Ind J Clin Biochem (Apr-June 2015) 30(2):167–173
DOI 10.1007/s12291-014-0428-2

ORIGINAL ARTICLE

Prevalence of Vitamin D Deficiency in a Pediatric Hospital of Eastern India

Surupa Basu · Ruchi Gupta · Monjori Mitra · Apurba Ghosh

Received: 3 October 2013 / Accepted: 13 March 2014 / Published online: 22 April 2014

Abstract Vitamin D deficiency is highly prevalent in Indian children of northern, western and southern states. Serum 25 hydroxy cholecalferol (ng/ml) was analyzed in 310 children and adolescents of pediatric hospital of Kolkata, India. Serum calcium (mg/dl), phosphorous (mg/dl) and alkaline phosphatase (IU/L) data was obtained. Median 25(OH)D was 19 ng/ml. 19.2 % of population had serum 25(OH)D < 10 ng/ml (severe deficiency), 52.9 % had <20 ng/ml (deficiency), 24.5 % had 20–29 ng/ml (insufficiency) and 22.6 % had >30 ng/ml (optimum). Deficiency was highest in adolescents (86.1 %), followed by school children (61.0 %), lowest in pre-school children (41.6 %). 25(OH)D concentrations was lowest in winters ($P = 0.002$) and spring ($P = 0.03$) compared to summer. There was no correlation with calcium ($P = 0.99$), phosphorous ($P = 0.23$) and ALP ($P = 0.63$). There is high prevalence of vitamin D deficiency in children and adolescents of eastern India. Prevalence was lower in younger subjects. 25(OH)D did not correlate with bone mineral markers.

Keywords Vitamin D · Pediatric · 25 hydroxy cholecalciferol · Prevalence · Calcium · Alkaline phosphatase

S. Basu (✉)
Department of Biochemistry, Institute of Child Health, 11, Dr. Biresh Guha Street, Kolkata 700029, West Bengal, India
e-mail: surupa.basu@rediffmail.com; basusurupa@gmail.com

R. Gupta · M. Mitra · A. Ghosh
Department of Pediatric Medicine, Institute of Child Health, Kolkata, India

Introduction

Vitamin D plays a key role in calcium and phosphate metabolism and is an essential micronutrient for bone health [1]. Following discoveries of ubiquitous presence of vitamin D receptors and enzyme machinery for vitamin D activation in most organs of the body, wider non-osseous roles for this steroid hormone have been proposed [2]. The universal prevalence of vitamin D deficiency [3], even in sunlit countries, along with various proposed health benefits from vitamin D supplementation [4], with increased availability of vitamin D estimation facilities [5] have catapulted screening for deficiency by several folds [6].

In the pediatric subjects, who are susceptible to deficiency and impaired bone mineral metabolism, screening for vitamin D deficiency is particularly recommended [7]. Epidemiological data from other countries, which lie in the sunny equatorial and tropical belts, and also from the northern, western and southern states of India, have consistently demonstrated the high prevalence of vitamin D in children and adolescents [3, 8–12]. This is surprising as exposure to sunlight is the main source of vitamin D, with diet contributing only 10–15 %. Modern day lifestyle changes, for example, more time spent indoors by children; cloud cover and pollution; traditional clothing; sunscreen usage are factors which affect optimum skin exposure to sunlight [7]. Genetic factors like higher prevalence of certain polymorphisms in the vitamin D binding protein associated with low vitamin D levels; darker skin tone compared to Caucasians; and dietary factors like low calcium and high fibre diet may additionally contribute to prevalence of deficiency states.

The optimal level of serum 25 hydroxycholecalciferol, or 25(OH)D (ng/ml) for general health in children is unknown, given very few outcome data for the group

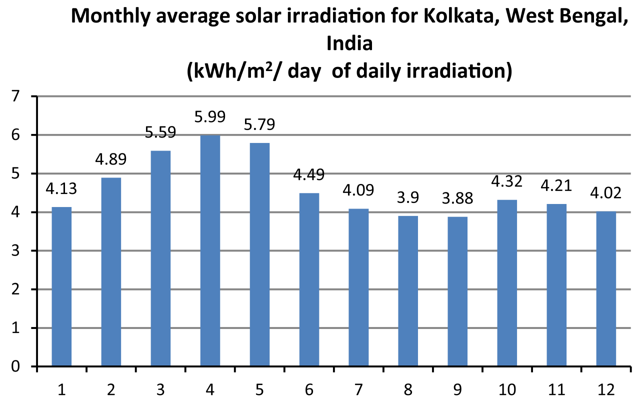

Fig. 1 Kilowatt hour per meter square of daily irradiation for Kolkata, West Bengal, India (from Ministry of New and Renewable Energy website, Govt. of India), monthly average data

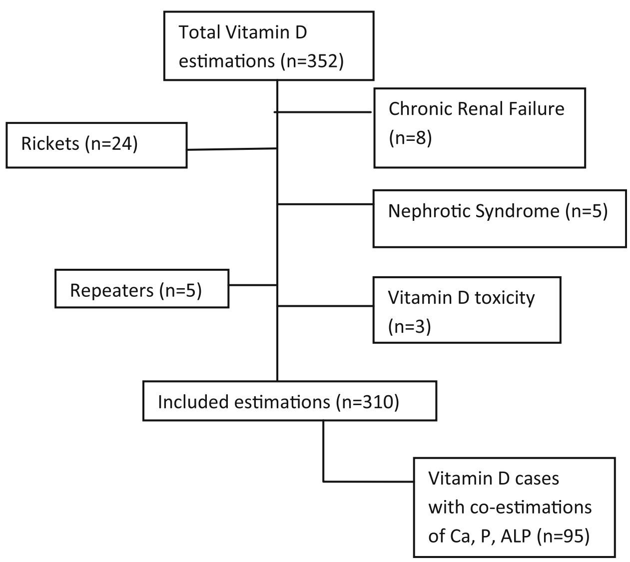

Fig. 2 Exclusion criteria and cases excluded

[13, 14]. By current definitions, though controversial, values less than 20 ng/ml is considered deficient, 20–30 ng/ml insufficient and optimal values are between 30–40 ng/ml [15, 16].

Prevalence of vitamin D deficiency in children has not been reported from the eastern region of India. In this paper, we have attempted to provide a cross-sectional overview of vitamin D concentrations in children and adolescents, along with data on biochemical correlates of vitamin D in bone metabolism. Kolkata, a major metropolitan city, situated at latitude of 22.5°N, and its neighbouring districts receive lower average annual solar irradiation compared to other regions of the country [17]. The monthly average solar irradiation data for Kolkata is provided in Fig. 1, with values ranging from ~4 kWh/m^2/day in the winter, to ~6 kWh/m^2/day in the summer.

Materials and Methods

Study Design

Retrospective analysis of serum 25(OH)D levels of pediatric patients of 1–16 years of age, who visited the outpatient clinics of a referral teaching trust hospital of Kolkata, India from Jan 2012–July 2013, was carried out. Demographic details, date of sampling, clinics attended were collated. Cases of rickets, nephrotic syndrome and chronic renal failure were excluded (Fig. 2). For cases that had repeat investigations, the first value was considered for analysis. Cases of toxicity (serum 25(OH)D concentration >100 ng/ml) were also excluded. Data was also collated for serum calcium, phosphorous and alkaline phosphatase (ALP) that were measured along with serum vitamin D. The study was approved by the institutional ethics committee.

Measurement of Vitamin D and Biochemical Correlates

Serum 25(OH)D metabolite is considered the most reliable biomarker of nutritional vitamin D status [18]. It was measured by the automated chemiluminiscence assay on the Roche immunoassay platform (Cobas e411, Roche Diagnostics), which measures both the D2 (from diet) and D3 (from UVB irradiation of the skin or supplementation) and has an assay range of 3–70 ng/ml. Values over70 ng/ml were suitably diluted to obtain absolute values. Satisfactory coefficient of variation (CV %) data were obtained for 25(OH)D (9.8 %) based on calculations of quality control (QC) data run at significant levels of measurement. Serum calcium (mg/dl), inorganic phosphate (mg/dl) and ALP (U/L) were measured on the Roche Integra 400 Plus chemistry analyzer (Roche Diagnostics, Manheim, Germany) and validated for reproducibility using immunoassay QC materials obtained from Bio-Rad (Irvin, CA, USA). Low levels of 25(OH)D were defined as "insufficient" if between 20–30 ng/ml and "deficient" if <20 ng/ml and "severely deficient" if <10 ng/ml, as cited in previous studies [15, 16]. Age-, gender- and method-specific laboratory reference ranges were used for calcium, phosphate and ALP.

Statistical Analysis

The distribution test for normality showed that the data was non-normal. Discrete data are presented as medians with interquartile range (IQR). The Mann–Whitney U test was used for computing group differences. The difference between categorical data was analyzed using Fisher's exact test. Spearman's rank correlation analysis was used to test for correlations (r_s) between variables. All analysis was

Table 1 Demographic characteristics of children and adolescents aged 1–16 years according to serum 25(OH)D level (ng/ml)

	Median, ng/ml (IQR)	<10 ng/ml Median, ng/ml (IQR) %severely deficient	10–19 ng/ml Median, ng/ml (IQR) % deficient	20–29 ng/ml Median, ng/ml (IQR) %insufficient	≥30 ng/ml Median, ng/ml (IQR) %optimum
Study population (1–16 years) ($n = 310$)	19 (11–28)	6 (4–8), 19.7	14 (12–17), 33.2	24 (22–27), 24.5	43 (32–53), 22.6
Ages 1–5 years ($n = 156$)	23 (13–31)	6 (3–8), 16.2	14(13–16), 25.4	25 (22–27), 27.6	42(32–53), 30.8
Ages 6–11 years ($n = 118$)	17 (11–25)	7 (6–8), 21.2	15(12–18), 39.8	24(22–27), 23.7	45 (41–60), 15.3
Ages 12–16 years ($n = 36$)	10 (8–17)	6 (3–9), 30.6	13 (11–17), 55.5	23 (NC), 13.9	None

NC not calculable, insufficient data

performed in Medcalc ver. 13.0.0.0 software (Mariakerke, Belgium). A P value of <0.05 was considered to be statistically significant.

Results

Subject Demographics and Distribution of Vitamin D Levels

A total of 310 cases (55 % males) were included for analysis (Fig. 2). Out of these, 95 had been investigated for serum calcium, phosphorus and ALP. Subjects were grouped into preschool stage (1–5 years) ($n = 156$), school (6–11 years) ($n = 118$) and adolescents (12–16 years) ($n = 36$). Table 1 demonstrates the distribution of median 25(OH)D concentrations and the prevalence (%) in the above age groups when classified into severely deficient (<10 ng/ml), deficient (10–19 ng/ml), insufficient (20–29 ng/ml) and sufficient or optimum (>30 ng/ml).

The median serum 25(OH)D for the population was 19 ng/ml (IQR 11–28). 19.7 % subjects were severely deficient (median 6 ng/ml), 52.9 % deficient (median 14 ng/ml) and 24.5 % insufficient (median 24 ng/ml).

The 25(OH)D levels decreased with increase in age ($r_s = -0.313$, $P < 0.0001$) (Fig. 3). Accordingly, the preschool group had the highest median 25(OH)D concentration (23 ng/ml), followed by school children (17 ng/ml) and lowest in adolescents (12 ng/ml). The adolescent group had the highest percentage of severely deficient (30.6 %) and deficient (86.1 %) subjects (Table 1).

No significant differences were found between serum 25(OH)D levels of boys and that of girls in any of the age groups (Table 2).

Changes in Vitamin D Levels with Season

The monthly median 25(OH)D levels changed with season showing a dip in winter months and higher values in the summer months (Fig. 4). The median 25(OH)D level in the summer months were significantly higher compared to those

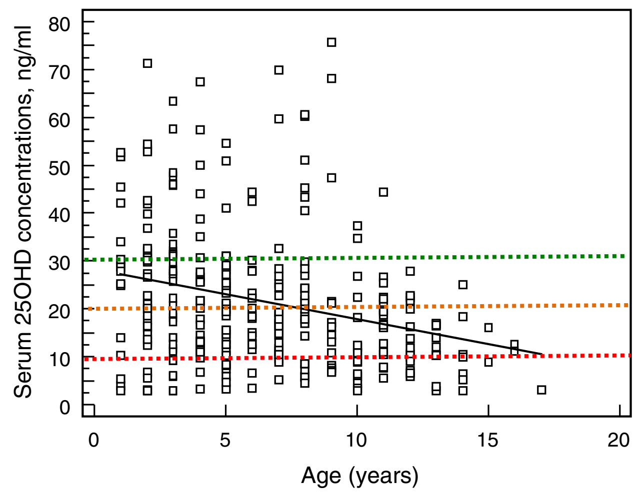

Fig. 3 Scatter diagram of serum 25(OH)D with age. Central regression line is the line of best fit ($r_s = -0.313$, $P < 0.0001$). *Dotted lines* represent 25(OH)D cut-offs to indicate severe deficiency (<10 ng/ml), deficiency (<20 ng/ml) and sufficiency (≥30 ng/ml)

in winter ($P = 0.002$)and spring ($P = 0.03$), but not significantly different from the median value obtained in the monsoon season ($P = 0.15$). The percentage of deficient and severely deficient subjects were high in the winter and spring months, and were the lowest in the summer months (Fig. 5).

Association of Vitamin D with Biochemical Correlates

In 95 subjects, median calcium, phosphorous and ALP levels were found to be 9.5 mg/dl, 5.0 mg/dl and 239 IU/L respectively. All were within the normal reference ranges for the population. The median 25(OH)D concentration of the group was 22 ng/ml (IQR 13–31). Serum 25(OH)D did not show any significant correlation with any of the above common parameters of bone health (Table 3).

Discussion

The present study provides a cross-sectional data of biochemical hypovitaminosis D across a pediatric population

Table 2 Serum 25(OH)D levels (ng/ml) in boys and girls

	Median, ng/ml (IQR)	*P* value*
Total 1–16 years		$P = 0.15$
Male($n = 172$)	19 (17–22)	
Female ($n = 139$)	17 (14–21)	
Ages 1–5 years		$P = 0.50$
Male ($n = 86$)	24 (19–28)	
Female ($n = 70$)	22 (16–28)	
Ages 6–11 years		$P = 0.13$
Male ($n = 63$)	19 (15–22)	
Female ($n = 55$)	16 (12–19)	
Ages 12–16 years		$P = 0.25$
Male ($n = 23$)	13 (10–17)	
Female ($n = 13$)	9 (4–23)	

* $P < 0.05$ was considered significant

residing in and around the metropolitan city of Kolkata, in eastern India. These data show that a large section of children and adolescents (53 %) are nutritionally deficient in vitamin D, as per current definitions, and require supplementation to replenish body stores and correct circulating levels to the optimal range [19]. Only 22 % of the study population had optimal levels of which none were adolescents, the group which was also the most deficient. Although adolescents were under-represented in the population, this finding is alarming, since maximal accrual of bone mass takes place during this growth phase [20].

The median vitamin D concentration in the preschool and school going children was found to be 23 and 17 ng/ml, respectively and deficiency was seen in 42 and 61 % subjects. Similar statistics have been seen in reports in children living in the northern, western and southern states of the country. Marwaha et al. [10] have shown that healthy

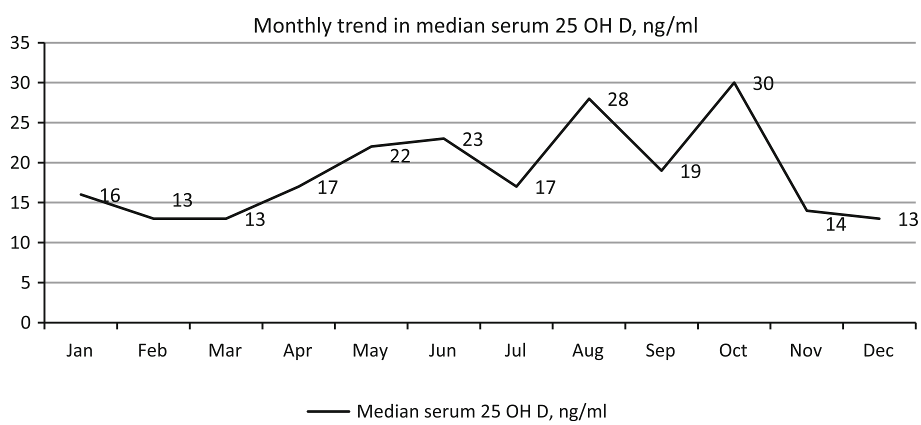

Fig. 4 Trend line depicting variation in 25(OH)D in children and adolescents ($n = 310$) with median monthly 25(OH)D (ng/ml)

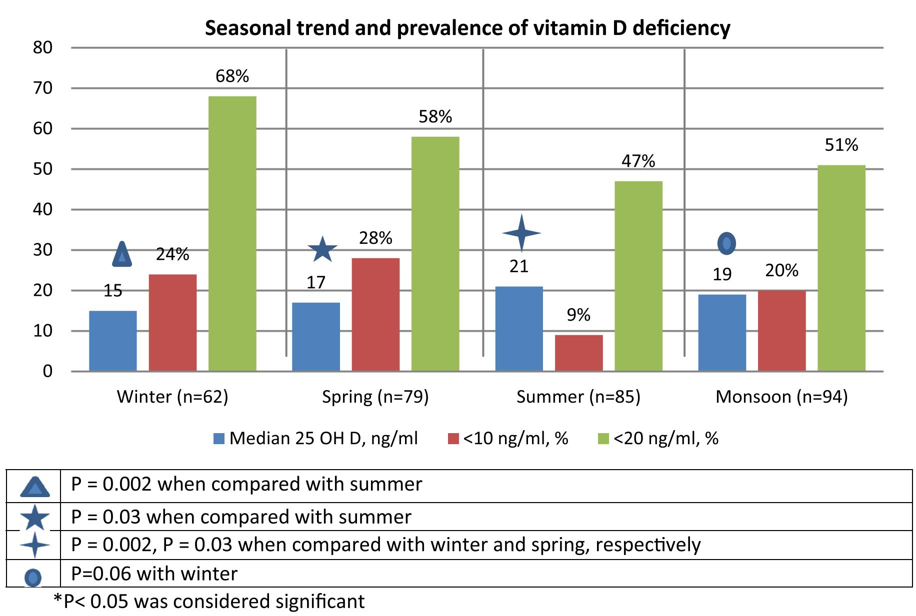

Fig. 5 Percentage of children with vitamin D deficiency during the four seasons of the year (*green bars*), percentage with severe deficiency (*red bars*), median 25(OH)D levels (ng/ml) for measurements made during the season (*blue bars*)

Table 3 Correlation of serum 25(OH)D (ng/ml) with serum calcium (mg/dl), inorganic phosphorous (mg/dl) and alkaline phosphatase levels (IU/L)

Total ($n = 95$)	Median value (IQR)	Spearman's rank correlation with serum 25(OH)D (ng/ml)
Serum calcium, mg/dl	9.5 (9.0–10.0)	$r_s = 0.001$ $P = 0.99$
Serum phosphorous, mg/dl	5.0 (4.6–5.5)	$r_s = 0.134$ $P = 0.23$
Serum alkaline phosphatase, IU/L	239 (186–300)	$r_s = -0.054$ $P = 0.63$

* $P < 0.05$ was considered significant

school children of northern India had mean serum level of 12 ng/ml, and over 90 % of children were vitamin D deficient. Harinarayan et al's study [12] in rural and urban children residing in the southern state of Andhra Pradesh report prevalence of 81.5 % in urban male children and 76.5 % in rural areas.

The study findings of high prevalence of subclinical vitamin D deficiency in children, as observed across the country, are relevant and calls for action. Adequate vitamin D is associated with absence of vitamin D deficiency rickets, maximal suppression of PTH, optimal calcium absorption from the gut and higher bone mineral content in children and adolescents [21]. Additionally, vitamin D has been recently shown to have various extra-skeletal roles such as immunomodulatory and antiproliferative effects on disease [22].

By global statistics, Indian children have far higher prevalence of vitamin D deficiency when compared to reports from US [23] or China [8]. This may be explained by the skin pigmentation of Indian children who are relatively dark compared to Caucasian counterparts. Cutaneous synthesis of vitamin D from the UVB rays of sunlight is reduced due to increased melanin content in darker individuals [7]. Additionally, these findings reflect lack of vitamin D supplementation guidelines in the country [24] including food fortification programmes [25]. Our study population consisted predominantly of children living in urban and semi-rural areas, and of lower socio-economic background, and therefore pollution, city dwelling, less access to playgrounds, and overall poor nutritional status may have influenced vitamin D concentrations, which have not been separately looked into, in the present study.

The prevalence of deficiency was markedly dependent on the season of blood sampling, being lowest in winters and highest in summers (Fig. 5). The variability in solar irradiation will significantly impress upon the serum levels of vitamin D since more than 90 % of the body requirements are derived by cutaneous synthesis from sunlight. The seasonal variations in the median vitamin D levels and the percentage of deficient cases are in agreement with the pattern of solar radiation received in Kolkata (Fig. 1). The month of August and October were a deviation as children had higher median vitamin D levels than expected from the solar radiation pattern, which may be due to sampling inadequacies. Moreover, October is a festive month in West Bengal and it may be hypothesized that more children may have upward shift in outdoor activities as well as in their diet. Of note is the fact that practitioners should consider the season of sampling during supplementation, keeping an additional margin to cover adequacy during the winter and spring months when sunlight availability is low [26].

We failed to find correlation of 25(OH)D (median, 22 ng/ml) with serum calcium, phosphorous and ALP levels, which were within normal ranges. A recent study [27] found that 86.1 % children demonstrated some abnormality in calcium, phosphorous, ALP or parathyroid hormone (PTH) only when serum 25(OH)D was below 10.8 ng/ml. This and another study [26] demonstrated PTH levels to increase when vitamin D values fall below 13.6 and 12 ng/ml, stating this cut-off to represent vitamin D deficiency in children. Though we did not evaluate PTH, our data on calcium, phosphorous and ALP implies that it may be inappropriate to assume cut off values of vitamin D based on adult studies, as a surrogate marker of bone health in children and adolescents. Another explanation for the non-correlation may have its basis in the concept of free or bioavailable vitamin D (not bound to carrier proteins), which has shown to better correlate with bone markers in comparison to the total serum 25(OH)D levels in both healthy and osteoporotic men [28, 29].

Our study has some limitations and findings must be interpreted accordingly. Our population consisted of children attending the outpatient department of the hospital who cannot be treated as "healthy" subjects. There may be influences on serum vitamin D concentrations due to any underlying acute or chronic pathologies, though we have excluded the major identifiable causes of vitamin D deficiency i.e. renal disease and rickets. This was a single centre urban hospital based study, and therefore the results cannot be made generalizable for all children living in the region. Factors relating to vitamin D status including intake of supplements, children's BMI, sunshine exposure, rural or urban residence, diet and the time of physical activities were not collected. However, this study was cross-sectional with an intention to report prevalence and not draw conclusions regarding the causality of vitamin D deficiency. A marginally lower prevalence of deficiency compared to other regions of India may be due to inclusion of children on vitamin D supplementation, although all repeat investigations and toxicity levels of vitamin D were excluded from the study. We were not able to obtain sufficient data for the PTH, a measure of bone health better associated with vitamin D status as compared to calcium, phosphorous or ALP.

This paper adds to the growing reports of high deficiency status of children in India. Pediatricians and health care practitioners caring for children should encourage children and adolescents to adopt a healthy lifestyle to maintain normal body mass index, including diet with vitamin D-containing foods (meat, fish, eggs, dairy products) and adequate outdoor activities for optimum sun exposure. Children at risk for deficiency such as obese or dark skinned may require more vitamin D than they are currently making from sunlight or consuming in their diet. It is also time for national authorities to evaluate the causes for high prevalence of vitamin D deficiency in Indian children and formulate appropriate measures such as adequate sun exposure, dietary recommendations, food fortification, and vitamin D supplementation in children and adolescents.

Conclusion

Vitamin D deficiency and insufficiency is highly prevalent amongst children and adolescents living in and around the city of Kolkata, India. Prevalence increases with age, but is independent of gender. There is marked seasonal variation in prevalence, being higher in winter and springs and lower in summers and monsoon. Serum concentrations are not correlated with commonly tested bone mineral markers. These data call for future investigations to evaluate the causality of high prevalence of vitamin D deficiency in Indian children in spite of living in a sunlit country and, to also redefine biochemical end points of 25(OH)D that correlates with bone metabolism in children and adolescents. In the meantime, using definitions of deficiency, insufficiency and optimum that have extrapolated from adult studies, we conclude that more Indian children require judicious exposure to sunlight, fortified foods and adequate supplementation to maintain bone health.

References

1. Lips P. Vitamin D physiology. Prog Biophys Mol Biol. 2006;92:4–8.
2. Wacker M, Holick MF. Vitamin D—effects on skeletal and extraskeletal health and the need for supplementation. Nutrients. 2013;5(1):111–48.
3. Mithal A, Wahl DA, Bonjour JP, Burckhardt P, Dawson-Hughes B, Eisman JA, IOF Committee of Scientific Advisors (CSA) Nutrition Working Group, et al. Global vitamin D status and determinants of hypovitaminosis D. Osteoporos Int. 2009;20(11):1807–20.
4. Vieth R, Bischoff-Ferrari H, Boucher BJ, Dawson-Hughes B, Garland CF, Heany RP, et al. The urgent need to recommend an intake of vitamin D that is effective. Am J Clin Nutr. 2007;85:649–50.
5. Wallace AM, Gibson S, de la Hunty A, Lamberg-Allardt C, Ashwell M. Measurement of 25-hydroxyvitamin D in the clinical laboratory: current procedures, performance characteristics and limitations. Steroids. 2010;75:477–88.
6. Marcus MB. Vitamin D tests soar as deficiency, diseases linked. USA Today, 13 July 2008. http://www.usatoday.com/news/health/2008-07-13-vitamin-d-tests_N.htm (2013). Accessed 1 Oct 2013.
7. Misra M, Pacaud D, Petryk A, et al. Vitamin D deficiency in children and its management: review of current knowledge and recommendations. Pediatrics. 2008;122:398–417.
8. Zhu Z, Zhan J, Shao J, Chen W, Chen L, Li W, et al. High prevalence of vitamin D deficiency among children aged 1 month to 16 years in Hangzhou, China. BMC Public Health. 2012;12:126–32.
9. Muhairi SJ, Mehairi AE, Khouri AA, Naqbi MM, Maskari FA, et al. Vitamin D deficiency among healthy adolescents in Al Ain, United Arab Emirates. BMC Public Health. 2013;13:33–9.
10. Marwaha RK, Tandon N, Reddy DHK, Agrawal R, Singh R, Sawhney RC, et al. Vitamin D and bone mineral density status of healthy school children in northern India. Am J Clin Nutr. 2005;82:477–82.
11. Ekbote VH, Khadilkar AV, Mughal MZ, Hanumante N, Sanwalka N, Khadilkar VV, et al. Sunlight exposure and development of rickets in Indian toddlers. Indian J Pediatr. 2010;77(1):61–5.
12. Harinarayan CV, Ramalakshmi T, Prasad UV, Sudhakar D. Vitamin D status in Andhra Pradesh: a population based study. Indian J Med Res. 2008;127(3):231–8.
13. Wagner CL, Greer FR. Prevention of rickets and vitamin D deficiency in infants, children, and adolescents. Pediatrics. 2008;122(5):1142–52.
14. Abrams SA. Vitamin D requirements in adolescents: what is the target? Am J Clin Nutr. 2011;93(3):483–4.
15. Holick MF, Vitamin D. Status: measurement, interpretation, and clinical application. Ann Epidemiol. 2009;19(2):73–8.
16. Pramyothin P, Holick MF. Vitamin D supplementation: guidelines and evidence for subclinical deficiency. Curr Opin Gastroenterol. 2012;28(2):139–50.
17. India solar resource maps from Solar Energy Centre, Ministry of New and Renewable Energy, Government of India on http://www.mnre.gov.in/sec/solar-resources.htm. Accessed 02 Oct 2013.
18. Zerwekh JE. Blood biomarkers of vitamin D status. Am J Clin Nutr. 2008;87(4):1087S–91S.
19. Holick MF. Resurrection of vitamin D deficiency and rickets. J Clin Invest. 2006;116:2062–72.
20. Bailey DA, Martin A, McKay H, Whiting S, Mirwald RL. Calcium accretion in girls and boys during puberty: a longitudinal analysis. J Bone Miner Res. 2000;15:2245–50.
21. Shaw NJ, Mughal MZ. Vitamin D and child health part 1 (skeletal aspects). Arch Dis Child. 2013;98(5):363–7.
22. Shaw NJ, Mughal MZ. Vitamin D and child health: part 2 (extraskeletal and other aspects). Arch Dis Child. 2013;98(5):368–72.
23. Mansbach JM, Ginde AA, Camargo CA Jr. Serum 25-hydroxyvitamin D levels among US children aged 1 to 11 Years: do children need more vitamin D?. Pediatrics. 2009;124:1404–10.
24. Khadilkar AV, Chiplonkar SA. Vitamin D supplementation in children: indian perspectives. In: Preedy VR, Srirajaskanthan R,

Patel VB, editors. Handbook of food fortification and health nutrition and health. New York: Humana; 2013. p. 373–81.

25. Babu US, Calvo MS. Modern India and the vitamin D dilemma: evidence for the need of a national food fortification program. Mol Nutr Food Res. 2010;54(8):1134–47.
26. Docio S, Riancho JA, Pres JM, Otmos JA, Amado JA, Gonzalez-Macìas J. Seasonal deficiency of vitamin D in children: a potential target for osteoporosis preventing strategies. J Bone Miner Res. 1998;13:544–8.
27. Atapattu N, Shaw N, Högler W. Relationship between serum 25-hydroxyvitamin D and parathyroid hormone in the search for a biochemical definition of vitamin D deficiency in children. Pediatr Res. 2013;. doi:10.1038/pr.2013.139.
28. Powe CE, Ricciardi C, Berg AH, Erdenesanaa D, Collerone G, Ankers E, et al. Vitamin D-binding protein modifies the vitamin D-bone mineral density relationship. J Bone Miner Res. 2011;26(7):1609–16.
29. Al-oanzi ZH, Tuck SP, Raj N, Harrop JS, Summers GD, Cook DB, et al. Assessment of vitamin D status in male osteoporosis. Clin Chem. 2006;52(2):248–54.