Relationship of serum osteoprotegerin levels with coronary artery disease severity, left ventricular hypertrophy and C-reactive protein


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ABSTRACT

OPG (osteoprotegerin) is an inhibitor of osteoclastogenesis and recent work suggests it has a role in atherosclerosis. Therefore we measured serum OPG levels in patients with coronary artery disease, compared the serum OPG levels among the different groups according to the number of stenotic vessels and determined whether there was any correlation with aortic calcification, LV (left ventricular) mass index and serum CRP (C-reactive protein) levels. Subjects (n = 100; mean age, 57 years) who underwent coronary angiograms were enrolled. Blood pressure, body mass index, fasting blood glucose, lipid profiles and CRP levels were measured and the LV mass indices were calculated using ECGs. Serum OPG levels were measured by ELISA. The presence of calcification in the aortic notch was checked by a chest X-ray. The subjects were divided into four groups according to the number of stenotic vessels. The mean serum OPG levels increased significantly as the number of stenotic vessels increased, and the mean serum OPG levels were higher in the group with three-vessel disease compared with the groups with no- or one-vessel disease. The mean serum CRP level was significantly higher in the group with three-vessel disease compared with the groups with no-, one- and two-vessel disease. Age and LV mass index showed significant positive correlations with serum OPG levels, although significance was lost after an adjustment for age. Serum CRP levels were positively correlated with serum OPG levels even after an adjustment for age. There were no differences in serum OPG levels according to the presence of fasting hyperglycaemia or aortic calcification. In conclusion, serum OPG level was related to the severity of stenotic coronary arteries and serum CRP levels. LV mass indices showed no significant correlation with OPG levels. The precise mechanism for the role of OPG in atherosclerosis needs to be investigated further.

Key words: atherosclerosis, coronary artery disease, C-reactive protein (CRP), left ventricular mass index, osteoprotegerin.

Abbreviations: BMI, body mass index; BP, blood pressure; CRP, C-reactive protein; DC, dendritic cell; ESRD, end-stage renal disease; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; LV, left ventricular; OPG, osteoprotegerin; RANK, receptor activators of NF-κB (nuclear factor κB); RANKL, RANK ligand; sRANKL, soluble RANKL; TC, total cholesterol; TNF, tumour necrosis factor; TRAIL, TNF-related apoptosis-inducing ligand; TG, triacylglycerol.

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INTRODUCTION

Cardiovascular disease ranks first among the causes of death in developed countries, and various risk factors are known to contribute to the pathogenesis of atherosclerosis, such as diabetes, hypertension, hyperlipidaemia and chronic vascular inflammation [1,2]. Osteoporosis and cardiovascular diseases are commonly found together in elderly people, and a recent population-based longitudinal study demonstrated that the progression of atherosclerotic calcification is associated with bone loss in women during the menopause [3]. The mechanisms of oestrogen deficiency and lipid oxidation with menopause have been proposed to explain this relationship and, conceptually, a shift of calcium from the skeleton towards the arterial wall can account for both of these disorders [4–6], but the underlying paracrine mechanisms that operate in bone metabolism and vascular homoeostasis have not been defined yet.

RANKL (RANK ligand) and OPG (osteoprotegerin) have well-established regulatory effects on bone metabolism [7]. RANKL binds to its receptor RANK to induce osteoclastogenesis and bone resorption. OPG is a member of the TNF (tumour necrosis factor)-receptor family, and it is a glycoprotein that acts in competition with the RANK on the surface of osteoclasts to bind as a decoy receptor on RANKL; this prevents osteoclast differentiation, and it has recently been proposed as the link between osteoporosis and atherosclerosis [8]. OPG-knockout mice not only present with early-onset osteoporosis, but they also have multiple arterial calcifications in the large arteries [8–10]. Some of the studies performed on subjects with coronary artery disease have shown strong associations between plasma levels of OPG and the presence and severity of coronary artery disease [11,12]. A very recent prospective study by Kiechl et al. [13] reported that a high serum level of OPG was an independent risk factor for the incidence of cardiovascular disease and mortality and for the incidence of vascular morbidity. These results suggest that OPG could participate not only in bone metabolism, but also in the progression of, and protection against, atherosclerosis.

The RANKL/OPG system has recently been studied intensively for its involvement in the pathogenesis of immunological diseases such as juvenile rheumatoid arthritis [14]. Recent reports on the pathogenesis of atherosclerosis suggest the crucial role of inflammation in the initiation and progression of atherosclerosis, and OPG might protect blood vessels against atherosclerosis through either the attenuation of vascular calcification or protection against inflammation, although the precise mechanisms are still elusive [15,16].

Therefore, in the present study, we compared the levels of serum OPG in four groups of patients with coronary artery disease of varying severity. We evaluated the association of OPG with aortic calcification and the extent of LV (left ventricular) hypertrophy, which is frequently mentioned as a predictor of cardiovascular mortality, and the serum CRP level, a novel marker for atherosclerosis [16,17].

MATERIAL AND METHODS

Study population

The study was performed on 100 patients with the main complaint of chest pain, who underwent coronary artery angiography at the cardiology department of Kangbuk Samsung Medical Center, Seoul, South Korea from May to September 2003. Those patients with medical illnesses such as acute infection, chronic renal failure (serum creatinine ≥ 2.0 mg/dl), osteoporosis and other malignancies and those taking steroids or other immunosuppressants were excluded from enrolment to the study. Written informed consent was obtained from each participant. The study protocol conforms to the ethical guidelines of the Declaration of Helsinki, and the study received approval by the Institution’s Human Research Committee.

Measurement of anthropometric data, blood chemistry and CRP levels

Height, weight, waist circumference and the systolic and diastolic BPs (blood pressures) were measured in duplicate and the results were averaged. BMI (body mass index) was calculated by dividing the weight (kg) by the square of height (m). Blood samples were taken after an overnight fast. Smoking history was considered positive if the subject had smoked, even if they had stopped smoking.

Fasting blood glucose, TC (total cholesterol), triacylglycerol (TG; triglyceride), HDL-C (high-density lipoprotein cholesterol) and LDL-C (low-density lipoprotein cholesterol) were measured. The hexokinase method was used to measure blood glucose levels and an enzymatic calorimetric test was used to measure TC and TG levels. The selective inhibition method was used to measure the level of HDL-C, and the homogeneous enzymatic calorimetric test was used to measure the level of LDL-C.

Serum CRP levels were measured by nephelometric assay using a BNII nephelometer (Dade Behring, Deerfield, IL, U.S.A.).

Measurement and calculation of LV mass index

Two-dimensional echocardiography data were available for 82 of the 100 patients. M-mode echocardiography was used to measure the wall thickness and the internal diameter, and both were measured at end-diastole. The
following equation was used to calculate LV mass [18]:

\[ \text{LV mass (g)} = 1.04 \times [(\text{LVED} + \text{IVST} + \text{LVPWT})^3 - (\text{LVED})^3] - 13.6 \]

where LVED is LV end-diastolic diameter, IVST is interventricular septal thickness, and LVPWT is LV posterior wall thickness. The LV mass index was calculated by dividing the LV mass with the BSA (body surface area; m²). The body surface area was calculated using the Mosteller formula [19]:

\[ \text{LV mass index (g/m}^2) = \frac{\text{LV mass (g)}}{\text{BSA (m}^2)}. \]

Measurement of serum OPG and confirmation of aortic calcification

Blood samples were taken after an overnight fast and before the coronary angiogram. The serum was separated and stored at \(-80^\circ\) C, and the OPG levels were measured by an ELISA system (Oscotec, Chunan, Korea). In brief, a monoclonal IgG antibody was used as the capture antibody and a biotin-labelled polyclonal anti-human OPG antibody was used as the detection antibody. All the samples were measured in duplicate and the results were then averaged. The intra-assay coefficient of variation for the OPG measurement was 6.9–9.0 %, and the inter-assay coefficient of variation was 6.0–9.0 %.

A radiologist evaluated the presence of aortic arch calcification on a chest X-ray film obtained from each patient.

Coronary artery angiography

Coronary artery angiography was performed on all patients. Significant stenosis was defined when the internal diameter decreased by more than 50 %. The patients were grouped according to the number of significantly stenotic vessels into no-, one-, two- and three-vessel disease groups.

Statistical analysis

SPSS for Windows (version 11.0) was used for the statistical analysis. All data were expressed as means \(\pm\) S.D. One-way ANOVA was used to determine the differences among the different groups according to the number of stenotic coronary vessels. ANCOVA (analysis of covariance) was used to compare the serum OPG levels among different groups according to the number of vessels involved after an adjustment was made for age. The Shapiro–Wilk test was used to test for normality for each variable. Pearson’s correlation analysis was used to determine the correlation between each variable and the OPG level, and partial correlations were used to adjust for age. \(P < 0.05\) was considered statistically significant.

### Table 1  General characteristics of the patients with documented coronary artery disease

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>n</th>
<th>(\pm) S.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>56.97</td>
<td>11.88</td>
</tr>
<tr>
<td>Sex (male/female)</td>
<td>59/41</td>
<td></td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>131.98</td>
<td>16.94</td>
</tr>
<tr>
<td>Diastolic BP (mmHg)</td>
<td>83.10</td>
<td>9.50</td>
</tr>
<tr>
<td>Smoking (%)</td>
<td>46</td>
<td></td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>25.01</td>
<td>2.89</td>
</tr>
<tr>
<td>Fasting glucose (mg/dl)</td>
<td>118.98</td>
<td>50.96</td>
</tr>
<tr>
<td>TC (mmol/l)</td>
<td>5.03</td>
<td>1.16</td>
</tr>
<tr>
<td>HDL-C (mmol/l)</td>
<td>1.27</td>
<td>0.28</td>
</tr>
<tr>
<td>LDL-C (mmol/l)</td>
<td>2.89</td>
<td>0.92</td>
</tr>
<tr>
<td>TG (mmol/l)</td>
<td>1.64</td>
<td>0.73</td>
</tr>
<tr>
<td>LV mass index (g/m²)</td>
<td>117.50</td>
<td>24.04</td>
</tr>
<tr>
<td>OPG (pg/ml)</td>
<td>680.80</td>
<td>374.21</td>
</tr>
<tr>
<td>CRP (mg/l)</td>
<td>0.51</td>
<td>1.02</td>
</tr>
<tr>
<td>Aortic calcification (%)</td>
<td>30</td>
<td></td>
</tr>
<tr>
<td>Fasting glucose (\geq 126) mg/dl (%)</td>
<td>24</td>
<td></td>
</tr>
<tr>
<td>Number of stenotic vessels (\ast)</td>
<td>45</td>
<td></td>
</tr>
<tr>
<td>None (%)</td>
<td>45</td>
<td></td>
</tr>
<tr>
<td>One-vessel (%)</td>
<td>30</td>
<td></td>
</tr>
<tr>
<td>Two-vessel (%)</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td>Three-vessel (%)</td>
<td>10</td>
<td></td>
</tr>
</tbody>
</table>

RESULTS

Characteristics of the study population

The characteristics of the study population are shown in Table 1. There was no significant gender difference in OPG levels (698.56 \(\pm\) 315.58 pg/ml in men compared with 655.23 \(\pm\) 201.41 pg/ml in women; \(P = 0.404\)) (Table 1). The highest percentage (45 %) of the patients had normal coronary angiography (no-vessel disease), whereas the lowest percentage of the patients (10 %) had three-vessel disease.

Comparison of cardiovascular disease risk factors according to the severity of coronary artery disease

We compared the mean values of cardiovascular disease risk factors by dividing the patients into a normal group (without significant stenosis) and groups with one-, two- or three-vessel disease (Table 2). There was a significant difference in the mean age across the groups, and the patients with two-vessel disease were the oldest (\(P < 0.01\)). The mean HDL-C level was significantly different among the groups with the mean value in the group with no-vessel disease being significantly higher compared with those in the groups with one- and
two-vessel disease ($P < 0.05$), as determined using a post-hoc analysis. No significant differences were observed among the groups for BMI, BP and smoking habits ($P > 0.05$).

The mean serum CRP level in the group with three-vessel disease was significantly higher than in the no-, one- and two-vessel disease groups ($P < 0.05$; Table 2).

Serum OPG level, the presence of aortic calcification and fasting hyperglycaemia according to the number of stenotic vessels

Serum OPG levels increased significantly as the number of stenotic coronary arteries increased and, by using post-hoc analysis, the group with three-vessel disease showed a significantly higher OPG level compared with the groups with no- or one-vessel disease ($P < 0.05$). After adjustment for age with ANCOVA, this significance was lost ($P = 0.088$).

The number of subjects with aortic calcification did not show a significant difference according to the number of vessels involved, although a trend was observed (Table 2; $P = 0.067$). When the serum OPG level was compared between the groups without and with aortic calcification, no significant difference was noted, although the level was somewhat higher in those with calcification than in those without (701.54 ± 199.62 compared with 668.64 ± 302.14 pg/ml respectively; $P = 0.623$).

When the serum OPG levels were compared between the groups without and with fasting hyperglycaemia (glucose $\geq 126$ mg/dl), no significant difference was observed (655.91 ± 202.05 compared with 688.66 ± 294.06 pg/ml respectively; $P = 0.541$).

The mean serum OPG level was higher in subjects with a positive smoking history than in those without, although this was not statistically significant (700.35 ± 308.93 compared with 659.75 ± 242.64 pg/ml respectively; $P = 0.466$).

Correlation between the level of serum OPG and risk factors of cardiovascular disease

Correlation analyses were performed between the level of serum OPG and the various risk factors of cardiovascular disease.
Table 3  Correlation analysis between serum OPG levels and cardiovascular risk factors before and after adjustment for age
Analyses were done with Pearson’s correlation and partial correlation analyses.

<table>
<thead>
<tr>
<th>Risk Factor</th>
<th>Before adjustment for age</th>
<th>After adjustment for age</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$r$</td>
<td>$P$</td>
</tr>
<tr>
<td>Age (years)</td>
<td>0.207</td>
<td>0.039</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>0.130</td>
<td>0.203</td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>0.101</td>
<td>0.318</td>
</tr>
<tr>
<td>Diastolic BP (mmHg)</td>
<td>0.060</td>
<td>0.552</td>
</tr>
<tr>
<td>TC (mg/dl)</td>
<td>0.016</td>
<td>0.874</td>
</tr>
<tr>
<td>HDL-C (mg/dl)</td>
<td>-0.034</td>
<td>0.736</td>
</tr>
<tr>
<td>LDL-C (mg/dl)</td>
<td>0.104</td>
<td>0.369</td>
</tr>
<tr>
<td>TG (mg/dl)</td>
<td>-0.083</td>
<td>0.415</td>
</tr>
<tr>
<td>CRP (mg/l)</td>
<td>0.224</td>
<td>0.022</td>
</tr>
<tr>
<td>LV mass index (g/m²)</td>
<td>0.240</td>
<td>0.039</td>
</tr>
</tbody>
</table>

Figure 2  Correlation between serum OPG and CRP levels in patients with coronary artery disease

![Correlation between serum OPG and CRP levels](image)

$r = 0.234, p = 0.022$

disease (Table 3). Positive correlations were observed between the serum OPG levels and patient age and the LV mass index, but the significance was lost after adjustment for age (Table 3; $P = 0.039$ before adjustment; $P = 0.465$ after adjustment for age).

The serum OPG level showed positive significant correlation with serum CRP levels, and the significance was consistent even after adjustment for age (Table 3 and Figure 2).

DISCUSSION

In the present study, we found that mean serum OPG levels increased significantly as the number of stenotic coronary arteries increased and the differences were more pronounced when compared with patients without significant stenosis, although the significance was lost after an adjustment for age. This result is consistent with previous reports that have suggested a correlation between OPG and cardiovascular disease [11–13]. The distinctive point of our present study was that we tried to uncover the discrete mechanism of OPG involvement in atherosclerosis by additionally analysing the relationship of LV mass index, serum CRP levels and serum OPG levels. It is interesting that serum CRP levels showed a positive association with serum OPG levels and the significance was consistent even after an adjustment was made for age.

The evidence for the action of OPG on aortic calcification was first suggested by the finding that OPG-knockout mice not only developed early-onset osteoporosis, but they also had life-threatening hypercalcaemia as well as arterial calcification in the large arteries [10]. These severe aortic calcifications and osteoporosis were completely prevented by cross-breeding the knockout mice with OPG transgenic mice in which the transgene had been delivered prenatally; thus this demonstrated that the OPG deficiency is crucial for the clinical manifestation of these two disorders [20]. It has also been reported that OPG is distributed not only in aortic media, but also in the smooth muscle cells and the vascular endothelial cells of the coronary artery, and OPG is involved in the regulation of cellular survival via the NF-κB pathway [21,22]. Dhole et al. [23] using autopsied specimens have reported that OPG and RANKL were present in non-diseased vessel walls and early atherosclerotic lesions but, in the advanced calcified lesions, OPG was present in the bony structure of the fibrocalcified plaques, whereas RANKL was only present in the extracellular matrix surrounding the calcium deposits, suggesting the regulatory role of OPG in advanced atherosclerosis. The involvement of OPG in the progression of atherosclerosis has been evident not only in animal studies, but also in humans, as was shown by two previous studies in which serum OPG levels were correlated proportionally with the severity of coronary artery disease [11,12]. The results of our present study are consistent with these previous results in that the patients with coronary artery disease have higher OPG serum levels than those patients without significant coronary stenosis.

Although many studies are currently under way, the precise role for OPG in human atherosclerosis is still elusive. Schoppet et al. [24] hypothesized that RANKL might be involved in these processes and so they measured sRANKL (soluble RANKL) serum levels in subjects who had undergone coronary angiograms. In contrast with OPG serum levels, sRANKL levels were significantly lower in patients with coronary artery disease compared with patients without the disease, but the serum levels were not correlated with the severity of coronary artery disease. Schoppet et al. [24] suggested a role of blood
such as rheumatoid arthritis that are characterized by inhibitory activity of OPG on osteoclastogenesis [32]. TRAIL can block the inducer of cytotoxic ligand TRAIL (TNF-related apoptosis-inducing ligand) inhibits TRAIL-induced apoptosis of killer T-cells and vice versa; moreover, TRAIL can block the binding of OPG to RANKL or other cytokines, a possible mechanism could be hypothesized in which the compensatory increase in serum OPG in atherosclerotic subjects occurred in an attempt to attenuate vascular calcification.

Then, is OPG involved in vascular protection only through the regulation of vascular calcification? According to the large-scale prospective study by Kiechl et al. [13], the relative risk of cardiovascular mortality was increased 3–4-fold in patients with high serum OPG levels, although the mortality due to non-vascular causes showed no association with OPG levels [13]. Thus we hypothesized that OPG might be involved in cardiovascular mortality through a mechanism separate from vascular calcification, for example, the cardiovascular system itself, and so we measured the LV mass index of the patients, which has been considered one of the predictors of cardiovascular mortality, and analysed its association with serum OPG levels [17]. Although we failed to show a precise correlation of serum OPG levels with LV hypertrophy, our present study is still meaningful as it is the first to examine the relationship between LV mass index and serum OPG levels.

The OPG/RANKL/RANK system seems to play a major role in modulating the immune system. RANKL-knockout mice show not only osteopetrosis, but also severe immunological abnormalities, and the binding of RANKL to RANK augments DC (dendritic cell) survival, enhances the immunostimulatory capacity of DCs and modulates activated T-cells [29,30]. In OPG-knockout mice, OPG was found to be critically involved in B-cell maturation and the generation of efficient antibody responses [31]. In addition, the binding of OPG to the cytotoxic ligand TRAIL (TNF-related apoptosis-inducing ligand) inhibits TRAIL-induced apoptosis of cells and vice versa; moreover, TRAIL can block the inhibitory activity of OPG on osteoclastogenesis [32]. Therefore the activation of the RANKL/RANK system is being proposed as the pathogenic mechanism for diseases such as rheumatoid arthritis that are characterized by both inflammation and destruction of bone [14]. Recent studies have suggested a role of vascular inflammation in the pathogenesis of atherosclerosis [15]. Thus we measured CRP, an acute-phase reactant that has been intensively studied as a novel marker of atherosclerosis, and evaluated whether there was any correlation between serum OPG and CRP levels [16]. Interestingly, the serum CRP level was positively correlated with the serum OPG level and this correlation was consistently significant even after adjustment for age, suggesting the possible involvement of OPG in the atherosclerotic inflammatory process. The association of CRP with OPG has been reported in one previous study by Kiechl et al. [13] in which serum CRP levels had a positive correlation with serum OPG levels, and this is consistent with our result. Whether the elevated OPG levels are related to CRP and atherosclerosis, or whether they are simply related to another inflammatory process such as ongoing infection that we could not detect, is not known. However, the fact that both serum OPG and CRP levels increased exclusively in patients with three-vessel disease, and that they correlated significantly with each other, could be interpreted as at least the possibility of OPG involvement in vascular protection through a different mechanism to that of vascular calcification attenuation. For example, they could be related through inhibition of the inflammatory process related to atherosclerosis. Further investigations are needed to clarify the precise mechanisms.

The present study has several limitations. First, the number of subjects was small and the subjects were not well characterized, i.e. there were significant differences in age among the groups. As mentioned previously, the results could have been affected by age, since serum OPG levels are known to increase with aging and the significance we observed was lost after adjustment for age [33]. However, the group with two-vessel disease was the oldest, but the mean serum OPG level was highest in the group with three-vessel disease. Therefore this limitation could not have affected the results. The second limitation is the possible hidden influence of metabolic bone disease that was incompletely evaluated by serum OPG levels. Some of the female participants with severe osteoporosis were excluded from the study; however, routine BMD (bone mineral density) evaluation in male patients was not possible for all the subjects. Future studies including BMD measurements on coronary artery disease patients with an adjustment for the presence of metabolic bone disease could reveal more definite results. Finally, in regard to the assessment of aortic calcification, we used a very approximate method to observe the presence of calcification by means of a simple chest X-ray. It would have been better if we had used a more specific method to measure calcification.

In conclusion, serum OPG levels showed a good correlation with the number of stenotic arteries in coronary...
artery disease patients. Although LV mass indices did not show significant correlations with OPG levels, serum CRP levels were positively correlated with serum OPG levels, suggesting the possible involvement of OPG in the process of atherosclerosis through the attenuation of inflammation related to CRP. Further investigations are needed to elucidate the precise role of OPG in cardiovascular mortality and atherosclerosis.

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