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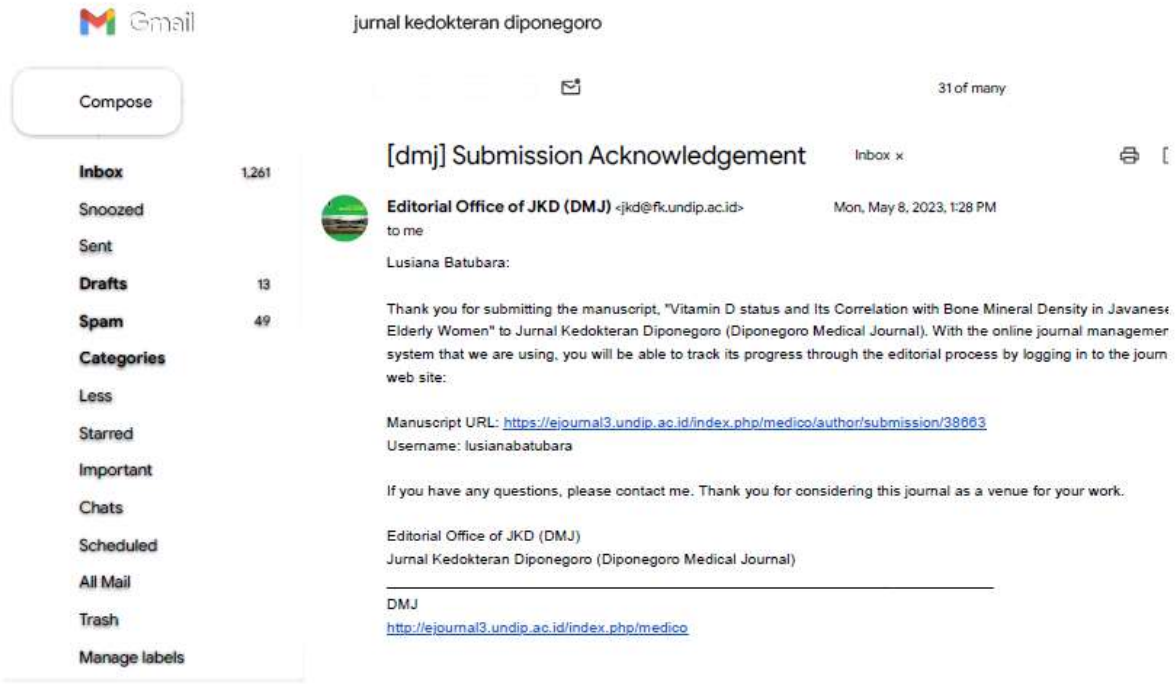

Vitamin D status and Its Correlation with Bone Mineral Density in Javanese Elderly Women

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Abstract

Background: Vitamin D (25(OH)D) is a fat-soluble secosteroid hormone that plays a major role in bone mineralization and other metabolic processes in the human body. A complex between biologically active vitamin D (1,25(OH)2D) and its receptor serves as a transcription factor for various genes involves in bone homeostasis. Several studies have been link vitamin D deficiency with a reduction in Bone Mineral Density (BMD), but the result was still conflicting. However, data regarding vitamin D deficiency in the Indonesian population are rarely available.
Objective: The study aims to assess vitamin D status and its correlation with Bone Mineral Density (BMD) among Javanese elderly women.
Methods: A cross-sectional study was conducted in 75 healthy Javanese elderly women aged 60-84 years old. Serum Vitamin D was measured by enzyme link immunoassay using 25(OH)D ELISA kit. BMD was measured by dual-energy X-ray absorptiometry (DXA).
Results: Mean±SD serum 25(OH)D level of the study population was 14.97±6.6ng/mL, we found that 73.3% were vitamin D deficient and 26.7% did not. There is no correlation between vitamin D and BMD lumbar, femoral neck or T-score (p=0.064, -0.215; p=0.443, -0.090; and p=0.109, -0.187 respectively). Lower BMD lumbar, femoral neck and T-score was 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, there is no correlation has been found between vitamin D status and bone mineral density in this population.

Keywords: BMD; DXA; elderly; vitamin D; 25(OH)D

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Introduction

Vitamin D is a fat-soluble secosteroid hormone that plays a major role in bone mineralization and other metabolic processes in the human body.(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 hydroxylated in the liver to form 25-hydroxyvitamin D (25(OH)D) and then in the kidney to its biologically active form 1,25-dihydroxyvitamin D (1,25(OH)2D).(3) The first step of 25-hydroxylation reaction was facilitated by 25-hydroxylases (cytochrome P450 (CYP) 2R1, 27A1 and 3A4) and the second step was facilitated by 1α-hydroxylase (CYP27B1).(4)

Vitamin D status is determined by measuring serum 25-hydroxyvitamin D (25(OH)D) level. Even though there is no general agreement on the adequate vitamin D status, most clinicians agree that serum 25(OH)D below 20 ng/mL was deficient.(5) Vitamin D deficiency has a high prevalence over the world, not only in four seasonal countries but also in tropical countries.(6–9) The elderly is one of several risk groups for vitamin D deficiency.(7) It is common among community-dwelling elderly in developed countries and very common among institutionalized elderly.(10) A study in 2004 demonstrated that from 74 elderly women living in the institutionalized care

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unit in Indonesia, 35.1% were vitamin D deficient.(11)

Osteoporosis is the most prevalent degenerative disease among elderly women. International Osteoporosis Foundation state that 1 in 3 women over fifty will experience osteoporotic fracture worldwide.(12) Osteoporosis is a multifactorial disorder characterized by low bone mass and enhanced skeletal fragility. Estimation of bone mineral density (BMD) by using dual-energy X-ray absorptiometry (DXA) regarded as the "gold standard" for diagnosing osteoporosis.(13) A complex between biologically active vitamin D (1,25(OH)2D) and its receptor serves as a transcription factor for various genes involves in bone homeostasis.(14) Several studies have been linked vitamin D deficiency with reduction in BMD leading to bone loss and increased risk of osteoporosis(15–17), but the result was still conflicting.(18–22)

The causal relation between serum vitamin D levels and bone mineral density remains to be understood. However, data regarding vitamin D deficiency in the community-dwelling population in Indonesia are rarely available and how its relation with BMD was still unknown. This study aims to analyze vitamin D levels among community-dwelling Javanese elderly women and investigate whether serum vitamin D levels were correlated with bone mineral density (BMD) in Javanese population.

Methods

Study Population

Healthy Javanese elderly women aged 60 or older were randomly recruited from elderly health service in Semarang, Central Java, Indonesia between May to October 2018. Community dwellers elderly woman with no history of diabetes, liver disease, chronic kidney disease, thyroid disorder and were not taking medication that affected calcium, vitamin D and bone metabolism were eligible for the present study.

Study Design

This study was an observational analytic study with a cross-sectional design. After signing the informed consent, each subject was required to complete a questionnaire to collect basic characteristic data and history of medical status and drug history. All subject underwent anthropometric measurement and blood sample were collected for the quantification of SGOT, SGPT, serum creatinine level, serum 25-hydroxyvitamin D (25(OH)D) level at the central laboratory of the institute and BMD measurement was conducted at Telogorejo Hospital Semarang.

Anthropometric measurements

Weight was measured in light clothing using calibrated digital scales accurate to 0.1 kg. Height was measured using wall-mounted stadiometer to the nearest ± 1 cm without shoes. Body Mass Index (BMI, kg/m2) was calculated as weight divided by height squared. BMI <18.5 were classified as underweight, BMI 18.5–24.9 were classified as normal weight, BMI 25.0–29.9 were classified as overweight and BMI ≥30 were classified as obese.

Biochemical measurements

Serum samples obtained from venous blood were stored at -80^{o}C until analyzed. Serum Glutamic Oxaloacetic Transaminase (SGOT), Serum Glutamic Pyruvic Transaminase (SGPT), and serum creatinine were measured by the IFCC method without pyridoxal phosphate (P-51-P). Vitamin D status was measured by enzyme-linked Immuno Sorbent Assay (ELISA) method by using the 25-hydroxyvitamin D (25(OH)D) ELISA kit (Diagnostics Biochem Canada Inc.). In this study, serum 25(OH)D level <20 ng/mL is considered to be vitamin D deficient, serum 25(OH)D level 20-29 ng/mL is considered to be insufficient and serum 25(OH)D level ≥30 ng/mL is considered to be sufficient.(23)

Assessment of Bone Mineral Density

Bone Mineral Density (BMD) was determined at the region of the hips and the lumbar spine using dual-energy X-ray absorptiometry (DXA) GE Prodigy lunar iDXA. The hip densitometry measurement was the femur neck region and the lumbar spine densitometry measurement included the vertebrae lumbar L1-L4. The BMD data (g/cm2) 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

The data were statistically analyzed using SPSS version 20 (SPSS Inc., Chicago, IL, USA). Continuous variables were reported as mean ± standard deviation (SD) and categorical variables were presented as frequencies and percentages. The mean difference between the two groups was analyzed using the Mann-Whitney U test. Correlation

of two numerical variables was analyzed using pearson or spearman test. p-value <0.05 were considered to be significant.

Results

Baseline characteristics of the study population are presented in table 1. There was 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 of it was older. Mean BMI of the study population was 25.54 ± 4.8 kg/m2 (range 16.4-40.7) and from table 2, we can see that most of the study population (45.3%) were have 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
25(OH)D (ng/mL)	14.97 ± 6.6	4.25	31.14
Bone Mineral Density			
Lumbar spine (g/cm^2)	0.91 ± 0.16	0.63	1.34
Femoral 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 was 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.

Mean (±SD) serum 25(OH)D level of the study population 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 (25(OH)D < 20 ng/mL) and not (25(OH)D ≥20 ng/mL). In this study, we found that there is no significant difference in baseline characteristics (age, weight, height, and BMI) between 25(OH)D deficient group and non-deficient group.

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,25(OH)D<20 ng/mL	55	73.3
Insufficient,25(OH)D 20-29 ng/mL	18	24
Sufficient,25(OH)D≥30 ng/mL	2	2.7

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

The study participants show a mean lumbar and femoral neck as follows 0.91±0.16 g/cm2 (range 0.63-1.34) and 0.73±0.10 g/cm2 (0.52-1.02). 50.7% from study population was osteoporosis, 33.3% has osteopeni and 16% was normal. There is no correlation between serum 25(OH)D levels and BMD at the lumbar spine (L1-L4, r = -0.215, p=0.064), femoral 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 lumbar spine, femoral 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 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).

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

Variable	BMD Lumbar spine	BMD Femoral Neck	T-score
25(OH)D levels	-0.215; 0.064	-0.090; 0.443	-0.187; 0.109

Table 5. Correlation between serum 25(OH)D levels and BMD with age and BMI (r; p-value)

Variable	Age	BMI
25(OH)D levels	-0.033; 0.780	-0.130; 0.265
BMD Lumbar spine	-0.238; 0.040*	0.525; 0.000*
BMD Femoral 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

Discussion

Sun exposure is an essential source of vitamin D production. When 7-dehydrocholesterol in the skin was exposed to UV-B (290-320 nm), it will absorb the energy of the UV-B radiation leading to the thermodynamically unstable molecule pre-vitamin D3. Pre-vitamin D3 then rapidly isomerizes into vitamin D3.(1) People living in the tropical and subtropical countries might be expected to have higher vitamin D levels compared with four-season countries due to plentiful sunshine. Nevertheless, several study has been reported incident of vitamin D deficiency even in a country where people can get adequate sun exposure like China, Korea, Thailand, and India.(9,24–26)

The elderly is one of several risk groups for vitamin D deficiency.(7) Studies from 170 community dwellers older than 65 years in rural areas of southern Taiwan demonstrated that 30.6% men and 57.7% women have low vitamin D status (25(OH)D < 30 ng/mL) and women were the majority of participants with low vitamin D status (65.3%).(27) A population-based cross-sectional study among non-institutionalized Chinese aged 50-70 years in Beijing and Shanghai China documented up to 69.2% of the study population were vitamin D deficient.(28) Another study from sunshine abundant Hyderabad metropolitan city, South India demonstrated the prevalence of vitamin D deficiency among the urban elderly population was 56.3%.(29) Our study which is conducted in Semarang city, the capital and largest city in Central Java, Indonesia (latitude 6°58′S) also demonstrated a high prevalence of vitamin D deficiency among elderly women up to 73.3%. From these studies, we can conclude that regardless of the latitude, vitamin D deficiency was a frequent finding among community-dwelling elderly.

Several studies have been conducted to evaluate the risk factor of vitamin D deficiency in the absence of seasonal variation in UV exposure. In this study, we did not find differences in baseline characteristics (age, weight, height, and BMI) between 25(OH)D deficiency group and non-deficiency group. This finding in line with a study among middle-aged and elderly Chinese (aged 45-74 years) in Singapore which found that there is no association between age and BMI with serum vitamin D concentration. This population-based prospective cohort study of 63,257 subjects demonstrated significant predictor of vitamin D concentration among women subjects were dietary vitamin D intake and genetic variation in enzyme cytochrome P450 (CYP) 2R1, 3A4 and vitamin D binding protein (GC).(30) Huang et al also reported that vitamin D status was not associated with age and BMI among the elderly in southern Taiwan, whereas inadequate sun exposure was the only predictable risk in elderly women.(27) Another study on 276 post-menopausal women in 2o N near Kuala Lumpur, Malaysia found that compare to Chinese women, Malay women had significantly lower mean vitamin D concentration (68.8 ± 15.7 and 44.4 ± 10.6 nmol/L, $p<0.05$ respectively). Besides have more skin pigmentation, Malay women also follow religious dress code using hijab and closed clothes that shall limit the sun exposure.(31) Only a few studies in Indonesia have focused on the status of the vitamin D deficiency in the elderly. The previous study in 2005 conducted in four Institutionalized care unit in Indonesia demonstrated a 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 as the result of various risk factors interacting in this population. Besides ecological factors (weather and season condition to latitude), lifestyle and individual factors such as genetic variation and skin pigmentation might be contributing to influence serum vitamin D level in elderly women.

In our study, we cannot find any significant correlation between serum vitamin D levels and BMD neither at the lumbar nor at the femoral neck sites among Javanese elderly women. This finding was inconsistent with the previous study which demonstrates a positive correlation between vitamin D levels and BMD in elderly.(16,32,33) However, previous study conducted in Hyderabad India observed 100 healthy postmenopausal women also

demonstrated no correlation between serum 25(OH)D and BMD both at the femoral neck (r = 0.11; p = 0.29) and the lumbar 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 level of 25(OH)D caused a decrease of biologically active vitamin D (1,25(OH)D) and consequently decreased calcium absorption in the intestine. Decreased serum calcium concentration stimulates parathyroid glands to increased expression, production, and secretion of parathyroid hormone (PTH) which help maintain serum calcium level by releasing calcium from the bone resorption.(17) However, several studies have been demonstrated that not all subjects with low vitamin D levels developed secondary hyperparathyroidism and therefore can not causes bone loss.(35)

The significant factor affected BMD in our study either at the lumbar spine and femoral neck sites were increased age and decreased BMI. This study was support previous finding in a longitudinal study that increasing ages and decreased BMI were associated with BMD loss and not affected by serum 25-OH vitamin D status.(36) Age-related bone loss was a complex mechanism involved many factor. It is not only due to hormonal factor, but also genetic, alterations in cellular components of the bone, biochemical and vasculature status and also affected by extrinsic factor such as nutrition, physical activity, history of comorbid medical condition and also 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 relation between serum vitamin D status and BMD in our study population. Second, information about food recall and physical activity is not available in this study as it was an important factor that can contribute to bone mass remodelling.

Conclusion

From this study, we concluded that vitamin D deficiency was prevalent among Javanese elderly women. However, there is no correlation has been found between vitamin D status and bone mineral density in this population.

Ethical approval

Ethical clearance was obtained with the approval and consideration of the Health Research Ethics Commission of the Faculty of Medicine, Diponegoro University. The subject was willingly join the study by signing informed consent.

Conflicts of interest

The authors declare no conflict of interest

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References

1. Hanel A, Carlberg C. Vitamin D and evolution: Pharmacologic implications. Biochem Pharmacol. 2020;173:113595.
2. Albahrani AA, Greaves RF. Fat-soluble vitamins: Clinical indications and current challenges for chromatographic measurement. Clin Biochem Rev. 2016;37(1):27–47.
3. Pike JW, Christakos S. Biology and mechanisms of action of the vitamin D hormone. Endocrinol Metab Clin North Am. 2017;46(4):815–43.
4. Jones G, Prosser DE, Kaufmann M. Cytochrome P450-mediated metabolism of vitamin D. J Lipid Res. 2014;55(1):13–31.
5. Holick MF. Vitamin D status: Measurement, interpretation, and clinical application. Ann Epidemiol. 2009;19(2):73–8.
6. Hilger J, Friedel A, Herr R, Rausch T, Roos F, Wahl DA, et al. A systematic review of vitamin D status in populations worldwide. Br J Nutr. 2014;111(1):23–45.
7. van Schoor N, Lips P. Global overview of vitamin D status. Endocrinol Metab Clin North Am. 2017;46(4):845–70.
8. van Schoor NM, Lips P. Worldwide vitamin D status. Best Pr Res Clin Endocrinol Metab. 2011;25(4):671–80.

9. Yu S, Fang H, Han J, Cheng X, Xia L, Li S, et al. The High Prevalence of Hypovitaminosis D in China. md J. 2015;94(8):1–6.

10. Mosekilde L. Vitamin D and the elderly. Clin Endocrinol. 2005;62(3):265–81.

11. Setiati S. Vitamin D status among Indonesian elderly women living in institutionalized Care units. Acta Med Indones-Indones J Intern Med. 2004;40(2):78–83.

12. Sözen T, Özışık L, Başaran NÇ. An overview and management of osteoporosis. Eur J Rheumatol. 2017;4(1):46–56.

13. World Health Organization. WHO scientific group on the prevention and management of osteoporosis: report of a WHO scientific group. Genewa, Switzerland; 2003. 54 p.

14. Pike JW, Lee SM, Meyer MB. Regulation of gene expression by 1,25-dihydroxy-vitamin D3 in bone cells: exploiting new approaches and defining new mechanisms. Bonekey Rep. Nature Publishing Group; 2014;3:482.

15. Lips P, Van Schoor NM. The effect of vitamin D on bone and osteoporosis. Best Pr Res Clin Endocrinol Metab. 2011;25(4):585–91.

16. Sadat-ali M, Al Elq A, Al-turki HA, Al-mulhim FA, Al-ali AK. Influence of vitamin D levels on bone mineral density and osteoporosis. Ann Saudi Med. 2011;31(6):602–8.

17. Reid IR, Bolland MJ, Grey A. Effects of vitamin D supplements on bone mineral density: a systematic review and meta-analysis. Lancet. Elsevier Ltd; 2014;383(9912):146–55.

18. Alkhenizan A, Mahmoud A, Hussain A, Gabr A, Alsoghayer S, Eldali A. The relationship between 25(OH)D Levels (Vitamin D) and Bone Mineral Density (BMD) in a Saudi Population in a Community-Based Setting. PLoS One. 2017;12(1):e0169122.

19. Kota S, Jammula S, Kota S, Meher L, Modi K. Correlation of vitamin D, bone mineral density and parathyroid hormone levels in adults with low bone density. Indian J Orthop. 2013;47(4):402–7.

20. Khashayar P, Meybodi HRA, Hemami MR, Keshtkar A, Dimai HP, Larijani B. Vitamin D status and its relationship with bone mineral density in a healthy Iranian population. Rev Bras Ortop. Sociedade Brasileira de Ortopedia e Traumatologia; 2016;51(4):454–8.

21. Kamineni V, Latha AP, Ramathulasi K. Association between serum 25- hydroxyvitamin D levels and bone mineral density in normal postmenopausal women. J MidLife Heal. 2016;7(4):163–8.

22. Chailurkit L, Kruavit A, Rajatanavin R. Vitamin D status and bone health in healthy Thai elderly women. Nutrition. Elsevier Ltd; 2011;27(2):160–4.

23. Alireza K, Eslamifar A, Gheini MH, Abachi M, Azizi E, Monir S, et al. Prevalence of Vitamin D deficiency among an elderly people. Int J Curr Res. 2016;8(11):41315–8.

24. Park J, Hong IY, Chung JW, Choi HS. Vitamin D status in South Korean population: Seven-year trend from the KNHANES. Medicine (Baltimore). 2018;97(26):e11032.

25. Chailurkit L, Aekplakorn W, Ongphiphadhanakul B. Regional variation and determinants of vitamin D status in sunshine-abundant Thailand. BMC Public Health. BioMed Central Ltd; 2011;11:853.

26. Babu US, Calvo MS. Modern India and the vitamin D dilemma: Evidence for the need of a national food fortification program. Mol Nutr Food Res. 2010;54:1134–47.

27. Huang C, Huang YA, Lai Y, Sun C. Prevalence and predictors of hypovitaminosis D among the elderly in subtropical region. PLoS One. 2017;12(7):e0181063.

28. Lu L, Yu Z, Pan A, Hu FB, Franco OH, Li H, et al. Plasma 25-hydroxyvitamin D concentration and metabolic syndrome among middle-aged and elderly chinese individuals. Diabetes Care. 2009;32(7):1278–83.

29. Suryanarayana P, Arlappa N, Sai Santhosh V, Nagalla B, Pondey LR, Undrajavarapu P, et al. Prevalence of vitamin D deficiency and its associated factors among urban elderly population in Hyderabad metropolitan city, South India. Ann Hum Biol. Taylor & Francis; 2018;45(2):133–9.

30. Robien K, Butler LM, Wang R, Beckman KB, Walek D, Koh W-P, et al. Genetic and environmental predictors of serum 25(OH)D

concentrations among middle-aged and elderly Chinese in Singapore. Br J Nutr. 2013;109(3):493–502.

31. Rahman SA, Chee WSS, Yassin Z, Chan SP. Vitamin D status among postmenopausal Malaysian women. Asia Pac J Clin Nutr. 2004;13(3):255–60.

32. Li S, Ou Y, Zhang H, Zhang Z, Zhou H, Liu L, et al. Vitamin D Status and Its Relationship with Body Composition , Bone Mineral Density and Fracture Risk in Urban Central South Chinese Postmenopausal Women. Ann Nutr Metab. 2014;64:13–9.

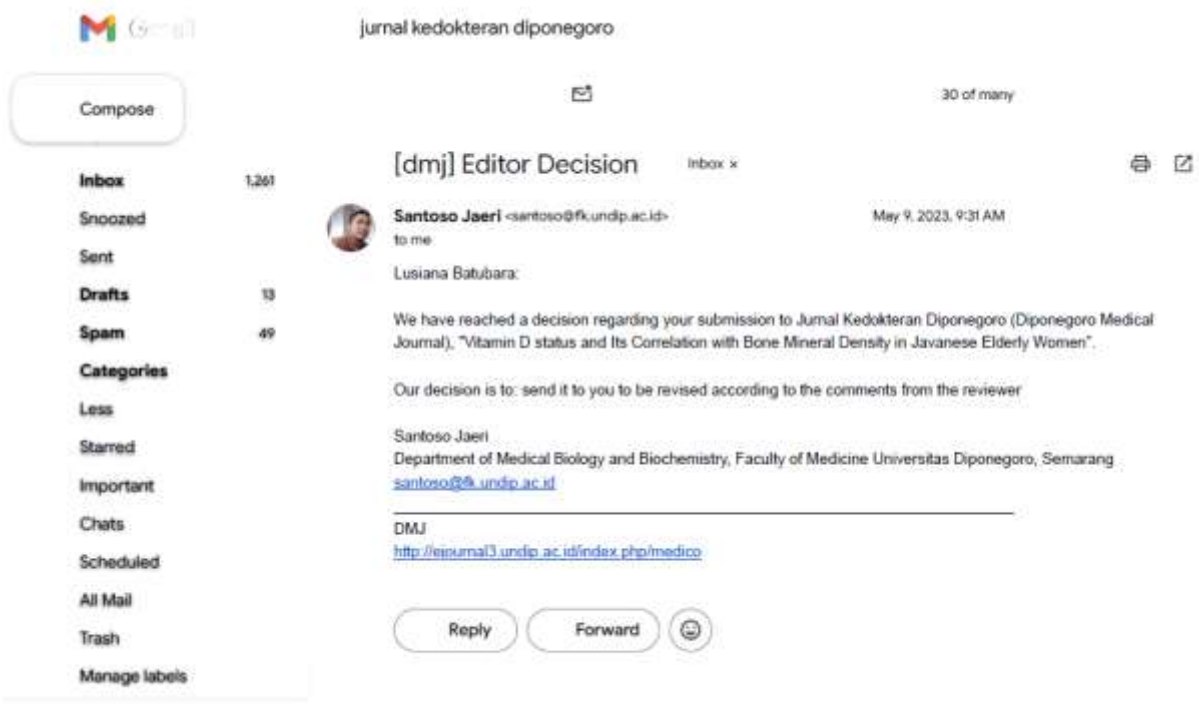
33. Labronici PJ, Blunck SS, Lana FR, Esteves BB, Franco JS, Fukuyama JM, et al. Vitamin D and its relation to bone mineral density in postmenopause women. Rev Bras Ortop. 2013;48(3):228–35.

34. Kruavit A, Chailurkit LO, Thakkinstian A, Sriphrapradang C, Rajatanavin R. Prevalence of Vitamin D insufficiency and low bone mineral density in elderly Thai nursing home residents. BMC Geriatr. BMC Geriatrics; 2012;12:49.

35. Arabi A, Baddoura R, El-Rassi R, Fuleihan GE-H. PTH level but not 25(OH) vitamin D level predicts bone loss rates in the elderly. Osteoporos Int. 2012;23:971–80.

36. Hannan MT, Felson DT, Dawson-hughes B, Tucker KL, Cupples LA, Wilson PWF, et al. Risk Factors for Longitudinal Bone Loss in Elderly Men and Women : The Framingham Osteoporosis Study. J Bone Min Res. 2000;15(4):710–20.

2. Editor Decision: send it to you to be revised according to the comments from the reviewer (9 May 2023)



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Abstract

Background: Vitamin D (25(OH)D) is a fat-soluble secosteroid hormone that plays a major role in bone mineralization and other metabolic processes in the human body. A complex between biologically active vitamin D (1,25(OH)D) and its receptor serves as a transcription factor for various genes involves in bone homeostasis. Several studies have been link vitamin D deficiency with a reduction in Bone Mineral Density (BMD), but the result was still conflicting. However, data regarding vitamin D deficiency in the Indonesian population are rarely available.
Objective: The study aims to assess vitamin D status and its correlation with Bone Mineral Density (BMD) among Javanese elderly women.
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Results: Mean±SD serum 25(OH)D level of the study population was 14.97±6.6ng/mL, we found that 73.3% were vitamin D deficient and 26.7% did not. There is no correlation between vitamin D and BMD lumbar, femoral neck or T-score (p=0.064, -0.215; p=0.443, -0.090; and p=0.109, -0.187 respectively). Lower BMD lumbar, femoral neck and T-score was 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, there is no correlation has been found between vitamin D status and bone mineral density in this population.

Keywords: BMD; DXA; elderly; vitamin D; 25(OH)D

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Introduction

Vitamin D is a fat-soluble secosteroid hormone that plays a major role in bone mineralization and other metabolic processes in the human body.(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 hydroxylated in the liver to form 25-hydroxyvitamin D (25(OH)D) and then in the kidney to its biologically active form 1,25-dihydroxyvitamin D (1,25(OH)2D).(3) The first step of 25-hydroxylation reaction was facilitated by 25-hydroxylases (cytochrome P450 (CYP) 2R1, 27A1 and 3A4) and the second step was facilitated by 1α-hydroxylase (CYP27B1).(4)

Vitamin D status is determined by measuring serum 25-hydroxyvitamin D (25(OH)D) level. Even though there is no general agreement on the adequate vitamin D status, most clinicians agree that serum 25(OH)D below 20 ng/mL was deficient.(5) Vitamin D deficiency has a high prevalence over the world, not only in four seasonal countries but also in tropical countries.(6–9) The elderly is one of several risk groups for vitamin D deficiency.(7) It is common among community-dwelling elderly in developed countries and very common among institutionalized elderly.(10) A study in 2004 demonstrated that from 74 elderly women living in the institutionalized care

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unit in Indonesia, 35.1% were vitamin D deficient.(11)

Osteoporosis is the most prevalent degenerative disease among elderly women. International Osteoporosis Foundation state that 1 in 3 women over fifty will experience osteoporotic fracture worldwide.(12) Osteoporosis is a multifactorial disorder characterized by low bone mass and enhanced skeletal fragility. Estimation of bone mineral density (BMD) by using dual-energy X-ray absorptiometry (DXA) regarded as the "gold standard" for diagnosing osteoporosis.(13) A complex between biologically active vitamin D (1,25(OH)2D) and its receptor serves as a transcription factor for various genes involves in bone homeostasis.(14) Several studies have been linked vitamin D deficiency with reduction in BMD leading to bone loss and increased risk of osteoporosis(15–17), but the result was still conflicting.(18–22)

The causal relation between serum vitamin D levels and bone mineral density remains to be understood. However, data regarding vitamin D deficiency in the community-dwelling population in Indonesia are rarely available and how its relation with BMD was still unknown. This study aims to analyze vitamin D levels among community-dwelling Javanese elderly women and investigate whether serum vitamin D levels were correlated with bone mineral density (BMD) in Javanese population.

Methods

Study Population

Healthy Javanese elderly women aged 60 or older were randomly recruited from elderly health service in Semarang, Central Java, Indonesia between May to October 2018. Community dwellers elderly woman with no history of diabetes, liver disease, chronic kidney disease, thyroid disorder and were not taking medication that affected calcium, vitamin D and bone metabolism were eligible for the present study.

Study Design

This study was an observational analytic study with a cross-sectional design. After signing the informed consent, each subject was required to complete a questionnaire to collect basic characteristic data and history of medical status and drug history. All subject underwent anthropometric measurement and blood sample were collected for the quantification of SGOT, SGPT, serum creatinine level, serum 25-hydroxyvitamin D (25(OH)D) level at the central laboratory of the institute and BMD measurement was conducted at Telogorejo Hospital Semarang.

Anthropometric measurements

Weight was measured in light clothing using calibrated digital scales accurate to 0.1 kg. Height was measured using wall-mounted stadiometer to the nearest ± 1 cm without shoes. Body Mass Index (BMI, kg/m2) was calculated as weight divided by height squared. BMI <18.5 were classified as underweight, BMI 18.5–24.9 were classified as normal weight, BMI 25.0–29.9 were classified as overweight and BMI ≥30 were classified as obese.

Biochemical measurements

Serum samples obtained from venous blood were stored at -80ºC until analyzed. Serum Glutamic Oxaloacetic Transaminase (SGOT), Serum Glutamic Pyruvic Transaminase (SGPT), and serum creatinine were measured by the IFCC method without pyridoxal phosphate (P-51-P). Vitamin D status was measured by enzyme-linked Immuno Sorbent Assay (ELISA) method by using the 25-hydroxyvitamin D (25(OH)D) ELISA kit (Diagnostics Biochem Canada Inc.). In this study, serum 25(OH)D level <20 ng/mL is considered to be vitamin D deficient, serum 25(OH)D level 20-29 ng/mL is considered to be insufficient and serum 25(OH)D level ≥30 ng/mL is considered to be sufficient.(23)

Assessment of Bone Mineral Density

Bone Mineral Density (BMD) was determined at the region of the hips and the lumbar spine using dual-energy X-ray absorptiometry (DXA) GE Prodigy lunar iDXA. The hip densitometry measurement was the femur neck region and the lumbar spine densitometry measurement included the vertebrae lumbar L1-L4. The BMD data (g/cm2) 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)

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Statistical Analysis

The data were statistically analyzed using SPSS version 20 (SPSS Inc., Chicago, IL, USA). Continuous variables were reported as mean ± standard deviation (SD) and categorical variables were presented as frequencies and percentages. The mean difference between the two groups was analyzed using the Mann-Whitney U test. Correlation

of two numerical variables was analyzed using pearson or spearman test. p-value <0.05 were considered to be significant.

Results

Baseline characteristics of the study population are presented in table 1. There was 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 of it was older. Mean BMI of the study population was 25.54 ± 4.8 kg/m2 (range 16.4-40.7) and from table 2, we can see that most of the study population (45.3%) were have 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
25(OH)D (ng/mL)	14.97 ± 6.6	4.25	31.14
Bone Mineral Density			
Lumbar spine (g/cm^2)	0.91 ± 0.16	0.63	1.34
Femoral 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 was 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.

Mean (±SD) serum 25(OH)D level of the study population 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 (25(OH)D < 20 ng/mL) and not (25(OH)D ≥20 ng/mL). In this study, we found that there is no significant difference in baseline characteristics (age, weight, height, and BMI) between 25(OH)D deficient group and non-deficient group.

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,25(OH)D<20 ng/mL	55	73.3
Insufficient,25(OH)D 20-29 ng/mL	18	24
Sufficient,25(OH)D≥30 ng/mL	2	2.7

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

The study participants show a mean lumbar and femoral neck as follows 0.91±0.16 g/cm2 (range 0.63-1.34) and 0.73±0.10 g/cm2 (0.52-1.02). 50.7% from study population was osteoporosis, 33.3% has osteopeni and 16% was normal. There is no correlation between serum 25(OH)D levels and BMD at the lumbar spine (L1-L4, r = -0.215, p=0.064), femoral 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 lumbar spine, femoral 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 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).

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

Variable	BMD Lumbar spine	BMD Femoral Neck	T-score
25(OH)D levels	-0.215; 0.064	-0.090; 0.443	-0.187; 0.109

Table 5. Correlation between serum 25(OH)D levels and BMD with age and BMI (r; p-value)

Variable	Age	BMI
25(OH)D levels	-0.033; 0.780	-0.130; 0.265
BMD Lumbar spine	-0.238; 0.040*	0.525; 0.000*
BMD Femoral 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

Discussion

Sun exposure is an essential source of vitamin D production. When 7-dehydrocholesterol in the skin was exposed to UV-B (290-320 nm), it will absorb the energy of the UV-B radiation leading to the thermodynamically unstable molecule pre-vitamin D3. Pre-vitamin D3 then rapidly isomerizes into vitamin D3.(1) People living in the tropical and subtropical countries might be expected to have higher vitamin D levels compared with four-season countries due to plentiful sunshine. Nevertheless, several study has been reported incident of vitamin D deficiency even in a country where people can get adequate sun exposure like China, Korea, Thailand, and India.(9,24–26)

The elderly is one of several risk groups for vitamin D deficiency.(7) Studies from 170 community dwellers older than 65 years in rural areas of southern Taiwan demonstrated that 30.6% men and 57.7% women have low vitamin D status (25(OH)D < 30 ng/mL) and women were the majority of participants with low vitamin D status (65.3%).(27) A population-based cross-sectional study among non-institutionalized Chinese aged 50-70 years in Beijing and Shanghai China documented up to 69.2% of the study population were vitamin D deficient.(28) Another study from sunshine abundant Hyderabad metropolitan city, South India demonstrated the prevalence of vitamin D deficiency among the urban elderly population was 56.3%.(29) Our study which is conducted in Semarang city, the capital and largest city in Central Java, Indonesia (latitude 6°58′S) also demonstrated a high prevalence of vitamin D deficiency among elderly women up to 73.3%. From these studies, we can conclude that regardless of the latitude, vitamin D deficiency was a frequent finding among community-dwelling elderly.

Several studies have been conducted to evaluate the risk factor of vitamin D deficiency in the absence of seasonal variation in UV exposure. In this study, we did not find differences in baseline characteristics (age, weight, height, and BMI) between 25(OH)D deficiency group and non-deficiency group. This finding in line with a study among middle-aged and elderly Chinese (aged 45-74 years) in Singapore which found that there is no association between age and BMI with serum vitamin D concentration. This population-based prospective cohort study of 63,257 subjects demonstrated significant predictor of vitamin D concentration among women subjects were dietary vitamin D intake and genetic variation in enzyme cytochrome P450 (CYP) 2R1, 3A4 and vitamin D binding protein (GC).(30) Huang et al also reported that vitamin D status was not associated with age and BMI among the elderly in southern Taiwan, whereas inadequate sun exposure was the only predictable risk in elderly women.(27) Another study on 276 post-menopausal women in 2o N near Kuala Lumpur, Malaysia found that compare to Chinese women, Malay women had significantly lower mean vitamin D concentration (68.8 ± 15.7 and 44.4 ± 10.6 nmol/L, $p<0.05$ respectively). Besides have more skin pigmentation, Malay women also follow religious dress code using hijab and closed clothes that shall limit the sun exposure.(31) Only a few studies in Indonesia have focused on the status of the vitamin D deficiency in the elderly. The previous study in 2005 conducted in four Institutionalized care unit in Indonesia demonstrated a 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 as the result of various risk factors interacting in this population. Besides ecological factors (weather and season condition to latitude), lifestyle and individual factors such as genetic variation and skin pigmentation might be contributing to influence serum vitamin D level in elderly women.

In our study, we cannot find any significant correlation between serum vitamin D levels and BMD neither at the lumbar nor at the femoral neck sites among Javanese elderly women. This finding was inconsistent with the previous study which demonstrates a positive correlation between vitamin D levels and BMD in elderly.(16,32,33) However, previous study conducted in Hyderabad India observed 100 healthy postmenopausal women also

demonstrated no correlation between serum 25(OH)D and BMD both at the femoral neck (r = 0.11; p = 0.29) and the lumbar 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 level of 25(OH)D caused a decrease of biologically active vitamin D (1,25(OH)D) and consequently decreased calcium absorption in the intestine. Decreased serum calcium concentration stimulates parathyroid glands to increased expression, production, and secretion of parathyroid hormone (PTH) which help maintain serum calcium level by releasing calcium from the bone resorption.(17) However, several studies have been demonstrated that not all subjects with low vitamin D levels developed secondary hyperparathyroidism and therefore can not causes bone loss.(35)

The significant factor affected BMD in our study either at the lumbar spine and femoral neck sites were increased age and decreased BMI. This study was support previous finding in a longitudinal study that increasing ages and decreased BMI were associated with BMD loss and not affected by serum 25-OH vitamin D status.(36) Age-related bone loss was a complex mechanism involved many factor. It is not only due to hormonal factor, but also genetic, alterations in cellular components of the bone, biochemical and vasculature status and also affected by extrinsic factor such as nutrition, physical activity, history of comorbid medical condition and also 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 relation between serum vitamin D status and BMD in our study population. Second, information about food recall and physical activity is not available in this study as it was an important factor that can contribute to bone mass remodelling.

Conclusion

From this study, we concluded that vitamin D deficiency was prevalent among Javanese elderly women. However, there is no correlation has been found between vitamin D status and bone mineral density in this population.

Ethical approval

Ethical clearance was obtained with the approval and consideration of the Health Research Ethics Commission of the Faculty of Medicine, Diponegoro University. The subject was willingly join the study by signing informed consent.

Conflicts of interest

The authors declare no conflict of interest

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References

1. Hanel A, Carlberg C. Vitamin D and evolution: Pharmacologic implications. Biochem Pharmacol. 2020;173:113595.
2. Albahrani AA, Greaves RF. Fat-soluble vitamins: Clinical indications and current challenges for chromatographic measurement. Clin Biochem Rev. 2016;37(1):27–47.
3. Pike JW, Christakos S. Biology and mechanisms of action of the vitamin D hormone. Endocrinol Metab Clin North Am. 2017;46(4):815–43.
4. Jones G, Prosser DE, Kaufmann M. Cytochrome P450-mediated metabolism of vitamin D. J Lipid Res. 2014;55(1):13–31.
5. Holick MF. Vitamin D status: Measurement, interpretation, and clinical application. Ann Epidemiol. 2009;19(2):73–8.
6. Hilger J, Friedel A, Herr R, Rausch T, Roos F, Wahl DA, et al. A systematic review of vitamin D status in populations worldwide. Br J Nutr. 2014;111(1):23–45.
7. van Schoor N, Lips P. Global overview of vitamin D status. Endocrinol Metab Clin North Am. 2017;46(4):845–70.
8. van Schoor NM, Lips P. Worldwide vitamin D status. Best Pr Res Clin Endocrinol Metab. 2011;25(4):671–80.

9. Yu S, Fang H, Han J, Cheng X, Xia L, Li S, et al. The High Prevalence of Hypovitaminosis D in China. md J. 2015;94(8):1–6.

10. Mosekilde L. Vitamin D and the elderly. Clin Endocrinol. 2005;62(3):265–81.

11. Setiati S. Vitamin D status among Indonesian elderly women living in institutionalized Care units. Acta Med Indones-Indones J Intern Med. 2004;40(2):78–83.

12. Sözen T, Özışık L, Başaran NÇ. An overview and management of osteoporosis. Eur J Rheumatol. 2017;4(1):46–56.

13. World Health Organization. WHO scientific group on the prevention and management of osteoporosis: report of a WHO scientific group. Genewa, Switzerland; 2003. 54 p.

14. Pike JW, Lee SM, Meyer MB. Regulation of gene expression by 1,25-dihydroxy-vitamin D3 in bone cells: exploiting new approaches and defining new mechanisms. Bonekey Rep. Nature Publishing Group; 2014;3:482.

15. Lips P, Van Schoor NM. The effect of vitamin D on bone and osteoporosis. Best Pr Res Clin Endocrinol Metab. 2011;25(4):585–91.

16. Sadat-ali M, Al Elq A, Al-turki HA, Al-mulhim FA, Al-ali AK. Influence of vitamin D levels on bone mineral density and osteoporosis. Ann Saudi Med. 2011;31(6):602–8.

17. Reid IR, Bolland MJ, Grey A. Effects of vitamin D supplements on bone mineral density: a systematic review and meta-analysis. Lancet. Elsevier Ltd; 2014;383(9912):146–55.

18. Alkhenizan A, Mahmoud A, Hussain A, Gabr A, Alsoghayer S, Eldali A. The relationship between 25(OH)D Levels (Vitamin D) and Bone Mineral Density (BMD) in a Saudi Population in a Community-Based Setting. PLoS One. 2017;12(1):e0169122.

19. Kota S, Jammula S, Kota S, Meher L, Modi K. Correlation of vitamin D, bone mineral density and parathyroid hormone levels in adults with low bone density. Indian J Orthop. 2013;47(4):402–7.

20. Khashayar P, Meybodi HRA, Hemami MR, Keshtkar A, Dimai HP, Larijani B. Vitamin D status and its relationship with bone mineral density in a healthy Iranian population. Rev Bras Ortop. Sociedade Brasileira de Ortopedia e Traumatologia; 2016;51(4):454–8.

21. Kamineni V, Latha AP, Ramathulasi K. Association between serum 25- hydroxyvitamin D levels and bone mineral density in normal postmenopausal women. J MidLife Heal. 2016;7(4):163–8.

22. Chailurkit L, Kruavit A, Rajatanavin R. Vitamin D status and bone health in healthy Thai elderly women. Nutrition. Elsevier Ltd; 2011;27(2):160–4.

23. Alireza K, Eslamifar A, Gheini MH, Abachi M, Azizi E, Monir S, et al. Prevalence of Vitamin D deficiency among an elderly people. Int J Curr Res. 2016;8(11):41315–8.

24. Park J, Hong IY, Chung JW, Choi HS. Vitamin D status in South Korean population: Seven-year trend from the KNHANES. Medicine (Baltimore). 2018;97(26):e11032.

25. Chailurkit L, Aekplakorn W, Ongphiphadhanakul B. Regional variation and determinants of vitamin D status in sunshine-abundant Thailand. BMC Public Health. BioMed Central Ltd; 2011;11:853.

26. Babu US, Calvo MS. Modern India and the vitamin D dilemma: Evidence for the need of a national food fortification program. Mol Nutr Food Res. 2010;54:1134–47.

27. Huang C, Huang YA, Lai Y, Sun C. Prevalence and predictors of hypovitaminosis D among the elderly in subtropical region. PLoS One. 2017;12(7):e0181063.

28. Lu L, Yu Z, Pan A, Hu FB, Franco OH, Li H, et al. Plasma 25-hydroxyvitamin D concentration and metabolic syndrome among middle-aged and elderly chinese individuals. Diabetes Care. 2009;32(7):1278–83.

29. Suryanarayana P, Arlappa N, Sai Santhosh V, Nagalla B, Pondey LR, Undrajavarapu P, et al. Prevalence of vitamin D deficiency and its associated factors among urban elderly population in Hyderabad metropolitan city, South India. Ann Hum Biol. Taylor & Francis; 2018;45(2):133–9.

30. Robien K, Butler LM, Wang R, Beckman KB, Walek D, Koh W-P, et al. Genetic and environmental predictors of serum 25(OH)D

concentrations among middle-aged and elderly Chinese in Singapore. Br J Nutr. 2013;109(3):493–502.

31. Rahman SA, Chee WSS, Yassin Z, Chan SP. Vitamin D status among postmenopausal Malaysian women. Asia Pac J Clin Nutr. 2004;13(3):255–60.

32. Li S, Ou Y, Zhang H, Zhang Z, Zhou H, Liu L, et al. Vitamin D Status and Its Relationship with Body Composition , Bone Mineral Density and Fracture Risk in Urban Central South Chinese Postmenopausal Women. Ann Nutr Metab. 2014;64:13–9.

33. Labronici PJ, Blunck SS, Lana FR, Esteves BB, Franco JS, Fukuyama JM, et al. Vitamin D and its relation to bone mineral density in postmenopause women. Rev Bras Ortop. 2013;48(3):228–35.

34. Kruavit A, Chailurkit LO, Thakkinstian A, Sriphrapradang C, Rajatanavin R. Prevalence of Vitamin D insufficiency and low bone mineral density in elderly Thai nursing home residents. BMC Geriatr. BMC Geriatrics; 2012;12:49.

35. Arabi A, Baddoura R, El-Rassi R, Fuleihan GE-H. PTH level but not 25(OH) vitamin D level predicts bone loss rates in the elderly. Osteoporos Int. 2012;23:971–80.

36. Hannan MT, Felson DT, Dawson-hughes B, Tucker KL, Cupples LA, Wilson PWF, et al. Risk Factors for Longitudinal Bone Loss in Elderly Men and Women : The Framingham Osteoporosis Study. J Bone Min Res. 2000;15(4):710–20.

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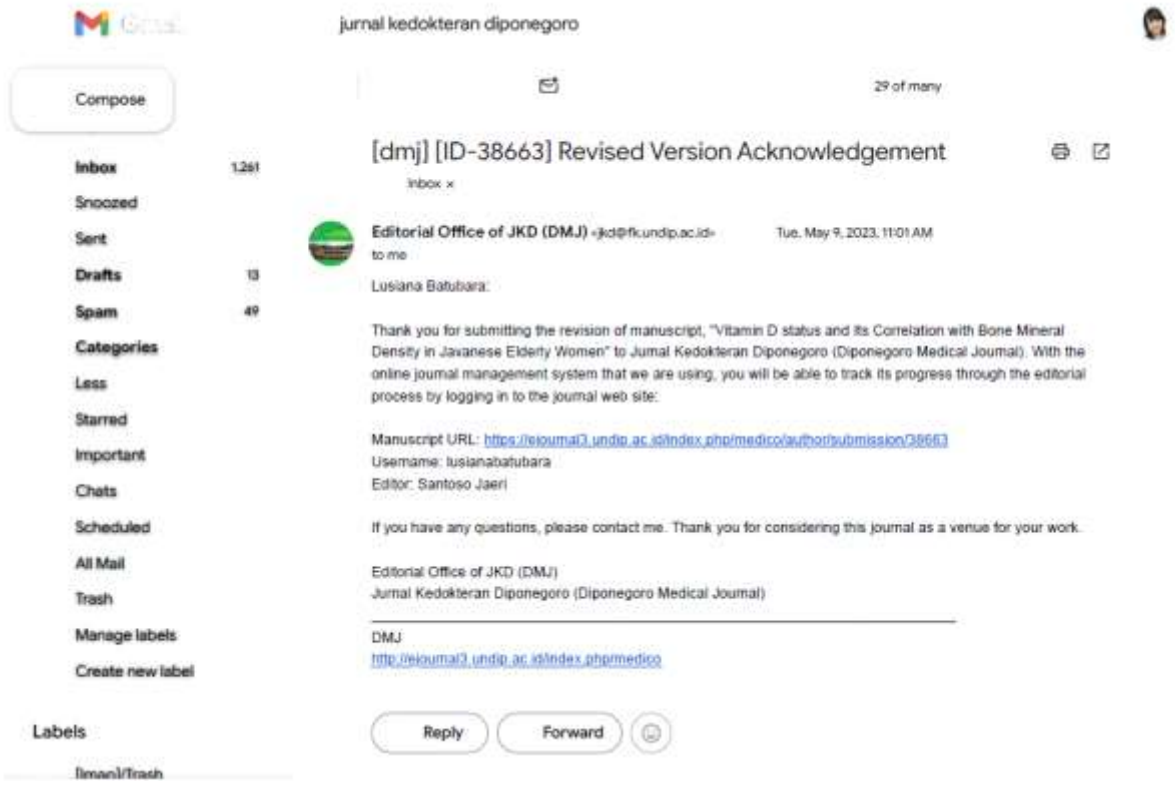

Vitamin D status and Its Correlation with Bone Mineral Density in Javanese Elderly Women

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Abstract

Background: Vitamin D (25(OH)D) is a fat-soluble secosteroid hormone that plays a major role in bone mineralization and other metabolic processes in the human body. A complex between biologically active vitamin D (1,25(OH)D) and its receptor serves as a transcription factor for various genes involves in bone homeostasis. Several studies have been link vitamin D deficiency with a reduction in Bone Mineral Density (BMD), but the result was still conflicting. However, data regarding vitamin D deficiency in the Indonesian population are rarely available.
Objective: The study aims to assess vitamin D status and its correlation with Bone Mineral Density (BMD) among Javanese elderly women.
Methods: A cross-sectional study was conducted in 75 healthy Javanese elderly women aged 60-84 years old. Serum Vitamin D was measured by enzyme link immunoassay using 25(OH)D ELISA kit. BMD was measured by dual-energy X-ray absorptiometry (DXA).
Results: Mean±SD serum 25(OH)D level of the study population was 14.97±6.6ng/mL, we found that 73.3% were vitamin D deficient and 26.7% did not. There is no correlation between vitamin D and BMD lumbar, femoral neck or T-score (p=0.064, -0.215; p=0.443, -0.090; and p=0.109, -0.187 respectively). Lower BMD lumbar, femoral neck and T-score was 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, there is no correlation has been found between vitamin D status and bone mineral density in this population.

Keywords: BMD; DXA; elderly; vitamin D; 25(OH)D

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Introduction

Vitamin D is a fat-soluble secosteroid hormone that plays a major role in bone mineralization and other metabolic processes in the human body.[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 hydroxylated in the liver to form 25-hydroxyvitamin D (25(OH)D) and then in the kidney to its biologically active form 1,25-dihydroxyvitamin D (1,25(OH)2D).[3] The first step of 25-hydroxylation reaction was facilitated by 25-hydroxylases (cytochrome P450 (CYP) 2R1, 27A1 and 3A4) and the second step was facilitated by 1α-hydroxylase (CYP27B1).[4]

Vitamin D status is determined by measuring serum 25-hydroxyvitamin D (25(OH)D) level. Even though there is no general agreement on the adequate vitamin D status, most clinicians agree that serum 25(OH)D below 20 ng/mL was deficient.[5] Vitamin D deficiency has a high prevalence over the world, not only in four seasonal countries but also in tropical countries.[6-9] The elderly is one of several risk groups for vitamin D deficiency.[7] It is common among community-dwelling elderly in developed countries and very common among institutionalized elderly.[10] A study in 2004 demonstrated that from 74 elderly

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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 state that 1 in 3 women over fifty will experience osteoporotic fracture worldwide.[12] Osteoporosis is a multifactorial disorder characterized by low bone mass and enhanced skeletal fragility. Estimation of bone mineral density (BMD) by using dual-energy X-ray absorptiometry (DXA) regarded as the "gold standard" for diagnosing osteoporosis.[13] A complex between biologically active vitamin D (1,25(OH)2D) and its receptor serves as a transcription factor for various genes involves in bone homeostasis.[14] Several studies have been linked vitamin D deficiency with reduction in BMD leading to bone loss and increased risk of osteoporosis[15-17], but the result was still conflicting.[18-22]

The causal relation between serum vitamin D levels and bone mineral density remains to be understood. However, data regarding vitamin D deficiency in the community-dwelling population in Indonesia are rarely available and how its relation with BMD was still unknown. This study aims to analyze vitamin D levels among community-dwelling Javanese elderly women and investigate whether serum vitamin D levels were correlated with bone mineral density (BMD) in Javanese population.

Methods

Study Population

Healthy Javanese elderly women aged 60 or older were randomly recruited from elderly health service in Semarang, Central Java, Indonesia between May to October 2018. Community dwellers elderly woman with no history of diabetes, liver disease, chronic kidney disease, thyroid disorder and were not taking medication that affected calcium, vitamin D and bone metabolism were eligible for the present study.

Study Design

This study was an observational analytic study with a cross-sectional design. After signing the informed consent, each subject was required to complete a questionnaire to collect basic characteristic data and history of medical status and drug history. All subject underwent anthropometric measurement and blood sample were collected for the quantification of SGOT, SGPT, serum creatinine level, serum 25-hydroxyvitamin D (25(OH)D) level at the central laboratory of the institute and BMD measurement was conducted at Telogorejo Hospital Semarang.

Anthropometric measurements

Weight was measured in light clothing using calibrated digital scales accurate to 0.1 kg. Height was measured using wall-mounted stadiometer to the nearest ± 1 cm without shoes. Body Mass Index (BMI, kg/m^2) was calculated as weight divided by height squared. BMI <18.5 were classified as underweight, BMI 18.5–24.9 were classified as normal weight, BMI 25.0–29.9 were classified as overweight and BMI ≥30 were classified as obese.

Biochemical measurements

Serum samples obtained from venous blood were stored at -80ºC until analyzed. Serum Glutamic Oxaloacetic Transaminase (SGOT), Serum Glutamic Pyruvic Transaminase (SGPT), and serum creatinine were measured by the IFCC method without pyridoxal phosphate (P-51-P). Vitamin D status was measured by enzyme-linked Immuno Sorbent Assay (ELISA) method by using the 25-hydroxyvitamin D (25(OH)D) ELISA kit (Diagnostics Biochem Canada Inc.). In this study, serum 25(OH)D level <20 ng/mL is considered to be vitamin D deficient, serum 25(OH)D level 20-29 ng/mL is considered to be insufficient and serum 25(OH)D level ≥30 ng/mL is considered to be sufficient.[23]

Assessment of Bone Mineral Density

Bone Mineral Density (BMD) was determined at the region of the hips and the lumbar spine using dual-energy X-ray absorptiometry (DXA) GE Prodigy lunar iDXA. The hip densitometry measurement was the femur neck region and the lumbar 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

The data were statistically analyzed using SPSS version 20 (SPSS Inc., Chicago, IL, USA). Continuous variables were reported as mean ± standard deviation (SD) and categorical variables were presented as frequencies and percentages. The mean difference between the two groups was analyzed using the Mann-Whitney U test. Correlation of two numerical variables was analyzed using

pearson or spearman test. p-value <0.05 were considered to be significant.

Results

Baseline characteristics of the study population are presented in table 1. There was 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 of it was older. Mean BMI of the study population was 25.54 ± 4.8 kg/m^2 (range 16.4-40.7) and from table 2, we can see that most of the study population (45.3%) were have 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
25(OH)D (ng/mL)	14.97 ± 6.6	4.25	31.14
Bone Mineral Density			
Lumbar spine (g/cm^2)	0.91 ± 0.16	0.63	1.34
Femoral 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 was 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.

Mean (±SD) serum 25(OH)D level of the study population 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 (25(OH)D < 20 ng/mL) and not (25(OH)D ≥20 ng/mL). In this study, we found that there is no significant difference in baseline characteristics (age, weight, height, and BMI) between 25(OH)D deficient group and non-deficient group.

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,25(OH)D<20 ng/mL	55	73.3
Insufficient,25(OH)D 20-29 ng/mL	18	24
Sufficient,25(OH)D≥30 ng/mL	2	2.7

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

The study participants show a mean lumbar and femoral neck as follows 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% from study population was osteoporosis, 33.3% has osteopeni and 16% was normal. There is no correlation between serum 25(OH)D levels and BMD at the lumbar spine (L1-L4, r = -0.215, p=0.064), femoral 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 lumbar spine, femoral 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 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).

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

Variable	BMD Lumbar spine	BMD Femoral Neck	T-score
25(OH)D levels	-0.215; 0.064	-0.090; 0.443	-0.187; 0.109

Table 5. Correlation between serum 25(OH)D levels and BMD with age and BMI (r; p-value)

Variable	Age	BMI
25(OH)D levels	-0.033; 0.780	-0.130; 0.265
BMD Lumbar spine	-0.238; 0.040*	0.525; 0.000*
BMD Femoral 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

Discussion

Sun exposure is an essential source of vitamin D production. When 7-dehydrocholesterol in the skin was exposed to UV-B (290-320 nm), it will absorb the energy of the UV-B radiation leading to the thermodynamically unstable molecule pre-vitamin D3. Pre-vitamin D3 then rapidly isomerizes into vitamin D3.[1] People living in the tropical and subtropical countries might be expected to have higher vitamin D levels compared with four-season countries due to plentiful sunshine. Nevertheless, several study has been reported incident of vitamin D deficiency even in a country where people can get adequate sun exposure like China, Korea, Thailand, and India.[9,24-26]

The elderly is one of several risk groups for vitamin D deficiency.[7] Studies from 170 community dwellers older than 65 years in rural areas of southern Taiwan demonstrated that 30.6% men and 57.7% women have low vitamin D status (25(OH)D < 30 ng/mL) and women were the majority of participants with low vitamin D status (65.3%).[27] A population-based cross-sectional study among non-institutionalized Chinese aged 50-70 years in Beijing and Shanghai China documented up to 69.2% of the study population were vitamin D deficient.[28] Another study from sunshine abundant Hyderabad metropolitan city, South India demonstrated the prevalence of vitamin D deficiency among the urban elderly population was 56.3%.[29] Our study which is conducted in Semarang city, the capital and largest city in Central Java, Indonesia (latitude 6°58′S) also demonstrated a high prevalence of vitamin D deficiency among elderly women up to 73.3%. From these studies, we can conclude that regardless of the latitude, vitamin D deficiency was a frequent finding among community-dwelling elderly.

Several studies have been conducted to evaluate the risk factor of vitamin D deficiency in the absence of seasonal variation in UV exposure. In this study, we did not find differences in baseline characteristics (age, weight, height, and BMI) between 25(OH)D deficiency group and non-deficiency group. This finding in line with a study among middle-aged and elderly Chinese (aged 45-74 years) in Singapore which found that there is no association between age and BMI with serum vitamin D concentration. This population-based prospective cohort study of 63,257 subjects demonstrated significant predictor of vitamin D concentration among women subjects were dietary vitamin D intake and genetic variation in enzyme cytochrome P450 (CYP) 2R1, 3A4 and vitamin D binding protein (GC).[30] Huang et al also reported that vitamin D status was not associated with age and BMI among the elderly in southern Taiwan, whereas inadequate sun exposure was the only predictable risk in elderly women.[27] Another study on 276 post-menopausal women in 2º N near Kuala Lumpur, Malaysia found that compare to Chinese women, Malay women had significantly lower mean vitamin D concentration (68.8 ± 15.7 and 44.4 ± 10.6 nmol/L, $p<0.05$ respectively). Besides have more skin pigmentation, Malay women also follow religious dress code using hijab and closed clothes that shall limit the sun exposure.[31] Only a few studies in Indonesia have focused on the status of the vitamin D deficiency in the elderly. The previous study in 2005 conducted in four Institutionalized care unit in Indonesia demonstrated a 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 as the result of various risk factors interacting in this population. Besides ecological factors (weather and season condition to latitude), lifestyle and individual factors such as genetic variation and skin pigmentation might be contributing to influence serum vitamin D level in elderly women.

In our study, we cannot find any significant correlation between serum vitamin D levels and BMD neither at the lumbar nor at the femoral neck sites among Javanese elderly women. This finding was inconsistent with the previous study which demonstrates a positive correlation between vitamin D levels and BMD in elderly.[16,32,33] However, previous study conducted in Hyderabad India observed 100 healthy postmenopausal women also

demonstrated no correlation between serum 25(OH)D and BMD both at the femoral neck (r = 0.11; p = 0.29) and the lumbar 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 level of 25(OH)D caused a decrease of biologically active vitamin D (1,25(OH)D) and consequently decreased calcium absorption in the intestine. Decreased serum calcium concentration stimulates parathyroid glands to increased expression, production, and secretion of parathyroid hormone (PTH) which help maintain serum calcium level by releasing calcium from the bone resorption.[17] However, several studies have been demonstrated that not all subjects with low vitamin D levels developed secondary hyperparathyroidism and therefore can not causes bone loss.[35]

The significant factor affected BMD in our study either at the lumbar spine and femoral neck sites were increased age and decreased BMI. This study was support previous finding in a longitudinal study that increasing ages and decreased BMI were associated with BMD loss and not affected by serum 25-OH vitamin D status.[36] Age-related bone loss was a complex mechanism involved many factor. It is not only due to hormonal factor, but also genetic, alterations in cellular components of the bone, biochemical and vasculature status and also affected by extrinsic factor such as nutrition, physical activity, history of comorbid medical condition and also 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 relation between serum vitamin D status and BMD in our study population. Second, information about food recall and physical activity is not available in this study as it was an important factor that can contribute to bone mass remodelling.

Conclusion

From this study, we concluded that vitamin D deficiency was prevalent among Javanese elderly women. However, there is no correlation has been found between vitamin D status and bone mineral density in this population.

Ethical approval

Ethical clearance was obtained with the approval and consideration of the Health Research Ethics Commission of the Faculty of Medicine, Diponegoro University. The subject was willingly join the study by signing informed consent.

Conflicts of interest

The authors declare no conflict of interest

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References

1. Hanel A, Carlberg C. Vitamin D and evolution: Pharmacologic implications. Biochem Pharmacol. 2020;173:113595.
2. Albahrani AA, Greaves RF. Fat-soluble vitamins: Clinical indications and current challenges for chromatographic measurement. Clin Biochem Rev. 2016;37(1):27–47.
3. Pike JW, Christakos S. Biology and mechanisms of action of the vitamin D hormone. Endocrinol Metab Clin North Am. 2017;46(4):815–43.
4. Jones G, Prosser DE, Kaufmann M. Cytochrome P450-mediated metabolism of vitamin D. J Lipid Res. 2014;55(1):13–31.
5. Holick MF. Vitamin D status: Measurement, interpretation, and clinical application. Ann Epidemiol. 2009;19(2):73–8.
6. Hilger J, Friedel A, Herr R, Rausch T, Roos F, Wahl DA, et al. A systematic review of vitamin D status in populations worldwide. Br J Nutr. 2014;111(1):23–45.
7. van Schoor N, Lips P. Global overview of vitamin D status. Endocrinol Metab Clin North Am. 2017;46(4):845–70.
8. van Schoor NM, Lips P. Worldwide vitamin D status. Best Pr Res Clin Endocrinol Metab. 2011;25(4):671–80.
9. Yu S, Fang H, Han J, Cheng X, Xia L, Li S, et al. The High Prevalence of Hypovitaminosis D in China. md J. 2015;94(8):1–6.
10. Mosekilde L. Vitamin D and the elderly. Clin Endocrinol. 2005;62(3):265–81.
11. Setiati S. Vitamin D status among Indonesian elderly women living in institutionalized Care units. Acta Med Indones-Indones J Intern

Med. 2004;40(2):78–83.
12. Sözen T, Özışık L, Başaran NÇ. An overview and management of osteoporosis. Eur J Rheumatol. 2017;4(1):46–56.
13. World Health Organization. WHO scientific group on the prevention and management of osteoporosis: report of a WHO scientific group. Genewa, Switzerland; 2003. 54 p.
14. Pike JW, Lee SM, Meyer MB. Regulation of gene expression by 1,25-dihydroxy-vitamin D3 in bone cells: exploiting new approaches and defining new mechanisms. Bonekey Rep. Nature Publishing Group; 2014;3:482.
15. Lips P, Van Schoor NM. The effect of vitamin D on bone and osteoporosis. Best Pr Res Clin Endocrinol Metab. 2011;25(4):585–91.
16. Sadat-ali M, Al Elq A, Al-turki HA, Al-mulhim FA, Al-ali AK. Influence of vitamin D levels on bone mineral density and osteoporosis. Ann Saudi Med. 2011;31(6):602–8.
17. Reid IR, Bolland MJ, Grey A. Effects of vitamin D supplements on bone mineral density: a systematic review and meta-analysis. Lancet. Elsevier Ltd; 2014;383(9912):146–55.
18. Alkhenizan A, Mahmoud A, Hussain A, Gabr A, Alsoghayer S, Eldali A. The relationship between 25(OH)D Levels (Vitamin D) and Bone Mineral Density (BMD) in a Saudi Population in a Community-Based Setting. PLoS One. 2017;12(1):e0169122.
19. Kota S, Jammula S, Kota S, Meher L, Modi K. Correlation of vitamin D, bone mineral density and parathyroid hormone levels in adults with low bone density. Indian J Orthop. 2013;47(4):402–7.
20. Khashayar P, Meybodi HRA, Hemami MR, Keshtkar A, Dimai HP, Larijani B. Vitamin D status and its relationship with bone mineral density in a healthy Iranian population. Rev Bras Ortop. Sociedade Brasileira de Ortopedia e Traumatologia; 2016;51(4):454–8.
21. Kamineni V, Latha AP, Ramathulasi K. Association between serum 25- hydroxyvitamin D levels and bone mineral density in normal postmenopausal women. J MidLife Heal. 2016;7(4):163–8.
22. Chailurkit L, Kruavit A, Rajatanavin R. Vitamin D status and bone health in healthy Thai elderly women. Nutrition. Elsevier Ltd; 2011;27(2):160–4.
23. Alireza K, Eslamifar A, Gheini MH, Abachi M, Azizi E, Monir S, et al. Prevalence of Vitamin D deficiency among an elderly people. Int J Curr Res. 2016;8(11):41315–8.
24. Park J, Hong IY, Chung JW, Choi HS. Vitamin D status in South Korean population: Seven-year trend from the KNHANES. Medicine (Baltimore). 2018;97(26):e11032.
25. Chailurkit L, Aekplakorn W, Ongphiphadhanakul B. Regional variation and determinants of vitamin D status in sunshine-abundant Thailand. BMC Public Health. BioMed Central Ltd; 2011;11:853.
26. Babu US, Calvo MS. Modern India and the vitamin D dilemma: Evidence for the need of a national food fortification program. Mol Nutr Food Res. 2010;54:1134–47.
27. Huang C, Huang YA, Lai Y, Sun C. Prevalence and predictors of hypovitaminosis D among the elderly in subtropical region. PLoS One. 2017;12(7):e0181063.
28. Lu L, Yu Z, Pan A, Hu FB, Franco OH, Li H, et al. Plasma 25-hydroxyvitamin D concentration and metabolic syndrome among middle-aged and elderly chinese individuals. Diabetes Care. 2009;32(7):1278–83.
29. Suryanarayana P, Arlappa N, Sai Santhosh V, Nagalla B, Pondey LR, Undrajavarapu P, et al. Prevalence of vitamin D deficiency and its associated factors among urban elderly population in Hyderabad metropolitan city, South India. Ann Hum Biol. Taylor & Francis; 2018;45(2):133–9.
30. Robien K, Butler LM, Wang R, Beckman KB, Walek D, Koh W-P, et al. Genetic and environmental predictors of serum 25(OH)D concentrations among middle-aged and elderly Chinese in Singapore. Br J Nutr. 2013;109(3):493–502.
31. Rahman SA, Chee WSS, Yassin Z, Chan SP. Vitamin D status among postmenopausal Malaysian women. Asia Pac J Clin Nutr. 2004;13(3):255–60.
32. Li S, Ou Y, Zhang H, Zhang Z, Zhou H, Liu L, et al. Vitamin D Status and Its Relationship with Body Composition , Bone Mineral Density and Fracture Risk in Urban Central South Chinese Postmenopausal Women. Ann Nutr Metab. 2014;64:13–9.
33. Labronici PJ, Blunck SS, Lana FR, Esteves BB, Franco JS, Fukuyama JM, et al. Vitamin D and its relation to bone mineral density in postmenopause women. Rev Bras Ortop. 2013;48(3):228–35.
34. Kruavit A, Chailurkit LO, Thakkinstian A,

Sriphrapradang C, Rajatanavin R. Prevalence of Vitamin D insufficiency and low bone mineral density in elderly Thai nursing home residents. BMC Geriatr. BMC Geriatrics; 2012;12:49.

35. Arabi A, Baddoura R, El-Rassi R, Fuleihan GE-H. PTH level but not 25(OH) vitamin D level predicts bone loss rates in the elderly. Osteoporos Int. 2012;23:971–80.
36. Hannan MT, Felson DT, Dawson-hughes B, Tucker KL, Cupples LA, Wilson PWF, et al. Risk Factors for Longitudinal Bone Loss in Elderly Men and Women : The Framingham Osteoporosis Study. J Bone Min Res. 2000;15(4):710–20.

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BONE MINERAL DENSITY AND VITAMIN D STATUS IN ELDERLY JAVANESE WOMEN

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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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REFERENCES

1. Hanel A, Carlberg C. Vitamin D and evolution: Pharmacologic implications. Biochem Pharmacol. 2020;173:113595.
2. Albahrani AA, Greaves RF. Fat-soluble vitamins: Clinical indications and current challenges for chromatographic measurement. Clin Biochem Rev. 2016;37(1):27–47.
3. Pike JW, Christakos S. Biology and mechanisms of action of the vitamin D hormone. Endocrinol Metab Clin North Am. 2017;46(4):815–43.
4. Jones G, Prosser DE, Kaufmann M. Cytochrome P450-mediated metabolism of vitamin D. J Lipid Res. 2014;55(1):13–31.
5. Holick MF. Vitamin D status: Measurement, interpretation, and clinical application. Ann Epidemiol. 2009;19(2):73–8.
6. Hilger J, Friedel A, Herr R, Rausch T, Roos F, Wahl DA, et al. A systematic review of vitamin D status in populations worldwide. Br J Nutr. 2014;111(1):23–45.
7. van Schoor N, Lips P. Global overview of vitamin D status. Endocrinol Metab Clin North Am. 2017;46(4):845–70.
8. van Schoor NM, Lips P. Worldwide vitamin D status. Best Pr Res Clin Endocrinol Metab. 2011;25(4):671–80.
9. Yu S, Fang H, Han J, Cheng X, Xia L, Li S, et al. The High Prevalence of Hypovitaminosis D in China. md J. 2015;94(8):1–6.
10. Mosekilde L. Vitamin D and the elderly. Clin Endocrinol. 2005;62(3):265–81.
11. Setiati S. Vitamin D status among Indonesian elderly women living in institutionalized Care units. Acta Med Indones-Indones J Intern Med. 2004;40(2):78–83.
12. Sözen T, Özışık L, Başaran NÇ. An overview and management of osteoporosis. Eur J Rheumatol. 2017;4(1):46–56.

JURNAL KEDOKTERAN DIPONEGORO
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JKD (DMJ), Volume 12, Number 3, May 2023 : 124-130

13. World Health Organization. WHO scientific group on the prevention and management of osteoporosis: report of a WHO scientific group. Genewa, Switzerland; 2003. 54 p.
14. Pike JW, Lee SM, Meyer MB. Regulation of gene expression by 1,25-dihydroxy-vitamin D3 in bone cells: exploiting new approaches and defining new mechanisms. Bonekey Rep. Nature Publishing Group; 2014;3:482.
15. Lips P, Van Schoor NM. The effect of vitamin D on bone and osteoporosis. Best Pr Res Clin Endocrinol Metab. 2011;25(4):585–91.
16. Sadat-ali M, Al Elq A, Al-turki HA, Al-mulhim FA, Al-ali AK. Influence of vitamin D levels on bone mineral density and osteoporosis. Ann Saudi Med. 2011;31(6):602–8.
17. Reid IR, Bolland MJ, Grey A. Effects of vitamin D supplements on bone mineral density: a systematic review and meta-analysis. Lancet. Elsevier Ltd; 2014;383(9912):146–55.
18. Alkhenizan A, Mahmoud A, Hussain A, Gabr A, Alsoghayer S, Eldali A. The relationship between 25(OH)D Levels (Vitamin D) and Bone Mineral Density (BMD) in a Saudi Population in a Community-Based Setting. PLoS One. 2017;12(1):e0169122.
19. Kota S, Jammula S, Kota S, Meher L, Modi K. Correlation of vitamin D, bone mineral density and parathyroid hormone levels in adults with low bone density. Indian J Orthop. 2013;47(4):402–7.
20. Khashayar P, Meybodi HRA, Hemami MR, Keshtkar A, Dimai HP, Larijani B. Vitamin D status and its relationship with bone mineral density in a healthy Iranian population. Rev Bras Ortop. Sociedade Brasileira de Ortopedia e Traumatologia; 2016;51(4):454–8.
21. Kamineni V, Latha AP, Ramathulasi K. Association between serum 25-hydroxyvitamin D levels and bone mineral density in normal postmenopausal women. J MidLife Heal. 2016;7(4):163–8.
22. Chailurkit L, Kruavit A, Rajatanavin R. Vitamin D status and bone health in healthy Thai elderly women. Nutrition. Elsevier Ltd; 2011;27(2):160–4.
23. Alireza K, Eslamifar A, Gheini MH, Abachi M, Azizi E, Monir S, et al. Prevalence of Vitamin D deficiency among an elderly people. Int J Curr Res. 2016;8(11):41315–8.
24. Park J, Hong IY, Chung JW, Choi HS. Vitamin D status in South Korean population: Seven-year trend from the KNHANES. Medicine (Baltimore). 2018;97(26):e11032.
25. Chailurkit L, Aekplakorn W, Ongphiphadhanakul B. Regional variation and determinants of vitamin D status in sunshine-abundant Thailand. BMC Public Health. BioMed Central Ltd; 2011;11:853.
26. Babu US, Calvo MS. Modern India and the vitamin D dilemma: Evidence for the need of a national food fortification program. Mol Nutr Food Res. 2010;54:1134–47.
27. Huang C, Huang YA, Lai Y, Sun C. Prevalence and predictors of hypovitaminosis D among the elderly in subtropical region. PLoS One. 2017;12(7):e0181063.
28. Lu L, Yu Z, Pan A, Hu FB, Franco OH, Li H, et al. Plasma 25-hydroxyvitamin D concentration and metabolic syndrome among middle-aged and elderly Chinese individuals. Diabetes Care. 2009;32(7):1278–83.
29. Suryanarayana P, Arlappa N, Sai Santhosh V, Nagalla B, Pondey LR, Undrajavarapu P, et al. Prevalence of vitamin D deficiency and its associated factors among urban elderly population in Hyderabad metropolitan city, South India. Ann Hum Biol. Taylor & Francis; 2018;45(2):133–9.
30. Robien K, Butler LM, Wang R, Beckman KB, Walek D, Koh W-P, et al. Genetic and environmental predictors of serum 25(OH)D concentrations among middle-aged and elderly Chinese in Singapore. Br J Nutr. 2013;109(3):493–502.
31. Rahman SA, Chee WSS, Yassin Z, Chan SP. Vitamin D status among postmenopausal Malaysian women. Asia Pac J Clin Nutr. 2004;13(3):255–60.
32. Li S, Ou Y, Zhang H, Zhang Z, Zhou H, Liu L, et al. Vitamin D Status and Its Relationship with Body Composition, Bone Mineral Density and Fracture Risk in Urban Central South Chinese Postmenopausal Women. Ann Nutr Metab. 2014;64:13–9.

JURNAL KEDOKTERAN DIPONEGORO
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JKD (DMJ), Volume 12, Number 3, May 2023 : 124-130

33. Labronici PJ, Blunck SS, Lana FR, Esteves BB, Franco JS, Fukuyama JM, et al. Vitamin D and its relation to bone mineral density in postmenopause women. Rev Bras Ortop. 2013;48(3):228–35.
34. Kruavit A, Chailurkit LO, Thakkinstian A, Sriphrapradang C, Rajatanavin R. Prevalence of Vitamin D insufficiency and low bone mineral density in elderly Thai nursing home residents. BMC Geriatr. BMC Geriatrics; 2012;12:49.
35. Arabi A, Baddoura R, El-Rassi R, Fuleihan GE-H. PTH level but not 25(OH) vitamin D level predicts bone loss rates in the elderly. Osteoporos Int. 2012;23:971–80.
36. Hannan MT, Felson DT, Dawson-Hughes B, Tucker KL, Cupples LA, Wilson PWF, et al. Risk Factors for Longitudinal Bone Loss in Elderly Men and Women : The Framingham Osteoporosis Study. J Bone Min Res. 2000;15(4):710–20.