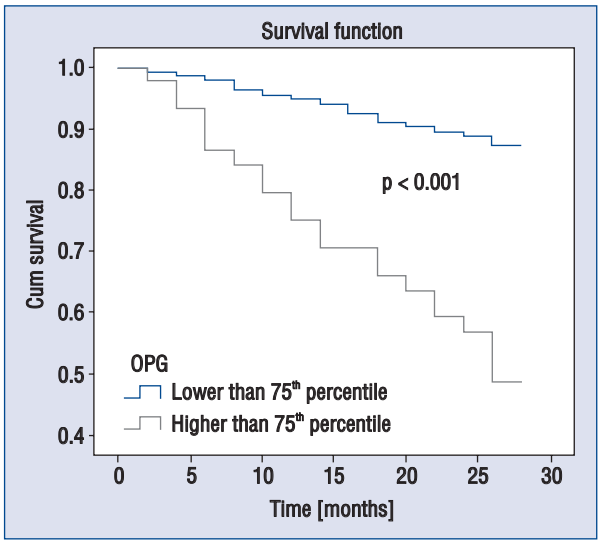

Figure 2. Kaplan-Meier event free survival curves for patients with osteoprotegerin (OPG) levels higher (gray) and lower (blue) than 75th percentile value of OPG (1485 pg/mL).

a higher OPG level in CAD patients compared to the others. The serum OPG level increased along with the increase in the severity of CAD. They finally concluded that serum OPG levels are associated with the presence and severity of CAD, suggesting that OPG may be involved in the progression of CAD. In the Copenhagen City Heart Study, Mogelvang et al. [12] showed that, after multivariate adjustment, for each doubling of the plasma OPG concentration, the risk for subclinical peripheral atherosclerosis increased by 50%. They concluded that OPG is independently associated with traditional risk factors of atherosclerosis, subclinical peripheral atherosclerosis, and clinical atherosclerotic disease such as ischemic heart disease and ischemic stroke. Anand et al. [13] implemented a study on 510 patients with type 2 diabetes mellitus without any manifestation in favor of CAD and showed that the serum OPG level could predict both subclinical CAD and near-term cardiovascular events. All these studies and many similar other reports [9, 14, 15] are strongly supporting an association between CAD and serum OPG level. We evaluated its sensitivity in a cohort of patients with suspected stable CAD. To the best of our knowledge, the applicability of serum OPG level as a diagnostic tool in comparison with other available modalities were not investigated before.

According to the cutoff point provided in our study, the frequency of patients with high serum OPG level (≥ 1412 pg/mL) was demonstrated to be higher among the CAD patients compared to the control group (even when controlled for age, sex, and history of smoking). The sensitivity and specificity of serum OPG level in differentiating among patient with and without CAD, was moderate (60% and 57.8%, respectively). The ETT was implemented for a subgroup of patients based on the attending physicians decision. The sensitivity of ETT was demonstrated to be 82.8% with a low specifity (38.1%) which can be attributed to the higher number of patients with normal coronary arteries resulted from a long list of our exclusion criteria. However the specifity for high OPG level was higher than ETT (57.8% vs. 38.1%). The concurrent use of ETT and serum OPG level was also studied with the aim to improve the patient selection for coronary angiography. Although the sens-

Table 3. Multivariate Cox analysis in which continuous covariates including age and body mass index were analyzed and interpreted per unit, but the serum osteoprotegerin (OPG) level was analyzed and interpreted per cutoff value. However according to the p values, only the OPG level and exercise tolerance test (ETT) result was demonstrated to have significant impact on the time of major adverse cardiovascular events occurrence.

	P	Hazard ratio (HR)	95% confidence interval for HR	
			Lower	Upper
Age	0.669	1.007	0.974	1.041
Body mass index	0.485	1.031	0.946	1.123
Smoking	0.291	1.586	0.673	3.734
Diabetes	0.236	0.602	0.260	1.394
Male sex	0.634	0.787	0.293	2.110
Hyperlipidemia	0.973	1.015	0.427	2.412
Higher OPG level	0.012	0.039	0.030	0.479
Positive ETT	0.022	0.045	0.030	0.642

itivity was increased significantly by this method, the specificity was decreased. Interestingly NPV was increased to about 80%. If we could prove this in larger studies it will help us to find a safe and cheap approach compared with scintigraphy or computed tomography angiography to evaluate the presence and risk of CAD especially in this low risk subgroup of patients with higher false positive results in conventional diagnostic modalities like ETT or myocardial perfusion imaging studies.

In our study the median of serum OPG level was significantly higher among CAD patients compared to the others. Reinhard et al. [16] showed that increased OPG level was an independent predictor of significant CAD. Some studies have reported a direct correlation between OPG level and the number of involved coronary arteries [5, 17]. We could not show a statistically significant correlation between mean serum OPG level and the number of involved coronary arteries (SVD, 2VD, 3VD). Previous studies demonstrated that the CAD scoring system is better than categorizing CAD patients according to number of involved coronary arteries in determining the severity of disease [18]. We showed a significant correlation between serum OPG level and CAD-score and we believe that CAS provides more accurate measurement of coronary atherosclerosis burden.

In our study during a midterm follow-up period of about 2 years, MACE, defined as cardiovascular death, ACS and HF occurred more commonly in patients with higher OPG levels. Most part of this difference was related to higher rate of ACS in patients with higher serum OPG levels, also there was a trend to higher rate of cardiovascular death in this group (Fig. 1). Vik et al. [19] followed 6265 subjects recruited from general population with a mean follow-up period of about 10.6 years and they found that increase in OPG level was associated with an increased future risk of myocardial infarction, total mortality, and mortality of ischemic heart disease independent of traditional cardiovascular risk factors. Similarly Pederson et al. [4] reported increased risk of all-cause mortality, cardiovascular mortality and myocardial infarction with increasing OPG levels among patients with suspected stable angina, but independent effects were mainly confined to OPG levels above the 90th percentile. Higher OPG level was an independent predictor of poor cardiac prognosis in patients with intermediate coronary lesions [2]. Also serum OPG level was a strong predictive of long-term mortality and HF development in patients with ACS, independent of conventional risk markers [20]. One proposed mechanism for this increased rate of plaque instability and poor cardiovascular outcome is up-regulated expression of OPG in endothelial cells, in the presence of proinflammatory cytokines, which in turn increases the expression of endothelial cell adhesion molecules, up-regulation of the inflammatory cells and increased activity of matrix metalloproteinase which leads to degradation of the extracellular matrix and reduced thickness of the fibrous cap, the erosion of which causes thrombus formation [1].

Limitations of the study

This was a single center study with limited sample volume. Non invasive diagnostic modalities were not used for all patients and the possibility of a selection bias by attending physicians could not be ruled out. To exclude possible confounding factors in measuring OPG levels we selected a relatively low risk group of patients and traditionally this may be associated with higher rate of false positive results in conventional tests. Samples were stored at −70°C until analysis, and the possibility of protein degradation cannot be excluded.

Conclusions

This study showed that serum OPG level may be a simple adjuvant to other noninvasive diagnostic modalities of CAD. Higher OPG levels are associated with extensive atherosclerosis burden, higher rate of plaque instability and cardiovascular events in midterm follow-up of patients with suspected CAD.

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Conflict of interest: none declared

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