



Serum osteocalcin and bone mineral density in postmenopausal women

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ABSTRACT

Since high bone turnover is associated with decreased bone mass, biochemical markers of bone remodeling, such as serum osteocalcin, may be used to assess osteoporosis and to predict fractures in elderly women, particularly those involving trabecular bone, and use of a combination of bone mineral density (BMD) and biochemical markers may improve fracture prediction. The serum levels of osteocalcin constitute a specific biochemical parameter of bone formation. Compared to imaging techniques, assays for osteocalcin are safe, noninvasive and easily performed. The aim of this study was to determine the relationship of serum osteocalcin and BMD in postmenopausal women. A cross sectional study was performed on 53 postmenopausal women in South Jakarta from February to April 2010. The subjects were assessed for anthropometric characteristics, serum osteocalcin levels and BMD. BMD was measured at the lumbar spine, right femoral neck and at the left distal radius by dual energy X-ray absorptiometry (DXA). Mean serum osteocalcin was 28.99 ± 10.02 ng/ml. The Pearson correlation test on all subjects indicated a significant inverse correlation between serum osteocalcin and femoral neck BMD ($r = -0.29$; $p=0.034$). By arranging the data into tertiles, a significant association was found in non-obese subjects between mean femoral neck BMD and serum osteocalcin ($p=0.036$). The Tukey posthoc multiple comparison test showed a significant mean difference in femoral neck BMD between the lowest and the highest tertiles of osteocalcin serum concentrations ($p=0.028$). Maintenance of body weight is important for maintaining BMD in postmenopausal women.

Key words: Serum osteocalcin, BMD, body weight, postmenopausal women

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INTRODUCTION

The increased knowledge of society on healthy living has resulted in an increase in the numbers of older persons worldwide,

including Indonesia. The 2010 population census indicates that older persons constitute approximately 10 percent of the Indonesian population, whilst the 15.5 million menopausal women in the year 2000 are predicted to

increase to 24 million in 2015.⁽¹⁾ Osteoporosis is one of the health problems arising in women entering menopausal age, and will presumably seriously affect the lives of postmenopausal women. Osteoporosis and the potentially serious consequences of osteoporotic fracture increase with advancing age of the population, making skeletal health assessment an important component of routine care in postmenopausal women.⁽²⁾

Osteoporosis-related fracture risk is usually assessed by bone mineral density (BMD) as a measure of bone mass and a predictor of fracture, since bone mass affects bone strength or the ability of a bone to withstand trauma. It is well established that 75-90% of the variance in bone strength is related to BMD. The risk of fracture is known to be higher in women with low BMD, with the risk doubling for a reduction of 1 standard deviation in BMD.⁽³⁾

However, since bone strength is also determined by other factors, such as bone geometry, microarchitecture, and size, BMD by itself is not a good predictor of fracture risk. Epidemiological data indicate that high body weight or high body mass index (BMI) are related to high bone mass, while a reduction in body weight may result in loss of bone mass.⁽⁴⁾ On the other hand, some are of the opinion that fat mass is negatively related to BMD.^(7,8) Hsu et al. showed that postmenopausal women with a higher percentage of body fat may have a higher risk for osteoporosis, osteopenia, and nonspine fractures.⁽⁹⁾

Decreased estrogen concentrations at menopause age lead to lower intestinal absorption of calcium, resulting in low serum calcium concentrations and increased osteoclastic resorption of bone. Both increase bone turnover and constitute risk factors for the development of osteoporosis.⁽⁹⁾ Menopause and aging are associated with accelerated loss of cortical bone. Bone loss is the result a negative remodeling balance due to impaired bone formation and/or increased bone

resorption.^(10,11)

Fractures may be predicted from bone turnover markers either via BMD, because low BMD is associated with high bone turnover,⁽¹²⁾ or independently of BMD, since increased bone turnover negatively affects bone microarchitecture and fragility.⁽¹³⁾

Currently biochemical markers of bone turnover are being used for predicting the rate of bone loss and for assessing the risk of fractures in postmenopausal women. Estimation of bone turnover rates may be obtained through determination of the serum or urinary concentrations of certain proteins that are representative of the bone remodeling process. These proteins may be divided into bone formation markers and bone resorption markers. The most specific and sensitive bone formation markers include osteocalcin and bone alkaline phosphatase, which are indicative of osteoblastic activity, whereas bone resorption markers, such as tartrate resistant acid phosphatase and cross-linked telopeptides, reflect osteoclastic activity.⁽¹⁴⁾

Osteocalcin (OC), also termed Gla (̑ carboxyglutamic acid) protein, is a bone matrix protein synthesized by mature osteoblasts, and constitutes approximately 15% of noncollagenous bone matrix proteins.⁽¹⁵⁾ Most of the osteocalcin (80-90%) is adsorbed to bone hydroxyapatite, with a minor percentage leaking into the circulation.⁽¹⁶⁾ Serum OC may be detected by various tests, such as assays using monoclonal antibodies against OC N-mid and N terminal OC fragments. Osteoporotic females have significantly higher levels of OC, bone alkaline phosphatase, and crosslinked telopeptide-C (CTX).⁽¹⁷⁾ In general, serum OC concentrations increase with age, such that women aged over 65 years of age have an approximately 2 fold higher OC concentration in comparison with women under 44 years of age.⁽¹⁸⁾ The aim of the present study was to investigate the relationship between serum osteocalcin and BMD values in postmenopausal women.

METHODS

Study design

From February to April 2010 a cross-sectional study was carried out at the Mampang Prapatan Community Health Center, South Jakarta.

Study subjects

The study sample was selected by cluster and simple random sampling from postmenopausal women in the Mampang Prapatan area meeting the following inclusion criteria: postmenopausal women in the age range of 47-60 years, duration of menopause of more than one year, not consuming steroids and bisphosphonates, actively mobile, able to communicate, and willing to participate in this study. The exclusion criteria were: a past history of hysterectomy, malignancies, hormonal therapy, diabetes mellitus, abnormality of liver and kidney functions, and acute infections. A total of 53 female subjects were included in this study. All participants gave written informed consent.

Questionnaire

The respondents filled out a questionnaire relating to demographic data, including age, education, employment, number of pregnancies, and duration of menopause.

Anthropometric assessments

Height was measured to the nearest 0.1 cm using a portable microtoise and weight to the nearest 0.1 kg using Sage portable scales. Body mass index (BMI) was calculated as the weight (kg) divided by the square of the height (m). For Asian populations, BMI classified into the following categories: underweight (<18.5 kg/m²), normal (18.5-22.9 kg/m²), overweight (23.0 -25 kg/m²), and obese (>25 kg/m²).⁽¹⁹⁾

BMD measurements

BMD measurements (g/cm²) for the lumbar spine (L1-L4), femoral neck and distal

radius were obtained by dual energy X ray absorptiometry (DXA) using a Lunar DPX Bravo Nomusa densitometer (GE Medical Systems). The BMD measurements were performed at Budi Jaya Hospital, South Jakarta.

Osteocalcin measurements

Measurement of OC was performed on fasting serum samples collected before 10 a.m. and stored at a temperature below -70 °C before analysis. Determination of serum OC concentration was performed at Prodia Laboratories, Jakarta, using the Elecsys N mid osteocalcin assay reagent kit (Roche Diagnostics, Cat No. 12 149 133). The Elecsys N mid osteocalcin assay utilizes two monoclonal antibodies, one specific for epitopes on the N mid OC fragment and the other specific for the N terminal OC fragment, and therefore detects the intact OC as well as the N-mid fragment. The detection range of the kit was 0.5 – 300 ng/ml, while the coefficient of variation was 2.28%. Serum OC concentrations were divided into tertiles, the lowest tertile ranging from 12 up to 24.39 ng/ml, while the middle and highest tertiles were 24.4 – 32.45 ng/ml and 32.46 – 70.59 ng/ml, respectively.

Ethical clearance

The protocol for this study was approved by the Research Ethics Committee, Medical Faculty, Trisakti University.

Statistical analysis

Data were entered to SPSS format using version 17.0. Categorical variables were summarized using frequencies and percentages, while continuous variables were summarized using mean and standard deviation. One-way analysis of variance (ANOVA) was used to compare the mean difference in BMD in the tertile serum osteocalcin levels. A significant ANOVA test was followed by a Tukey post hoc multiple comparison test to determine which specific

Table 1 Characteristics of the subjects (n=53)

Characteristic	n (%)
Age (yrs)	53.49 ± 3.36 ^a
Duration of menopause (yrs)	4.55 ± 2.38 ^a
Education	
Did not finish senior high school (SMA)	45 (84.9)
Senior high school graduate	7 (13.2)
Academy/university	1 (1.9)
Employment	
Unemployed	31 (58.5)
Self-employed (informal sector)	22 (41.5)
Number of pregnancies	
0-3 pregnancies	19 (35.8)
> 3 pregnancies	34 (64.2)
Osteocalcin (ng/ml)	28.99 ± 10.02 ^a
Tertiles of serum osteocalcin (ng/ml)	
Lowest	17 (32.1)
Mid	18 (34)
Highest	18 (34)
Nutritional status	
Non obese	41 (77.4)
Obese	12 (22.6)
BMD values	
Lumbar BMD (g/cm ²)	0.90 ± 0.12 ^a
Femoral neck BMD (g/cm ²)	0.78 ± 0.12 ^a
Radial BMD (g/cm ²)	0.53 ± 0.08 ^a

^aMean ± SD

groups were significantly different. Statistical significance was set at $p < 0.05$.

RESULTS

In this study there were 53 women meeting the inclusion criteria, for whom the major characteristics are shown in Table 1. The mean age of the respondents was 53.49 ± 3.36 years, while the duration of menopause was 2 - 7 years. A total of 41 subjects (77.4 %) was

non-obese and the mean of femoral neck BMD was 0.78 ± 0.12 g/cm².

In non-obese postmenopausal women, femoral bone mass was found to be significantly higher (0.82 ± 0.89 g/cm²) in the lowest serum OC tertile, in comparison with that in the mid (0.77 ± 0.11 g/cm²) and higher tertiles and (0.71 ± 0.13 g/cm²) respectively (Table 2). However, for the lumbar and radial bones, the mass was not significantly different between lower serum OC tertile and the other two tertiles.

Table 2. Mean of bone marrow density by tertiles of serum osteocalcin in non-obese subjects

	Tertiles of serum osteocalcin (ng/ml)			p
	Lowest (n=13) 12 – 24.39	Mid (n=14) 24.4 – 32.45	Highest (n=14) 32.46 – 70.59	
BMD (g/cm ²) ^a				
Lumbar	0.91 ± 0.11 ^a	0.86 ± 0.10	0.85 ± 0.11	0.364
Femoral neck	0.82 ± 0.89	0.77 ± 0.11	0.71 ± 0.13	0.036
Radial	0.56 ± 0.09	0.51 ± 0.08	0.51 ± 0.05	0.271

^aMean ± SD

Table 3. Mean differences and SEs of femoral neck BMD in non-obese subjects
(Tukey multiple comparisons procedures)

Tertile of serum osteocalcin		Femoral neck BMD ^a	p
Lowest	Mid	0.051 ± 0.041	0.444
	Highest	0.112 ± 0.041	0.028
Mid	Lowest	-0.051 ± 0.041	0.444
	Highest	0.061 ± 0.041	0.305

^aMean ± SD

The results of the Tukey post hoc multiple comparison test indicated a significant mean difference in femoral neck BMD between the lowest and highest tertiles of serum osteocalcin concentrations ($p=0.028$) (Table 3).

The results of the Pearson correlation test showed a significant inverse correlation between serum osteocalcin concentrations and femoral neck BMD ($r = -0.29$; $p = 0.034$) (Table 4).

DISCUSSION

In the present study, femoral neck BMD had a significant negative correlation with serum osteocalcin. Since there is a complex relationship between bone turnover and bone mass, such that high bone turnover is associated with decreased bone mass,⁽²¹⁾ it has been suggested that bone markers can predict fractures in elderly women, particularly those involving trabecular bone,⁽²²⁾ and that the use of a combination of BMD and bone markers can improve fracture prediction.⁽²³⁾ A case control study of 90 postmenopausal women with mean age of 66 ± 8 years showed results

that were consistent with the results of the present study, in that there is a significant inverse correlation of proximal femur BMD with osteocalcin.⁽²⁴⁾ However, a study in Pakistan, involving 50 postmenopausal women with mean age of 54.36 ± 0.81 , obtained inconclusive results indicating lack of a significant association between BMD and serum osteocalcin concentrations.⁽²⁵⁾ Biochemical markers of bone turnover, such as osteocalcin, may also be used for early evaluation of the effects of treatment. Additionally, serial measurements of bone markers may assist in evaluating the patients' responses to specific antiresorptive therapy.⁽²⁶⁾ Serum OC is considered a specific marker of osteoblast function, as its levels have been shown to correlate with bone formation rates. However, since it is also released from bone matrix during bone resorption, it reflects the overall turnover of bone and is considered as a bone turnover marker. OC has a high affinity for calcium and has a compact α helical conformation that is calcium dependent. The α carboxyglutamic acid (Gla) residues of OC are capable of binding to bone matrix hydroxyapatite, thus leading to bone mineralization. Calcium- and phosphorus-deficient osteoporotic women may have a decreased rate of bone mineralization due to a reduction in hydroxyapatite crystal formation. In this condition, free OC may be present in the circulation, thus explaining the increased serum OC concentration in osteoporotic postmenopausal women.^(2,12,18)

Table 4. Correlation between serum osteocalcin and BMD in all subjects (n=53)

BMD	Serum osteocalcin r values	p
Lumbar	- 0.079	0.572
Femoral neck	-.0.290	0.034
Radial	-.0.093	0.502

The current study showed that non-obese postmenopausal women in the high osteocalcin tertile had the lowest mean femoral neck BMD values. These results are similar to those of a population survey that included women in the age range of 20–69 years, indicating that women with high OC concentrations had the lowest mean BMD values.⁽²⁷⁾ Other studies on elderly women have shown low body weight to be a major predictor of BMD and bone loss, and this association may be mediated by increased bone turnover.^(28,29) In the study by Zhao et al.⁽⁸⁾ it was found that fat mass is negatively related to bone mass when adjusted for body weight.⁽⁸⁾ In another study, there was a significant risk of osteoporosis, osteopenia, and non spine fractures in subjects with high weight-adjusted body fat percentages.⁽¹²⁾ BMD in postmenopausal women is determined by peak bone mass and the amount of bone lost since menopause. In several studies, body weight or BMI was shown to be inversely associated with bone turnover markers, where persons with a low body weight have high bone turnover, whereas overweight and obese persons had a lower serum OC than those of normal weight.⁽³⁰⁻³²⁾ Apparently, the effect of obesity on fracture risk depends on its definition. If defined on the basis of BMI or body weight, obesity may be protective against bone mineral loss or vertebral fracture. However, if obesity is based on the percentage of body fat, obesity may be a risk factor for osteoporosis. In this connection, both metabolic syndrome and hyperglycemia could be risk factors for osteoporosis. High HDL protects against osteoporosis, increasing BMD and lowering fracture risk.⁽³³⁾

While biochemical markers of bone turnover may be able to predict bone loss and thus fracture risk, they may also predict fracture risk independently of BMD. High bone turnover can disrupt the trabecular architecture by increasing the incidence of trabecular perforation and buckling, thus reducing bone strength, without necessarily appreciably

affecting BMD. Because of the overlap in BMD distribution of normal and fracture populations, the BMD criteria used are useful in population-based measurements but less useful in assessing individual risk.⁽³⁴⁾ Similarly, a single measurement of a biochemical marker of bone turnover may be unable to predict even short-term individual fracture risk. However, there is increasing evidence that a combination of a biochemical marker and BMD may be a better predictor of fracture than BMD alone. Biochemical markers of bone turnover cannot substitute for serial BMD measurements, but may be useful when considered in conjunction with BMD measurement. The present study admittedly has several limitations. Firstly, this study performed only investigations on bone formation without investigating bone resorption. Secondly, the study design was cross-sectional and therefore cannot depict a causal relationship between OC concentrations and BMD. Lastly, the age of the respondents was relatively homogenous in the 50–60 year range, while the duration of menopause was 2–7 years, such that this study can only describe bone changes occurring during early menopause.

CONCLUSIONS

This study demonstrates a significant inverse correlation between serum osteocalcin and femoral neck BMD. In non-obese subjects there is a significant difference in femoral neck BMD values between the lowest and the highest tertiles of osteocalcin serum concentrations.

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