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Vitamin D and bone mineral density status, and their correlation with bone turnover markers in healthy children aged 6–14 in Vietnam

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Abstract

Background: Hypovitaminosis D is arising as a major public health problem causing adverse effects on bone mineral density (BMD), especially on the pediatric skeletal system. Bone turnover markers (BTMs) are used to assess bone health due to their fracture prediction ability.

Objectives: We aimed to assess the status of vitamin D and BMD and their correlation with BTMs in healthy children.

Materials and Methods: A cross-sectional study was conducted on 794 school-going children, from 6 to 14, recruited in Can Tho, Vietnam. The subjects' BMD and serum concentration of 25-hydroxyvitamin D (25OHD), N-terminal procollagen of type l collagen (P1NP), and β-isomerized C-terminal telopeptides (β-CTx) were measured. Linear regression analysis was carried out to assess the correlation.

Results: The mean concentration of 25OHD was 67.39 nmol/L, which shows that 30.6% of the subjects had vitamin D insufficiency and deficiency. Low BMD was found in 12.7% of the subjects. Hypovitaminosis D was common in girls (35.2%) or overweight/obese children (42.04%), while low BMD was prevalent among stunting children (15.46%). When analyzing BMD and 25OHD levels by ages, BMD was found to increase with age, and vitamin D in the 6–10 age group was significantly higher than the 11–14 age group. A negative correlation between BMD, 25OHD, P1NP, and β-CTx was found.

Conclusion: The high prevalence of hypovitaminosis D among Vietnamese children was confirmed. We found a high prevalence of BMD and its negative correlation with 25OHD, BTMs. Strategies to improve vitamin D and BMD status need to be considered urgently.

Keywords: Vitamin D, BMD, Bone turnover markers, P1NP, β-CTx.

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Introduction

Vitamin D is a fundamental hormone that affects physiological metabolism in many human organs. Recent studies have shown the association between vitamin D and brain health, which suggests a causality relationship between vitamin D deficiency and Alzheimer's disease and/or depression [1]. Vitamin D is reported to maintain the neuroprotection [2] in early life and the cognitive function in adults [3]. Vitamin D also interacts with growth hormones. An improvement in the level of insulin-like growth factor 1 (IGF-1) is seen after a vitamin D therapy for children with growth hormone deficit [4]. In addition, vitamin D expresses its receptors on immune cells and thus has the capability to modulate immune responses [5].

Stimulation of calcium absorption from the intestine is the principal function of vitamin D [6]. This process plays a vital role in promoting the development of the pediatric skeletal system since vitamin D deficiency is found associated with severe rickets. Recent studies have shown a high prevalence of hypovitaminosis D in childhood. Research on a sample of 2062 children in the 10–18 age group by Kim et al. in 2012 has shown that 68.1% of the participants have 25OHD concentration below 50 nmol/L [7]. The other study, performed among obese adolescents in New Delhi, reported that the prevalence of vitamin D deficiency could go up to 100% of the subjects [8]. Moreover, the significant association between vitamin D and bone mineral density (BMD) clearly shows the negative effects of hypovitaminosis D on the bone mass [9].

An increased risk of 89% of fracture per decreasing standard deviation (SD) of BMD is proven in children with 9.9 years of age [10], indicating an alarming consequence of vitamin D deficiency on the onset of puberty. In addition to BMD, bone turnover markers (BTMs) are reliable indicators to assess bone

health by their ability to predict the risk of hip and vertebral fracture [11]. Among various BTMs reflecting the activity of osteoclasts and osteoblasts, N-terminal procollagen of type l collagen (P1NP) and β-isomerized C-terminal telopeptides (β-CTx) are specific markers to reflect the bone formation and resorption, respectively [12].

In Vietnam, the high prevalence of hypovitaminosis D among healthy children was previously reported. The study conducted by Laillou et al. [13] showed a prevalence of more than 50% of children having serum 25OHD concentration below 50 nmol/L. However, the data might not generalize to vitamin D status throughout the year since this study was carried out during the sunniest months. In addition, data on BMD status and the correlation between BMD and 25OHD concentration, BTMs in children have been very limited, especially in Vietnam. Therefore, we aimed to evaluate BMD and vitamin D status and their relationship with BTMs, including P1NP and β-CTx, in children aged 6–14 in Can Tho, Vietnam.

Materials and Methods

Study population

This research was a cross-sectional study carried out in Can Tho, Vietnam from November 2012 to April 2016. The city of Can Tho is located at 10°02′N, 105°47′E in the southern region of Vietnam. It has a tropical climate with only 2 distinct seasons, the rainy and the dry seasons. The rainy season takes place between May and October, with an annual average rainfall of 1,674 mL (65.9 inches). The dry season usually lasts from November to April. The annual average temperature is 24°C–28°C (75.2°F–82.4°F), with the mean sunshine hours of 2,561 hours monthly [14]. The amount of sunshine is significantly associated with the synthesis of vitamin D and serum 25OHD concentration [15].

In the research, 794 children aged between 6 and 14 were randomly recruited from 3 elementary schools and 2 secondary schools by the probability proportional to size sampling and stratified random sampling. Children were excluded from the study if any following criteria were present: (a) chronic diseases that can lower BMD e.g., malabsorption syndrome, Cushing's syndrome, hyperthyroidism, primary hyperparathyroidism and multiple myeloma, (b) acute diseases at the time of recruitment, (c) participants in different interventions or (d) refusals to participate (Figure 1).

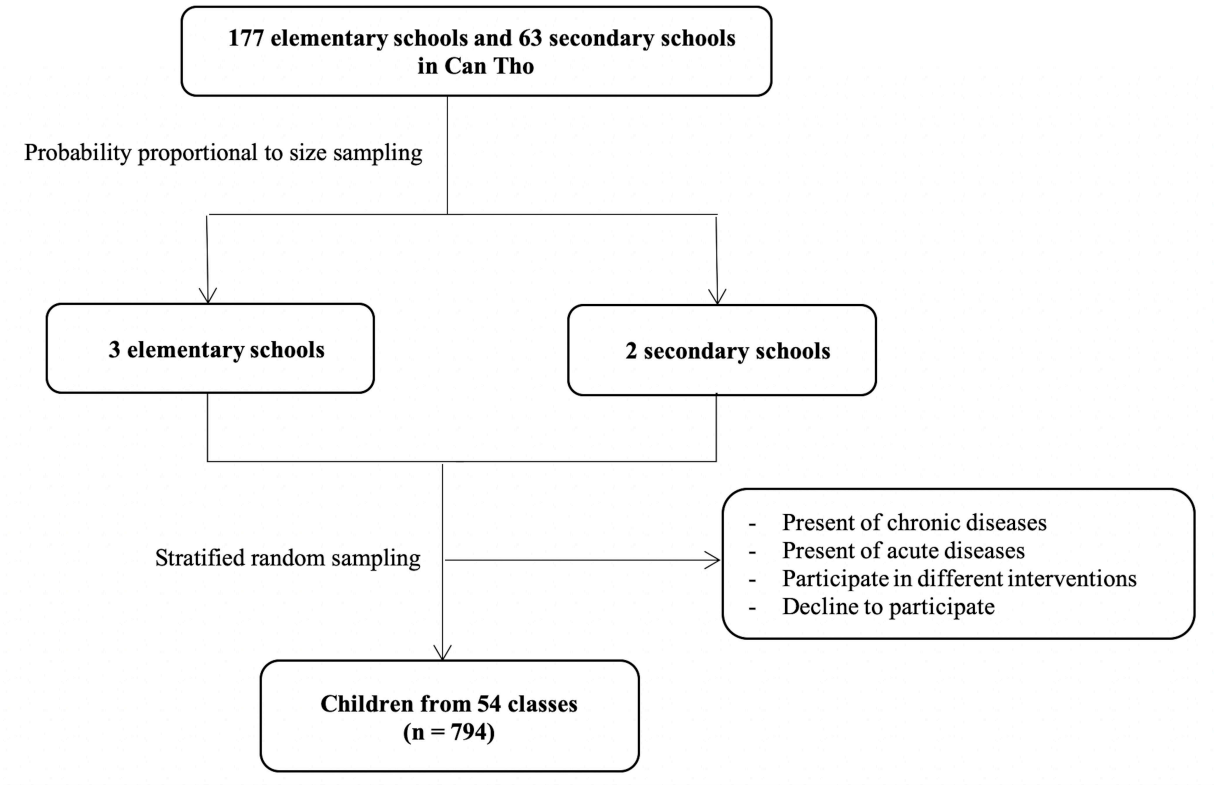

Figure 1. *Flowchart of the study population.*

The Institutional Review Board of Can Tho University of Medicine and Pharmacy, Can Tho, Vietnam approved our study protocol. Prior to enrollment, all children and their parents were informed about the procedures and goals of the research. Written informed consent was fully obtained from all parents of the participants.

Anthropometry

Selected children were weighed without shoes/sandals and wearing light clothes by using an electronic UNICEF scale with a precision of 0.1 kg (SECA 890, Hamburg, Germany). Height was measured using a wooden height board (UNICEF) to the nearest of 0.1 cm. Body weight and height data were

adapted for calculating body mass index (BMI). Anthropometric status was evaluated by the following classifications, according to 2007 World Health Organization (WHO) growth reference: height-for-age < -2 SD for stunting, BMI-for-age > +2 SD for overweight and BMI-for-age > +3 SD for [16].

Blood analysis and BMD examination

In the research, 3 mL of venous blood was taken from each subject by medical technicians. The blood samples were extracted for 500 µl serum samples, which were then kept frozen at – 20°C at Can Tho University Hospital, Can Tho before being transferred (within 2 days) to the Medic Medical Center, Ho Chi Minh City for analysis. High-Performance Liquid Chromatography-Mass Spectrometry (HPLC/MS) (Thermo Scientific Ultimate 3000 HPLC, Thermo Fisher Scientific Inc., Massachusetts, USA) was used to measure the serum concentration of 25OHD which is considered the best marker for vitamin D status. The obtained concentrations were categorized as one of three vitamin D status, including normal, insufficiency and deficiency for 25OHD level of ≥ 50 nmol/L, 37.5-50 nmol/L and ≤ 37.5 nmol/L, respectively [17–19]. 25OHD is considered the best marker for human vitamin D status since it is not tightly regulated by PTH compared to circulating 1,25-dihydroxyvitamin D [1,25(OH)2D] [20]. The serum concentration of PTH and BTMs, including P1NP and β-CTx, were measured on a Roche Elecsys 2010 (Roche Diagnostics, Indiana, USA). In addition, BMD of all subjects was examined at the distal third forearm, using the GE Lunar Prodigy DXA (Massachusetts, USA). The BMD values were defined as one of two BMD status, encompassing normal for Z-score > -1 SD and low for Z-score < -1 SD [21].

Table 1. *Participant characteristics.*

Characteristic	Boy	Girl	Test
Gender, N (%)	393 (49.5)	401 (50.5)	-
Age (years), mean ± SD	10.1 ± 2.5	10.2 ± 2.7	P=0.725
Location, N (%)			P=0.953
Urban	156 (19.6)	160 (20.2)	
Rural	241 (30.4)	237 (29.8)	
Height (cm), mean ± SD	134.4 ± 15.9	132.2 ± 15.3	P=0.003
Weight (kg), mean ± SD	32.4 ± 13.5	29.8 ± 11.8	P=0.049
BMI (kg/m^2)	17.27 ± 4.2	16.37 ± 3.4	P=0.001
Nutritional status, N (%)			P<0.001
Stunting	81 (10.2)	126 (15.9)	
Normal healthy	249 (31.4)	250 (31.5)	
Overweight/obesity	63 (7.9)	25 (3.1)	
Vitamin D status, N (%)			P <0.05
Normal	291 (74.1)	260 (64.8)	
Insufficiency	50 (12.7)	72 (18)	
Deficiency	52 (13.2)	69 (17.2)	
BMD, N (%)			P=0.055
Normal	352 (89.6)	341 (85)	
Low	41 (10.4)	60 (15)	
25OHD, nmol/L	72.23 ± 30.58	63.64 ± 27.23	P<0.001
BMD, g/cm^2	0.3118 ± 0.0511	0.3003 ± 0.0614	P=0.004
P1NP (ng/mL)	534.16 ± 263.41	463.85 ± 241.69	P<0.001
β-CTx (ng/mL)	1029.41 ± 413.58	909.81 ± 424.25	P<0.001
PTH (pg/mL)	31.46 ± 17.8	34.08 ± 11.6	P=0.322

25OHD, 25-hydroxyvitamin D; BMD, bone mineral density; BMI, Body Mass Index; P1NP, procollagen type I N-terminal propeptide; β-CTx, β-isomerized C-terminal telopeptides.

Statistical analysis

The analysis was carried out by using WHO Anthro-Plus and SPSS 20.0 (SPSS Inc., Chicago, Illinois, USA). Categorical variables were analyzed using the Chi-square test and were presented as the percentage and frequency. Continuous variables were described as means ± SD. Independent sample T-test was used to compare the mean between two groups and one-way analysis of variance (ANOVA) was used to analyze data of 3 factors. Differences with a P value < 0.05 were significantly considered.

Ethical approval

This study is approved by the Institutional Review Board of Can Tho University of Medicine and Pharmacy (number: 44/QD-DHYDCT.NCKH).

Results

A total of 794 children were taken into the analysis, characteristics of the subjects stratified as genders were summarized in Table 1. There were no significant differences in age and location between boys and girls in our study. However, the mean BMI, weight, and height among boys were significantly higher than that of girls. There was also a difference in nutritional status between 2 genders. More boys had overweight and obesity compared to girls, while stunting was more observed among girls rather than boys (P<0.001). In addition, the mean 25OHD concentration and BMD of both genders were 67.39 ± 29.16 nmol/L and 0.306 ± 0.0568 g/cm^2. The prevalence of abnormal vitamin D status was 30.6% of the subjects, in which 35.2% of girls had abnormal value compared to only 25.9% of boys (P<0.05). The prevalence of low BMD was 12.7% and no significant differences in BMD status were observed in different genders (P=0.055).

In Pearson's correlation coefficient and multivariate linear regression analyses, BMD was inversely correlated with 25OHD concentration (r =−0.2165, P<0.001). This model could explain 4.69% of the total variance of BMD. There was also an inverse correlation between the level of P1NP and β-CTx with BMD, with r=−0.1209 (P < 0.0016) and r=−0.1725 (P < 0.001), respectively. All three variables, including 25OHD, P1NP, and β-CTx, could explain 7.35% of the total variance of BMD.

When analyzing the data of BMD and vitamin D by nutritional status, mean BMD was highest among children with overweight/obesity (P<0.001), while no significant difference was noted in the average concentration of 25OHD between three nutritional status (P=0.031). However, we found significant differences in BMD and vitamin D status among children with different nutritional status. Low BMD was observed in stunting and normal children, with a prevalence of 15.46% and 13.83%, respectively. In overweight/obese children, low BMD status was not found (P=0.001), but the prevalence of abnormal 25OHD level (42.04%) was highest compared to the other nutritional groups (P<0.001) (Table 2).

Table 3 presents the BMD and 25OHD levels by age groups. Data were significantly different between age groups (P<0.001). BMD gradually increases with age, from a mean of 0.269 g/cm^2 in children aged 6 to 0.366 g/cm^2 among 14-year-old children, except for a slight decrease in the 9-year-old group. 25OHD level was significantly higher in the 6–10 age group compared to the 11–14 age group (P <0.001).

Table 2. *BMD and vitamin D status divided according to nutritional status.*

Characteristic	Stunting	Normal	Overweight/ Obesity	Test
BMD, (g/cm^2)	0.3012 ± 0.0601	0.3005 ± 0.0513	0.3483 ± 0.0613	P<0.001
BMD, N (%)				P=0.001
Normal	175 (84.54)	430 (86.17)	88 (100)	
Low	32 (15.46)	69 (13.83)	0 (0)	
25OHD, (nmol/L)	69.79 ± 32.74	67.82 ± 26.94	59.32 ± 31.3	P=0.031
Vitamin D, N (%)				P<0.001
Normal	144 (69.57)	356 (71.34)	51 (57.95)	
Insufficiency	34 (16.43)	80 (16.03)	7 (7.95)	
Deficiency	29 (14.01)	63 (12.63)	30 (34.09)	
25OHD, 25-hydroxyvitamin D; BMD, bone mineral density				

Table 3. *BMD and 25OHD concentration stratified by age groups.*

Age (years)	N	BMD (g/cm^2)	25OHD (nmol/L)
6	89	0.269 ± 0.049	86.297 ± 32.736
7	87	0.286 ± 0.033	79.782 ± 20.795
8	80	0.292 ± 0.04	78.736 ± 19.421
9	75	0.287 ± 0.033	80.788 ± 21.415
10	81	0.301 ± 0.039	87.428 ± 31.042
11	102	0.303 ± 0.045	50.846 ± 24.016
12	96	0.307 ± 0.044	57.351 ± 24.007
13	97	0.336 ± 0.076	45.4 ± 20.187
14	87	0.366 ± 0.065	50.041 ± 20.988

Discussion

In this research, we analyze the BMD and vitamin D status as well as the correlation between PTH, BTMs, and BMD. Regarding vitamin D, 30.6% of the participants had insufficiency and deficiency status. A higher prevalence of abnormal vitamin D was noted among girls and among overweight/obese children compared to boys and those with the other nutritional status, respectively. Moreover, the mean 25OHD concentration of the subjects was 67.39 nmol/L, in which children in the 6–10 age group had significantly higher 25OHD concentration than the 11 – 14 age group. This difference may indicate a decrease in the 25OHD level with age. Regarding BMD, the mean BMD of the subjects was 0.3118 g/cm^2 and 0.3003 g/cm^2 among boys and girls, respectively. The BMD was found to increase with age, except for a slight decrease at the age of 9. Low BMD was significantly higher in children with stunting compared to those with normal or overweight/obesity. We also discovered an inverse correlation between 25OHD level, P1NP, or β-CTx with BMD, with r=−0.2165, r=−0.1209, and r=−0.1725, respectively.

In terms of vitamin D, the mean concentration of 25OHD in children from Can Tho, Vietnam was consistent with researches from other geographic regions. Soininen et al. [22] showed a mean 25OHD level of 68.5 nmol/l among 374 Finnish children aged 6–8, compatible with a mean of 67.39 nmol/L in this study. In addition, Kim et al. [7] measured the 25OHD concentration in the other age group, 10-18-year-old, reported an average 25OHD of 44.2 nmol/L, compatible to that of 10–14 age group, but significantly lower compared to the 6–10 age group in our study. Although the decrease of vitamin D status with age was proven in elderly people [23], this hypothesis occurred in children who needs further investigation.

Despite using the same cut-offs for vitamin D insufficiency and deficiency as our study, Khor et al. [24] carried out a study in Malaysia, found that the prevalence of abnormal vitamin D was 72.4%, significantly higher than the prevalence of 30.6% as found in our study. Moreover, abnormal vitamin D status is prevalent among schoolgirls rather than schoolboys and among children with normal nutritional status rather than children with stunting and overweight/obesity. The difference in the percentage of hypovitaminosis D between our study and study of Khor et al. may be due to several reasons. Firstly, the measurement methods used in our study were HPLC/MS while Malaysian study used chemiluminescent immunoassay (CLIA). Secondly, clothing style is also an important risk factor for a high prevalence of hypovitaminosis D as the majority population in Malaysia wears veils due to cultural reasons [25,26]. Thirdly, Malaysian schoolchildren have darker pigmentation compared to Vietnamese schoolchildren, which requires much more sun exposure time to synthesize an equivalent amount of vitamin D [27]. Fourth, the differences in the ingredients of daily consumed food may also lead to the differences in the mean 25OHD concentration between Vietnamese and Malaysian children.

Many previous studies have reported the increase of BMD with age. Glastre et al. [28] reported an increase of BMD from 0.446 ± 0.048 g/cm^2 in children aged 1 to 0.891 ± 0.123 g/cm^2 in children age 15. Arabia et al. [29] also showed an increase from 0.49 g/cm^2 in 10–11 age group to 0.68 g/cm^2 among boys and 0.63 g/cm^2 among girls in the 17–18 age group. On the other hand, very few studies assess BMD of healthy children by nutritional status and the correlation between BMD and 25OHD, BTMs have been carried out so far. A national survey on 4002 Egyptian adolescents found a significant low BMD among stunted participants compared to subjects with normal height [29], which is in agreement with our findings. There is a negative correlation between forearm BMD and 25OHD level which is inconsistent with previous studies. Marwaha et al. [9] measured forearm BMD of 664 healthy schoolgirls aged 7–17 in Delhi but no significant correlation with vitamin D was observed. However, the other study in Western India reported a significant correlation (r=0.786) between BMD and vitamin D [30]. In terms of P1NP and β-CTx, the study including data from 520 post-menopause women showed that BTMs were negatively correlated with formal neck, hip, and lumbar spine BMD [12].

Our study has both strengths and limitations that should be taken into consideration. On the one hand, to the best of our knowledge, this is the first research reports the BMD status and the correlation between BMD and 25OHD concentration, BTMs in Vietnamese children. Moreover, the HPLC/MS used to measure 25OHD concentration in our study is considered a gold standard due to its reliable specificity and sensitivity compared to chemiluminescent immunoassay (CLIA) and radioimmunoassay (RIA) [31]. On the other hand, the subjects in this study are solely recruited from Can Tho, Vietnam, and thus cannot generalize to all Vietnamese children.

This study offers reliable data that could be adopted in national strategies of pediatric bone health improvement. Although the obtained percentage of hypovitaminosis D children in this study was significantly lower than the prevalence reported by Laillou et al. among Vietnamese children, strategies to improve the vitamin D and BMD status need to be established urgently to deal with this health issue. In addition, the interaction between BMD and nutritional status as well as the correlation between BMD and 25OHD, BTMs needs to be investigated in a more nationally representative sample. The difference in 25OHD concentration between the 6–10 and 11–14 age groups in this study suggests a possibility of age interaction to 25OHD level in children. Further studies need to be carried out to confirm this finding.

Conclusion

In conclusion, our study reports the first data on pediatric BMD status and the correlation between BMD and 25OHD, BTMs as well as confirms the high prevalence of hypovitaminosis D among healthy children in Vietnam. BMD was found to increase with age. Abnormal vitamin D is prevalent in girls or overweight/obese children, but low BMD is common among stunting children. A higher 25OHD concentration in the 6–10 age group than that of 11–14 age group as well as a negative correlation between BMD and 25OHD, BTMs need to be confirmed in further research. These findings provide evidence for strategies for bone health improvement in Vietnamese children.

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