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Circulating osteoprotegerin levels are associated with age, waist-to-hip ratio, serum total cholesterol, and low-density lipoprotein cholesterol levels in healthy Korean women

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Abstract

Osteoprotegerin (OPG) is a recently identified cytokine that acts as a decoy receptor for the receptor activator of nuclear factor κB ligand. Osteoprotegerin has been shown to be an important inhibitor of osteoclastogenesis and arterial calcification in animal models. Recently, OPG has been proposed as a link molecule between osteoporosis and arterial calcification, but the relationship between circulating OPG levels and cardiovascular disease in human populations is unclear. Thus, the aim of this study was to investigate the relationship between circulating OPG levels and cardiovascular risk factors in healthy Korean women. The subjects were 286 women aged 37 to 73 (mean ± SD, 51.5 ± 6.9 years). We examined blood pressure, body mass index, and waist-to-hip ratio. Serum concentrations of OPG were determined by enzyme-linked immunosorbent assay. Fasting plasma glucose levels, serum lipid profiles, insulin levels, and serum follicle-stimulating hormone (FSH) levels were determined by standard methods and homeostasis model assessment of insulin resistance was calculated. We observed a significant association between serum OPG levels and age, waist-to-hip ratio, total cholesterol, low-density lipoprotein cholesterol, and FSH levels ($P < .05$). Among the metabolic components, the older, obese, and hypercholsterolemic subjects had higher serum OPG levels ($P < .05$). However, no significant relationship was found between serum OPG levels and blood pressure and fasting plasma glucose levels. We found that mean serum OPG levels were about 11% greater in postmenopausal women (mean ± SD, 1358.5 ± 380.0 pg/mL) than in premenopausal women (mean ± SD, 1228.8 ± 407.7 pg/mL, $P < .001$). In multiple regression analysis with OPG as the dependent variable, serum FSH and low-density lipoprotein cholesterol levels were the significant predictor for serum OPG level ($R^2 = 0.051$, $P < .05$). In conclusion, our results show that circulating OPG levels are partly associated with cardiovascular risk factors and menopausal status in healthy Korean women. Out findings suggest that OPG may be an important paracrine factor of cardiovascular disease in the female population.

1. Introduction

Osteoprotegerin (OPG) is a soluble glycoprotein that belongs to the tumor necrosis factor (TNF) receptor superfamily. It was identified almost simultaneously and independently by several groups of investigators [1-3]. Osteoprotegerin acts as a decoy receptor of the receptor activator of nuclear factor κB (NF-κB) ligand (RANKL), which is a key regulator of osteoclastogenesis and is known

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to inhibit osteoclastogenesis by binding to RANKL, thus, preventing RANKL from binding to the receptor activator of NF-κB on osteoclasts [4,5].

Recently, OPG has shown promise not only as an inhibitor of osteoclastogenesis, but also as a preventive mediator of cardiovascular diseases, such as arterial calcification and atherogenesis. It has been reported that OPG is highly expressed in the bones, heart, and major arteries [1]. Moreover, OPG-deficient mice exhibited severe osteoporosis and vascular calcification of the aorta and renal arteries [6]. The intravenous injection of recombinant OPG protein and the transgenic overexpression of OPG in OPG-deficient mice effectively rescued them from osteoporosis and prevented the formation of arterial calcifications [7]. It was shown that arterial calcification induced by warfarin or vitamin D treatment was inhibited by treating the mice with recombinant OPG [8]. Osteoprotegerin has been proposed to be a link molecule between osteoporosis and arterial calcification in an animal model. In addition, it has been suggested to be an explanation for the increased coincidence of arterial calcification and osteoporosis in human populations [9-11].

There have been reports that serum OPG levels are associated with the severity of coronary artery disease and cardiovascular mortality, and that these are increased in the patients with diabetes. Increased serum OPG levels may be a compensatory self-defense response to the progression of atherosclerosis [12-14]. However, the associations between serum OPG levels and cardiovascular risk factors in healthy populations are not known. To investigate the relationship between serum OPG levels and cardiovascular risk factors in women, we examined serum OPG levels, age, blood pressure, body mass index (BMI), waist-to-hip ratio (WHR), fasting plasma glucose levels, insulin resistance, and serum lipid profiles in healthy Korean women.

2. Subjects and methods

The study population consisted of 286 healthy Korean women (mean ± SD age, 51.5 ± 6.9 years; range, 37-73) who entered Miz Medi Hospital Healthcare Center for medical checkups from January 1, 2002, to December 31, 2002. Women with diabetes mellitus, ischemic heart disease, cerebrovascular disease, thyroid dysfunction, chronic liver, and renal diseases were excluded, but patients with hypertension were included in the analysis. We also excluded the patients taking medicine that can affect bone metabolism.

The subjects included 137 postmenopausal women (mean ± SD age, 54.4 ± 6.0 years; mean years since menopause, 4.6 ± 0.4 years) and 149 premenopausal women (mean ± SD age, 48.9 ± 6.6 years). The menopause was defined as the absence of menstruation for at least 12 months and serum FSH levels of higher than 40 IU/L. The protocol used was approved by the Institutional Review Board of Miz Medi Hospital, and informed consent was obtained from all participants.

We measured height, weight, and systolic and diastolic blood pressures (SBP and DBP, respectively). Body mass index was calculated as weight (kg) divided by height (m) squared and was used as an index of overall adiposity (kg/m^2). Waist circumference was measured midway between the lowest rib and the iliac crest, and hip circumference was taken over the widest part of the gluteal region. Waist-to-hip ratio was used as measure of central obesity.

Blood samples were taken after an overnight fast. Serum was separated, stored at −80°C, and serum OPG levels were measured by enzyme-linked immunosorbent assay (Oscotec, Korea). In brief, a monoclonal IgG antibody was used as a capture antibody, and a biotin-labeled polyclonal antihuman OPG antibody was used as a detection antibody. All samples were measured in duplicate, and the results were averaged. The intra-assay coefficient of variation (CV) for the OPG measurement was 6.9% to 9.0% and the interassay CV was 6.0% to 9.0%.

Fasting plasma glucose, serum total cholesterol (TC), triglyceride (TG), and high-density lipoprotein (HDL) levels were determined by colorimetry (Vitros, Ortho-Clinical Diagnostics, Puritan, NJ), and serum low-density lipoprotein (LDL) levels were calculated using the Friedewald equation.

Serum follicle-stimulating hormone (FSH) levels were measured by chemiluminescent sandwich immunoassay (ADVIA Centaur Estradiol Assay, Bayer Corp, Tarry town, NY). The intra-assay CV for FSH measurement was 2.0% to 2.9%, and the interassay CV was 0.3% to 2.7%. Plasma insulin levels were assayed by immunoradiometric assay (RIABEAD II, Abbott, Tokyo, Japan). Insulin resistance were calculated according to the homeostasis model assessment (HOMA-IR) as follows: HOMA-IR = fasting insulin (μU/mL) × fasting glucose (mmol/L)/22.5 [15].

According to the guideline of the Third Report of the National Cholesterol Education Program Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (NCEP-ATPIII) [16], the cardiovascular risk factors were defined as follows: age ≥55 years, hypertension (blood pressure ≥140/90 mm Hg), serum LDL cholesterol (LDL-C) ≥160 mg/dL, TC ≥240 mg/dL, TG ≥200 mg/dL, and HDL cholesterol (HDL-C) <40 mg/dL. A patient was considered as diabetic with a fasting plasma glucose level ≥126 mg/dL, obese if BMI ≥30 kg/m^2, or centrally obese if WHR ≥0.9.

All results are presented as means ± SDs. SPSS (version 10.0; Chicago, IL) for Windows was used for statistical analysis. We used Student *t* tests to determine statistical differences in means of average serum levels of OPG between each normal and high-risk group according to the NCEP-ATPIII guidelines. Correlation analyses between serum OPG levels and each cardiovascular risk factor were performed using the Pearson correlation analysis. Multiple

Table 1
General characteristics of the subjects

Characteristics	Means ± SDs
Age (years)	51.53 ± 6.91
BMI (kg/m^2)	24.06 ± 2.91
WHR	0.85 ± 0.06
SBP (mm Hg)	125.10 ± 18.70
DBP (mm Hg)	75.57 ± 12.05
Serum glucose levels (mg/dL)	90.85 ± 20.43
Seum TC levels (mg/dL)	201.29 ± 39.10
Serum TG levels (mg/dL)	122.03 ± 68.87
Serum HDL-C levels (mg/dL)	59.44 ± 14.17
Serum LDL-C levels (mg/dL)	117.42 ± 35.40
Serum creatinine levels (mg/dL)	0.70 ± 0.09
Serum TSH levels (mIU/L)	2.38 ± 5.32
Serum FSH levels (mIU/mL)	40.58 ± 31.48
Serum OPG levels (pg/mL)*	1289.6 ± 398.6
Premenopausal serum OPG levels (pg/mL)	1226.3 ± 405.9
Postmenopausal serum OPG levels (pg/mL)	1358.5 ± 380.0

TSH indicates thyroid-stimulating hormone.
* $P < .001$ for comparison of means between pre- and postmenopausal values.

Table 3
Bivariate correlation analysis between cardiovascular risk factors and serum OPG levels

Characteristics	Bivariate correlation	
	r	P
Age	0.157	.008
BMI	−0.063	.292
WHR	0.134	.023
SBP	0.089	.132
DBP	0.092	.122
Glucose	0.012	.844
TC	0.175	.003
LDL-C	0.176	.003
TG	0.020	.735
HDL-C	0.023	.702
FSH	0.176	.003
Insulin	−0.091	.125
HOMA-IR	−0.074	.214

Correlation coefficients and P values were calculated using Pearson correlation analysis.

regression analysis was performed with stepwise method to see the correlations between variables, adjusting for the confounding factors. P values of less than .05 were considered significant.

3. Results

3.1. General characteristics

The study group's main clinical and laboratory features are shown in Table 1. The number of women ≥55 years was 80 (28.0%). The number of obese women with BMI ≥30 kg/m^2 was 8 (2.8%). The number of centrally obese women with WHR ≥0.9 was 63 (22.0%).

There were 58 (20.3%) and 9 (3.1%) subjects with hypertension and diabetes, respectively, and the numbers of subjects with high serum TC (≥240 mg/dL), high TG (≥200 mg/dL), high LDL-C (≥160 mg/dL), and low HDL-C levels (<40 mg/dL) were 48 (16.8%), 34 (11.9%), 32 (11.2%), and 16 (5.6%), respectively.

3.2. Mean serum OPG levels according to cardiovascular risk factors

The mean value of serum OPG in all subjects was 1289.6 ± 398.6 pg/mL. Serum OPG levels were significantly higher in the older (age ≥55 years) subjects than in younger (age <55 years) subjects (1366.4 ± 400.5 vs 1259.8 ± 394.8 pg/mL, $P < .05$) (Table 2).

The mean serum OPG level was higher in those with WHR ≥0.9 than in those with WHR <0.9 (1378.4 ± 369.7 vs 1262.9 ± 403.8 pg/mL, $P < .05$) (Table 2).

No significant difference was found between the mean serum OPG levels of those with BMI ≥30 kg/m^2 and those with BMI < 30 kg/m^2. Diabetes and hypertension were not associated with the serum OPG level (Table 2).

The mean serum OPG levels tended to be higher in those with high serum TC (TC ≥240, OPG = 1392.4 ± 456.5 pg/mL; TC <240 mg/dL, OPG = 1268.8 ± 384.4 pg/mL, $P = .050$) and LDL-C levels (LDL-C ≥160 mg/dL, OPG = 1417.2 ± 452.0 pg/mL; LDL-C <160 mg/dL, OPG = 1273.5 ± 390.1 pg/mL, $P = .055$). However, serum TG and

Table 2
Serum OPG levels according cardiovascular risk factors

Characteristics	Serum OPG (pg/mL) (mean ± SD)	Characteristics	Serum OPG (pg/mL) (mean ± SD)	P
Age <55 years	1259.8 ± 394.8	Age ≥55 years	1366.4 ± 400.5	.042
BMI <30 kg/m^2	1290.2 ± 401.0	BMI ≥30 kg/m^2	1267.8 ± 324.5	.876
WHR <0.9	1262.9 ± 403.8	WHR ≥0.9	1378.4 ± 369.7	.042
Normotension	1291.8 ± 406.1	Hypertension	1281.0 ± 370.5	.854
Serum glucose <126 mg/dL	1288.0 ± 397.8	Serum glucose ≥126 mg/dL	1339.0 ± 464.4	.707
Serum TC <240 mg/dL	1268.8 ± 384.4	Serum TC ≥240 mg/dL	1392.4 ± 456.5	.050
Serum TG <200 mg/dL	1285.7 ± 404.7	Serum TG ≥200 mg/dL	1318.7 ± 354.0	.651
Serum HDL-C >40 mg/dL	1290.3 ± 396.7	Serum HDL-C ≤40 mg/dL	1276.8 ± 442.2	.895
Serum LDL-C <160 mg/dL	1273.5 ± 390.1	Serum LDL-C ≥160 mg/dL	1417.2 ± 452.0	.055

P values were calculated using Student t test.

Table 4
Multiple regression analysis with serum OPG level as the dependent variable

Characteristics	β	P
Age	.077	.244
WHR	.088	.144
SBP	.022	.723
FSH	.144	.016
TC	.031	.837
LDL-C	.146	.015
R^2	.051	

Multiple regression analysis was done with stepwise method.

HDL-C level were found to have no relationship to the mean serum OPG level (Table 2).

Postmenopausal women had a significantly higher mean serum OPG level than premenopausal women (1358.5 ± 380.0 vs 1226.3 ± 405.9 pg/mL, $P < .001$) (Table 1).

3.3. Association among serum OPG levels, insulin resistance index, and cardiovascular risk factors

Bivariate correlation analysis of serum OPG levels with cardiovascular risk factors were performed (Table 3). Serum OPG levels were positively correlated with age, WHR, and serum TC, LDL-C, and FSH levels. There was no significant correlation between OPG and insulin levels or HOMA-IR (Table 3).

To exclude the effect of each variable on serum OPG, multiple regression analysis was performed with serum OPG level as the dependent variable (Table 4). Among the variables that had significant correlation with serum OPG level in bivariate correlation analysis, serum FSH and LDL-C levels were the significantly positive predictor for serum OPG level ($R^2 = 0.051$, $P < .05$).

4. Discussion

This study demonstrates that serum OPG levels are positively correlated with age, WHR, and serum TC, LDL-C, and FSH levels in healthy women. Among these, serum FSH and LDL-C level were the consistent predictors for serum OPG level after adjustment for other confounding factors.

Previous reports have presented similar results about the association between serum OPG levels and the age in women and men [17-19]. Bone matrix OPG levels also showed age-related increases both in cortical and trabecular bones [20]. Old age per se is a risk factor for cardiovascular disease and osteoporosis, and the incidences increase with aging. The increasing serum levels of OPG with aging could be interpreted as a compensatory mechanism to counteract the acceleration of atherosclerosis and bone resorption [17]. Further studies on the changes of production and clearance of OPG with aging are required.

Despite the lack of a significant association after adjustment for other confounding factors, we found a positive correlation between the serum OPG level and WHR, and the mean value of serum OPG levels in women with WHR ≥0.9 was significantly higher than in women with WHR <0.9. Furthermore, serum OPG levels were higher in hypercholesterolemic subjects. The mechanism by which centrally obese women with metabolic components have increased serum OPG levels is not clear. Obesity is regarded as an inflammatory disorder and mediates a systemic inflammatory response [21]. Interleukin 6 (IL-6), TNF-α, leptin, and peroxisome proliferator–activated receptor γ (PPARγ) may be the principal explanatory variables for the association of WHR and OPG. IL-6 and TNF-α are potent osteoclastic cytokines. Osteoprotegerin completely blocked TNF-mediated bone loss by increasing bone mineral density and bone volume in a transgenic mouse model [22]. Moreover, IL-6 and TNF-α were correlated with obesity, and this could underlie the association of insulin resistance with endothelial dysfunction, coagulopathy, and coronary heart disease [23,24]. Although the precise association of OPG with PPARγ is not clearly confirmed, increasing evidence indicates the PPARγ activation is of some importance in modulating the development and progression of atherosclerosis [25,26], and it was reported that the OPG expression in human aortic smooth muscle cells was significantly reduced in response to PPARγ activation [27]. One could speculate that during the process of vessel damage, OPG and PPARγ activation could act in concert to prevent the acceleration of atherosclerosis in proinflammatory milieu caused by obesity. Therefore, increased serum OPG levels in centrally obese subjects with metabolic syndrome might be partly explained as compensatory response to increased inflammatory reaction, which have harmful effects in the pathogenesis of atherosclerosis, which needs further research.

The hypothesis that the RANKL/OPG system could link osteoporosis and arterial calcification is underlined by high clinical prevalence and the coincidence of arterial calcification and cardiovascular disease in postmenopausal women and elderly people with osteoporosis [9-11]. In addition, atherosclerotic calcification shares features with bone calcification [28]. In the vascular system, OPG is produced by the smooth muscle and endothelial cells in vitro and acts as a survival factor for endothelial cells [29]. One recent study demonstrated RANKL and OPG immunoreactivity in a nondiseased vessel wall and in an early atherosclerotic lesion in human tissues, whereas in advanced calcified lesions, only RANKL was detected in the extracellular matrix surrounding calcium deposits [28]. Proresorptive inflammatory cytokines, such as IL-1α and TNF-α were found to elevate OPG expression in human microvascular endothelial cells, and microvascular endothelial cell–derived OPG might serve as an autocrine signal to inhibit blood vessel calcification [30]. In more detail, OPG is an α(v)β(3)-induced, NF-κB–dependent survival factor for endothelial cells [29]. It blocks endothelial cell apoptosis by binding TNF-related apoptosis-inducing ligand and prevents its interaction with death-inducing TNF-related apoptosis-inducing ligand receptors [31]. At present, there is no information about the main

sources and the regulatory mechanism of circulating OPG. It is still questionable whether the serum OPG levels represent the tissue or cellular OPG levels. Further studies are required to assess the contribution of RANKL and OPG in vascular diseases and to analyze their role as biochemical markers of vascular diseases.

In the present study, the mean serum OPG level was about 11% greater in postmenopausal women than in premenopausal women, and serum FSH levels were found to be a significant determinant for serum OPG levels, which is consistent with the results reported by other groups [17,18]. The precise mechanism for increased serum OPG levels in postmenopausal women is not clear, but it could be speculated to be elevated as a compensatory response to the acceleration of atherosclerosis and osteoporosis after menopause caused by estrogen-deficient status. As serum FSH levels cannot be the sole determinant marker for menopausal status, we defined menopause by combining clinically absence of menstruation for at least 12 months and elevation of FSH level at the same time. To date, the association of estrogen and OPG in human subjects is still controversial. No significant association was found between serum estrogen levels and serum OPG levels by one group [18], but there is a positive relationship between circulating OPG and serum estradiol by other groups [19,20]. One in vitro study has revealed that estrogen acts on osteoblastic cells to increase the secretion of OPG, suggesting the possibility of the role OPG in the antiresorptive action of estrogen on bone [32]. It could also be interpreted that higher serum OPG levels in postmenopausal women is related to the compensatory production of OPG to the acceleration of atherosclerosis caused by estrogen-deficient status. However, based on the results from recently published, large, randomized-controlled studies such as those from Women's Health Initiative, the traditionally believed vasculo-protective effects of estrogen are no longer convincing [33,34]. Because serum estradiol levels were not measured, the direct relationship between serum OPG levels and serum estradiol could not be evaluated, which could be a limitation to our study. The precise relationship of estrogen status and OPG needs further research.

In this study, serum OPG levels were higher in hypercholesterolemic subjects, and serum LDL-C level was the significant determinant for serum OPG levels after adjusting other confounding variables. Serum LDL-C level is considered as the treatment target for lipid-lowering therapy in subjects with cardiovascular diseases according to NCEP-ATPIII guidelines [16], and the elevated LDL-C levels are known to promote atherogenesis through oxidative modification within the artery wall, promoting formation of foamy cells and providing a stimulus for inflammation [35]. Previous studies have failed to prove any relationships of serum OPG levels with lipid profiles, although the studies reported positive correlations of OPG with atherosclerosis [12,13]. Although it could be hypothesized that serum OPG levels have elevated as the protective mechanism to early atherosclerotic changes that might have been caused by elevated LDL-C, more studies are needed to clarify the exact relationship of serum OPG level and lipid profiles.

In conclusion, our data show that circulating OPG is partially associated with age, central obesity, hypercholesterolemia, and the menopausal status in healthy Korean women. These findings suggest that OPG may be an important paracrine factor of cardiovascular disease related with menopause within the human female population.

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