# Prevalence of Vitamin D deficiency and associated risk factors among children residing at high altitude in Shimla district, Himachal Pradesh, India

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## ABSTRACT

**Introduction:** Vitamin D is important for bone development in children. A high prevalence of Vitamin D deficiency (VDD) has been documented from different parts of India. However, limited data are available on VDD among children residing at high altitude region of country. **Objectives:** To assess the prevalence of VDD and associated risk factors among children in the age group of 6–18 years in Shimla, Himachal Pradesh. **Methods:** A community-based cross-sectional study was conducted in the year 2014–2015. A total of 626 children in the age group of 6–18 years were enrolled from 30 clusters which were identified using population proportionate to size sampling method. A minimum of 20 children in the age group of 6–18 years per cluster were selected using random number tables. The data on socioeconomic status, physical activity, sunlight exposure, and biochemical parameters of bone and mineral metabolism were assessed. **Results:** Ninety-three percent of school-age children were found Vitamin D deficient as per serum 25(OH) D levels of <20 ng/ml. The prevalence was significantly higher among females. **Conclusion:** A high prevalence of VDD was found in children residing in high altitude region.

Key words: Himachal Pradesh, School age children, Vitamin D deficiency

### INTRODUCTION

Vitamin D is important for bone development in children.<sup>[1]</sup> Beyond bone integrity and calcium absorption, Vitamin D is involved in various physiological and pathological processes.<sup>[2]</sup> Vitamin D has many noncalcemic functions, which includes immune, cardiovascular, endocrine, neuropsychological functions, neuromuscular performance, cellular differentiation, and anticancer actions.<sup>[3-7]</sup>

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Studies conducted in India have documented high prevalence (85–98%) of Vitamin D deficiency (VDD) among school age children.<sup>[8-13]</sup> Majority of these studies have been conducted in plain regions of country. The common risk factors identified as associated with VDD are low exposure to sun,<sup>[14]</sup> atmospheric pollution,<sup>[15]</sup> darker skin pigmentation,<sup>[16]</sup> low physical activity,<sup>[17]</sup> indoor confinement of children during the day and high rise buildings.<sup>[14]</sup> These risk factors are infrequent at high altitudes regions as compared to plains areas. There was a lack of evidence on the status of VDD among children from high altitude region. Thus, the present study was conducted with an objective to assess the prevalence of

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VDD and associated risk factors among children in the age group of 6–18 years in Shimla, Himachal Pradesh.

# **Methods**

#### **Subjects**

A community-based cross-sectional study was conducted in the year 2014-2015 in district Shimla, Himachal Pradesh. The district is situated at an altitude of more than 2000 meters. A total of 626 children in the age group of 6-18 years were enrolled from 30 clusters. The clusters were identified using population proportionate to size sampling method. The inclusion and exclusion criterion adopted is shown in Figure 1. All children in the age group of 6–18 years in the cluster (school) were enlisted. A minimum of 20 children in the age group of 6-18 years per cluster were selected with the help of random number tables. The ratio of children included in the study in the age group of 6-11 and 12-18 years was 6:14. As more than 90% of children in the age group of 6-18 years attended the school in the district, thus, the children studying in the school were taken as a proxy of the children in the community. Hence, school



Figure 1: Operational flow diagram for the study. \*Data were not collected from children in the age group of 6-11 years

based approach was adopted for the study. The subjects in the age group of 6–11 years were not included for the assessment of socio-economic status (SES), physical activity, sunlight exposure, and dietary pattern as these children were unable to provide valid information on these parameters.

The study was approved by ethical committee of All India Institute of Medical Sciences, New Delhi. The written consent was obtained from the parents of each child before data collection.

#### Assessment of socio-economic status

A pretested semi-structured questionnaire was administered to elicit information on identification data and socio-demographic profile. Assessment of SES was done using Kuppuswamy's SES scale.<sup>[18]</sup>

#### Anthropometric profile

Standing height was recorded with barefoot, using a standard stadiometer (SECA 213) to the nearest 1 mm. Subjects were weighed using a clinical balance (SECA 813) to the nearest 0·1 kg, wearing minimal clothing and without shoes. Body mass index (BMI) was calculated as weight (kg)/height (m<sup>2</sup>). The International Obesity Task Force BMI cut-offs were used to determine obesity among children.<sup>[19]</sup>

#### **Clinical examination**

All the subjects were examined to detect clinical signs and symptoms of VDD. The subjects were observed while walking and movement of limbs were noticed for the diagnosis of bowleg (genu varum) and knock-knee (genu valgum). The forehead was examined for frontal bossing. The subjects were also observed while getting up from a sitting position and vice-versa for the diagnosis of proximal muscle weakness.

#### **Physical activity level**

Physical activity assessment was done by administering a detailed questionnaire. The data on the type of physical activity performed along with duration (in minutes) in the last 24 h of the survey was collected during school and home. The activities were then categorized as light, moderate, or heavy activity based on metabolic equivalent of task values. The physical activity level (PAL) value for each subject in the age group of 12–18 years was calculated using standard methodology.<sup>[20]</sup>

#### Assessment of exposure to sunshine

A questionnaire was utilized to elicit information regarding time spent in the sunshine in last 24 h during routine daily activities from the subjects in the age group of 12–18 years. Area of body exposed to sunlight was calculated with the help of Wallace' Rule of Nine.<sup>[21]</sup> The type of clothing worn by the subjects during various activities was also assessed. The total duration in minutes and body area (percentage) under direct sunshine was recorded.

#### **Dietary profile**

The pattern of dietary consumption of foods was collected by administering the food frequency questionnaire. This method is utilized to elicit retrospective qualitative information on the frequency of consumption of food items in various major food groups such as cereals, pulses, green leafy vegetables, roots and tubers, fruits, milk and milk products, eggs, flesh foods, and sugar. The pattern of food items consumed during the preceding 1 year in various groups was assessed in the subjects. The children in the age group of 12–18 years were included for assessment of dietary profile of the subjects.

#### **Biochemical profile**

Blood samples were collected from venipuncture from median cubital veins of subjects. After collection, blood samples were centrifuged within 2 h, and serum samples were stored at  $-20^{\circ}$ C until transported to the central laboratory, All India Institute of Medical Sciences, New Delhi. The sera were collected in screw capped serum storage vials and was stored at  $-70^{\circ}$  until analysis.

All the biochemical parameters were assessed using standard laboratory procedures (autoanalyzers). The biochemical estimation of serum 25-hydroxy Vitamin D ((25OH) D) and intact parathyroid hormone (PTH) was done using chemiluminescence immunoassay using kits (DiaSorin Co., Italy).

The serum calcium (o-cresolphthalein method), phosphorus (molybdate method), albumin (BCG method) and alkaline phosphatase (PNPP method) were measured by colorimetry using kits (Roche Cobas Co. Germany).

The US Endocrine Society classification was utilized for the determination of VDD among children. Serum ((25OH) D) level of 30 ng/ml and above, 20–29 ng/ml, and <20 ng/ml were classified as sufficient, insufficient, and deficient, respectively.<sup>[22]</sup>

#### Sample size and statistical analysis

The sample size of the study was calculated keeping in view the anticipated prevalence of Vitamin D as 25%, a confidence level of 75%, absolute precision of 5.0 and a design effect of 2.0. A total sample size of 600 was calculated. However, we included 626 children in the present study.

IBM Statistical Package for Social Sciences (SPSS) version 20.0, Armonk, NY: IBM corp was used for statistical analysis of data. Quantitative data (serum (25OH) D)) values expressed in mean  $\pm$  SD; quantitative data was expressed in frequency and percentage. Chi-square test/ Fischer exact test was used to establish the association between different parameters with Vitamin D levels. The P < 0.05 was considered as statistical significant.

### RESULTS

A total of 626 children were included, 198 children (95 males; 103 females) from the age group of 6–11 years and 428 (203 males; 225 females) from 12 to 18 years. The mean age of the subjects enrolled was  $13.1 \pm 3.4$  years (male) and  $13.2 \pm 3.2$  years (female), respectively.

#### Socio-economic status

The 165 (38.5%), 138 (32.3%), and 125 (29.2%) subjects belonged to upper, middle, and lower SES, respectively. Subjects belonging to upper SES had higher deficiency of Vitamin D, followed by children from middle and lower SES [Table 1].

#### Anthropometry

The mean BMI in children of 6–11 and 12–18 years was  $16.5 \pm 2.9 \text{ kg/m}^2$  and  $18.3 \pm 2.3 \text{ kg/m}^2$ , respectively. No significant association was found between VDD and BMI of subjects studied.

#### **Clinical examination**

All children were assessed for the clinical symptoms of VDD. It was found that children had back pain (n = 16), joint pain (n = 9), muscle pain (n = 10), and knock-knee (n = 1). The symptoms such as muscle pain, knock knees, back pain, and joint pain were observed higher in children who had lower serum Vitamin D level (<20 ng/ml) as compared to subjects with sufficient Vitamin D levels.

#### **Physical activity level**

Children with sedentary PAL had higher prevalence of VDD (88.8%) as compared to children with low physical activity [Table 1].

#### **Sunshine exposure**

It was found that children who were exposed to sunlight for <150 min had higher prevalence of VDD [Table 1].

#### **Dietary profile**

Sixty-five percent of children were vegetarian, 28.9% were nonvegetarian, and 6.3% were eggetarian. VDD was more common in vegetarian subjects as compared to nonvegetarian. The frequency of consumption of Vitamin

Table 1: Relationship between serum Vitamin D levels with different parameters in children 6-18 years (n=626)						
Parameters	Deficient (<20)		Insufficient/s	Insufficient/sufficient (≥20)		
	n (%)	Mean±SD	n (%)	Mean±SD		
Age group (years)						
6-11	178 (30.6)	10.5±4.3	20 (45.5)	24.4±3.5	0.45	
12-18	404 (69.4)	10.7±4.3	24 (54.5)	25.5±5.2		
Gender						
Male	266 (45.7)	12.1±4.1	32 (72.7)	24.8±4.0	< 0.001	
Female	316 (54.3)	9.4±4.0	12 (27.3)	25.5±5.7		
Socio-economic status						
Upper	157 (38.9)	10.9±4.3	8 (33.3)	24.5±5.8	0.85	
Middle	130 (32.2)	10.7±4.2	8 (33.3)	26.2±5.1		
Lower	117 (28.9)	10.9±4.3	8 (33.3)	25.7±5.2		
Sunlight exposure time (min)						
0-60	110 (27.2)	9.3±4.2	9 (37.5)	24.9±3.9	0.25	
61-150	221 (54.7)	10.9±4.2	11 (45.8)	26.8±6.3		
>150	73 (18.1)	12.2±4.1	4 (16.7)	24.0±4.5		
Sunscreen use						
Yes	24 (5.9)	11.0±5.5	1 (8.3)	31.7	0.84	
No	381 (94.1)	11.3±4.8	22 (91.7)	33.8±4.2		
Physical activity level						
Sedentary life style (<1.4)	357 (88.8)	10.7±4.3	23 (95.8)	25.6±5.2	0.87	
Low physically active life style (1.4-1.54)	35 (8.7)	11.0±4.4	1 (4.2)	21.6		
Physical activity (≥1.55)	10 (2.4)	12.5±3.9	0	-		
Serum intact PTH (pg/ml)						
PTH (<15)	16 (2.8)	9.9±4.9	1 (2.3)	24.0	0.27	
PTH (15-65)	491 (84.5)	11.1±4.2	41 (93.2)	25.1±4.6		
PTH (>65)	74 (12.7)	8.0±3.5	2 (4.5)	23.2±0.9		
Serum total calcium (mg/dl)						
Calcium (8·10-10·4)	387 (66.5)	10.5±4.3	32 (72.7)	25.4±4.8	0.51	
Calcium (>10.4)	195 (33.5)	10.9±4.3	12 (27.3)	23.9±3.5		
Serum inorganic phosphorus (mg/dl)						
Phosphorus (<2.5)	8 (1.4)	12.5±4.4	1 (2.3)	20.5	0.37	
Phosphorus (2·5-4.8)	492 (84.5	10.7±4.4	40 (90.9)	24.6±4.0		
Phosphorus (>4.8)	82 (14.1)	10.4±3.7	3 (6.8)	31.4±7.0		
Serum albumin (g/dl)						
Albumin (<4.0)	23 (4.0)	12.5±4.5	4 (9.1)	24.8±1.6	0.12	
Albumin (4.0-5.5)	559 (96.0)	10.6±4.3	40 (90.9)	25.0±4.7		
Serum alkaline phosphatase (IU/I)						
Alkaline phosphatase (<180)	211 (36.3)	10.4±4.3	14 (31.8)	24.7±5.2	0.63	
Alkaline phosphatase (180-1200)	371 (63.7)	10.8±4.3	30 (68.2)	25.1±4.2		

Figure in parentheses denotes percentages. PTH: Parathyroid hormone, SD: Standard deviation

D rich foods was found lower in the Vitamin D deficient children compared to Vitamin D sufficient children.

#### **Biochemical profile**

#### Vitamin D deficiency

It was found that the distribution of children with sufficient (30 ng/ml and above), insufficient (20–29 ng/ml), and deficient (<20 ng/ml) Vitamin D levels was 7 (1.1%), 37 (5.9%) and 582 (93%), respectively.

The overall prevalence of VDD in the age group of 6–11 years and 12–18 years was 89.9 and 94.4%, respectively. Females had higher prevalence of VDD compared to males [Table 2].

#### Serum intact parathyroid hormone

PTH levels were found within normal range among subjects having sufficient serum Vitamin D levels (≥30 ng/ml). An

ascending trend in PTH values was seen with increase in the severity of VDD. Twelve percent (n = 74) children suffering from VDD had PTH levels of more than 65 pg/ml indicating possible involvement of skeletal system [Table 1].

The mean PTH was higher in females compared to males. The low serum ((25OH) D) levels and high PTH levels in females could be due to low exposure of body to sunlight [Table 3].

# Serum calcium, phosphorous, serum albumin, and alkaline phosphatase levels

Sixty-six percent of children with VDD had deficient serum calcium levels. No significant difference was observed between Vitamin D status and serum calcium, phosphorous, serum albumin, and alkaline phosphatase levels.

to age and gender of the children in 6-18 years ( $n=626$ )							
Age group (years)	Male ( <i>n</i> =298) <i>n</i> (%) (≥20 ng/ml)		Female ( <i>n</i> =328) <i>n</i> (%) (≥20 ng/ml)		Total deficient		
	Deficient	Insufficient/ sufficient	Deficient	Insufficient/ sufficient	n (%)		
6-11	81 (85.3)	14 (14.7)	97 (94.2)	6 (5.8)	178 (89.9)		
12-18	185 (91.1)	18 (8.9)	219 (97.3)	6 (2.7)	404 (94.4)		
Total	266 (89.3)	32 (10 7)	316 (96.3)	12 (3.7)	582 (93.0)		

Figure in parentheses denotes percentages

 Table 3: Comparison of different biochemical

 parameters according to gender and age group (n=626)

Categories	Male	Female	Р
Serum 25(OH) D (ng/ml) (years)			
6-11	12.8±6.5	11.1±5.3	0.07
12-18	13.8±5.2	9.5±4.9	< 0.0001
Serum intact PTH (pg/ml) (years)			
6-11	36.7±19.8	42.9±18.6	0.01
12-18	42.9±21.8	43.0±21.9	0.57
Serum total calcium (mg/dl) (years)			
6-11	10.1±0.5	10.1±0.5	0.82
12-18	10.3±0.5	10.2±0.5	0.82
Serum inorganic phosphorus			
(mg/dl) (years)			
6-11	4.4±0.5	4.4±0.5	0.92
12-18	4.0±0.7	3.8±0.6	0.92
Serum albumin (g/dl) (years)			
6-11	4.3±0.3	4.4±0.3	0.56
12-18	4.5±0.3	4.5±0.3	0.56
Serum alkaline phosphates			
(IU/I) (years)			
6-11	270.9±73.7	288.4±78.2	0.12
12-18	249.6±118.6	178.8±114.0	< 0.0001

PTH: Parathyroid hormone, 25(OH) D: 25-hydroxyvitamin D

# DISCUSSION

VDD is now recognized as a pandemic. The major cause of VDD is the lack of sun exposure in moderation. Sunlight is the major source of Vitamin D for most humans. Very few foods naturally contain Vitamin D, and foods that are fortified with Vitamin D are often inadequate to satisfy child's Vitamin D requirement.<sup>[23]</sup>

VDD causes poor mineralization of the collagen matrix in young children's bones leading to growth retardation and bone deformities known as rickets.<sup>[24]</sup>

In this study, we found 93% of children living at high altitude regions had VDD (<20 ng/ml). We found that subjects had a low exposure to sunlight in spite of abundant sunlight in the district. An earlier study conducted among adults of 18–40 years residing in high altitude region of Kashmir valley also reported that 83% of the subjects had VDD.<sup>[25]</sup>

In India, studies from different parts of the country have reported a high prevalence of VDD 96.9% (adolescents),<sup>[9]</sup>

90.8% (6–18 years),<sup>[10]</sup> 93.7% (school girls),<sup>[11]</sup> 92.3% (10– 14 years)<sup>[12]</sup> and 87% (college students),<sup>[13]</sup> respectively. The cut-offs to define VDD, however, varied between the studies. Studies utilizing higher cut-offs (75 nmol/L or 30 ng/ml) to diagnose VDD have reported a near universal presence of VDD among different age groups, including pregnant and lactating women, newborns, adolescents, and health professionals.<sup>[26]</sup>

In this study, the mean serum ((25OH) D) concentration in males and females was  $12.1 \pm 4.1$ ,  $9.4 \pm 4.0$ , respectively. The male children had higher mean serum ((25OH) D) levels. This was possibly due to higher sun exposure among them due to more outdoor lifestyle and activities as they have more freedom.

It was also found that subjects belonging to upper SES had higher prevalence of VDD (38.9%), followed by subjects from middle (32.2%) and low (28.9%) SES. Similar results have been reported in an earlier study which found 91.9% of the subjects from upper SES and 89.6% from lower SES had VDD.<sup>[10]</sup> The children in low SES are comparatively poorly dressed possibly leading to more body area exposed to sun as compared to high SES.

Daytime outdoor physical activity may act as a surrogate indicator for sun exposure, however, exercise in itself may contribute to the maintenance of Vitamin D status, other than merely by increasing exposure of skin to sunlight.<sup>[27]</sup> The physical activity increases local bone mass, reduces calcium excretion and raises absorption efficiency,<sup>[28]</sup> thus increasing serum calcium which results in sparing serum Vitamin D.

In this study, it was found that children with a sedentary lifestyle had higher prevalence of VDD compared to children with higher physical activity lifestyle. A study conducted in Saudi reported that VDD was common among children and was influenced by physical activity pattern.<sup>[29]</sup>

The findings of the present study revealed that a high prevalence of VDD in hilly area at an altitude of more than 2000 meters was similar to plain areas. Although the hilly areas at high altitude have adequate sunshine with low air pollution compared to plain area. Promotion of an active outdoor lifestyle and activities among children in both homes and schools may counteract the VDD.

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#### **Conflicts of interest**

There are no conflicts of interest.

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