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Association between the Serum Osteoprotegerin Level and Target Lesion Calcium in Coronary Artery Disease

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ABSTRACT

Background and Objectives : Osteoprotegerin (OPG) is a decoy receptor for receptor nuclear factor-kB ligand (RANKL). We sought to evaluate the association between the serum OPG level and the target lesion calcium (TLC) in those patients suffering with coronary artery disease (CAD). **Subjects and Methods :** We assayed the serum OPG levels in 65 CAD patients (mean age: 62±10 yrs, M : F=46 : 19) with using enzyme immunoassay, and these patient underwent intravascular ultrasound (IVUS) examinations of their target lesions. The degree of TLC was estimated by the maximum arc of calcium and also the calcified plaque surface area that was calculated from the serial cross-section IVUS images. **Results :** The median serum OPG levels were greater in the subjects with TLC than in the subjects without TLC (1.36 vs 0.95 ng/mL, respectively, $p<0.05$). Significant correlation was noted between the serum OPG levels and the maximum arc of calcium ($r=0.274$, $p=0.027$). The median serum OPG levels were significantly increased more in the subjects who had a maximum arc of calcium ranging from 90 to 180 degrees than in those subjects who had a maximum arc of calcium less than 90 degrees (1.63 vs 1.14 ng/mL, respectively, $p<0.05$) and the median serum OPG levels were also increased more in the subjects who fell within the second tertile of the calcified plaque surface area than that in those subjects who fell within the first and third tertile (0.96, 1.53, 1.40 ng/mL for the first, second, third tertile, respectively, $p<0.05$). On the stepwise multivariate logistic regression analysis, the serum OPG level remained a risk factor for TLC after adjustment was made for the other risk factors such as age, diabetes mellitus, HbA1C and a smoking history ($p=0.019$, odds ratio 5.208 [95% confidence interval: 1.308-20.744]). **Conclusion :** In patients with CAD, an increased serum OPG level is associated with target lesion calcification. **(Korean Circulation J 2006;36:337-342)**

KEY WORDS : Osteoprotegerin ; Coronary artery disease.

Introduction

When performing percutaneous coronary intervention (PCI), the presence of coronary calcification is associated with other unfavorable lesion morphologies, and it is related to procedural complications and failure for successful balloon dilation.[1)2)] Target lesion calcification is also associated with suboptimal stent expansion[3)] and stent thrombosis.[4)] Coronary calcification is associated with the patients' age and with the presence and severity of atherosclerosis.[5-9)] In addition, endochondral bone formation in coronary arteries has been suggested as a possible mechanism of coronary calcification,[10)] and many bone regulatory factors have been identified in calcified atherosclerotic lesion, which suggests a regulatory role of the osteogenic factors for vascular calcification.[11)12)]

Osteoprotegerin (OPG) and receptor activator of nuclear factor-κB ligand (RANKL) are both novel members of the tumor necrosis factor (TNF) receptor signaling family, and this family constitutes the final effectors of osteoclast function. Therefore, they represent the essential regulators of bone mass and bone homeostasis.[13)]

Even though OPG deficient mice developed both severe osteoporosis and medial arterial calcification of the aorta and renal arteries,[14)] the association between

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coronary calcification in humans and OPG remains unclear. Therefore, we evaluated the relationship between the serum OPG level and target lesion calcium in those patients with coronary artery disease (CAD) by assessing the lesions with intravascular ultrasound (IVUS) while we were performing PCI.

Subjects and Methods

From March 2003 to August 2004, we recruited 65 sequential patients, including 7 cases of acute myocardial infarction, 11 cases of unstable angina and 47 cases of stable angina, and all these patients underwent intravascular ultrasound (IVUS) examination before their PCI procedures. 59 lesions were de novo and 6 were restenosis lesions. The patients' blood pressure, body mass index (BMI) and their hematological and biochemical profile were determined at the time of their physical examination. The patients with a past history or present illness of thyroid disease, parathyroid disease, a nephropathy condition with a serum creatinine level >2.0 mg/dl or a tumor involving bone, and also those patients with abnormal serum calcium and phosphorous levels were excluded from the present study.

The patients were considered as having type 2 diabetes mellitus (DM) if they were being treated with insulin or oral hypoglycemic agents, or if they had a fasting glucose level ≥126 mg/dL. The subjects with persistent elevated blood pressure (≥140/90 mmHg) or those subjects who were taking antihypertensive medications were classified as hypertensive. This study was approved by our institutional review committee; the subjects were informed of the investigative nature of this study and a written consent was obtained before study entry.

Evaluation of target lesion calcium (TLC) with using intravascular ultrasound (IVUS)

IVUS was performed with a standard 3.2 Fr, 30-MHz ultrasound catheter (Cardiovascular Imaging Systems, SciMed/Boston Scientific, Minneapolis, Minnesota) that was advanced over a 0.014-inch guidewire. The IVUS catheter uses a movable transducer within the catheter sheath that allowed accurate, reproducible translation of the transducer at the tip of the catheter when this was used in conjunction with a motorized pullback device (Cardiovascular Imaging Systems, SciMed/Boston Scientific).

For the patients suffering with acute myocardial infarction, the target lesion was the infarct-related lesion that was identified by the combination of left ventricular wall motion abnormality, the ECG findings and the angiographically determined lesion morphology. For the patients with multivessel disease, the lesions with a more severe diameter stenosis that were eligible for PCI were selected as the target lesions, but any chronic total occlusions were excluded. The lesion site selected for analysis was that lesion-image slice having the largest arc of calcium. Calcium was identified by its distinctly bright echogenic appearance with the corresponding acoustic shadowing (Fig. 1B, C). The degree of TLC was estimated by measuring the largest arc of calcium with a protractor that was centered on the lumen. When multiple calcium deposits were observed and/or the arcs of calcium were interrupted, all the arc angles were summed (Fig. 1D). To quantify the TLC, we also measured the calcified plaque surface area as reported by Scott et al.[15] The calcified plaque-lumen circumferential lengths were measured from the digitalized serial IVUS images that were taken 1.0 mm apart; with using Simpson's method, the calcified plaque surface area was then calculated (Fig. 2).

Serum OPG measurement

Blood samples were collected and stored at -80℃ until use. The serum OPG levels were determined by using a sandwich ELISA (DuoSet ELISA, R & D system, Minneapolis, Minnesota). Mouse anti-human OPG was used to capture the OPG from the serum. The captured OPG was then detected with biotinylated goat antihuman OPG monoclonal antibody and a tetramethylbenzidine substrate. All the samples were measured in duplicate and the results were averaged.

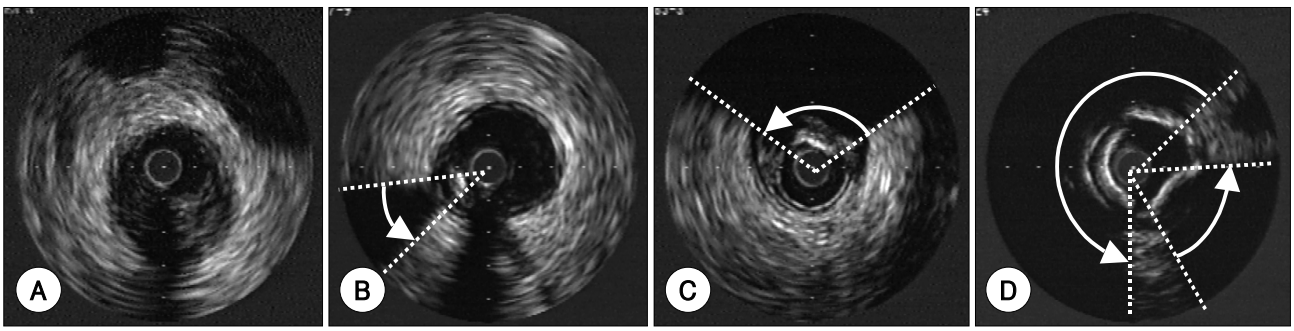

Fig. 1. Measurement of the maximum arc of calcium on the most severely calcified image slices of the target lesions with using a protractor centered on the lumen. Fig. A shows the non calcified soft plaque. In Figs. B and C, the maximum arcs of calcium were 15° and 115°, respectively. If multiple calcium deposits were found, all the arcs were summed, as is shown in Fig. D and the summed arc of calcium was 295°.

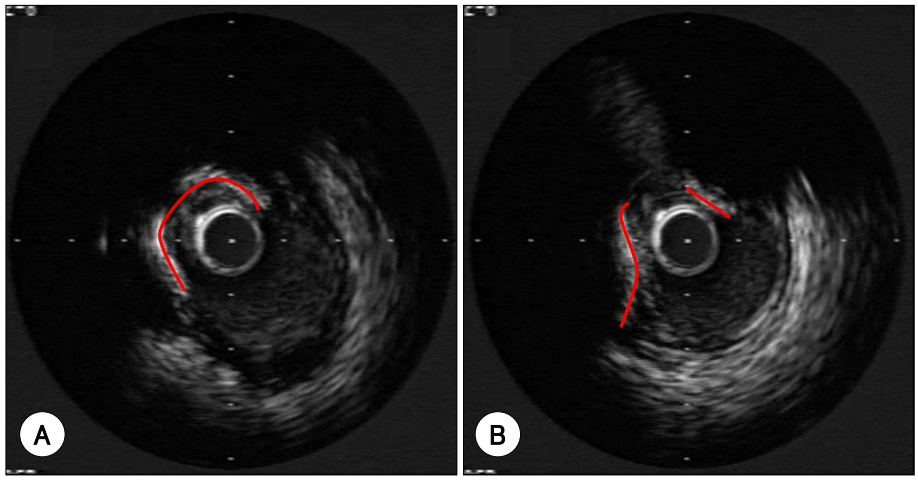

Fig. 2. Measurement of calcified plaque surface area. Fig. A and B are serial IVUS image slices 1.0 mm apart. The red lines represent the calcified plaque-lumen circumference. The calcium surface area is measured with the sum of the calcified plaque-lumen circumference length in the target lesion. IVUS: intravascular ultrasound.

Statistical analysis

The results were expressed as means±SDs or as medians with inter-quartile ranges (IQR), according to the characteristics of the continuous variables. Comparisons between the groups for the study variables were done with using unpaired Student's t tests or the Mann-Whitney test. The relationships between the largest arcs of TLC and serum OPG levels were evaluated by bivariated correlation analysis, and Pearson's correlation coefficient was then calculated. According to the maximum arc of the TLC (≤90°, >90° and ≤180°, >180°) and also the calcified plaque surface area (first, second or third tertile), the lesions were subdivided and analyzed by performing Kruskal-Wallis test. Finally, stepwise logistic regression analysis was used to compute the odds ratios (OR) and the 95% confidence intervals (CI) with the presence of TLC as the dependent variable and the serum OPG levels as the explanatory variable. All the statistical analyses were conducted using SPSS 13.0 for the Windows package.

Results

The study group included 46 men and 19 women, and their ages ranged from 28 to 84 years (mean age: 62±10 years). The mean value of the BMI was 25.0±2.6 kg/m^2. On the basis of the IVUS evaluation, 16 patients were categorized as being subjects without TLCs, and 49 patients were with TLCs. The characteristics of the 2 groups are given in Table 1. The patients with multivessel disease had a higher prevalence of TLC than those patients with one vessel disease (51% vs 19%, respectively, p<0.05). However, there were no significant differences in the other risk factors, including age, BMI, DM, hypertension, a smoking history, the lipid profile and the hemoglobin A1c (HbA1c) levels between the two groups.

Table 1. Patients' characteristics according to target lesion calcium

Variables	No TLC (n=16)	TLC (n=49)	p
Age (yrs)	57±13	63±9	0.093
Female gender (%)	4 (25)	15 (31)	0.361
BMI (Kg/m^2)	25.5±2.3	24.8±2.8	0.381
DM (%)	1 (6)	14 (29)	0.091
HTN (%)	9 (56)	25 (51)	0.779
Smoking history (%)	9 (56)	15 (31)	0.080
TC (mg/dL)	188±55	183±42	0.699
TG (mg/dL)	115±80	124±58	0.664
HDL (mg/dL)	34±6	38±10	0.187
HbA1c (%)	5.7±1.0	6.3±1.2	0.099
LAD (%)	14 (88)	46 (94)	0.509
Left main disease (%)	2 (13)	9 (18)	0.718
Multivessel disease (%)	3 (19)	25 (51)	0.022*

Data are means±SDs. BMI: body mass index, DM: diabetes mellitus, HTN: hypertension, TC: total cholesterol, TG: triglyceride, HDL: high density lipoprotein cholesterol, LAD: left anterior descending coronary artery, TLC: target lesion calcium

The median serum OPG levels were significantly higher in the patients with TLCs than in those patients without TLCs (median-IQR [ng/mL]: 0.95-0.67 to 1.25 vs. 1.36-0.89 to 1.81, respectively, p<0.05) (Fig. 3). The serum OPG levels were significantly positively correlated with the target lesions' maximum arcs of calcium (r=0.274, p<0.05) (Fig. 4). However, according to the subdivision of the maximum arcs of calcium (≤90°, >90° and ≤180°, >180°), the difference was only significant between the group having arcs less than 90° and those patients with arcs more than 90° and less than 180° (median-IQR [ng/mL]: 1.14-0.84 to 1.44 vs. 1.63-1.12 to 2.07, respectively, p<0.05) (Fig. 5). According to the subdivision of the calcified plaque surface area, the serum OPG level was significantly increased in the second tertile group more than that of first or third tertile groups (median-IQR [ng/mL]: 0.96-0.69 to 1.32 in

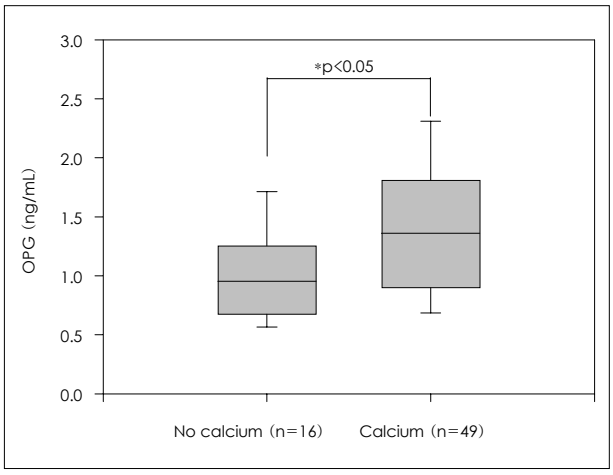

Fig. 3. The serum levels of osteoprotegerin according to the presence or absence of target lesion calcium. The central line represents the distribution median, the boxes span from the 25th to the 75th percentiles, and the error bars extend from the 10th to the 90th percentiles. OPG: osteoprotegerin.

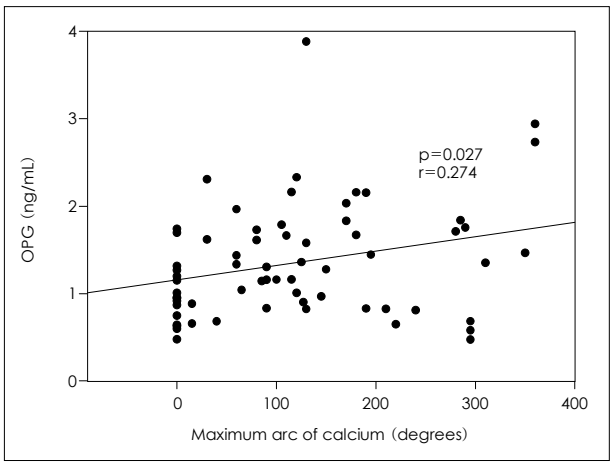

Fig. 4. The correlation between the maximum arcs of calcium in the target lesions and serum osteoprotegerin levels. OPG: osteoprotegerin.

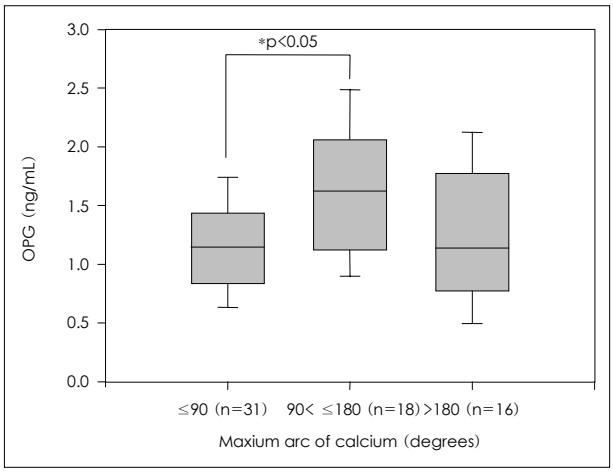

Fig. 5. The serum osteoprotegerin levels subdivided by the maximum arcs of calcium (≤90°, 90° and ≤180°, >180°) of the target lesions. The central line represents the distribution median, the boxes span from the 25th to the 75th percentiles, and the error bars extend from the 10th to the 90th percentiles. OPG: osteoprotegerin.

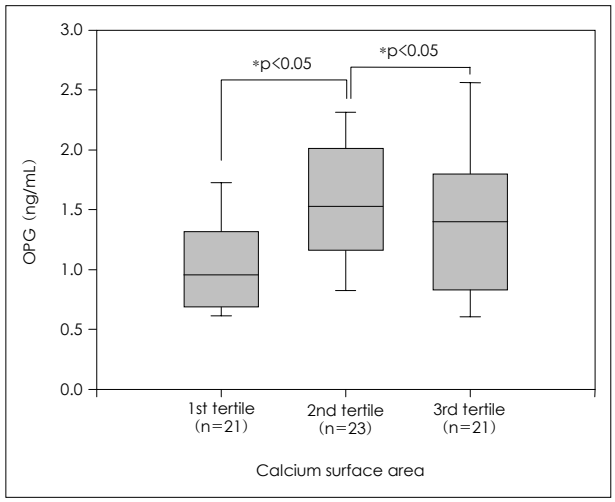

Fig. 6. The serum osteoprotegerin levels according to the subdivision by the calcified plaque surface area of the target lesions. The central line represents the distribution median, the boxes span from the 25th to the 75th percentiles, and the error bars extend from the 10th to the 90th percentiles. OPG: osteoprotegerin.

Table 2. Stepwise multiple regression analysis for the variables associated with TLC

	Odds ratio (95% CI)	p
Independent variables		
OPG	5.208 (1.308-20.744)	0.019
Excluded variables		
Age		0.142
DM		0.124
HbA1C		0.153
Smoking history		0.098

CI: confidence interval, OPG: osteoprotegerin, DM: diabetes mellitus, HbA1C: glycosylated hemoglobin, TLC: target lesion calcium

the first tertile group vs 1.53-1.16 to 2.02 in second tertile group vs 1.40-0.83 to 1.80 in the third tertile group, $p<0.05$) (Fig. 6). On the stepwise multiple regression analysis, the serum OPG levels were independently associated with TLC, and with an OR of 5.21 (95% CI: 1.31 to 20.74; $p=0.019$), after adjusting for age, type 2 DM, HbA1c and a smoking history (Table 2).

Discussion

The OPG and RANKL system plays an important role in bone homeostasis, and its role in vascular biology has recently received more attention. OPG is produced by a variety of tissues, including the cardiovascular system (heart, arteries and veins), lung, kidney, immune tissues and bone,[13)16)] and elevated serum OPG levels have been associated with cardiovascular death in elderly women,[17)] the presence and severity of CAD[18)19)] and the progression of vascular calcification in hemodialysis patients.[20)21)] The major finding of the present study is that the serum OPG levels were independently associated

with TLC after adjusting for age, which is known to be a strong predictor of coronary calcification.

Even though the serum level of OPG is often increased with vascular disease, the tissue level of OPG seems to be quite different. The expression of OPG has been reported to be decreased in calcific arotic valves[22] and in human calcified atheroslcerotic arteries;[12)23)] further, OPG and TRAIL(TNF related apoptosis inducing ligand) have recently been concurrently detected in the calcified regions of atherosclerotic arteries.[24]

Until now, it is unclear why the serum and tissue levels of OPG are different, and it is also unclear how OPG is associated with the vascular calcification. In the present study, the serum OPG levels were not increased more in those patients having a TLC greater than 180 degrees compared with those patients having a TLC between 90 and 180 degrees, and a similar finding was also noted when the TLCs were analyzed by the calcified plaque surface area. This bimodal distribution is consistent with the nonlinear sigmoidal progression of arterial calcification that has been proposed by Yoon et al,[25] and it suggests that OPG production is dynamic rather than constant in the course of vascular calcification. Furthermore, it might suggest that OPG can be a protective factor for vascular calcification, and the elevation of the serum OPG concentration is only a reflection of incomplete compensation of the OPG for vascular calcification.

In this study, the LDL cholesterol level and the HDL cholesterol level were not associated with the TLC, which is different from previous reports,[26)27)] and this is because calcification would be expected to correlate with the duration of exposure to hyperlipidemia, as measured in cholesterol-years.[28]

This is the first study that has shown the relationship between coronary calcification and the serum OPG level with performing direct visualization of the coronary arteries by using IVUS. However, there are some limitations in our study. First, because we performed IVUS evaluation only in the targeted PCI vessels, we could not estimate the calcification that might exist in the other coronary arteries in the same patient, and this makes it difficult to extend our results to the relationship between the total amount of coronary artery calcium and the serum OPG level. Second, even though IVUS is the most sensitive in vivo imaging modality to detect coronary calcium because it uses a high frequency transducer in close proximity to the high echogenic calcium,[29] the current IVUS method that is used to quantify calcium typically measures the arc of calcium in only a single plane at the target lesion and/or at the reference site.[8] Thus, it does not assess the calcium along the vessel and it may be an insufficient measure of the total calcium. In addition, even within a single slice of the artery, the depth-wise thickness of the calcium from a deposit within the lumen is difficult to ascertain due to acoustic shadowing.

In conclusion, an elevated serum OPG level is a strong predictor of TLC in the patients suffering with CAD, but further investigation is needed to reveal whether OPG plays an active role or if the increment of serum OPG concentration is only an incomplete counterregulatory mechanism of vascular calcification.

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