

## Vitamin D status of apparently healthy early adolescents in Amravati City of Maharashtra, India

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### Abstract

**Introduction/Background:** Although vitamin D deficiency has been documented as a frequent problem in studies of young adults, elderly persons and children in other countries, there are limited data on the prevalence of this nutritional deficiency among healthy Indian adolescents. This study was conducted to determine the prevalence of vitamin D status in adolescents aged 13 to 15 years.

**Objective:** To determine the prevalence of vitamin D deficiency in healthy adolescents in India.

**Methods/Study Design:** Observational study with Clinical evaluation for evidence of vitamin D deficiency. The study was conducted in 62 apparently healthy school adolescents (33 Male & 29 Female) of 13 to 15 years of age from Amravati City in months of January 2013. Serum calcium, alkaline phosphates & 25-hydroxyvitamin D [25(OH) D] measured randomly in 62 subjects

**Results/Findings:** Fifty-nine participants (95.16%) were vitamin D deficient (serum 25OHD level < 20 ng/dl. Using a cutoff level of 25(OH) D of  $\leq 20$  ng/ml [ $\leq 50$  nmol/l] three participants (4.8 %) were having normal vitamin D level. Overall 95.2% of study participants were either vitamin D deficient or insufficient. The prevalence of vitamin D deficiency varied between boys (95%) and girls (96.5%).

**Discussion/Conclusion:** Vitamin D deficiency and insufficiency were highly prevalent in adolescents, and more common in girls. This might be an important public health problem and in addition to its effect on the growing skeleton, hypovitaminosis D may affect other organ systems adversely. Our findings support a recommendation for vitamin D supplementation for adolescents. Vitamin D supplementation is an efficient and feasible way to maintain serum 25(OH) D levels. This supplementation is not for the prevention of hypovitaminosis D only, but also as a prophylaxis for osteoporosis.

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**Key words:** Hypovitaminosis, Vitamin D deficiency, Adolescents, prevalence

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## **Introduction/Background**

Vitamin D deficiency is common in the developing countries and exists in both childhood and adult life. In addition to skeletal effects, including maintenance of normal bone turnover, mineralization during adulthood, and prevention of rickets in children, vitamin D may confer protection against health problems such as type 1 diabetes mellitus, hypertension, multiple sclerosis, and cancer.<sup>1</sup> Recent data indicates that vitamin D deficiency is pandemic, even the healthy and the young are not spared. High prevalence rates are reported in otherwise healthy infants, children and adolescents<sup>2-5</sup>, and also from diverse countries around the world including India<sup>6,7</sup> There are growing data from studies of young adolescents and youth in other countries that vitamin D deficiency is an unrecognized and prevalent health problem.<sup>8-12</sup> Optimal bone mineral health during childhood and adolescence leads to adequate peak bone mass which acts as a safeguard against osteoporosis and susceptibility to fractures in adulthood and old age. About 40-50 per cent of total skeletal mass is accumulated during childhood and adolescence. The findings from hospital-based studies show evidence that vitamin D deficiency is present in a significant proportion of general population in India.<sup>13-15</sup> It has also been noted that osteoporotic fractures occur 10-20 years earlier in Indian men and women, compared to Caucasians in the West<sup>[16]</sup>. Clinical examination to detect overt cases of vitamin D deficiency would represent only the 'tip of an iceberg' of vitamin D insufficiency.<sup>17</sup> While severe vitamin D deficiency, usually associated with 25(OH) D levels <5.0 ng/ml, results in rickets and osteomalacia, even less severe deficiency has been associated with a number of negative skeletal consequences including Secondary hyperparathyroidism, increased bone turnover, enhanced bone loss and fracture risk.<sup>18, 19</sup>

High prevalence of vitamin D insufficiency in healthy children and adolescents has been reported worldwide in the past few years.<sup>20-25</sup> However, data on vitamin D deficiency among Indian children and adolescents is scarce.<sup>26</sup> Pubertal age groups are more susceptible to vitamin D deficiency disorders. Early adolescence is the critical period of rapid physical growth and changes in body composition, physiology and endocrine.<sup>27</sup>

Metabolic bone disorders secondary to vitamin D deficiency continue to be prevalent in Indian subcontinent as reported by hospital-based studies. The earliest description of adolescent rickets in India dated back to 1925.<sup>28</sup> In children of Indian origin residing in South Africa, the prevalence of knock knees and bow legs with gaps of 2.5 cm or more was 6.1-19.4 per cent.<sup>29</sup> Adolescent period is prone to vitamin D deficiency state due to increased mineral demands of the growing skeleton.<sup>30,31</sup> Several studies have demonstrated low serum vitamin 25 (OH)D levels in populations across India.<sup>32-34</sup> In North India (27°N), 91% of healthy school girls<sup>35</sup> and 78% of healthy hospital staff have hypovitaminosis D.<sup>32</sup>

In developing countries like India, data on clinical and sub clinical vitamin D deficiency status among adolescents are scarce. In that context the present study was carried out with objective to know the Vitamin D status in apparently healthy early adolescents in Amravati City.

## **Objective**

To determine the prevalence of vitamin D deficiency in healthy school adolescents in Amravati District of Maharashtra, India.

## **Methods/Study Design**

The Observational study with Biochemical & Clinical evaluation for evidence of vitamin D deficiency. The study was conducted in 62 apparently healthy school adolescents (33 Male & 29 Female) of 13 to 15 years of age. Study carried out in months of January 2013. The parents of each participant were informed about the study protocol and gave written informed consent to their children's participation. The study protocol was approved by the Institutional Ethics Committee (IEC). Exclusion criteria included a chronic illness and use of medications known to affect bone metabolism; those who were being seen for a sick on visit, those who were not having blood drawn.

## **Anthropometric Measurement**

A training workshop on standardizing the method of anthropometric measurement was conducted by qualified trainers prior to data collection. Height was recorded without shoes, using a wall stadiometer to the nearest 1 mm. Subjects were weighed using a bathroom weighing machine with an accuracy of 100gms was used to record the weight with wearing light clothing and without shoes. BMI was calculated as weight (in kg)/height (in m<sup>2</sup>). The BMI cutoffs provided by Cole et al.<sup>36</sup> were used to classify children as normal, overweight or obese. Every morning, the scale and stadiometer were calibrated with standard weight and height, respectively. All measurements were performed at the same time of the day (i.e. early morning between 8 and 11.30 am) for all participants

## **Biochemical Profile**

A five milliliter venous blood sample was obtained from the subjects by qualified nurses using standardized tubes. Blood samples were sent to High Tech Pathological laboratory within two hours of blood draw where they underwent standardized (quality controlled) analyses.

Blood samples were collected from subjects in the fasting state at 8.00 hours without venostasis under basal conditions for estimation of 25-hydroxyvitaminD (25(OH)D). The most commonly used and sensitive index in assessing vitamin D status of an individual is 25(OH) D. The serum concentration of 25(OH)D were measured by Vit D level measured by Fully automated Chemi Luminescent Immuno Assay (C.L.I.A) technology from Thyrocare Technologies Limited Laboratory. Serum Ca were measured by a colorimetric method and alkaline phosphatase by a liquid kinetic method. The normal laboratory range for serum Ca is 9.2–11 mg/dl). The normal laboratory range for serum alkaline phosphatase is 104–390 IU/l.

The definition of hypovitaminosis D was based on two criteria: firstly, serum concentration of 25(OH)D below 50 nmol/l (20 ng/ml) In adolescents, serum 25(OH) below 20 ng/mL may be considered the cutoff point for vitamin D deficiency, and between 20–30 ng/mL may be considered the range for vitamin D insufficiency.<sup>37,38</sup> However, these values are not unanimously accepted by all researchers. Furthermore, optimal vitamin D function in a determined organ or tissue may need a different vitamin D set point. And, secondly, recommended by Lips<sup>39</sup>, as mild (25–50 nmol/l or 10-20 ng/mL), moderate (12.5–25 nmol/l or 5- 10ng/mL) and severe hypovitaminosis D (12.5 nmol/l or <5 ng/mL) Exact cut – offs for “deficiency” and “insufficiency” remain controversial.

## Statistical Analysis

All data were normally distributed. Descriptive statistics (frequencies, means, standard deviations, percentage) were used to estimate serum 25(OH) D concentrations by age groups, gender & nutritional status. The statistic analyses such as one-way Anova or t-test were not used in present study. Data entry done in Microsoft excel programme as per questionnaires variables. The analyses were conducted using SPSS (v.16.0) version.

## Results/Findings

Total 62 adolescent were selected for present study 33 boys & 29 girls. The mean age of study participants  $14.5 \pm 0.5$  in boys &  $14.3 \pm 0.47$  in girls. The mean of anthropometric measurement including Height, weight & BMI was  $163.7 \pm 7.1$  cm,  $47.36 \pm 9.36$  kg &  $17.5 \pm 2.83$  kg/m<sup>2</sup> in boys and  $156.07 \pm 4.8$  cm,  $44.97 \pm 6$  kg &  $18.49 \pm 2.64$  kg/m<sup>2</sup> in girls. Serum levels of 25-OHD in the total group ranged between  $7.44 \pm 3.44$  to  $31.15 \pm 4.23$  ng/ml. The mean level of 25-OHD was lower in girls compared to boys (M;  $12.99 \pm 3.44$ , F;  $11.40 \pm 4.23$ ) The mean of biochemical parameter including Serum calcium & serum alkaline phosphatase was  $9.14 \pm 0.47$  mg/dl &  $215 \pm 75.62$  IU/L in boys. In girls  $9.36 \pm 0.69$  mg/dl &  $115 \pm 42.5$  IU/L respectively. [Table 1]

Prevalence of vitamin D deficiency (serum 25OHD Level < 20 ng/mL) was 95.2% in the total group. As per lips classification prevalence of Hypovitaminosis D was 95% in total children. The mild hypovitaminosis 27.3%, moderate hypovitaminosis 67.7% and 5% was in normal vitamin D level . Prevalence of vitamin D deficiency in female students, a seen in Table 2, was more than in males. (**Figure 1**). There was no significant difference in prevalence between adolescent girls and boys ( $93.9\%$  vs  $96.6\%$ ,  $P=0.10$ ). Of 29 girls, 15 (7%) showed decreased ALP value. There was no significant difference in ALP values between normocalcemic and hypocalcemic girls ( $P > 0.05$ ). 35 (56.5%) subject out of 62 showed hypocalcemia (serum calcium < 9.2 mg/dl). 63.3% boys & 48.4% of girls found to hypocalcemic. [Table 3]

## Discussion/Conclusion

Serum 25-Hydroxyvitamin D [25(OH)D] is the precursor of the active metabolite calcitriol, and its concentrations reflect body stores of vitamin D. Serum 25(OH)D levels are dependent on vitamin D intake, and cutaneous synthesis in the skin upon exposure to solar UV-B radiation (UVB). The vital role of vitamin D in bone mineralization depends on its critical role in the absorption of calcium and phosphorus in the intestine as well as the differentiation of cells in the osteoblastic lineage (Holick, 2003).<sup>40</sup>

We found a high prevalence of hypovitaminosis D (serum 25OHD Level < 20 ng/mL) in this study in 59 subjects (95.2 %). However as per Lips classification prevalence of hypovitaminosis D was 95% in total children of that 27.3% showed mild hypovitaminosis & 67.7% showed moderate hypovitaminosis as shown in Table 2.

A comparison of serum vitamin D data with other studies may not be entirely appropriate, given the fact that different studies were conducted in different seasons and using different assays. Nonetheless, according to the Lips classification (Lips, 2001). Raman *et al.*<sup>41</sup> found that hypovitaminosis D (< 50 nmol/L) was seen in 88 % of apparently healthy adolescents in New Delhi, India. Puri S *et al.*<sup>35</sup> found that 91% of healthy school girls have hypovitaminosis D. Both studies in India will compare with 95.2 % of adolescents in the present study. Other studies reported low prevalence of Vitamin D deficiency as compare to present study among healthy adolescents it was reported as 59.4% in Turkey<sup>42</sup>, one study reported high rates of subclinical vitamin D deficiency (65%) in adolescent girls who wear concealing clothing in the Kocaeli region.<sup>[43]</sup> other studies showed 78% in France,<sup>44</sup> 42.5% in Beijing,<sup>45</sup> 47% in Greece.<sup>46</sup> In other study conducted in north India demonstrated clinical evidence of vitamin D deficiency in 10.8 per cent of apparently healthy adolescents.<sup>47</sup> In Asian migrants in the United Kingdom, the prevalence of clinical vitamin D deficiency in children and adolescents was shown to be 12.5 to 66 per cent.<sup>48, 49</sup>

There were no consistent definitions of hypovitaminosis D; values corresponding to vitamin D deficiency ranged from less than 5 ng/mL to less than 12 ng/mL, and those for vitamin D insufficiency ranged from less than 10 ng/mL to less than 32 ng/mL (to convert 25 hydroxyvitamin D concentrations to nanomoles per liter, multiply by 2.496).

Serum 25(OH) D is our best available laboratory aid for diagnosing frank vitamin D deficiency, which causes rickets in children and osteomalacia in adults. Vitamin D deficiency is typically associated with 25(OH)D levels below 25 nmol/L. However, identification of inadequate levels of vitamin D for optimal bone health-vitamin D "insufficiency"- is likely of greater clinical importance, which defined as serum 25-OHD level between 25 and 50 nmol/L (10-20 ng/mL).<sup>50</sup>

In our study the mean serum concentration of 25(OH) D was  $12.25 \pm 3.88$ . it was higher than the study on children from northern India was ( $11.8 \pm 7.2$  ng/ml)<sup>[ 26]</sup>. The mean 25(OH)D concentration in adolescents in our study was lower than that reported in China ( $13.9 \pm 9.6$ ) [45]. Recent studies on Vitamin D receptor (VDR) in knockout mice revealed that the effects of vitamin D on skeletal health depend exclusively on intestinal Ca absorption.<sup>51</sup> Therefore, the assumption that in various tissues the effect of vitamin D deficiency below the level affecting Ca homeostasis is mediated through VDR, is controversial. However, in many

epidemiological studies hypovitaminosis D is associated with chronic diseases and malignancy.<sup>52, 53</sup>

## **Conclusion**

We conclude that there is a high prevalence of biochemical hypovitaminosis D in apparently healthy schoolchildren in India. The observation suggests that low daily calcium intake and vitamin D acquirement are two important problems in Indian early adolescents both boys & girls during rapid growth at puberty. Therefore, calcium and vitamin D supplementation should be added to their daily diets by changing their dietary status and sunlight exposure by a sequential program. This study provides useful tools for public health and health policy planning.

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## **Authors' Contributions:**

Conceived, designed and implemented the experiments by: Dr.Rajendra Dhore.

Conceived, designed, analyzed the data, wrote the paper by Dr.Vinod Wasnik

Both authors read and approved the final manuscript.

## **Ethical Considerations:**

The study protocol was submitted to the Institutional Ethical Committee and clearance was obtained. The parents of each participant were informed about the study protocol and gave written informed consent to their children's participation before initiation of the study.

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**Table 1:** Baseline characteristics and biochemical parameters of 62 Study Participants.  
(Mean values and standard deviations)

Variables	Male (n = 33)		Female (n =29)	
	Mean	Standard Deviation	Mean	Standard Deviation
Age (Years)	14.55	0.5	14.31	0.47
Height (cm)	163.73	7.1	156.07	4.8
Weight (kg)	47.36	9.36	44.97	6.0
BMI ( kg/m <sup>2</sup> )	17.5	2.83	18.49	2.64
Vit D ( ng/ml)	12.99	3.44	11.40	4.23
Serum calcium ( mg/dl)	9.14	0.47	9.36	0.69
Serum alkaline Phosphatase ( IU/L)	215	75.62	115.3	42.5

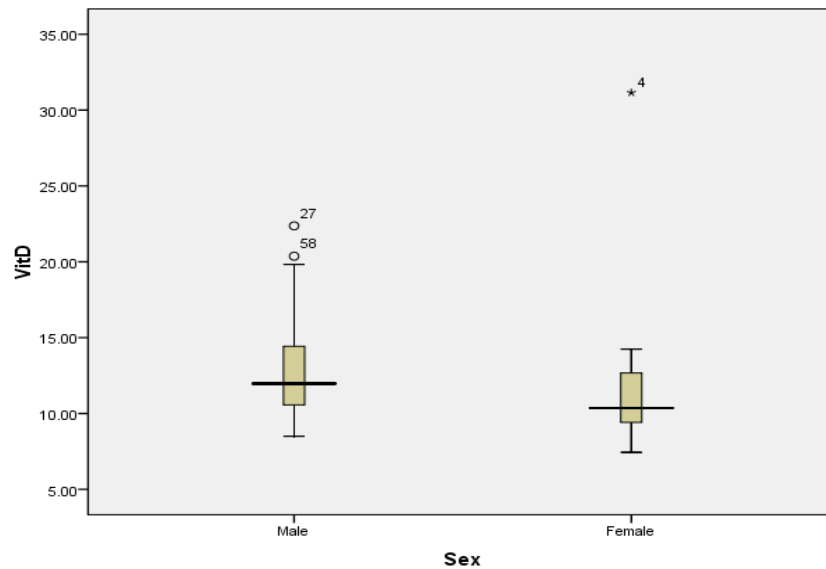
**Table 2:** Vitamin D status among healthy adolescents

Vit D Status	Male (n-33)	Female ( n-29)	Total (n-62)
<b>As per Previous study*</b>			
Deficients (<20 ng/ml)	31 (93.9)	28(96.6)	59 (95.2)
Insufficients(20-30 ng/ml)	2(6.1)	1(3.4)	3(4.8)
<b>As per Lips P **</b>			
Mild Hypovitaminosis (5-10 ng/ml)	6 (18.2)	11(37.9)	17(27.3)
Moderate Hypovitaminosis ( 10-20 ng/ml)	25(75.8)	17(58.6)	42(67.7)
Normal (>20 ng/ml)	2(6.1)	1(3.4)	3(4.8)

Figures in parenthesis indicate percentage

\* Calatayud M et al [38]; *Endocrinol Nutr.* 2009; 56:164–169.

\*\* Lips P [39] *Endocr Rev*; 2001. 22, 477–501



**Figure 1:** Box plot of gender variation in serum 25-hydroxyvitamin D (25OHD) level. The mean 25OHD level was significantly higher among males than females. The center line in the box indicates the median; +, the mean; the top and bottom of the box, quartile boundaries; vertical bars, minimum and maximum values within 1.5 times the interquartile range of the quartile boundary; and circles, more extreme values.

**Table 3:** Biochemical parameter among healthy adolescents

Biochemical Parameter	Male (n-33)	Female ( n-29)	Total (n-62)
<b>Serum Calcium Level</b>			
Below (< 9.2 mg/dl)	21(63.6)	14(48.4)	35(56.5)
Normal ( 9.2--11 mg/dl)	12(36.4)	15(51.6)	27(43.5)
<b>Serum Alkaline Phosphatase</b>			
Below (< 104 IU / L)	1(3)	15(51.7)	16(25.8)
Normal ( 104—390 IU/L)	32(97)	14(48.3)	46(74.2)

Figures in parenthesis indicate percentage