ORIGINAL ARTICLE

Correlation of Vitamin D With Bone Mineral Density by Dual Energy X-ray Absorptiometry (DXA) Scan Among Healthy Malay Adult

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ABSTRACT

Introduction: Scarcity of data found in regard to association of vitamin D level with bone mineral density (BMD). Our study aimed to determine the correlation of vitamin D with BMD and intact parathyroid hormone (iPTH) among healthy Malay adult. **Methods:** This cross-sectional study recruited 126 healthy Malay volunteers (aged 21–45 years old) from Kota Bharu, Malaysia. Serum total calcium, albumin, phosphorus, 25-hydroxyvitamin D (25(OH)D), and iPTH were measured. BMD was assessed with dual energy X-ray absorptiometry (DXA) scan over left hip (right hip in case of problem with left hip) and lumbar spine (L1 – L4 vertebrae). **Results:** The mean serum 25(OH)D was 38.91 ± 14.07 nmol/L. Out of 126 study subjects, 104 subjects (82.5%) had insufficient level of vitamin D (< 50 nmol/L). Mean hip and lumbar BMD were 0.952 ± 0.145 g/cm2 and 1.006 ± 0.133 g/cm2 respectively. According to T-score, 93 subjects (73.8%) had normal T-score of ≥ -1, 33 subjects (26.2%) had osteopenia (T-score -2.5 to -1) and none had osteoporosis (T-score ≤ -2.5). Significant positive correlation between serum 25(OH)D and hip BMD ($r = 0.234$, $p = 0.009$) was observed, but no correlation was found between serum 25(OH)D and lumbar BMD. Meanwhile, significant inverse correlation between serum 25(OH)D and iPTH was observed ($r = -0.324$, $p < 0.001$). **Conclusion:** High prevalence of vitamin D insufficiency was observed among healthy Malay population, but majority had normal bone density. Nonetheless, serum 25(OH)D was positively correlated with BMD and inversely correlated with iPTH. Our findings support the role of vitamin D for maintaining bone health.

Keywords: Adult, Vitamin D, Bone mineral density

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INTRODUCTION

Vitamin D deficiency is a worldwide epidemic across all age groups, particularly common in risk groups such as elderly, children and pregnant women (1, 2). Despite being a continent with low latitude, prevalence of vitamin D deficiency is high in Asia especially in India and China (3). In Malaysia, although sunlight is ample throughout the year, several studies on the three major ethnics (Malays, Chinese and Indians) show that vitamin D insufficiency is common, particularly in Malays compared to the other two ethnics (4-9). Although Malays have higher prevalence of vitamin D insufficiency, limited numbers of studies on osteoporotic fracture in Malaysia show Chinese have lower BMD and high osteoporotic fracture rate compared to Malays and Indians (23, 24).

Vitamin D plays a vital role in maintenance of adequate serum calcium and phosphate concentrations, and bone health. Vitamin D deficiency can lead to secondary hyperparathyroidism, causing an increase in the bone turnover and bone loss, therefore a major risk factor for osteoporosis (10, 11). However, the association between serum 25-hydroxyvitamin D (25(OH)D) level and bone mineral density (BMD) remains controversial across studies conducted among different group of populations at different places. Among young subjects, there were significant association between 25(OH)D with bone mineral density (12). Significant association between 25(OH)D and BMD were also observed in studies

among postmenopausal women in Saudi, Central South China, Sicilian, Arab and in selected senile osteoporotic fracture patients respectively (13, 14, 15, 16, 17, 18). However, contradicting finding observed among adult patients seen in osteoporotic clinic, Singapore with low BMD (19), among healthy Bangladeshi women aged above 45 years old seen in urban hospital (20) and among Indian patient attending osteoporotic clinic with low BMD (21). Only 5 out of 11 studies among Chinese population in Asia showed association between 25(OH) D with BMD (22).

Majority of previous studies conducted were within menopausal groups of population and among patients with mineral bone disease and showed inconsistent association between serum 25(OH)D with BMD. Therefore, the current study aimed to determine the correlation between 25(OH)D level with BMD and intact parathyroid hormone (iPTH) level among healthy Malay adult at younger age (21 to 45 years old) in Kota Bharu, Kelantan, Malaysia.

MATERIALS AND METHODS

Subjects

This cross-sectional study was conducted at Hospital Universiti Sains Malaysia (HUSM), Kota Bharu, Kelantan, Malaysia. The largest sample size obtained from estimation of sample size was 126 after adding 20% anticipated dropout rate based on single mean formula with population standard deviation of 13.1 (7) and estimated difference was 2.5 at 1.96 level of significance. Total of 126 healthy Malay adult aged 21 to 45 years old were enrolled within 3 months duration, from August to October 2017. This study used adult ages 21 to 45 years old as source population. As according to National Institute of Arthritis and Musculoskeletal and Skin Diseases, by age 20 in boys and 18 in girls, up to 90 percent of peak bone mass is acquired (25). After menopause, women start to loose bone mass continuously throughout the postmenopausal years (25). A study in Malaysia showed the mean age at menopause in the Malaysian women was 47.96 years (26).

The participants were volunteers recruited from hospital staffs and family members that were accompanying patients through advertisement. Demographic data were taken, and one day dietary recall calcium intake were obtained based on validated food and calcium intake questionnaire (27) to take into consideration of confounder for PTH level. Subjects with chronic illnesses such as liver failure, diabetes mellitus, chronic kidney disease, thyroid disorders and established bone mineral disease were excluded from this study. Subjects on drugs that affect vitamin D and bone turnover such as oral contraceptive pill, hormone replacement therapy, vitamin D supplements, and steroids were also excluded. Besides, subjects who had recent fracture within past 1 year, pregnant, lactating and menopausal women were also excluded. The subjects were screened based on the inclusion and exclusion criteria. This study was conducted with the approval of Human Research Ethics Committee USM (HREC) No. 17030151 and written informed consent was obtained from all subjects.

Biochemical measurements

Laboratory tests included serum total calcium, albumin, phosphorus, 25(OH)D and iPTH. 10 ml of venous blood was collected in the morning after an overnight fast of 10-12 hours. After centrifugation, serum was stored at -70°C until analysis. Serum total calcium, albumin and phosphorus were determined by spectrophotometric analysis using ARCHITECT C 800 analyzer by Abbott Diagnostics. Albumin-corrected calcium was calculated using the following formula: corrected calcium (mmol/L) = measured total calcium (mmol/L) + 0.02 (40 – serum albumin (g/L)).

Serum 25(OH)D was determined by paramagnetic particle, chemiluminescent immunoassay competitive principle using Access 2 Immunoassay System analyzer by Beckman Coulter. The total imprecision (within and between run precision) was 6.1 – 7.5%. This method is traceable to a Joint Committee for Traceability in Laboratory Medicine (JCTLM)-approved isotope dilution mass spectrometry (ID-LC-MS/MS) reference method procedure (RMP) developed at Ghent University (28, 29). This RMP is further traceable to the NIST SRM 2972 (30). According to World Health Organization (WHO), as well as Institute of Medicine (IOM), the threshold of optimum vitamin D level is 50 nmol/L (20 ng/mL) (31, 32). Based on serum 25(OH)D level, the subjects were classified as vitamin D sufficiency (≥ 50 nmol/L) and insufficiency (< 50 nmol/L) in this study. The serum 25(OH)D < 30 nmol/L is defined as vitamin D deficiency and this level is associated with an increased risk of osteomalacia or ricket and requires higher dose of vitamin D supplementation than in subjects with vitamin D insufficiency (33).

Serum iPTH was determined by electrochemiluminescence immunoassay (ECLIA) sandwich principle using Elecsys Cobas e601 analyzer by Roche Diagnostic. The CV for repeatability was 1.1 – 2.0%, while the intermediate precision was 1.7 – 3.4%. Level < 1.6 pmol/L was taken as low, 1.6 – 6.9 pmol/L as normal, and > 6.9 pmol/L as high.

Bone mineral density

BMD was assessed by DXA scan using Bone Densitometer Hologic Discovery A fan beam (Waltham, USA) over left hip (right hip in case of problem with left hip) and lumbar spine (L1 – L4 vertebrae). The hip region includes entire femoral head, neck, and approximately 3 inches of the shaft. The in vivo coefficient variation of BMD measurement was 0.442%. Absolute BMD is the measured parameter from bone mineral content in grams over two-dimensional projected area in cm^2 of

the bone, thus the unit of BMD is g/cm2 (31). BMD values were also interpreted as WHO T-score. T-score was calculated based on of normal reference values for age- and sex-matched populations (using included software – Hologic Discovery QDR software version 13.4.2 provided by DXA manufacturer to calculate T-score relative to native Japanese reference data). The T-score of total hip or total lumbar spine between ≤ -2.5 was defined as osteoporosis, -2.5 to -1 as osteopenia and ≥ -1 as normal (34).

Statistical analysis

Data analysis was performed using Statistical Package for Social Science (SPSS) version 24. Numerical data were expressed as mean ± standard deviation (SD) for normally distributed data or as median ± interquartile range (IQR) for skewed data. Bivariate correlation analyses were conducted using either Pearson correlation (if bivariate normal distribution assumption is met) or Spearman's rank correlation (if bivariate normal distribution assumption is not met). Categorical data were expressed as frequency and percentage, while Pearson's Chi-square test was performed to test for correlation. A probability value of $P < 0.05$ was taken as statistically significant.

RESULTS

A total of 126 Malay adult were included in the study, 55 (43.7%) were male and 71 (56.3%) were female. The mean age was 30.9 ± 7.0 years. General characteristics of study subjects are shown in Table I. The mean serum 25(OH)D level of total subjects was 38.91 ± 14.07 nmol/L. There was a statistically significant difference in mean 25(OH)D level between male and female (47.47 ± 14.70 nmol/L versus 32.37 ± 9.20 nmol/L, respectively; $p < 0.001$, 95% CI 10.96, 19.34).

Table I: General characteristics of study subjects (N=126)

Characteristic	n(%)	Mean±SD
Gender		
Male	55 (43.7)	
Female	71 (56.3)	
Age (years)		30.9 ± 7.0
Weight (kg)		64.8 ± 14.9
Height (m)		1.61 ± 0.09
BMI (kg/m^2)		24.70 ± 4.78
Calcium (mmol/L)		2.31 ± 0.09
Phosphorus (mmol/L)		1.16 ± 0.18
iPTH (pmol/L)		4.48 ± 1.64
low (< 1.6)	0 (0)	
normal (1.6 – 6.9)	113 (89.7)	
high (> 6.9)	13 (10.3)	
25(OH)D (nmol/L)		38.91 ± 14.07
Hip BMD (g/cm^2)		0.952 ± 0.145
Lumbar BMD (g/cm^2)		1.006 ± 0.133
T-score		
Normal (≥ -1)	93 (73.8)	
Osteopenia (-2.5 to -1)	33 (26.2)	
Osteoporosis (≤ -2.5)	0 (0)	
Daily calcium intake (mg/day)		351 ± 227

SD: standard deviation, BMI: body mass index, iPTH: intact parathyroid hormone, 25(OH)D: 25-hydroxyvitamin D, BMD: bone mineral density

Out of the 126 subjects, only 22 subjects (17.5%) had sufficient 25(OH)D level (≥ 50 nmol/L) while 104 subjects (82.5%) had insufficient level (< 50 nmol/L) and 92 subjects (73.0%) had vitamin D < 30 nmol/L. Among the 22 subjects who have sufficient 25(OH)D level, majority are male; and among the 104 subjects who have insufficient 25(OH)D level, majority are female as show in Table II.

Table II: Correlation between vitamin D status and gender (N=126)

Serum 25(OH)D	Gender [n (%)]		p value
	Male	Female	
Sufficiency	20 (90.9)	2 (9.1)	<0.001
Insufficiency	35 (33.7)	69 (66.3)	

25(OH)D: 25-hydroxyvitamin D, p value < 0.05 was considered as statistically significant, 25(OH)D ≥ 50 nmol/L was considered as sufficiency, 25(OH)D < 50 nmol/L was considered as insufficiency

Mean hip BMD was lower than mean lumbar BMD (0.952 ± 0.145 g/cm2 and 1.006 ± 0.133 g/cm2, respectively). According to T-score, 93 subjects (73.8%) had normal T-score (≥ -1), 33 subjects (26.2%) had osteopenia (T-score -2.5 to -1) and none had osteoporosis (T-score ≤ -2.5). The mean serum iPTH level was 4.48 ± 1.64 pmol/L and majority (89.7%) had normal iPTH levels.

Pearson correlation showed a statistically significant positive correlation between serum 25(OH)D with hip BMD ($r = 0.234$, $p = 0.009$)[Table III]. However there was no significant correlation between serum 25(OH)D with lumbar BMD. Besides being positively correlated with hip BMD but not with lumbar BMD, serum 25(OH) D had a statistically significant positive correlation with age ($r = 0.350$, $p < 0.001$) and statistically significant inverse correlation with gender (female) ($r = -0.538$, $p < 0.001$) and iPTH ($r = -0.324$, $p < 0.001$). No correlation between serum 25(OH)D with BMI was observed.

Both hip and lumbar BMD showed positive correlation with BMI ($r = 527$, $p < 0.001$ and $r = 527$, $p < 0.001$ respectively). As for gender, both hip and lumbar BMD showed significant inverse correlation with gender (female) ($r = -0.513$, $p < 0.001$ and $r = -0.272$, $p = 0.002$ respectively). It was also observed that there was no correlation between daily calcium intake and iPTH with both hip and lumbar BMD.

DISCUSSION

There is no consensus on the definition of optimal vitamin D level. There are controversies among different experts. Some experts defined optimal vitamin D level using parathyroid hormone (PTH) suppression as a determinant of optimal 25(OH)D levels, which resulted in a wide range of estimation, from 20 - 110 nmol/L (8 - 44 ng/mL), with most fell between 75 - 110 nmol/L (30 – 44 ng/mL) (12). According to WHO, the suppression of PTH with vitamin D supplementation only occurs in individuals with a baseline serum 25(OH)

Table III: Correlation between serum 25(OH)D and other variables (N=126)

Variable	1	2	3	4	5	6	7	8
1. Gender (female)	1							
2. Age	-0.112	1						
3. BMI	-0.104	0.285**	1					
4. Calcium intake	-0.111	0.008	0.056	1				
5. iPTH	0.264**	-0.092	-0.093	0.058	1			
6. Hip BMD	-0.513**	0.014	0.587**	0.001	-0.107	1		
7. Lumbar BMD	-0.272**	0.174	0.257**	-0.057	-0.105	0.719**	1	
8. 25(OH)D	-0.538**	0.350**	0.056	0.048	-0.324**	0.234**	0.092	1

BMD: bone mineral density, 25(OH)D: 25-hydroxyvitamin D, BMI: body mass index, iPTH: intact parathyroid hormone, *p < 0.05, **p < 0.001.

D level less than 50 nmol/L (20 ng/mL) (34). While according to Endocrine Society, optimal serum 25(OH) D level is defined as 75 nmol/L (>30 ng/mL) (35). Other experts use bone health outcomes to determine optimal serum 25(OH)D level, for example in 2011, Institute of Medicine (IOM) concluded that a vitamin D level of at least 50 nmol/L is sufficient for optimum bone health in majority (97.5%) of the population (32). Our study used cut off of 50 nmol/L as threshold.

This study revealed that the majority of subjects (82.5%) had vitamin D insufficiency, with mean serum 25(OH) D level of 38.91 nmol/L, below the recommended vitamin D level. The mean of 25(OH)D in this study was a bit lower than a study among premenopausal and postmenopausal women in Kelantan (43.3 nmol/L) (36) but higher than previous study among healthy adult aged 18-50 years old from several selected subdistricts in Kota Bharu which was 23.5 nmol/L (9). The possible reason for the variation of vitamin D level most likely due to gender, different target age of subjects between studies, different time of blood collection, and different method of 25(OH)D measurement.

This study also showed that vitamin D insufficiency is more prevalent in female. These observations are consistent with findings from previous studies done on Malay populations in Malaysia by Moy (5) and Ismail et al., (9). This could be explained by indoor activities, inadequate sun exposure due to clothing style (wearing long sleeves, long skirts and pants, and veil) and sun protection use especially in female, improper timing of sun exposure and genetic factors.

Adequate level of vitamin D has an important effect on bone mass no matter young or old. It influences calcium metabolism, osteoclast development, osteoblast activity, matrix ossification, bone remodelling, and hence BMD (10). Vitamin D deficiency can cause secondary hyperparathyroidism, leading to an increase in the bone turnover and bone loss, hence an important risk factor for osteoporosis (11). Severe vitamin D deficiency causes osteomalacia in adults and rickets in children. On the other hand, adequate vitamin D level has been shown to prevent osteoporotic fracture (11).

The association between serum 25(OH)D and BMD, as well as association between serum 25(OH)D and PTH remain controversial. There are studies showing positive correlation between BMD and 25(OH)D. Meanwhile, other studies do not support this correlation. In our study, a statistically significant positive fair correlation between serum 25(OH)D and hip BMD was found, but not with lumbar BMD. Bone consists of trabecular and cortical bone mass and the volume of cortical and trabecular compartment differ base on site. The lumbar spine consists mostly of trabecular bone as it comprises block of trabecular bone covered by a thin of cortical bone. The trabecular bone is relatively stable when there is high PTH level compared to cortical bone (37) and this might explain why the lumbar was less affected than hip bone. The hip consists mostly of cortical bone which affected more in metabolic bone disease (38), thus might explain the association of 25(OH)D with hip BMD. Another possible reason of significant association between 25(OH)D with BMD of hip is vitamin D affect cortical bone of hip more than trabecular bone of lumbar (39). There was study showed lower vitamin D level was associated with hip bone loss (40).

Our findings are consistent with data from the National Health and Nutrition Survey (NHANES) III which showed positive association between 25(OH)D and BMD particularly among white young and older males (12). Similarly, studies done in Saudi Arabia by Sadat-Ali et al., (13) and in its neighbouring country, Qatar, by Bener et al., (16) also reported positive association between vitamin D level and BMD. Study by Napoli et al., on postmenopausal women in Italy demonstrated that subjects with low serum 25(OH)D showed lower BMD T-score independently of used cut-off, but this difference was lost on femoral neck when cut-off at 75 nmol/L was used (15). However, a review by Man et al., on 11 studies found inconsistent relationship between serum 25(OH)D level and BMD in the Chinese population, whereby five studies reported an association

and six studies did not (22).

A study done in Singapore, neighbouring country of Malaysia, by Chandran et al., (18) showed no association between serum 25(OH)D and BMD, which is contradicting to our study. This might be due to their study subjects were not randomly recruited from general population, but were all patients suspected to have osteoporosis and osteopenia, and thus the low BMD ranging 0.580 – 0.747 g/cm2 reported in their study. Similarly, study in India by Kota et al., also revealed serum 25(OH)D levels not directly correlated with BMD, and their study subjects were all patients with osteoporosis and osteopenia with low BMD ranging 0.72 – 0.79 g/cm2 (21). Two studies on postmenopausal women by Ahmed et al., (19) and Labronici et al, (20) in which majority of their study subjects were also patients with osteoporosis and osteopenia, showed no correlation between serum 25(OH)D levels and BMD. Hence it is worth mentioning that prevalence of osteopenia and osteoporosis in study subjects could confound the correlation between vitamin D levels and BMD.

As for PTH, a negative correlation between serum 25(OH)D and PTH and has been noted in both Caucasian and non-Caucasian populations (4, 6, 9, 13, 15, 18, 21). However, few studies show that not all patients with vitamin D deficiency develop secondary hyperparathyroidism (14, 17). In our study, there was a statistically significant inverse fair correlation between serum 25(OH)D and iPTH ($r = -0.324$, $p < 0.001$). These observations are consistent with finding from previous study done on in Malaysia by Rahman et al., (4) whereby the inverse correlation between PTH and serum 25(OH) D in Malay population was driven by subjects with serum 25(OH)D of 30 nmol/L or less. Another study done in Malaysia by Ismail et al., (9) showed a steep increase in PTH level when vitamin D levels reach 20 nmol/L.

There are two studies by Chandran et al., (18) and Kota et al., (21) which showed a negative correlation between serum 25(OH)D and iPTH when serum 25(OH)D was < 75nmol/L. Napoli et al., (15) found a significant change in PTH levels for 25(OH)D at 65 – 75 nmol/L, but an inflection point was not clear. Meanwhile, Ahmed et al., (19) reported no association between vitamin D and iPTH, which could be explained by small number of study subjects tested for iPTH (only 38 out of 133 subjects tested for iPTH). Likewise, Li et al., (14) also found vitamin D status not showing any relationship with PTH levels, but the PTH levels were measured only in less than half of the study subjects.

Although our study showed a positive correlation between serum 25(OH)D and hip BMD, and negative correlation between serum 25(OH)D and iPTH, there was no correlation between iPTH and BMD, either hip or lumbar. We speculate that it can be due to the majority of our subjects had normal iPTH level and normal T-score (89.7% and 73.8% of study subjects, respectively). These controversial findings regarding association of vitamin D with BMD and PTH among all the studies including ours can be partially explained by heterogeneity in: participants' ethnicity, populations' geography and climate, age group, study size, inclusion and exclusion criteria, diet, physical activity, threshold of optimal serum 25(OH)D value, and prevalence of osteopenia and osteoporosis in study subjects.

Despite of high proportion of vitamin D insufficiency, the bone density among the study subjects were normal. The discrepancy has not been frequently reported in other research, but may be explained by the fact that mineral bone disease may less affect young adult compared to older adult. The current lack of agreement on optimal vitamin d level for maintaining bone health possibly another reason to have normal bone density in subject categorized under vitamin D insufficiency (<50 nmol/L). Among Premenopausal women in Pakistani, level of 16 ng/ml (39.9 nmol/L able to keep iPTH within normal range which might support the lower level of 25(OH)D to maintain healthy bone (41). Based on bone markers changes, level of 20-35 nmol/L is required for bone health (9).

Obesity is known to be associated with vitamin D insufficiency due to sequestration of vitamin D by adipose tissue (42). However our study showed no correlation between serum 25(OH)D and BMI. This could be due to mean BMI of our subjects (24.70 ± 4.78 kg/m2) was normal, similar to findings by Zhang et al., (43) and Baradaran et al., (44) (mean BMI 25.0 ± 0.1 kg/m2 and 24.2 ± 3.8 kg/m2, respectively).
Interestingly, our study also showed that both hip and lumbar BMD were positively correlated with BMI. Our findings are consistent with studies done in various Asian populations, such as in Malaysia by Yahya et al., (45), in India by Kota et al., (21) and in China by Wu et al., (46). We postulate that this can be explained by higher mechanical load on bones and better nutritional status in higher BMI.

We did not find any correlation between BMD and daily calcium intake. It could be due to low daily calcium intake among our subjects (351 ± 227 mg/day), which was significantly lower than recommended daily calcium intake of 1000 mg/day by IOM (31). A low level of calcium intake also was observed among women from low income family in Kelantan but their level was higher than our study subjects (492.9±316.51 mg/day) (36). Even though the dietary calcium intake was assessed by validated questionnaire, it could still be subjected to subjects' recall bias. It probably also affected by trained or untrained interviewer.

Other limitation of this study includes small sample

size, single ethnicity and single locality. Besides, all the subjects were healthy and relatively young volunteers, hence our study results may not be representative of a normal general population. Also, confounding factors such as physical activity and exposure to sunlight were not assessed in this study.

CONCLUSION

This study confirmed a high prevalence of vitamin D insufficiency in healthy Malay population particularly in female, even though Malaysia is a country with ample sunlight throughout the year. Fortification of food, oral vitamin D and calcium and regular sunlight exposure are ways to improve vitamin D level. Although vitamin D insufficiency prevailed, majority of the healthy Malay population had normal bone density. Nonetheless, our study showed a positive correlation between serum 25(OH)D and hip BMD, and inverse correlation between serum 25(OH)D and iPTH, which affirmed the role of vitamin D in maintaining optimal bone health.

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REFERENCES

1. Edwards MH, Cole ZA, Harvey NC, Cooper C. The global epidemiology of vitamin D status. J Aging Res Clin Prac. 2014;3:148-58.
2. 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;111:23-45.
3. Palacios C, Gonzalez L. Is vitamin D deficiency a major global public health problem? J Steroid Biochem Mol Biol. 2014;144:138-45.
4. Rahman SA, Chee WS, Yassin Z, Chan SP. Vitamin D status among postmenopausal Malaysian women. Asia Pac J Clin Nutr. 2004; 13: 255-60.
5. Moy FM. Vitamin D status and its associated factors of free living Malay adults in a tropical country, Malaysia. J Photochem Photobiol B. 2011;104:444-8.
6. Green TJ, Skeaff CM, Rockell JE, Venn BJ, Lambert A, Todd J, et al. Vitamin D status and its association with parathyroid hormone concentrations in women of child-bearing age living in Jakarta and Kuala Lumpur. Eur J Clin Nutr. 2008;62:373-8.
7. Chin KY, Ima-Nirwana S, Ibrahim S, Mohamed IN, Wan Ngah WZ. Vitamin D status in Malaysian men and its associated factors. Nutrients. 2014;6:5419-33.
8. Al-Sadat N, Majid HA, Sim PY, Su TT, Dahlui M, Bakar MF, et al. Vitamin D deficiency in Malaysian adolescents aged 13 years: findings from the Malaysian Health and Adolescents Longitudinal Research Team study (MyHeARTs). BMJ open. 2016;6:e010689.
9. Ismail TS, Muda BC, Rosdi RA, Zainuddin A, Isa S, Mustapaha Z. Determining the Required Vitamin D Level for Bone Health Based on Bone Turnover Markers. Korean J Clin Lab Sci. 2017;49:337-44.
10. Laird E, Ward M, McSorley E, Strain JJ, Wallace J. Vitamin D and bone health; Potential mechanisms. Nutrients. 2010;2:693-724.
11. Lips P, Van Schoor NM. The effect of vitamin D on bone and osteoporosis. Best Pract Res Clin Endocrinol Metab. 2011;25:585-91.
12. Bischoff-Ferrari HA, Dietrich T, Orav EJ, Dawson-Hughes B. Positive association between 25-hydroxy vitamin D levels and bone mineral density: a population-based study of younger and older adults. Am J Med. 2004;116:634-9.
13. Sadat-Ali M, Al Elq AH, Al-Turki HA, Al-Mulhim FA, Al-Ali AK. Influence of vitamin D levels on bone mineral density and osteoporosis. Ann Saudi Med. 2011;31:602-8.
14. 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.
15. Napoli N, Strollo R, Sprini D, Maddaloni E, Rini GB, Carmina E. Serum 25-OH vitamin D in relation to bone mineral density and bone turnover. Int J Endocrinol. 2014;2014:487463.
16. Bener A, Saleh NM. Low Vitamin D, and bone mineral density with depressive symptoms burden in menopausal and postmenopausal women. J Midlife Health. 2015;6:108-14.
17. Wu J, Zhao F, Ma F, Guan T, Liu P. Relationship analysis of 25-Hydroxy-vitamin-D and parathyroid hormone in bone mineral density from senile osteoporotic fracture patients. Int J Clin Exp Med. 2017;10:13571-6.
18. 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;48:228-35
19. Chandran M, Hoeck H, Wong H, Zhang R, Dimai H. Vitamin D status and its relationship with bone mineral density and parathyroid hormone in Southeast Asian adults with low bone density. Endocr Pract. 2010;17:226-34.
20. Ahmed AS, Haque WM, Uddin KN, Abrar FA, Afroz F, Huque HF, et al. Vitamin D and bone mineral density status among postmenopausal Bangladeshi women. IMC J Med Sci. 2018;12:44-9.
21. 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;47:402-7.
22. Man PW, van der Meer IM, Lips P, Middelkoop BJ. Vitamin D status and bone mineral density in the

Chinese population: a review. Arch Osteoporos. 2016;11:14.
23. Lim PS, Ong FB, Adeeb N, Seri SS, Noor-Aini MY, Shamsuddin K, et al. Bone health in urban midlife Malaysian women: risk factors and prevention. Osteoporos Int. 2005;16:2069-79.
24. Lee JK, Khir AS. The incidence of hip fracture in Malaysians above 50 years of age: variation in different ethnic groups. APLAR J Rheumatol. 2007;10:300-5.
25. Osteoporosis: Peak Bone Mass In Women | NIH Osteoporosis And Related Bone Diseases National Resource Center. Bones.Nih.Gov 2015.
26. Jahanfar S, Abdul RB, Shah RB, Nor AB, Sharifah NB, Siti AB. Age of menopause and menopausal symptoms among Malaysian women who referred to health clinic in Malaysia. Shiraz E Med J. 2006;7:1-9.
27. Montomoli M, Gonnelli S, Giacchi M, Mattei R, Cuda C, Rossi S, Gennari C. Validation of a food frequency questionnaire for nutritional calcium intake assessment in Italian women. European Journal of Clinical Nutrition. 2002 Jan;56(1):21
28. Stepman HC, Vanderroost A, Van Uytfanghe K, Thienpont LM. Candidate reference measurement procedures for serum 25-hydroxyvitamin D3 and 25-hydroxyvitamin D2 by using isotope dilution–liquid chromatography–tandem mass spectrometry. Clin Chem. 2011;57:441-8.
29. Thienpont LM, Stepman HC, Vesper HW. Standardization of measurements of 25-hydroxyvitamin D3 and D2. Scand J Clin Lab Invest. 2012;72:41-9.
30. Certificate of analysis, Standard Reference Material 2972: 25-hydroxyvitamin D2 and D3 calibration solutions. Gaithersburg, MD: Standard Reference Materials Program, NIST; 2009.
31. Ross AC, Manson JE, Abrams SA, Aloia JF, Brannon PM, Clinton SK, et al. The 2011 report on dietary reference intakes for calcium and vitamin D from the Institute of Medicine: what clinicians need to know. J Clin Endocrinol Metab. 2011;96:53-8.
32. Garg MK, Kharb S. Dual energy X-ray absorptiometry: pitfalls in measurement and interpretation of bone mineral density. Indian J Endocrinol Metab. 2013;17:203-10.
33. Munns CF, Shaw N, Kiely M, Specker BL, Thacher TD, Ozono K, Michigami T, Tiosano D, Mughal MZ, Mλkitie O, Ramos-Abad L. Global consensus recommendations on prevention and management of nutritional rickets. Hormone research in paediatrics. 2016;85(2):83-106.
34. WHO Scientific Group on Prevention, Management of Osteoporosis, World Health Organization. Prevention and management of osteoporosis: report of a WHO scientific group. WHO 2003.
35. Holick MF, Binkley NC, Bischoff-Ferrari HA, Gordon CM, Hanley DA, Heaney RP, et al. Evaluation, treatment, and prevention of vitamin D deficiency: an Endocrine Society clinical practice guideline. J Clin Endocrinol Metab. 2011;96:1911-30.
36. Hawa M, Sakinah H, Hermizi H. Calcium and Vitamin D Status of Kelantanese Malay Women from Low Income Family: A Population-Based Study. J Aging Res Clin Pract. 2013;2(2):191-6.
37. Christodoulou S, Goula T, Ververidis A, Drosos G. Vitamin D and bone disease. BioMed research international. 2013;2013.
38. Ott SM. Cortical or trabecular bone: what's the difference?. American journal of nephrology. 2018 Jul 1;47(6):373-6.
39. Nakamura K, Tsugawa N, Saito T, Ishikawa M, Tsuchiya Y, Hyodo K, et al. Vitamin D status, bone mass, and bone metabolism in home-dwelling postmenopausal Japanese women: Yokogoshi Study. Bone. 2008;42:271-7.
40. Ensrud KE, Taylor BC, Paudel ML, Cauley JA, Cawthon PM, Cummings SR, Fink HA, Barrett-Connor E, Zmuda JM, Shikany JM, Orwoll ES. Serum 25-hydroxyvitamin D levels and rate of hip bone loss in older men. The Journal of Clinical Endocrinology & Metabolism. 2009 Aug 1;94(8):2773-80.
41. Masud F. Vitamin D levels for optimum bone health. Singapore medical journal. 2007 Mar 1;48(3):207.
42. Wortsman J, Matsuoka LY, Chen TC, Lu Z, Holick MF. Decreased bioavailability of vitamin D in obesity. Am J Clin Nutr. 2000;72:690-3.
43. Zhang Y, Zhang X, Wang F, Zhang W, Wang C, Yu C, et al. The relationship between obesity indices and serum vitamin D levels in Chinese adults from urban settings. Asia Pac J Clin Nutr. 2016;25:333-9.
44. Baradaran A, Behradmanesh S, Nasri H. Association of body mass index and serum vitamin D level in healthy Iranian adolescents. Endokrynol Pol. 2012;63:29-33.
45. Yahya NF, Makbul IA, Daud NM, Aziz QA. Relationship Between Body Mass Index, Calcium Intake and Vitamin D Status with Bone Mineral Density among Young Adults: A Preliminary Investigation. Pak J Nutr. 2018;17:156-62.
46. Wu SF, Du XJ. Body mass index may positively correlate with bone mineral density of lumbar vertebra and femoral neck in postmenopausal females. Med Sci Monit. 2016;22:145-51.