Turkish Journal of Endocrinology and Metabolism, (2002) 3 : 111-116 **ORIGINAL ARTICLE**

Lower Levels of Osteoprotegerin in Subjects with Arterial Calcification and an Inverse Correlation Between Osteoprotegerin and Lipoprotein (a) Levels

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Osteoporosis an d vascular disease are common and important causes of morbidity and mortality in elderly people. We aimed to evaluate serum osteoprotegerin (OPG) levels and their correlation with radial artery calcification and lipid profile in 36 patients undergone coronary artery bypass graft surgery. We found radial artery calcification in 4 subjects and all had medial calcification. OPG levels of subjects with arterial calcification were significantly lower than subjects without arterial calcification (184.06 ± 18.30 vs 86.25 ± 13.79 pg/mL; p = 0.044). We did not observe any significant difference between the groups regarding age, sex, body mass index, hypertension, diabetes mellitus, glucose metabolism, lipid profile, smoking and alcohol intake. There was an inverse correlation between OPG and lipoprotein {Lp}(a) levels (r = -0.567; *p* < 0.0001). This inverse correlation was seen in both diabetic and non-diabetic subjects (r = -0.522; *p* = 0.031 and r = -0.597; *p* = 0.007). As a conclusion, in patients with coronary artery disease (CAD), OPG levels are lower in subjects with radial arterial calcification than in subjects without radial arterial calcification and there is an inverse correlation between OPG and Lp(a). Therefore, OPG may play an important role in arterial calcification and endothelial dysfunction.

Key words: **Osteoprotegerin, arterial calcification, lipoprotein (a), coronary artery bypass graft surgery**

Introduction

Osteoporosis and vascular disease are common and important causes of morbidity and mortality, frequently found together, especially in elderly people. The commonest of these associations is the presence of atherosclerosis and arterial calcification in osteoporotic patients, and there is a close relation between vascular calcification and osteoporosis (1-3).

Osteoprotegerin (OPG) is a soluble member of the tumor necrosis factor receptor family that acts as a paracrine factor within the bone microenviroment to decrease bone resorption (4). Receptor activator of NF κB-ligand (RANKL)/OPG ratio is an essential determinant of osteoclast and ostoblast biology (5,6). Any agent that regulates RANKL, OPG or both, is likely to indirectly modulate osteoclast and

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osteoblast differentiation, activation, and apoptosis. In contrast to RANKL, whose expression is mainly restricted to the skeletal and immune system, OPG is expressed in high concentration by a variety of tissues and cell types (4,6-9). High levels of OPG mRNA have been detected in lung, kidney, heart, liver, stomach, intestine, skin, brain and spinal cord, thyroid gland, and bone (7,8). OPG may also play a role in vascular calcification and atherosclerosis, because OPG is produced by vascular smooth muscle cells and endothelial cells, and severe arterial calcification is detected in OPG deficient mice (10-13). OPG represents an anti-apoptotic signal for endothelial cells, which suggests an important role for OPG in maintaining the integrity of the luminal surface of the vascular wall (13). OPG deficient mice had profound calcification of the large arteries by 2 months of age that progressed to marked medial calcification of the aorta and the renal arteries, profound intimal and medial proliferation, and partial aortic dissection by 4 months (11).

Vascular effects of OPG in humans are not known. Browner et al. reported that serum levels of OPG were greater in women with diabetes and in those who subsequently died of cardiovascular disease (14). Recently, Szulc et al. reported an age dependent increase in serum OPG in men (15). In this study, we aimed to evaluate serum OPG levels and their correlation with radial artery calcification as a measure of general arterial involvement in patients undergone coronary artery bypass graft (CABG) surgery. We also evaluated the correlation between OPG levels and lipid profile.

Materials and Methods

This prospective study was conducted at Baskent University Adana Hospital. Thirty six consecutive patients, in whom radial artery graft was utilized during CABG, were included in the study. Diabetic subjects met the American Diabetes Association criteria for type 2 diabetes. Hypertension was defined as systolic blood pressure ≥ 140 mmHg and/or diastolic blood pressure ≥ 90 mmHg or treatment with an antihypertensive medication.

Body weight and height were measured while subjects wore light clothing without shoes. Samples for the measurement of lipid profile, plasma glucose, fibrinogen, uric asid and osteoprotegerin levels were drawn after an overnight fast. Levels of plasma glucose, total cholesterol, high density lipoprotein (HDL) cholesterol, and triglycerides were determined by the calorimetric method using a Cobas Mira Plus autoanalyzer (Roche Diagnostics, Mannheim, Germany). Low-density lipoprotein (LDL) cholesterol level was calculated by the Friedwald formula. Apolipoprotein (Apo) A1, Apo B, and lipoprotein (a) (Lp[a]) were quantitated by the immunoturbidimetric method and uric asid by the calorimetric method in a Roche/Hitachi 912 autoanalyzer (Roche Diagnostics, Mannheim, Germany). Fibrinogen (Dade Behring, Marburg, Germany) was measured by modified Clauss method as described by the producer in a Dade Behring coagulometer. OPG (Immundiagnostik, Bensheim, Germany) was measured in Medistek ESR (Roche Diagnostics, Mannheim, Germany) using the enzyme-linked immunosorbent assay. The lower limit of detection of this assay is 4 pg/mL. Intra- and interassay variabilities are less than 10%. All samples were measured in duplicate and averaged; results differing by more than 20% were reassayed.

The radial artery was harvested as described by Reyes et al. (16). Soon after harvesting, the distal 5 mm portion of the radial artery graft was sent to the pathology laboratory. The radial arter specimens were fixed by 10% formaline and embedded in paraffin blocks. Each block was cut into 5 µm sections. Slides were stained by hematoxylin–eosin, Masson trichrome and elastic Van Giason. All slides were evaluated by a single pathologist and recorded as presence or absence of calcification and atherosclerosis.

Data are expressed as means ± SE. The Student's t-test or Mann-Whitney U test was used to assess significant differences between values in various groups of patients where appropriate. Homogeneity of variances were calculated by Levene's test. Pearson's correlation coefficient was used to evaluate associations between OPG and various parameters. A value of $p < 0.05$ was considered statistically significant. Data were analyzed using the SPSS for Windows (version 10.0; SPSS, Inc., Chicago, Illinois, USA).

Results

Characteristics of 36 subjects are summarized in Table 1. Seventeen of the subjects were diabetic and 19 were non-diabetic. The only difference between

Table 1. Characteristics of subjects.

Characteristics (mean ± SE)	Subjects (n = 36)
Age (year)	59.83 ± 1.22
BMI (kg/m^2)	28.79 ± 0.63
Osteoprotegerin (pg/mL)	173.19 ± 17.10
Fasting blood glucose (mg/dL)	124.78 ± 10.96
Total cholesterol (mg/dL)	212.57 ± 9.01
HDL cholesterol (mg/dL)	35.46 ± 1.61
LDL cholesterol (mg/dL)	134.56 ± 8.15
Triglyceride (mg/dL)	214.20 ± 26.01
Lipoprotein (a) (mg/dL)	43.08 ± 4.12
Uric asid (mg/dL)	5.72 ± 0.23
Fibrinogen (g/L)	3.06 ± 0.10

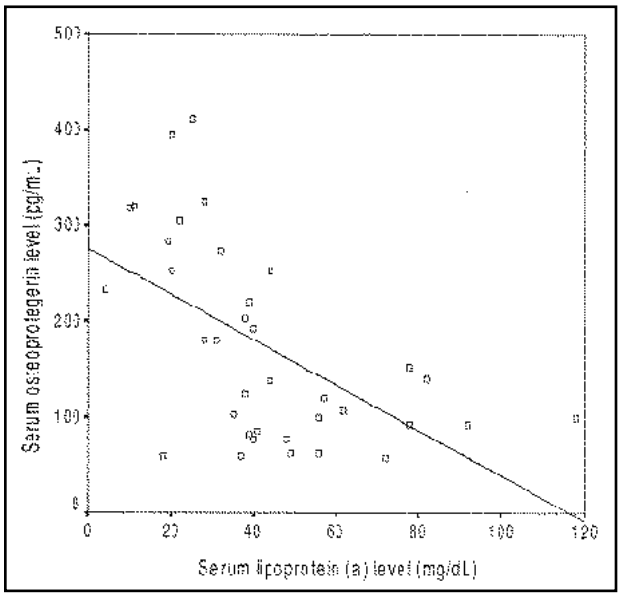

Figure 1. Scatter plot of serum osteoprotegerin levels and serum lipoprotein (a) levels in 36 subjects (r = -0.5.67; $p < 0.0001$)

diabetic and non-diabetic patients was fasting blood glucose levels (Table 2).

We found radial artery calcification in 4 subjects and all had medial calcification. OPG levels of subjects with arterial calcification were significantly lower than that of subjects without arterial calcification (184.06 ± 18.30 vs 86.25 ± 13.79 pg/mL; $p = 0.044$) We did not observe any significant difference between groups regarding age, sex, BMI, hypertension, diabetes mellitus, glucose metabolism, lipid profile, smoking and alcohol intake.

We found an inverse correlation between OPG and Lp(a) levels (r = -0.567; $p < 0.001$) (Figure 1). This inverse correlation was seen in both diabetic and non-diabetic subjects (r = -0.522; $p = 0.031$ and r = -0.597; $p = 0.007$).

Discussion

Principal finding of the present study is that patients with radial artery calcification have lower serum levels of OPG than patients without radial artery calcification. Previous animal study has shown that OPG deficient animals develop premature arterial calcification which is mainly in the media of large arteries (11). In our study, we found arterial calcification in 4 subjects and all of them were in the media of radial artery, which is a confirming finding of the animal study and previous studies in humans (11,17,18). As reported by Min and collagues, premature arterial calcification in OPG deficient animals is completely preventable by restoration of the gene, thus demonstrating that OPG

Table 2. Characteristics of diabetic and non-diabetic subjects.

Characteristics (mean ± SE)	Diabetic Subjects (n = 17)	Non-diabetic Subjects (n = 19)	*p* value
Age (year)	58.12 ± 1.69	61.37 ± 1.72	NS
BMI (kg/m^2)	28.59 ± 0.71	28.96 ± 1.04	NS
Osteoprotegerin (pg/mL)	167.94 ± 21.27	177.89 ± 26.73	NS
Fasting blood glucose (mg/dL)	160.76 ± 19.49	92.58 ± 4.31	< 0.0001
Total cholesterol (mg/dL)	221.81 ± 14.04	204.79 ± 11.69	NS
HDL cholesterol (mg/dL)	37.06 ± 2.94	34.11 ± 1.64	NS
LDL cholesterol (mg/dL)	132.80 ± 12.21	135.45 ± 11.24	NS
Triglyceride (mg/dL)	262.13 ± 50.72	173.84 ± 18.72	NS
Lipoprotein (a) (mg/dL)	45.18 ± 5.85	41.21 ± 5.91	NS
Uric asid (mg/dL)	5.40 ± 0.34	5.93 ± 0.31	NS
Fibrinogen (g/L)	2.91 ± 0.18	3.18 ± 0.12	NS

NS: Not significant

deficiency is crucial for the clinical manifestation of the disorder (19).

We did not find any difference between OPG levels of diabetic and non-diabetic subjects. In contrast to our study, Browner et al. reported higher OPG levels in diabetic subjects and in those who died of cardiovascular disease during the follow-up than in control group (14). Also we did not find any correlation between OPG levels and age, but Szulc et al. reported an age dependent increase in serum OPG in men (15). Reasons for these differences may lie in the fact that all of our subjects had coronary artery disease (CAD). Also our patients were older, the number of subjects in our current study is smaller and we did not have any control group. If we had control group and younger subjects, we may also have found similar differences between the normal subjects and patients with CAD, diabetes and/or hypertension. The mean OPG level of our subjects is higher than the mean OPG level of the patients reported by Szulc et al. (173 vs 62 pg/mL) (15). Interestingly, the mean OPG level of our subjects with arterial calcification is greater than that of the previous study (86 vs 62 pg/mL), showing a relatively increased OPG levels in patients with CAD in our study (15). A possible explanation for increased OPG concentrations in our subjects may be a compensatory mechanism to keep immune mechanisms contributing to arterial calcification under control. When this compensatory mechanism is incomplete, medial calcification may develop. A similar interpretation was made by Yano et al. when they found that women with osteoporosis and increased biochemical markers of bone turnover had higher serum concentrations of osteoprotegerin than did age-matched controls without osteoporosis (20).

Our study is the first to demonstrate an inverse correlation between serum levels of OPG and Lp (a). Lp(a) is a highly heterogeneous lipoprotein, due to variations in the size of apolipoprotein (a), and the density of the apoB100 containing particles to which apo(a) is linked (21). Although high plasma levels of Lp(a) have been associated with an increased risk for CAD, the mechanism underlying this association is still largely undetermined (21-26). Furthermore, elevated Lp(a) levels were associated with endothelial dysfunction even when atherosclerotic lesions were not recognizable by angiography (27-29). In Framingham Offspring Study, age adjusted Lp(a) values were 8% greater in postmenopausal women than in premenopausal women (30). Previous studies have shown that hormone replacement therapy lowers Lp(a) levels in postmenopausal women (26,31,32). OPG production declines with estrogen deficiency and increases during estrogen replacement therapy (33-35). Arterial smooth muscle cells and endothelial cells produce OPG, which represents an anti-apoptotic signal for endothelial cells. OPG may play an important role in the maintenance of the integrity of the vascular wall's luminal surface (11-13,19). Further studies are needed to document whether OPG and Lp(a) do have a bilateral action or their interaction is controlled by a third party.

As a conclusion, in patients with CAD, OPG levels are lower in subjects with arterial calcification than in subjects without arterial calcification and there is an inverse correlation between OPG and Lp(a). Therefore, OPG may play an important role in arterial calcification and endothelial dysfunction.

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