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BONE MINERAL DENSITY AND VITAMIN D STATUS IN ELDERLY JAVANESE WOMEN

Lusiana Batubara[1*], Faizah Fulyani[1], Dwi Ngestiningsih[1;2], Hermina Sukmaningtyas[3], Ainun Rahmasari Gumay[4], Etisa Adi Murbawani[5], Enny Probosari[5]

[1]Department of Medical Biology and Biochemistry, Faculty of Medicine, Universitas Diponegoro, Semarang, Indonesia
[2]Department of Internal Medicine, Faculty of Medicine, Universitas Diponegoro, Semarang, Indonesia
[3]Department of Medical Radiology, Faculty of Medicine Universitas Diponegoro, Semarang, Indonesia
[4]Department of Physiology, Faculty of Medicine Universitas Diponegoro, Semarang, Indonesia
[5]Department of Clinical Nutrition, Faculty of Medicine, Universitas Diponegoro, Semarang, Indonesia
* Corresponding Author : E-mail: tj.batubara@fk.undip.ac.id

ABSTRACT

Background: Vitamin D (25OHD) works as a lipid-soluble secosteroid hormone essential for metabolic activities, including bone mineralization. 1,25OHD and its receptor are transcription factors for various bone homeostasis genes. Numerous studies have connected vitamin D insufficiency with a decline in bone mineral density (BMD), but the results are still conflicting. However, information on vitamin D insufficiency among Indonesian individuals is hardly available. **Objective:** The study aims to evaluate vitamin D levels and their correlation with BMD among Javanese elderly women. **Methods:** 75 healthy Javanese elderly women between 60 and 84 participated in a cross-sectional study. An enzyme-linked immunoassay kit for 25OHD was used to measure the level of serum vitamin D. DXA, or dual-energy X-ray absorptiometry, was used to measure BMD. **Results:** The study population's mean serum 25OHD level was 14.97±6.6ng/mL. We found that 73.3% were deficient in vitamin D, and 26.7% were not. There is no correlation between vitamin D and BMD L-spine, F-neck, or T-score (p=0.064, -0.215; p=0.443, -0.090; and p=0.109, -0.187, respectively). Lower BMD L-spine, F-neck and T-score were correlated with increased age (r = -0.238, p = 0.040; r = -0.377, p = 0.001; and r = -0.295, p = 0.010, respectively) and decreased BMI (r = 0.525, p = 0.000; r = 0.516, p = 0.000; and r = 0.520, p = 0.000, respectively). **Conclusion:** From this study, we concluded that vitamin D deficiency was prevalent among Javanese elderly women. However, the vitamin D level in this population and bone mineral density do not appear to be correlated.

Keywords: *BMD; DXA; elderly; vitamin D; 25OHD*

INTRODUCTION

Vitamin D, a lipid-soluble secosteroid hormone, significantly influences the human body's metabolism and bone mineralization.[1,2] Vitamin D made in the skin (cholecalciferol) during ultraviolet-B (UVB) exposure of 7-dehydrocholesterol or vitamin D ingested in the diet (ergocalciferol) is biologically inactive and requires two sequential steps of hydroxylations. It is converted to its physiologically active form, 1,25OHD, after being hydroxylated in the liver to generate 25OHD.[3] Cytochrome P450 (CYP) 2R1, 27A1, and 3A4 are 25-hydroxylases that aided the first step of the 25-hydroxylation reaction, and CYP27B1 is a 1-hydroxylase that helped the second step.[4]

Vitamin D status is assessed using serum 25OH)D levels. Although there is disagreement among clinicians as to what constitutes an acceptable vitamin D status, most believe that a serum 25OHD level below 20 ng/mL indicates a deficiency.[5] Vitamin D deficiency has a high prevalence worldwide, not only in four seasonal countries but also in tropical countries.[6-9] One of the groups at risk for vitamin D insufficiency is the elderly.[7] Elderly people in industrialized nations who live in their communities and those who are institutionalized frequently experience it.[10] A study in 2004 demonstrated that of 74 elderly women living in the institutionalized care unit in Indonesia, 35.1% were vitamin D deficient.[11]

Osteoporosis is the most prevalent degenerative disease among elderly women. International Osteoporosis Foundation states that one in three women over 50 will experience osteoporotic fractures worldwide.[12] Low bone mass and increased skeletal fragility are two characteristics of the multifactorial condition osteoporosis. The "gold standard" for detecting osteoporosis is dual-energy X-ray absorptiometry (DXA), which estimates BMD.[13] A complex between 1,25OHD and its receptor is a transcription factor for various genes involved in bone homeostasis.[14] A lack of vitamin D has been associated in several studies with a decline in BMD, which causes bone loss and an increased

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risk of osteoporosis[15-17], but the result was still conflicting.[18-22]

It is still unclear how serum vitamin D levels are related to bone mineral density. However, data regarding vitamin D deficiency in the community-dwelling population in Indonesia are rarely available, and its relation with BMD is still unknown. This study aims to measure the vitamin D levels of elderly Javanese women living in their community and determine whether blood 25OHD levels and BMD in the Javanese population are related.

METHODS

Study Population

Javanese women over 60 who were in good health were chosen at random from an elderly health service in Semarang, Central Java, Indonesia, between May to October 2018. Community dwellers elderly women with no history of diabetes, liver disease, chronic kidney disease, or thyroid disorder and were not taking medication that affected calcium, vitamin D, and bone metabolism were eligible for the present study.

Study Design

Cross-sectional observational analytical design was employed in this work. Each subject was requested to fill out a questionnaire after giving their informed consent to gather basic information about them, their medical history, and their drug use history. All subjects underwent anthropometric measurement, and samples of blood were drawn to quantify SGOT, SGPT, serum creatinine level, and serum 25OHD concentration at the central laboratory of the institute and BMD measurement was conducted at Telogorejo Hospital Semarang.

Anthropometric measurements

With light clothing on, calibrated digital scales were used to measure weight that were precise to 0.1 kg. Without shoes, using a wall-mounted stadiometer, height was calculated to the nearest 1 cm. The Body Mass Index (BMI, kg/m2) was computed by dividing the weight by the square of the height. BMI 18.5 was considered underweight, 18.5-24.9 was considered normal weight, 25.0-29.9 was considered overweight, and BMI 30 was deemed obese.

Biochemical measurements

Serum samples obtained from venous blood were stored at -80^{o}C until analyzed. The IFCC method without pyridoxal phosphate (P-51-P) was used to quantify blood creatinine, serum glutamic oxyaloacetic transaminase (SGOT), and serum glutamic pyruvic transaminase (SGPT). An enzyme-linked immunoassay kit for 25 (OH)D was used to measure the serum vitamin D level (Diagnostics Biochem Canada Inc.). In this study, Serum 25OHD levels below 20 ng/mL are considered deficient in 25OHD, while levels between 20 and 29 ng/mL are considered insufficient. Serum 25OHD levels above 30 ng/mL are sufficient.[23]

Assessment of Bone Mineral Density

Dual-energy X-ray absorptiometry (DXA) GE Prodigy lunar iDXA was used to measure the bone mineral density (BMD) in the hip and lumbar spine regions. The hip densitometry measurement was the femur neck (F-neck) region, and the lumbar spine (L-spine) densitometry measurement included the vertebrae lumbar L1-L4. The BMD data (g/cm^2) obtained from the DXA test were used to calculate T-score, which defines osteoporotic status as follows: T-score ≥-1 was considered normal, T-score between -1 to -2.5 was considered osteopenia, and T-score ≤-2.5 was considered osteoporotic.[13]

Statistical Analysis

Statistical data analysis was performed using SPSS version 20 (SPSS Inc., Chicago, IL, USA). While categorical variables were shown as frequencies and percentages, continuous variables were represented as mean, standard deviation (SD). The mean difference between the two groups was assessed using the Mann-Whitney U test. The correlation of two numerical variables was analyzed using the Pearson or Spearman test. P-values <0.05 were considered to be significant.

RESULTS

Table 1 lists the general characteristics of the study population. There were 75 Javanese elderly women recruited in this study, with a mean age of 65.66 ± 5.6 (range 60-84). 78.7% of subjects were between 60-69 years old, and the rest were older. The mean BMI of the study population was 25.54 ± 4.8

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kg/m^2 (range 16.4-40.7), and Table 2 shows that most of the study population (45.3%) had a normal weight.

Table 1. Baseline characteristic of the study population

Variable	Mean ± SD	Min.	Max.
n = 75			
Age (years)	65.66 ± 5.6	60	84
Weight (kg)	57.42 ± 9.6	38	80
Height (m)	1.50 ± 0.05	1.35	1.65
BMI (kg/m^2)	25.54 ± 4.8	16.4	40.7
Laboratory Parameters			
SGOT (U/L)	21.28 ± 3.7	13.4	28.3
SGPT (U/L)	14.06 ± 5.2	7	34.8
Creatinine (mg/dL)	0.68 ± 0.13	0.45	1.06
25OHD (ng/mL)	14.97 ± 6.6	4.25	31.14
Bone Mineral Density			
L-spine (g/cm^2)	0.91 ± 0.16	0.63	1.34
F-Neck (g/cm^2)	0.73 ± 0.10	0.52	1.02
T-score	-2.2 ± 1.3	-4.7	1.2

Mean SGOT and SGPT were 21.28 ± 3.7 U/L (range 13.4 -28.3) and 14.06 ± 5.2 U/L (range 7-34.8), respectively and mean serum creatinine level was 0.68 mg/dL (range 0.45 - 1.06) indicated that all of the study participants have no liver abnormality or kidney failure.

Table 2. General description of categorical variables

Variable	n	%
Age (years)		
60-69	59	78,7
70-79	14	18.7
80-89	2	2.7
BMI groups		
Underweight	2	2.7
normal weight	34	45.3
Overweight	24	32
Obese	15	20
BMD classification		
Normal	12	16
Osteopenia	25	33.3
Osteoporosis	38	50.7
Vitamin D status		
Deficiency	55	73.3
Insufficient	18	24
Sufficient	2	2.7

The study population's average serum 25OHD level was 14.97 ng/mL. We found that 73.3% (n=55) of the study population had vitamin D deficiency, and 26.7% (n=20) did not (table 2). Table 3 depicts the characteristics of the study populations classified as vitamin D deficient (25OHD < 20 ng/mL) and not (25OHD ≥20 ng/mL). This study found no significant difference in baseline characteristics (age, weight, height, and BMI) between the 25OHD deficient and non-deficient groups.

Table 3. Comparison of baseline characteristics between vitamin D deficient and non-deficient group

Variable	deficient group (n=55)	Non-deficient group (n=20)	p-value*
Age, years (mean±SD)	65.41 ± 5.6	65.85 ± 6.3	0.86
Weight (kg)	58.15 ± 10.3	55.40 ± 7.3	0.26
Height (m)	1.50 ± 0.05	1.52 ± 0.06	0.35
BMI (kg/m^2)	25.8 ± 4.7	24.7 ± 5.0	0.25

*Mann-Whitney U test

Table 4. Correlation between serum 25OHD levels and BMD (r; p-value)

Variable	BMD L-spine	BMD F-Neck	T-score
25OHD levels	-0.215; 0.064	-0.090; 0.443	-0.187; 0.109

Table 5. Serum 25OHD levels and BMD with age and BMI (r; p-value)

Variable	Age	BMI
25OHD levels	-0.033; 0.780	-0.130; 0.265
BMD L-spine	-0.238; 0.040*	0.525; 0.000*
BMD F-Neck	-0.377; 0.001*	0.516; 0.000*
T-score	-0.295; 0.010*	0.520; 0.000*

*Correlation is significant at the 0.05 level

The study participants show a mean L-spine and F-neck of 0.91±0.16 g/cm^2 (range 0.63-1.34) and 0.73±0.10 g/cm2 (0.52-1.02). 50.7% of the study population had osteoporosis, 33.3% had osteopenia, and 16% were normal. There is no correlation between serum 25OHD levels and BMD at the L-spine (L1-L4, r = -0.215, p=0.064), F-neck (r=-0.090, p=0.443) or T-score (r=-0.187 p=0.109) (table 4). However, in this study, we found a weak negative correlation between BMD at the L-spine, F-neck and T-score with age (r = -0.238, p=0.040; r=-0.377, p=0.001; and r=-0.295, p=0.010 respectively) and

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moderate positive correlation with BMI (r=0.525, p=0.000; r=0.516, p=0.000; and r=0.520, p=0.000 respectively) (table 5).

DISCUSSION

The primary source of vitamin D generation is sunlight exposure. When 7-dehydrocholesterol in the skin is exposed to UV-B (290-320 nm), it will absorb the energy of the UV-B radiation resulting in pre-vitamin D3, a molecule with unstable thermodynamics. Pre-vitamin D3 then rapidly isomerizes into vitamin D3.[1] Given the abundance of sunshine, it makes sense that those who live in tropical and subtropical regions would have higher vitamin D levels than those who reside in the four-season areas. Nevertheless, several studies have reported a lack of vitamin D even in nations where people can obtain enough sun exposure, like China, Korea, Thailand, and India.[9,24-26]

One of the groups at risk for vitamin D insufficiency is the elderly.[7] Studies conducted on 170 community residents over 65 in rural southern Taiwan demonstrated that 30.6% of men and 57.7% of women have low vitamin D status (25OHD < 30 ng/mL). Participants with poor vitamin D levels were primarily women (65.3%).[27] A cross-sectional population-based study conducted among Chinese non-institutionalized between the ages of 50 and 70 in Beijing and Shanghai, China, documented Up to 69.2% of the participants in the study had vitamin D deficiency.[28] Another study from sunshine-abundant Hyderabad metropolitan city, South India, showed that 56.3% of the senior population in urban areas were vitamin D deficient.[29] Our study conducted in Semarang city, the capital and largest city in Central Java, Indonesia (latitude 6°58′S), showed a significant frequency of vitamin D insufficiency in older women., up to 73.3%. From these studies, we can conclude that regardless of latitude, elderly people who lived in communities frequently had vitamin D deficiencies.

Numerous studies have assessed the vitamin D deficiency risk factor without considering seasonal variations in UV exposure. This study did not find differences in baseline characteristics (age, weight, height, and BMI) between the 25OHD deficiency and non-deficiency groups. This conclusion is consistent with research conducted in Singapore among Chinese adults (aged 45 to 74), which discovered no connection between age, BMI, and the level of serum vitamin D. This 63,257 subject population-based prospective cohort research identified important indicators of vitamin D concentration in female subjects: dietary vitamin D intake and genetic variation in enzyme cytochrome P450(CYP)2R1, 3A4 and vitamin D binding protein (GC).[30] In addition, Huang et al. showed that vitamin D status was unrelated to age or BMI among older people in southern Taiwan. In contrast, inadequate sun exposure was the only predictable risk in elderly women.[27]

Malay women had a considerably lower mean vitamin D concentration than Chinese women, according to the study results on 276 postmenopausal women in 2º N near Kuala Lumpur, Malaysia. (68.8 ± 15.7 and 44.4 ± 10.6 nmol/L, p<0.05 respectively). Besides having more skin pigmentation, Malay women follow religious dress codes using hijab and closed clothes that limit sun exposure.[31] Only a few studies in Indonesia have focused on the status of vitamin D deficiency in the elderly. The previous study in 2005 conducted in four Institutionalized care units in Indonesia demonstrated low sun exposure as a possible risk for vitamin D deficiency, but no statistical association was reported.[11] Vitamin D deficiency is commonly seen in elderly women due to various risk factors interacting in this population. Besides ecological factors (weather and season conditions to latitude), lifestyle and individual factors such as genetic variation and skin pigmentation might influence serum vitamin D levels in elderly women.

Our study found no significant correlation between serum vitamin D levels and BMD at the L-spine or F-neck sites among Javanese elderly women. This finding was inconsistent with the previous study demonstrating a positive correlation between vitamin D levels and BMD in the elderly.[16,32,33] However, a previous study conducted in Hyderabad, India, observed 100 healthy postmenopausal women also demonstrated no correlation between serum 25OHD and BMD both at the F-neck (r = 0.11; p = 0.29) and the L-spine (r = 0.09; p = 0.35) sites.[21] This study was also in line with another study in India[19], Saudi Arabia[18], and Thailand.[34]

Vitamin D deficiency has been known to cause bone loss via secondary hyperparathyroidism. Low levels of 25OHD reduced the amount of 1,25OHD, which in turn reduced intestinal absorption of

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calcium. Reduced serum calcium concentration induces parathyroid glands to enhance parathyroid hormone (PTH) expression, synthesis, and release, which helps maintain serum calcium levels by releasing calcium from bone resorption.[17] Nevertheless, numerous investigations have shown that not all individuals with low vitamin D levels experienced secondary hyperparathyroidism, negating its ability to cause bone loss.[35]

The significant factors that affected BMD in our study at the L-spine and F-neck sites were increased age and decreased BMI. This study supported a previous finding in a longitudinal study that increasing ages and decreased BMI was associated with BMD loss and not affected by serum 25-OH vitamin D status.[36] Age-related bone loss was a complex mechanism involving many factors. It is not only due to hormonal factors but also genetic alterations in cellular components of the bone, biochemical and vasculature status. It is also affected by extrinsic factors such as nutrition, physical activity, history of comorbid medical conditions and drugs used.

There were some limitations of our study. First, we did not measure serum PTH levels. The absence of serum PTH data in this study limits the analysis of the relationship between serum vitamin D status and BMD in our study population. Second, information about food recall and physical activity is unavailable in this study as it is an important factor contributing to bone mass remodelling.

CONCLUSION

From this study, we concluded that vitamin D deficiency was prevalent among Javanese elderly women. However, the vitamin D level in this population and bone mineral density do not appear to be correlated.

ETHICAL APPROVAL

Ethical clearance was achieved with the consent and assessment of the Health Research Ethics Commission of the Faculty of Medicine, Diponegoro University. The subject willingly joined the study by signing informed consent.

CONFLICTS OF INTEREST

The authors declare no conflict of interest

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