Archives of Osteoporosis (2019) 14:13
https://doi.org/10.1007/s11657-019-0568-3

ORIGINAL ARTICLE

Bone mineral density and its correlation with vitamin D status in healthy school-going children of Western India

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Received: 2 October 2018 / Accepted: 22 January 2019

Abstract

Summary Studies on vitamin D deficiency status and its impact on bone health in a developing country are limited. We assessed the bone mineral density and vitamin D levels in healthy school-going children of Western India. Around 65% children had vitamin D deficiency and vitamin D levels had good correlation with bone mineral density.

Purpose Vitamin D is an important substrate in the metabolism of calcium homeostasis and skeletal metabolism. Epidemiological studies on vitamin D deficiency status and its impact on bone mineral metabolism in healthy children in a developing country are limited. We assessed the bone mineral density and vitamin D levels in healthy school-going children of Western India.

Methods We measured serum levels of 25-hydroxy vitamin D (25(OH)D) in healthy school-going children, aged 60 to 120 months. Dual-energy X-ray absorptiometry scan was done to assess the bone mineral density (BMD). The BMD status was compared with the levels of 25(OH)D and serum parathormone hormone in all the children.

Results A total of 100 school-going children were examined for evidence of vitamin D deficiency and 65% had deficiency (less than 50 nmol/L). The mean age was 90 months in males and 89 months in females. In the vitamin D-deficient group, the mean BMD (gm/cm^2) measurements for the lumbar spine was 0.439 ± 0.098 ($p < 0.001$) and the mean BMD (gm/cm^2) in the normal group was 0.606 ± 0.071 ($p < 0.001$). Pearson's and Spearman's rank correlation coefficients between vitamin D levels and BMD z score showed a significant positive correlation ($r = 0.82$, $\rho = 0.924$).

Conclusions We confirmed the high prevalence of vitamin D deficiency among healthy school-going children. The serum levels of vitamin 25(OH)D has a good correlation with bone mineral density.

Keywords Vitamin D deficiency · Bone mineral density · Bone mineral disease · 25-Hydroxy vitamin D · Nutrition · Developing country

Introduction

Vitamin D is synthesized in the skin in the presence of sunlight and it is an important substrate in the skeletal metabolism and calcium homeostasis [1, 2]. Calcium, vitamin D, and parathyroid hormone (PTH) are biochemically interlinked. Vitamin D deficiency causes poor intestinal calcium absorption and mobilization of calcium from bones to maintain circulatory levels, leading to bone mineral deficit and osteomalacia in adults and rickets in children [3–5]. The nutritional status of vitamin D is reflected by serum 25-hydroxy vitamin D (25(OH)D) levels [6]. The serum concentration of vitamin D (25(OH)D) is influenced by age, sex, diet, race, ethnicity, pubertal status, body mass index, climate, season, and place of living [7]. A study by Marwaha et al. [8] observed that clinical and biochemical hypovitaminosis D was highly prevalent in apparently healthy school children in northern India. It has also been reported from the rest of the world about the high prevalence of vitamin D deficiency which has a major implication in the bone health of children and later in their adulthood, which ultimately has an impact on the growth of the child [9–11]. Studies from adult population on the etiology of osteomalacia and osteoporosis have somewhat linked the

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Published online: 02 February 2019

evidence to poor vitamin D status in childhood [12]. There has been recent evidence that hypovitaminosis D is a common feature in childhood. Bellone et al. [13] evaluated vitamin D status in children living in Northern Italy and found out that almost half of the study population who were obese had vitamin D deficiency. Another study by Puri et al. [14] assessed the vitamin D status in healthy school-going girls in Delhi in relation to their nutrition and lifestyle and they found that prevalence of hypovitaminosis D was present in 90.8% of girls. Their study highlights the importance of lifestyle factors like time spent in outdoors and sunlight exposure to the vitamin D status in healthy school-going girls.

There is a strong correlation between vitamin D deficiency and risk for bone mineral disease and it has been shown that deficiency of vitamin D has an adverse effect on bone mass [15]. Low serum levels of calcium and vitamin D increases PTH secretion which causes high bone turnover, elevated PTH results in mineral changes in bone separate from osteoclast/osteoblast activity [5]. Many factors play a role in bone health of children including child health, physical activity, diet, calcium intake, and vitamin D status. Ardawi et al. [9] studied vitamin D levels and its correlation with lumbar spine bone mineral density (BMD) and they found a significant correlation ($p = 0.023$). However, an Indian study [16] did not show any correlation between vitamin D and hip or lumbar spine BMD ($p = 0.473$ and 0.353).

Data on the status of vitamin D levels and its correlation with bone mineral density in pediatric population is scarce. Epidemiological studies on vitamin D deficiency status and its impact on bone mineral metabolism in healthy children in a developing country are limited. The objective of our study was to assess the vitamin D status and its correlation with bone mineral density in healthy school-going children in a western state of India.

Material and methods

Study design and participants

This was a cross-sectional study, conducted in 100 apparently healthy school-going children of both sexes, aged between 60 and 120 months, studying in Government Primary School from north western state of India, in the month of May to July 2011. The children were selected randomly and most of them belonged to low socioeconomic community. The parents or guardians of each of the participant were informed regarding the nature of the study and informed written consent was taken for all the enrolled participants. The study protocol was approved by the Institutional Ethics Committee of Sawai Man Singh (SMS) Medical College, Jaipur. The inclusion criteria were apparently healthy children with body mass index (BMI) between 5th to < 85th percentile according to the Centers for Disease Control and Prevention (CDC) guidelines [17]. We excluded children with chronic systemic illness (endocrine, hepatic, and renal disorders), history of drug intake which would affect the bone metabolism like steroids, anticonvulsants, or any other known skeletal disease. All children were clinically examined and signs of vitamin D deficiency (frontal bossing, widening of wrist, knock knees, bow legs) was noted.

Anthropometric assessment

Weight was recorded by electronic weighing scale, wearing light cloths. A morning standing height without shoes was measured with the help of stadiometer and BMI was calculated by a standard formula (weight in kilos divided by height in square meters). The children were defined as underweight (< 5th percentile), normal (5th to < 85th percentile), overweight (85th to < 95th percentile), and obese (≥ 95th percentiles) using CDC percentiles [17].

Biochemical assessment

Blood samples were collected and serum levels of total calcium (mg/dL), phosphorus (mg/dL), and alkaline phosphatase (IU/L) were measured by calorimetric assay. The serum levels of immunoreactive PTH (pg/mL) and vitamin D (25 OH) (nmol/L) were measured by immunoradiometric assay. Vitamin D deficiency state was defined as levels less than 50 nmol/L [9]. Deficiency was further classified as mild (≥ 25 to < 50 nmol/L), moderate (≥ 12.5 to < 25 nmol/L), and severe (< 12.5 nmol/L).

Bone mineral density measurements

Bone mineral density (gm/cm^2) of lumbar spine (L1–L4) (anteroposterior view) was measured by dual-energy X-linked absorptiometry (DEXA) (Hologic DQR-1000/w scan). The BMD z score was calculated using the standard formula [18].

Sample size and statistical analysis

Previous studies have shown the prevalence of vitamin D deficiency ranging from 30 to 90% [8, 10, 11, 13, 14, 19–21]. We assumed expected prevalence around 50%. Keeping confidence interval at 95% and precision at 7%, we enrolled 100 children. Results were presented as mean ± SD, median, and range and categorical variables are expressed as frequencies. The correlation between continuous variables was ascertained by Pearson's and Spearman's rank correlation coefficients. Depending upon the distribution of continuous variables, Student's t test was used for parametric variables. For skewed data, Levene's test for equality of variance was used. Differences with p value of 0.05 or lower was considered significant.

Table 1 Biochemical and bone mineral density characteristics of subjects

Characteristics (mean ± SD)	Total (n = 100)	Male (n = 55)	Female (n = 45)	p value
Mean age (months) (range)	90 (60–120)	90 (64–120)	89 (60–120)	0.74
BMI Median (IQR)	16.1 ± 0.9 16.1 (15.4–16.9)	15.9 ± 0.8 15.9 (15.2–16.5)	16.3 ± 1.1 16.2 (15.5–17.3)	0.02
S. calcium (mg/dL) Median (IQR)	9.1 ± 0.9 9.1 (8.6–9.5)	9.1 ± 1 9.2 (8.5–9.5)	9.1 ± 0.8 9.1 (8.8–9.4)	0.78
S. phosphorus (mg/dL) Median (IQR)	4.5 ± 0.8 4.6 (4.1–5.1)	4.5 ± 0.9 4.6 (3.8–5.1)	4.5 ± 0.8 4.6 (4.2–5)	0.95
Alkaline phosphatase (U/L) Median (IQR)	191 ± 113 165 (116–244)	204 ± 133 155 (116–272)	177 ± 82 167 (114–218)	0.22
S. PTH[a] (pg/mL) Median (IQR)	57.4 ± 77.7 28.2 (16–68.4)	71.2 ± 96.6 46.2 (18–76.4)	40.7 ± 40.4 24.1(13.8–52.7)	0.03
25-Hydroxyvitamin D (nmol/L) Median (IQR)	44.13 ± 24.78 42.45 (22.85–57.73)	40.69 ± 21.45 37.66 (21.4–56.9)	48.33 ± 27.99 44.8 (24.1–63.5)	0.12
LS BMD (g/cm^2) Median (IQR)	0.498 ± 0.12 0.505 (0.387–0.60)	0.486 ± 0.123 0.498(0.361–0.593)	0.511 ± 0.115 0.514(0.396–0.61)	0.31
LS BMD (z score)[a] Median (IQR)	0.89 ± 1.6 1.7 (− 0.8–2.17)	0.72 ± 1.79 1.7(− 1.05–2.1)	1.08 ± 1.52 1.8 (− 0.05–2.2)	0.27

Normal ranges: calcium, 8.8–10.2 mg/dL; phosphorus, 2.7–4.9 mg/dL; alkaline phosphatase, 42–128 U/L; parathyroid hormone (PTH), 10–65 pg/mL; 25-hydroxyvitamin D, 51–200 nmol/L

[a] Skewed data, Levene's test for equality of variance was used and the p value from the unpaired t test was accordingly ascertained

Results

This school-based cross-sectional study was conducted at SMS Medical College, Jaipur, from May to July 2011. During the study period, 126 children were assessed for eligibility. Twenty-six children were excluded (under nutrition, 16; overweight, 5; on phenytoin, 2; obese, 1; and nephrotic syndrome in remission, 1). A total of 100 children (55 boys and 45 girls) were included in the study. The mean age was 90 months in males and 89 months in females. The mean BMI of study group was 16.1 ± 0.9 (male 15.9 ± 0.8, female 16.3 ± 1.1; $p < 0.02$) (Table 1).

Biochemical profile

The mean values of serum calcium, phosphorous, and alkaline phosphatase were 9.1 ± 0.9, 4.5 ± 0.8, and 191 ± 113 respectively and were comparable in males and females; however, higher value of alkaline phosphatase was seen in males (p-values 0.78, 0.95 and 0.22 respectively) (Table 1). The serum PTH had skewed data and the adjusted mean (median) were 71.2 (46.2) and 40.7 (24.1) in males and females respectively ($p = 0.03$).

Sixty-five percent of children had vitamin D deficiency. There was no significant gender difference in the prevalence of vitamin D deficiency. Boys had a prevalence of 65% and girls had 64%. Out of 65 deficient children, 34 had mild, 29 had moderate, and 2 had severe deficiency (Table 2). None of the children had any clinical evidence of vitamin D deficiency. The overall mean value of 25(OH)D in both the groups was 44.13 ± 24.78. There was no statistical significant difference between males and females ($p = 0.12$). Females had comparatively higher levels of 25(OH)D compared to males (48.33 ± 27.99 vs 40.69 ± 21.45).

Bone mineral density parameters

The mean lumbar spine BMD (gm/cm^2) of study participants was 0.498 ± 0.12 and there was no statistically significant difference in males and females ($p = 0.31$). The median BMD z

Table 2 Vitamin D levels in study subjects

	Total (n = 100) N (%)	Male (n = 55) N (%)	Female (n = 45) N (%)
Normal	35 (35)	19 (35)	16 (35)
Deficient	65 (65)	36 (65)	29 (64)
Mild	34 (52)	17 (47)	17 (59)
Moderate	29 (45)	18 (50)	11 (38)
Severe	2 (3)	1 (3)	1 (3)

Table 3 Bone mineral density and PTH levels according to vitamin D status

Vitamin D levels (*n*)	BMD (gm/cm^2)		BMD (*z* score)		Serum PTH	
	(mean ± SD)	Median(IQR)	(mean ± SD)	Median (IQR)	(mean ± SD)	Median (IQR)
Normal (35)	0.606 ± 0.071	0.620 (0.563–0.654)	2.36 ± 0.47	2.3 (2.1–2.6)	17.79 ± 11.83	14.6 (11.5–18.6)
Deficient (65)	0.439 ± 0.098	0.429 (0.358–0.507)	0.09 ± 1.54	0.2 (− 1.25–1.75)	78.84 ± 89.21	57.1 (25.65–91.9)
p value	< 0.001		< 0.001		< 0.001	
Mild deficiency (34)	0.500 ± 0.0870	0.505 (0.451–0.546)	1.19 ± 1.01	1.15 (0.57–1.92)	39.13 ± 27.71	26.85 (18.3–57.1)
Moderate deficiency (29)	0.374 ± 0.059	0.361 (0.334–0.404)	− 1.0 ± 0.99	− 1.2 (− 1.75 to − 0.5)	109.35 ± 88.26	69.6 (60.5–144.9)
Severe deficiency (2)	0.340 ± 0.053	0.340 (0.302–0.340)	− 2.65 ± 0.63	− 2.65 (− 3.1 to − 2.65)	311.5 ± 280	311.5 (113–311)

score was 1.7 (Table 1). The BMD status was compared with the levels of 25(OH)D and serum PTH. In the vitamin D-deficient group, the mean BMD (gm/cm^2) measurements for the lumbar spine was 0.439 ± 0.098 and the mean BMD (gm/cm^2) in the normal group was 0.606 ± 0.071 ($p < 0.001$). The mean serum PTH level in the normal group was 17.79 ± 11.83; however, the levels were elevated in the deficient group (78.84 ± 89.21, median 57.1) ($p < 0.001$) (Table 3). Due to the skewed data, Levene's test for equality of variance was used and the *p* value from the unpaired *t* test was accordingly ascertained for the serum PTH levels in the deficient group. Vitamin D levels and lumbar spine BMD (gm/cm^2 and *z* score) showed a positive correlation (Pearson's correlation coefficient (*r*) 0.786 and 0.820 respectively) (Spearman's rank correlation coefficient (ρ) 0.849 and 0.924 respectively) (Table 4 and Fig. 1). Vitamin D levels had a negative correlation with serum PTH levels ($r = -0.524$; $\rho = -0.846$) (Table 4, Fig. 2).

Discussion

In our study, around 65% children had vitamin D deficiency (52% had mild deficiency, 45% had moderate deficiency, and 3% had severe deficiency). Various studies had shown high prevalence of vitamin D deficiency, ranging from 30 to 90% in healthy children worldwide [8, 10, 11, 13, 14, 19–21]. Puri et al. [14] studied the prevalence of vitamin D deficiency in school-going girls from Delhi, aged from 6 to 18 years and found that 90.8% participants were deficient and 11.5% had clinical evidence of hypovitaminosis D. Another study showed that 35.7% children had vitamin D deficiency and 10.8% had clinical symptoms [8]. They emphasized the risk of bone mineral metabolic disturbances in this group of subjects when exposed to factors that cause imbalances to the bone mineral homeostasis. None of our children had clinical evidence of vitamin D deficiency. In our study, females had a higher 25(OH)D levels compared to males which was not the case in a study from Delhi [8] where males had a higher 25(OH)D levels than females. The probable reason for gender-based difference in vitamin D levels might be due to difference in outdoor activity and dietary habits. Most of the children in our study belong to low socioeconomic and rural background. The females from this background used to help their parents in household activity and agriculture-related work and probably had higher sunlight exposure. The major contributors for high prevalence of vitamin D deficiency are lack of outdoor activities, exposure to sunlight, and dietary deficiency of vitamin D. In the summer months, due to extreme weather conditions, children prefer staying indoors which culminates in inadequate exposure to sunlight. The other important factor to be considered is the poor dietary intake as the children belonged to low socioeconomic class and they lack access to healthy balanced diet. However, in our study, we did not study the average duration of exposure to sunlight and daily dietary calcium intake. Intake of vitamin D in the daily diet is important to maintain vitamin D homeostasis when exposure to sunlight and outdoor activities are minimal. It has been studied that there is a significantly higher serum levels of 25(OH)D levels with the fortification of dairy products [22]. The typical Indian food is found to be not a good source for vitamin D and mostly consists of cereal-based diet which is rich in phytates and hampers the absorption of vitamin D and calcium in the intestine [23]. Thus, fortification of

Table 4 Pearson and Spearman's rank correlation coefficients between vitamin D levels and bone mineral density and PTH levels

	BMD (*z* score)	BMD (gm/cm^2)	Serum PTH
Vitamin D (Pearson's *r*)	0.820	0.786	− 0.524
Vitamin D (Spearman's ρ)	0.924	0.849	− 0.846

Correlation is significant at the 0.01 level (two-tailed)

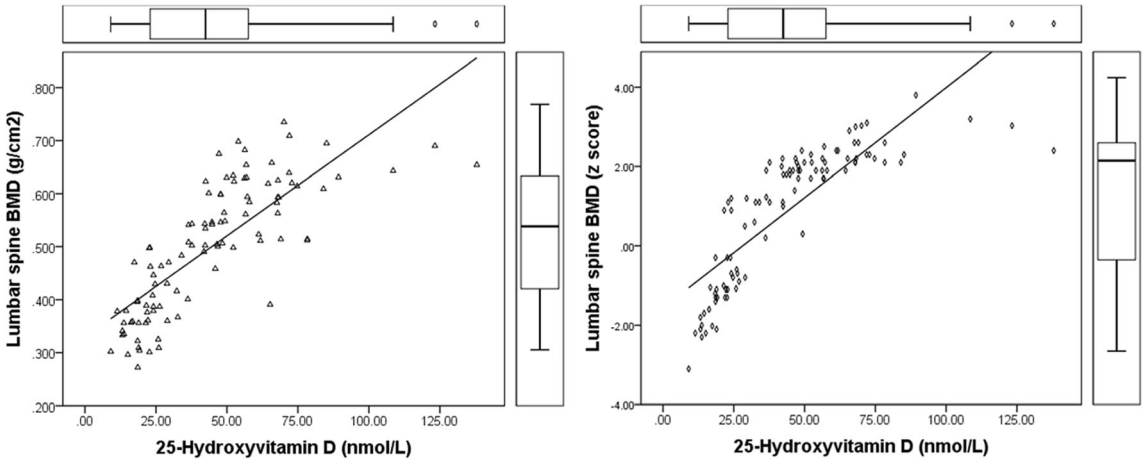

Fig. 1 Correlation of vitamin D levels with lumbar spine BMD (g/cm^2) ($r = 0.786$; $\rho = 0.849$) and z score ($r = 0.820$; $\rho = 0.924$)

food with vitamin D is essential to ensure adequate levels of vitamin D in the absence of exposure to sunlight and lack of outdoor activities.

We found a negative correlation between vitamin D and PTH ($r = -0.524$ and $\rho = -0.846$) and our results showed strong agreement with other studies [8, 12].

In our present study, we found a significant correlation between 25(OH)D levels and lumbar spine BMD ($r = 0.786$; $\rho = 0.849$ for gm/cm^2 and $r = 0.820$; $\rho = 0.924$ for BMD z score), which suggest that low BMD in these group of children is associated with deficiency of vitamin 25(OH)D. Marwaha et al. [23] studied BMD of the forearm and calcaneum in healthy Indian children but they did not correlate it with vitamin D status. Ardawi et al. [9] studied vitamin D levels and its correlation with lumbar spine BMD and the found a significant correlation ($p = 0.023$). However, an Indian study [16] did not show any correlation between vitamin D and hip or lumbar spine BMD (p = 0.473 and $p =$ 0.353). There is a strong correlation between vitamin D deficiency and risk for bone mineral disease and it has been shown that deficiency of vitamin D has an adverse effect on bone mass [15].The serum levels of vitamin 25(OH)D is a good marker for vitamin D status in children and its deficiency is associated with increased risk for bone mineral disease such as rickets. However, one must be careful about the overzealous use of vitamin D supplementations and its adverse effects [24, 25].

The association of dietary calcium intake and bone mineral density in children has long been debated on whether increasing the calcium intake affects the bone mineral density or not. A systematic review and meta-analysis on calcium intake and bone mineral density concluded that increasing calcium intake either by supplements or dietary sources produced a small non-progressive increase in bone mineral density. They also concluded that risk of fractures is also less likely to be significantly reduced [26]. However, a recently published multicentric study showed that adequate calcium intake for long duration in childhood improves bone mineral density and reduces the risk of osteopenia [27].

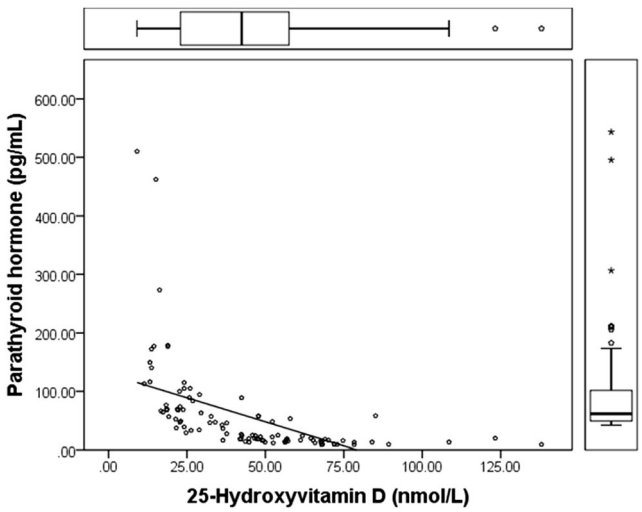

Fig. 2 Correlation of vitamin D levels with parathyroid hormone levels ($r = -0.524$; $\rho = -0.846$)

Conclusions

Our study demonstrates a significantly higher prevalence of vitamin D deficiency and its positive correlation with lumbar spine BMD in healthy school-going children of rural area of Western India. Our results suggest the importance of public awareness to vitamin D deficiency and its implication on bone health in children.

Limitations

We did not inquire about daily sunlight exposure and dietary calcium intake. Small sample size and wide age range are another limitations.

Compliance with ethical standards

Conflicts of interest None.

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