# Vitamin D Status of School Children in and around Guwahati

#### Dipti Sarma, Uma K. Saikia, Abhamoni Baro

Department of Endocrinology, Gauhati Medical College and Hospital, Guwahati, Assam, India

### Abstract

**Context:** Peripubertal and adolescent children are vulnerable to vitamin D deficiency as this is the period of rapid skeletal growth. **Aims:** This study was done to assess the vitamin D status in school children between the age of 8–14 years attending the government schools in rural and urban areas of Assam in Northeast India. **Settings and Design:** This was a cross-sectional observational study. **Materials and Methods:** About 500 students (350 from rural and 150 from urban areas) were recruited in the study. Serum 25-hydroxy vitamin-D [25(OH)D], parathyroid hormone (PTH), calcium, phosphorus and alkaline phosphatase were measured in fasting state. Daily nutrition intake and sunlight exposure were assessed. **Statistical Analysis:** Student's *t*-test and Pearson correlation test were done to assess the association between different variables. *P* value <0.05 was considered significant. **Results:** The prevalence of vitamin D deficiency was 8.4% and vitamin D insufficiency was 14.2%. There was no significant difference of mean 25(OH)D levels and sun exposure between rural and urban children. Out of 42 children with vitamin D deficiency, 36 (85.7%) had sun exposure <20% and 41 (97.6%) had calcium intake < 1000 mg/day. The rural children had a higher calcium intake as compared to urban children (*P* = 0.005). There was a significant positive correlation of mean 25(OH)D levels with serum calcium, sun exposure and calcium intake. **Conclusion:** The prevalence of vitamin D deficiency in peripubertal and adolescent age group children in and around Guwahati city of Assam is comparatively lower than that in other parts of the country.

Keywords: Guwahati, school children, vitamin D

## INTRODUCTION

Vitamin D deficiency has become a major public health problem in India.<sup>[11]</sup> Body complexion, inadequate sun exposure, vegetarian diet and unavailability of vitamin D fortified foods are important attributing factors.<sup>[2]</sup> Till date, there are numerous studies on prevalence of vitamin D status from different parts of India in different age groups in rural and urban areas.<sup>[1,2]</sup> However, there is a remarkable paucity of such studies in adolescent and preadolescent age group. Peripubertal age group has increased demand for vitamin D due to growth spurt and bone mass accrual during this period and hence they are prone to vitamin D deficiency.<sup>[3]</sup> With this background, the present study was carried out in healthy school children between the age of 8–14 years in the government schools in and around Guwahati, Assam to assess the extent of vitamin D deficiency in the period of rapid skeletal growth.

## **SUBJECTS AND METHODS**

The primary objective was to assess the vitamin D status in healthy children aged 8–14 years attending government schools in both rural and urban areas in Assam. This was a

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cross-sectional observational study done from 2011 to 2013 and was conducted in the city of Guwahati situated in the North-Eastern region of India. Guwahati has a geographical location of 26°11′0″ North and 91°44′0″ East. The average duration of cloud-free sunshine in this region is around 8–10 h per day throughout the year and the UV index at the given latitude is 7–12. Winter is short with a lowest temperature of 12°C and highest of 22°C. There is a little seasonal variation of the peak intensity of sunlight. An inclusion criteria was healthy children in the age group of 8–14 years attending government schools in both rural and urban areas in and around Guwahati city of Assam. We distributed invitation letters in all the government schools and students carried them home to show their parents. The parents of students who consented for the study returned the invitation letters with their signature. All

> Address for correspondence: Dr. Dipti Sarma, Department of Endocrinology, Gauhati Medical College and Hospital, Kamrup Metro, Guwahati, Assam - 781 035, India. E-mail: dipti\_sarma2003@yahoo.com

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these students with their parents were asked to come to school on a Sunday where the parents were interviewed regarding diet, sun exposure, any underlying illness that might interfere with vitamin D synthesis or any intake of drugs or vitamin supplements. Exclusion criteria were children with comorbid conditions that affect vitamin D synthesis and metabolism like diseases of skin, liver, kidney and gastrointestinal system. Children on vitamin D supplementation and on drugs including multivitamins, anticonvulsants, steroids, thyroxine, anti-tuberculosis treatment and antimetabolites were also excluded from the study.

The study was approved by the Institutional Ethical Committee. Another approval was sought and granted by the Indian Council for Medical Research (ICMR) and the study was funded by the ICMR. A team from the Department of Endocrinology of Gauhati Medical College and Hospital, Assam, went to each school after a prior intimation. A written consent was taken from all the parents of students who consented to participate in the study. Detailed history and body measurements were taken and almost all students were accompanied by their mothers for clarity in answering questions. Data were collected on their food habits, physical activity and sun exposure. Height and weight were measured, and body mass index (BMI) was calculated. The parents were asked to provide a rough estimate of the average daily time their child spent under the sun in the past month. Physical activity was evaluated by asking the parent to provide a rough estimate of their child's level of physical activity by engaging in active playing, running, jumping, climbing, playing football or basketball or other activities. Sunlight exposure was assessed by documenting average duration of exposure and percentage of the surface area of the body exposed daily. The dressing manner of the subjects was unrestricted and was not therefore considered. The percentage of body surface area exposed was assessed by the Wallace rule of nine. Sunshine exposure was calculated as

Sunshine exposure (%) = hours of exposure/day  $\times$  percentage of body surface area exposed.<sup>[4]</sup>

They were examined for clinical features of vitamin D deficiency like widening of wrist, double malleolus, genu varum, genu valgus, proximal muscle weakness and leg pain.

Puberty was assessed using the Tanner's classification.<sup>[5]</sup> Fasting blood samples were taken for serum 25-hydroxy vitamin D [25(OH)D], serum calcium (Ca), albumin, phosphorus, alkaline phosphatase and intact parathyroid hormone (PTH). The samples were separated in a refrigerated centrifuge at 1200 rotation/minute for 15 min at 4°C and were divided into five aliquots and stored at -20°C until analysed. Serum 25(OH) D was measured using the radioimmunoassay (RIA) according to the manufacturer's protocol. The sensitivity of this assay is 2.5 ng/ml. The intra-assay coefficient of variation (CV) is 9.1% at 22.7 ng/ml. Vitamin D deficiency was defined as 25(OH)D levels of <20 ng/ml and insufficiency was defined as 25(OH)D levels from 21 to 29 ng/ml as per the Endocrine Society Clinical Practice Guidelines.<sup>[6]</sup> Serum PTH were measured by immunoradiometric assay (IRMA) according to the manufacturer's protocol (N-TACT PTH IRMA kit USA) in Automatic Gamma Counter (Gamma 10, version 2.0). Normal range of PTH is 13-54 pg/ml. Serum calcium was measured by the photometric test and normal values of calcium were taken as 8.1-10.4 (mg/dl). Serum phosphorus was measured by using a photometric UV test and the normal values of phosphorus were taken as 4-7 mg/dl. Serum alkaline phosphatase was measured using the Orthophosphoric Monoester Phosphohydrolase Method. Serum albumin was measured by the photometric colorimetric test method and corrected calcium was calculated using the formula: Corrected Ca = (4 - albumin) 0.8 + estimated calcium. Dietary nutritionintake was assessed by estimating the average composition of the daily diet in terms of calcium from a 24 h recall of the food intake utilising a published data on nutritive value of Indian food.<sup>[7]</sup>

#### **Statistical analysis**

Statistical software SAS 9.3 was used to analyse the data. Quantitative data are presented as mean  $\pm$  standard deviation. Student's t-test was performed on different variables to compare between two groups. Association of 25(OH)D with different variables was analysed with the Chi-square test and the pattern of correlation was determined by the Pearson correlation. A *P* < 0.05 was considered as statistically significant.

### RESULTS

Five hundred children in the age group of 8–14 years were included in the study, out of which 350 children were from rural area and 150 children were from urban area. The age distribution of the study subjects is shown in Table 1.

Out of 500, 246 (49.2%) were males and 254 (50.8%) were females. The heights, weight and body mass index (BMI) of the subjects were within the normal range for age and sex. Children from urban area had greater height, weight and BMI than the rural counterparts, whereas rural children had significantly higher sun exposure [Table 2]. There was no significant difference of serum calcium, phosphorus and alkaline phosphatase levels in between the rural and urban group. The mean 25(OH)D levels in both the rural and urban children were within the normal range of  $\geq$  30 ng/ml and there was no significant difference of 25(OH)D levels between the rural and urban areas. The mean levels of serum PTH of the children were within the normal range of 13–54 pg/ml. A significantly higher sun exposure levels were seen in children of rural area of 12-13.99 years age group in both males and females. Rural females of the age group 8-9.99 years also had significantly higher sun exposure than the urban females of same age group [Table 3].

The prevalence of vitamin D deficiency in our study was 8.4% and that for vitamin D insufficiency was 14.2%. Of the vitamin D deficient group (n = 42), 23 (54.76%) had BMI <18.5 kg/m<sup>2</sup>, 36 (85.7%) had sun exposure <20%,

Table 1: Distribution	of	school	children	according to	1	age
and area						

Age group	Rural ( <i>n</i> =350)		Urban ( <i>n</i>	Total	
(years)	Female	Male	Female	Male	
8-9.99	34	30	7	4	75
10-11.99	52	50	9	11	122
12-13.99	56	57	3	8	124
14-14.99	34	37	59	49	179
Total	176	174	78	72	500

Each box represents the number of children in each group

 Table 2: Comparison of different parameters among rural and urban students

Variables	Rural ( <i>n</i> =350) (mean±SD)	Urban ( <i>n</i> =150) (mean±SD)	Р
Height (cm)	140±0.70	148.4±1.03	< 0.001*
Weight (kg)	33.5±0.48	39.9±0.82	< 0.001*
BMI (kg/m <sup>2</sup> )	16.87±0.14	17.93±0.24	0.001*
Calcium (mg/dl)	8.78±0.04	8.77±0.07	0.94
Phosphorus (mg/dl)	3.83±0.09	$3.96 \pm 0.05$	0.41
Alkaline phosphatase (IU/L)	386.4±7.58	362.2±4.85	0.11
25(OH)D (ng/ml)	38.81±0.63	36.60±1.21	0.07
PTH (pg/ml)	26.13±0.67	21.49±0.73	< 0.001*
Sun exposure (%)	38.78±9.41	35.78±7.19	0.28
Calcium intake (mg/day)	725.5±7.58	674.1±13.77	0.005*

\*Data are presented as mean±SD for each variable. \**P*<0.05 was considered significant. BMI: Body mass index, 25(OH)D: 25-hydroxy vitamin D, PTH: Parathyroid hormone, SD: Standard deviation

41 (97.6%) had calcium intake <1000 mg/day and 27 (64.2%) children were vegetarian. Of the 42 children with deficiency, 24 (57.14%) had a serum calcium level of <8.1 mg/dl, 31 (73.8%) had phosphorus levels <4 mg/dl, 29 (69%) had alkaline phosphatase of >400 IU/L and 5 (11.90%) children had PTH levels of >54 pg/ml [Table 4]. Pearson correlation analysis showed significant positive correlation of 25(OH)D levels with height, serum calcium, time and percentage of sun exposure and calcium intake [Table 5]. There was a significant negative correlation of 25(OH)D levels with BMI, alkaline phosphatase and PTH [Table 5].

## DISCUSSION

Our study is the first to address the problem of vitamin D deficiency in the North-East region of the country in healthy adolescent and pre-adolescent children. The adolescent group deserves special consideration, as this is the period of accrual of peak bone mass and because of increased demands of accelerated skeletal growth, vitamin D deficiency is likely to occur. There is a dearth of large-scale community-based studies to assess vitamin D status in adolescent age group. The first of the two large studies were done by Marwaha *et al.* in 2005 on 5137 healthy school children of urban New Delhi.<sup>[8]</sup> The study included children from lower and upper socioeconomic class where children from lower socioeconomic group had

significantly lower 25(OH)D levels. The second study by Puri et al. in 2008 on 3217 healthy school girls of Delhi demonstrated clinical vitamin D deficiency in 11.5% and biochemical hypovitaminosis D (serum 25-hydroxy vitamin D <50 nmol/l) in 90.8% of girls.<sup>[9]</sup> Our study did not consider the socioeconomic status and included 500 children from government schools of rural and urban region. The prevalence of vitamin D deficiency and vitamin D insufficiency in our study was found to be overall 8.4% and 14.2%, respectively. There was no difference of mean 25(OH)D levels between boys and girls. The prevalence of vitamin D deficiency was lower in our study as compared to the other parts of India as documented in various studies.[8-10] Mandlik et al. in their study in Maharashtra on school children between age of 6-12 years reported a prevalence of vitamin D insufficiency in 71% despite a sun exposure of 2 h.[10] This low prevalence of vitamin D deficiency in our subjects in Northeast India may be attributed to several factors. The first reason is the geographical location which ensures adequate sunshine throughout the year. The average duration of cloud-free sunshine in this region is around 8-10 h per day which translates into ample sunshine reaching the population throughout the year.<sup>[11]</sup> The most common Indian skin type has been found to be of type V followed by type IV and the estimated minimal erythemal dose (MED) for UVB for Indian skin is  $61.5 \pm 17.25$  J/cm.<sup>[12]</sup> However, this data are from southern India. The northeast Indian population are now considered to originate from central and eastern Asia (e.g. Korean, Chinese, Japanese or Philippino roots) according to the new six genetico-racial categories, which is predominantly Fitzpatrick skin type III.<sup>[13,14]</sup> Fitzpatrick skin type III have been shown to have lower MED compared to type IV-VI.<sup>[12]</sup> Since winter is short here, there is a little seasonal variation of the peak intensity of sunlight in northeast India. Moreover, our study was conducted in a different region and latitude of 26°11'0" North and 91°44'0" East and therefore the prevalence of vitamin D deficiency found in our study reflects the status unique to this region. Secondly, the diet of the people in this region which is predominantly non-vegetarian diet may have contributed to the low prevalence of vitamin D deficiency in this region. There is daily intake of eggs, meat (pork, chicken and lamb) and fish which are natural sources of vitamin D. Vitamin D content of meat is approximately 23 µg/kg for various meat products.<sup>[15]</sup>

The vitamin D deficient group had lower serum calcium, phosphorus and higher alkaline phosphatase. They also had lower intake of calcium and sun exposure. We found no significant difference of mean 25(OH)D levels and mean total sun exposure between children in the rural and urban area. There was no history of applying sunscreen in all the subjects. The rural children had a significantly higher calcium intake. This is because the rural children have better access to fresh vegetables as well as dairy products than the urban children. However, the mean calcium intake in both rural and urban children was lower than 1000 mg/day. The recommended daily allowance (RDA) of calcium for children aged 8–14 years

Variables	Age	Male ( <i>n</i> =179)			Female ( <i>n</i> =254)			
(years)		Rural (mean±SD)	Urban (mean±SD)	Р	Rural (mean±SD)	Urban (mean±SD)	Р	
Corrected Ca	8-9.99	8.56±0.14	9.12±0.31	0.39	9.02±0.18	9.08±0.38	0.98	
(mg/dl)	10-11.99	8.95±0.10	8.49±0.38	0.41	8.56±0.10	8.98±0.45	0.39	
	12-13.99	8.67±0.09	8.70±0.43	0.28	$8.80{\pm}0.08$	9.63±0.76	0.84	
	14-14.99	8.80±0.11	8.44±0.11	0.84	8.91±0.12	8.97±0.10	0.46	
Phosphorus	8-9.99	3.81±0.07	3.17±0.11	0.34	3.90±0.07	3.74±0.12	0.76	
(mg/dl)	10-11.99	3.72±0.07	3.05±0.18	0.5	3.92±0.07	3.28±0.40	0.86	
	12-13.99	3.67±0.08	3.76±0.24	0.62	3.68±0.07	3.96±0.33	0.35	
	14-14.99	3.54±0.09	3.81±0.09	0.13	4.69±0.95	4.01±0.09	0.59	
Alkaline	8-9.99	362.7±35.8	357±77	0.98	328.08±28.4	411.14±55.5	0.25	
phosphatase	10-11.99	393.3±20.1	593±50.6	0.49	427.6±22.2	571.4±42.2	0.66	
(IU/L)	12-13.99	384.9±16.2	512.6±118.9	0.33	387.6±16	368.6±78.7	0.6	
	14-14.99	389.2±19.5	312.7±21.3	0.46	391.1±17.8	302.1±16.3	0.42	
Vitamin D	8-9.99	36.92±2.21	38.26±3.20	0.22	37.28±2.05	36.32±3.74	0.67	
(ng/ml)	10-11.99	36.55±1.93	36.32±3.31	0.38	35.38±1.75	34.51±2.59	0.98	
	12-13.99	35.24±1.25	36.37±6.38	0.91	42.97±1.55	41.08±19.9	0.86	
	14-14.99	36.20±1.26	35.74±2.25	0.59	43.00±1.37	42.42±1.91	0.78	
PTH (pg/ml)	8-9.99	28.76±2.17	26.58±2.31	0.001	24.38±1.52	22.28±3.58	0.006*	
	10-11.99	26.70±1.69	22.42±3.18	0.008	25.23±2.10	19.37±2.26	< 0.001*	
	12-13.99	26.28±1.84	20.23±3.04	0.007	26.58±1.69	34.12±17.1	< 0.001*	
	14-14.99	25.81±1.79	20.64±2.27	< 0.001	25.72±2.27	21.21±1.11	< 0.001*	
Sun	8-9.99	26±1.2	28±1	0.09	27±1	24±2	< 0.001*	
exposure (%)	10-11.99	22±0.9	22±1	0.98	21±0.8	22±1	0.78	
	12-13.99	23±0.7	19±1	< 0.001*	22±0.7	17±2	< 0.001*	
	14-14.99	22±0.8	22±8	0.02*	21±0.9	22±0.7	0.39	

Table 3: Comparison of	biochemical and hormonal	profiles between urban an	d rural children among	different age groups

Data are presented as mean $\pm$ SD for each variable. \**P*<0.05 was considered significant. BMI: Body mass index, 25(OH)D: 25-hydroxy vitamin D, PTH: Parathyroid hormone, SD: Standard deviation

			D status of school children	
Variables	Range	Vitamin D deficiency (n=42) (%)	Vitamin D insufficiency (n=71) (%)	Vitamin D sufficiency (n=387) (%)
BMI (kg/m <sup>2</sup> )	18.5-24.9	18 (42.8)	27 (38)	110 (28.4)
	>25	1 (2.38)	1 (1.4)	6 (1.55)
	<18.5	23 (54.76)	34 (476.88)	280 (72.3)
Sun exposure (%)	<20	36 (85.7)	46 (64.78)	92 (23.77)
	>20	6 (14.28)	16 (22.5)	304 (78.55)
Calcium intake	<1000	41 (97.6)	60 (84.5)	382 (98.7)
(mg/day)	>1000	1 (2.38)	2 (2.8)	14 (3.61)
Diet	Vegetarian	27 (64.2)	26 (36.6)	72 (18.6)
	Non-vegetarian	15 (35.7)	36 (50.7)	324 (83.72)
Calcium (mg/dl)	8.1-10.4	17 (40.4)	38 (53.5)	343 (88.63)
	<8.1	24 (57.1)	22 (30.98)	50 (12.9)
	>10.4	1 (2.38)	2 (2.8)	3 (0.77)
Phosphorus (mg/dl)	<4	31 (73.8)	42 (59.15)	223 (57.62)
	>4	11 (26.19)	20 (28.16)	173 (44.7)
Alkaline	<400	13 (30.9)	30 (42.2)	236 (60.98)
phosphatase (IU/L)	>400	29 (69)	32 (45)	160 (41.34)
PTH (pg/ml)	13-54	32 (76.19)	56 (78.87)	375 (96.89)
	<13	5 (11.9)	5 (7)	13 (3.35)
	>54	5 (11.9)	1 (1.4)	8 (2.06)

Data are presented as number and percentage of children in each group. BMI: Body mass index, 25(OH)D: 25-hydroxy vitamin D, PTH: Parathyroid hormone

ranges from 600 to 800 mg/day.<sup>[6,16]</sup> In the study by Harinarayan *et al.* in Andhra Pradesh, dietary calcium intake was lower in

rural areas than urban areas and dietary phytate content was higher for rural areas but there was no significant difference

# Table 5: Pearson correlation analysis between vitamin D levels and other variables

Variables	<b>Correlation coefficient</b>	Р
Height (cm)	0.19	< 0.0001*
Weight (kg)	-0.025	0.57
BMI (kg/m <sup>2</sup> )	-0.1	0.02*
Corrected Ca (mg/dl)	0.26	< 0.0001*
Phosphorus (mg/dl)	-0.01	0.76
Alkaline phosphatase (IU/L)	-0.17	< 0.0001*
PTH (pg/ml)	-0.07	0.11
Time of exposure to sun	0.5	< 0.0001*
Sun exposure (%)	0.58	< 0.0001*
Calcium intake (mg/day)	0.24	< 0.0001*

\*P<0.05 was considered significant. BMI: Body mass index,

25(OH)D: 25-hydroxy vitamin D, PTH: Parathyroid hormone

in 25(OH)D levels of rural and urban children which is in concordance with our study.<sup>[17]</sup>

In our study, the urban children had a significantly higher BMI than rural children. This may be attributed to the lack of open space and lesser physical activity resulting in greater weight in urban children. However, the mean weights of all the subjects were within normal limit for their age and sex.<sup>[18]</sup> Of the vitamin D deficient children, 54.76% had BMI <18.5 kg/m<sup>2</sup>, whereas 42.85% had normal BMI of  $18.5-24.9 \text{ kg/m}^2$ . We also examined the relationship between 25(OH)D with BMI, serum calcium, calcium intake, sun exposure, serum alkaline phosphatase and PTH. Significant positive correlation of 25(OH)D levels with serum calcium, sun exposure and calcium intake were found. Significant negative correlation was seen between 25(OH)D levels with BMI, alkaline phosphatase and PTH. The finding is in accordance with the study of Puri et al. where they found significant correlation of vitamin D levels with sun exposure.[9]

# CONCLUSION

In our study prevalence of vitamin D deficiency was 8.4% and vitamin D insufficiency was 14.2% in apparently healthy adolescent and preadolescent school children in and around the city of Guwahati in North-Eastern India which is comparatively lower than the prevalence found in other parts of the country and reflects the status unique to our region. There was no difference of mean 25(OH)D levels between the rural and urban population in the study.

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#### **Conflicts of interest**

There are no conflicts of interest.

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