RESEARCH ARTICLE

Osteoprotegerin Serum Level is Associated with Severity of Coronary Artery Calcification in Non Diabetic Centrally Obese Men

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Abstract

BACKGROUND: Osteoprotegerin (OPG) is produced by a variety of tissues including those of the cardiovascular system. Recent clinical studies have suggested a significant correlation between elevated OPG serum level and cardiovascular mortality. Since coronary artery calcification (CAC) is positively associated with cardiovascular disease (CVD) events, we carried out a study to investigate whether OPG serum level is associated with the severity of CAC in non diabetic centrally obese men.

METHODS: A cross sectional study was done on seventy non diabetic centrally obese men. CAC score was determined by using dual source computed tomography (DSCT). OPG serum level was measured by enzyme-linked immunosorbent assay (ELISA) method. Statistical analysis was done with SPSS for windows ver 16. ANOVA was performed to analyze mean, maximum, minimum value, and Standard of Deviation (SD). Spearman correlation test was performed to determine the correlation between OPG serum level and CAC score. Significance value was defined as alpha level= 0.05 based on two-tailed tests.

RESULTS: OPG serum level was significantly correlated with CAC score. The severity of CAC increased with the increase of OPG level. Age was significantly correlated with OPG serum level and CAC score.

CONCLUSIONS: Our data show that serum OPG level was associated with the severity of CAC, which highlights that OPG could be involved in the progression of CAC in non diabetic obese men.

KEYWORDS: obesity, vascular calcification, osteoprotegerin, coronary artery calcification

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Introduction

Obesity poses a major health problem in the world including Indonesia. Based on the latest report from the Riset Kesehatan Dasar (RISKESDAS) 2007, the National Basic Health Research 2007, the prevalence of central obesity was 18.8% (1). One of the most devastating and insidious conditions associated with obesity is cardiovascular disease, which is predicted to contribute to the deaths of 23.6 million people by 2030. (2) Epidemiological data suggest that obesity, cardiovascular disease and vascular calcification are coexisting, implying a potential link between obesity and vascular calcification. This suggests an interdependent link between cytokines, which interact on various levels of multiple tissue types, namely an "osteo-adipose-vascular" network landscape (3).

Since its initial discovery as a key regulator in bone metabolism, osteoprotegerin (OPG) has gained profound interest in its potential association with vascular disease and calcification as well. Although *in vitro* and animal studies have suggested that OPG inhibited vascular calcification,

clinical studies have suggested that serum OPG level was increased in association with vascular calcification, coronary artery disease, stroke and future cardiovascular events. This contradiction has led to extensive debate on the potential of OPG as a biomarker for vascular disease. (4) Therefore, our current study was conducted in order to elucidate the correlation of OPG with coronary artery calcification (CAC) in non-diabetic centrally obese men.

Methods

SUBJECT RECRUITMENT

This study was approved by the Health Research Ethics Committee, Faculty of Medicine, Hasanuddin University (No: UH10070157). Written informed consents were obtained from all of the study participants. Seventy Indonesian obese men (waist circumferences ≥ 90 cm) aged 45-71 years, were recruited. Subjects with diabetes, symptoms of infection, or any underlying acute inflammation were excluded. Height, weight, blood pressure and waist circumference were measured and body mass index (BMI) was calculated.

DSCT MEASUREMENT

All partcipants were scanned using a Somatom Definition DSCT scanner (Siemens Medical Solutions, Forcheim, Germany). The system is equipped with two X-ray tubes and two corresponding detectors mounted on a single gantry with an angular offset of 90° and a gantry rotation time of 330 ms. Datasets were reconstructed using a single-segmental reconstruction algorithm: slice thickness 0.75 mm; increment 0.4 mm; medium-to-smooth convolution kernel (B26); resulting in a spatial resolution of 0.6–0.7 mm in-plane and 0.5 mm through-plane.

SERUM HANDLING

Blood was collected by venipuncture in 10 mL serum tubes with sodium citrate (BD Vacutainer Systems) and stored for 20 minutes at room temperature before centrifugation. Serum and plasma were sub-sampled in aliquots and frozen at -70°C.

ELISA

Serum OPG was assayed with a sandwich ELISA kit (Biomedica Gruppe, Wien, Germany). Briefly, the kit contains two polyclonal antibodies, which bind to two different epitopes of the molecule. The lower limit of detection is 0.14 pmol/L, with intra and inter-assay variation of 4% and 8%, respectively. Our normal range median = 1.8 pmol/L.

HEKSOKINASE METHOD

Measurements of fasting plasma glucose was carried out by heksokinase methods (Roche diagnostics). A highly specific method for determining the concentration of glucose in serum or plasma by spectrophotometrically measuringthe NADP formed from hexokinase catalyzed transformations of glucose and various intermediates. Assay was performed according to the manufacturer's instruction.

ENZYMATIC METHOD

Creatinine level was measured by enzymatic method, which utilizes a multi-step approach ending with a photometric end-point reaction. The enzyme creatinine amidohydrolase is used to convert creatinine to creatine. Creatine is broken down to sarcosine and urea by creatine amidinohydrolase. Further enzyme linked steps with sarcosine oxidase and peroxidase yield a colored chromogen read at 545 nm. Assay was performed according to manufacturers' instruction.

STATISTICAL ANALYSIS

Statistical analysis was performed with SPSS for Windows ver 16. ANOVA was performed to analyze mean, maximum, minimum value and SD. Spearman correlation was performed to determine the correlation between OPG serum level and CAC score. Significant value were defined as alpha level= 0.05 based on two-tailed tests.

Results

All recruited subjects' characteristics including age, body mass index, waist circumference, blood pressure, creatinine serum, fasting plasma glucose were collected, as shown in Table 1. Possible correlations of age, waist circumference, OPG level and CAC score were analyzed, from which we found significant correlations between age and OPG level ($r = 0.294$; $p = 0.007$), age and CAC score ($r = 0.278$; $p = 0.010$), OPG level and CAC score ($r = 0.226$; $p = 0.030$) (Table 2).

Further analysis was conducted by dividing CAC score in some extents. As shown in Figure 1, we observed an increase of OPG level along with the increment of the extent of CAC score, showing a positive correlation between OPG level and CAC score.

Table 1. Characteristics of 70 non-diabetic centrally obese men involved in the study

BMP2 (pg/mL)	(min-max)	Mean ± SD
Age (year)	45 - 71	55 ± 7
Waist circumference (cm)	90 - 125	100 ± 8
Systolic Blood Pressure (mmHg)	100 - 183	129 ± 15
Diastolic Blood Pressure (mmHg)	60 - 100	82 ± 9
SGPT (U/L)	11 - 73	36 ± 15
GFR (ml/min/1.73m^2)	60 - 142	90 ± 18
Fasting Plasma Glucose (mg/dL)	81 - 123	98 ± 10
OPG level (pmol/L)	2.12 - 6.87	3.99 ± 0.97
CAC score (Agatston Unit)	0 - 2006	196 ± 314

Table 2. Correlation between age, OPG level, CAC score and waist circumference

Variable	OPG (pmol/L) r	CAC score (AU) p
Age (year)	0.294 *	0.278*
Waist Circumference (cm)	0.162	0.137
OPG (pmol/L)		0.226*

* p-value < 0.05.

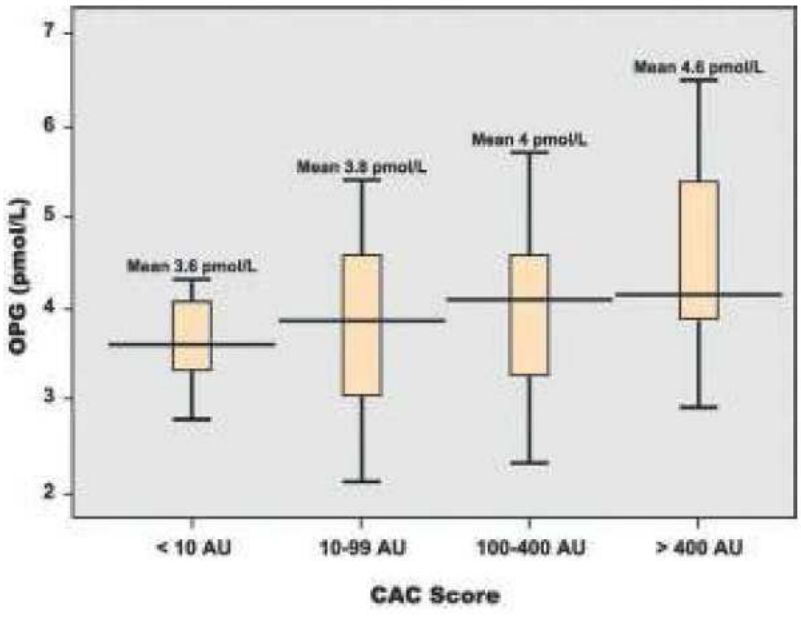

Figure 1. Comparison of OPG levels according to the extent of CAC scores.

Dicussion

Various OPG study in correlation to age were reported. Among them, an *in vitro* cell study reported a marked decrease of OPG expression from older cell; animal study reported decrease of *in situ* OPG expression with old rat (5); however, other clinical studies reported human OPG serum remained nearly constant with the increase of age or possibly increased along with aging in healthy population (6). Our results showed that aging significantly correlated with OPG serum level, in agreement with previous reports.

Calcium and phosphorus contents of vascular wall are increased with age. The vascular calcification linked to age is specific for arteries (7). Calcification that correlated with age and gender is first detected mostly in men at around age 40 (8), and in our study the youngest subject was 45 years old. Similarly, in our present study we found a positive correlation between age and CAC score among the 40-71 years old subjects.

In OPG knocked out mice, premature osteoporosis and arterial calcification were observed (9). It has been shown that administration of recombinant OPG protein to rats caused inhibition of arterial calcification induced by warfarin and vitamin D (10). Interestingly, the administration of recombinant OPG protein has shown to reverse osteoporosis and prevent vascular calcification, so it was concluded that OPG might play a role as an inhibitor of the calcification process (11). Our current results showed that OPG level increased along with CAC score. Some reports mentioned OPG level was increased in CVD (12,13,14,15,16). OPG level that was increased along with the increased severity of CAC might be due to the role of OPG in inhibiting RANKL, so differentiation of progenitor cells into osteoclasts was inhibited (17,18,19,20). In addition, OPG might indirectly prevent vascular calcification through its role in reducing the number of apatite nucleation in circulation. Association of OPG to TRAIL contributes to cell survival or prevents apoptosis in vascular smooth muscle cells (SMC) (21,22,23). In atherosclerosis, apoptotic body can be formed by nucleation of apatite (24). OPG increment could be a response to atherosclerosis, so that apoptosis in SMC could be prevented. Our current results showed a significant positive correlation between OPG and CAC where the high scores CAC reflected the severity of atherosclerosis and cardiovascular events (25,23,26). All of the above clinical and experimental data suggest an active role for OPG in vascular pathophysiology.

Conclusions

Our data show that serum OPG level was associated with the severity of CAC, which highlights that OPG could be involved in the progression of CAC in non diabetic obese men.

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