

Regional and seasonal variations in ultraviolet B irradiation and vitamin D synthesis in India

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Abstract

Summary Evaluation of ultraviolet B index (UVBI) and its impact on vitamin D synthesis is important. We observed the maximum UVBI between 11 am and 1 pm. There was no increase in serum 25(OH)D levels following sun exposure during winter as the UVBI was significantly low, emphasizing the need for vitamin D supplementation during these months. **Introduction** The amount of vitamin D3 synthesizing UVB irradiation (290–320 nm) reaching the earth's surface at different altitudes and seasons in different parts of India and its impact on vitamin D synthesis has not been well studied. **Methods** The hourly UVB index (UVBI) from 10 am to 3 pm everyday for 12 months was measured by a solar meter in 4 different zones (North, Northeast, West and South) of the country. To study the impact of sun light exposure on vitamin D

synthesis during winter, healthy school children aged 10–15 years were exposed to sunlight for a period of 30 min per day, between 11 am to 12 noon with 10 % body surface area, for 4 weeks. The main outcome measures were serum 25(OH)D, PTH, calcium, phosphate, and alkaline phosphatase levels before and after sun exposure.

Results The mean UVBI was highest between 11 am and 1 pm throughout the year in all locations. The highest UVBI was recorded from the North zone ($4.5 \pm 2.7 \mu\text{W}/\text{Cm}^2$), while the least was recorded in the Northeast zone ($2.1 \pm 1.2 \mu\text{W}/\text{Cm}^2$). UVBI readings in the Northeast zone were consistently low throughout the year, while all the other three zones showed significant seasonal fluctuations.

Surprisingly, we observed a significant decrease in serum 25(OH)D levels from baseline (6.3 ± 4.6 to $5.1 \pm 2.7 \text{ ng/mL}$; $p < 0.001$) despite sun exposure.

Conclusion The mean UVBI was highest between 11 am and 1 pm throughout the year in all locations. No increase in the serum 25(OH)D levels was observed following sun exposure in winter, emphasizing the need for vitamin D supplementation during these months.

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Keywords India · Ultraviolet B · Vitamin D

Introduction

India is a tropical country extending from 8.4° N to 37.6° N latitude. Majority of the population live in areas of ample sunlight throughout the year, and hence there was disbelief that vitamin D deficiency is uncommon in India. However, published data on bone mineral health from different parts of India have shown widespread vitamin D deficiency (25 (OH) D < 20 ng/mL) in up to 90 % of healthy subjects in all age groups [1–6]. It may be related to poor vitamin D formation,

probably as a result of inadequate sun exposure due to lifestyle modification, crowded houses with limited sun exposure, work culture of staying indoors, dark skin color, atmospheric pollution, and lack of national policy on fortification of foods with vitamin D.

Sun exposure in the present scenario may be able to alleviate the burden of vitamin D deficiency as more than 90 % of endogenous vitamin D is synthesized through solar ultraviolet B (UVB) irradiation of the skin [1, 6]. The maximum conversion of 7-dehydrocholesterol (7-DHC) to previtamin D₃ occurs at about UVB 298 nm with wavelength on either side of 298 nm becoming progressively less efficient in driving the conversion. About 60 % of vitamin D is produced between 290 and 300 nm [7].

We had recently shown that the effective vitamin D forming UVB irradiation in Delhi (28.61° N) is maximum between 11 am and 1 pm during the months of March–June and September–October. Thirty minutes of sun exposure during summer between 11 am and 12 noon with 15–30 % body surface area for a period of 4 weeks is able to increase serum 25(OH)D levels by 3.5 to 4.9 ng/mL [8]. However, no study till date has evaluated the UVB index (UVBI) across the country to see its regional and seasonal variations, before advocating sun exposure.

The present study is conceptualized to quantify the amount of vitamin D₃ synthesizing UVB irradiation reaching earth's surface at different altitudes and seasons in different parts of the country and to study the impact of sun exposure on vitamin D synthesis during winter.

Materials and methods

Measurement of vitamin D₃ synthesizing UVB irradiation

The amount of vitamin D₃ synthesizing UVB irradiation reaching the earth's surface was measured by Solarmeter 6.4 (Solartech Inc, Michigan, USA), specifically standardized to measure only UVB rays of 280–320 nm [9]. Hourly UVBI ($\mu\text{W}/\text{cm}^2$) and weather status were recorded from 10 am to 3 pm everyday for 12 months (March 2014 through February 2015). The UVBI was measured in free field areas at a fixed point in all these centers, with the sensor pointing straight upward toward the sun. Four different zones of the country, South [Madurai (Oddanchatram, Dindigul), Hyderabad], North [Delhi and Leh], Northeast [Guwahati], and West [Pune and Goa], were studied. The seasons were divided into summer (March–June), autumn (July–October), and winter (November–February). The latitude, longitude, altitude, and the distance from the equator of all these centers are depicted in Fig. 1 and Table 1. The central co-coordinating body in the Department of Dermatology of All India Institute of Medical

Sciences, New Delhi, reviewed monthly UVBI data from all the seven regional centers.

Impact of sunlight exposure on vitamin D synthesis during winter

To study the impact of sunlight exposure on vitamin D synthesis during winter, apparently healthy school children (50 boys and 155 girls) in the age group of 10 to 15 years from two schools located in New Delhi were recruited after obtaining permission from the school management. Children from all sections of class 8 from both schools, whose parents gave written consent, were included in the study. Children with any systemic illnesses or those taking medications or supplements that interfere with the evaluation of bone mineral metabolic parameters like anticonvulsants, antitubercular drugs, barbiturates, vitamin D, and steroids were excluded. The institute ethics committee approved the protocol.

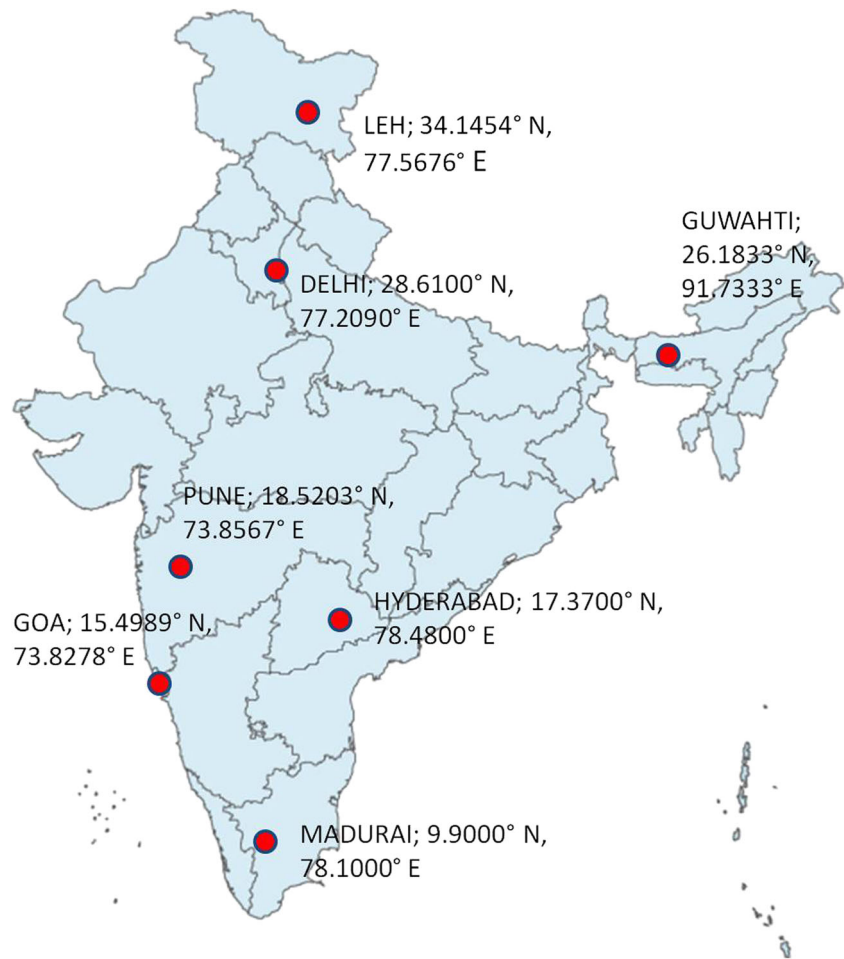
Intervention

Children were allowed to play in an open sunny ground in school during the usual recess for 30 min, between 11 am and 12 noon everyday for 6 days in a week. Participants were instructed to expose themselves at home for a similar duration at the same time on Sundays/holidays. Based on the school uniform, they had approximately 10 % body surface area (hands, neck, and face) exposed. The participants were instructed not to use sunscreen during the study period. UVBI and weather status were recorded everyday for the entire study period. Blood samples collected at baseline and after 4 weeks of sun exposure, were analyzed for serum 25(OH)D and parathyroid hormone (PTH) by Chemiluminescence assay (Diasorin, Stillwater, MN, USA) and calcium, phosphates and alkaline phosphatase by an autoanalyzer.

Statistical analysis

UVBI data were summarized, and average values depicted diagrammatically. Since the maximum UVBI was recorded between 11 am and 1 pm in our earlier report, in the present study, we recorded peak UVBI as a mean of 3 (hourly) readings between 11 am and 1 pm. Generalized estimating equation analysis was used to compare seasonal differences in UVBI. Paired *t* test was used to compare the mean levels of 25(OH)D, PTH, calcium, phosphate, and alkaline phosphatase, before and after sun exposure. *P* value of <0.05 was considered significant. All statistical analysis was implemented on Stata 12.1 (Stata Corp, College Station, TX, USA).

Fig. 1 Map of India depicting the study centers and their coordinates



Results

UVB Index at different geographic locations

The mean UVBI was highest between 11 am and 1 pm throughout the year in all locations as shown Fig. 2. The

highest UVBI was recorded in the north ($4.5 \pm 2.7 \mu\text{W}/\text{cm}^2$), while the least was recorded in the northeast zone ($2.1 \pm 1.2 \mu\text{W}/\text{cm}^2$). South and west zones recorded UVBI of $4.2 \pm 1.7 \mu\text{W}/\text{cm}^2$. The UVBI in the northeast zone was consistently low throughout the year, while the other zones showed significant seasonal fluctuations (Fig. 3 and Table 2).

Table 1 Geographic parameters of different study centers

City	Zone	Latitude	Longitude	Distance from equator	Altitude (MSL)
Goa	West	15.4989° N	73.8278° E	1714 km	+1022 ft
Guwahati	East	26.1833° N	91.7333° E	2915 km	+182 ft
Hyderabad	South	17.3700° N	78.4800° E	1935 km	+1778 ft
Madurai	South	9.9000° N	78.1000° E	1104 km	+331 ft
Leh	North	34.1454° N	77.5676° E	3803 km	+11,562 ft
Delhi	North	28.6100° N	77.2090° E	3190 km	+709 ft
Pune	West	18.5203° N	73.8567° E	2062 km	+1840 ft

MSL mean sea level

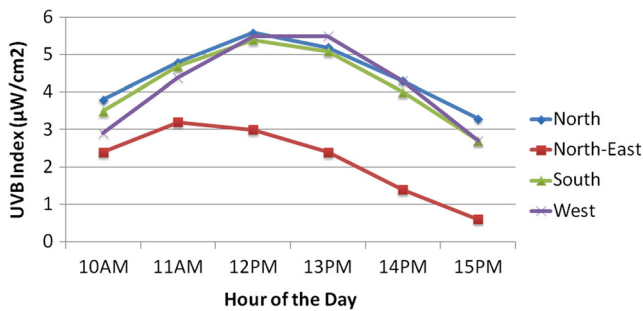


Fig. 2 Hourly pattern of mean UVB index at different geographic locations

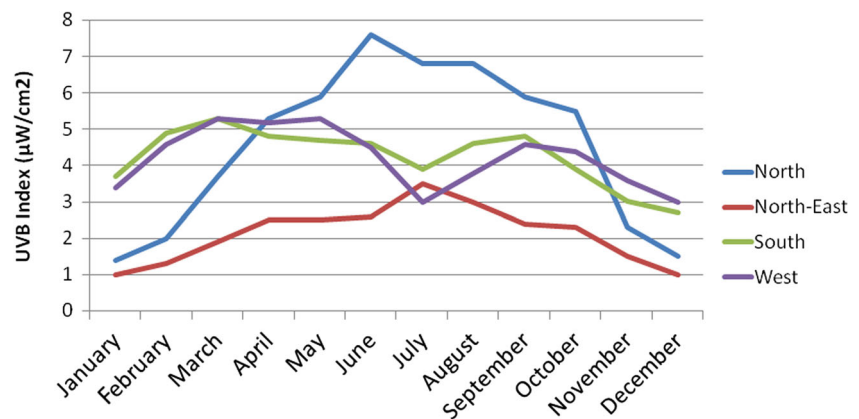
Seasonal differences in UVBI readings

The maximum UVBI was recorded during monsoon/autumn in the north ($6.3 \pm 2.4 \mu\text{W}/\text{Cm}^2$) and northeast ($2.8 \pm 1.3 \mu\text{W}/\text{Cm}^2$) zones, while south ($4.9 \pm 1.3 \mu\text{W}/\text{Cm}^2$) and west ($5.1 \pm 1.3 \mu\text{W}/\text{Cm}^2$) zones recorded maximum UVBI during summer. In contrast, the maximum UVBI was observed in the west zone ($3.6 \pm 1.2 \mu\text{W}/\text{Cm}^2$), and the least UVBI was recorded in northeast zone ($1.2 \pm 0.5 \mu\text{W}/\text{Cm}^2$) during winter. When the peak hour UVBI (11 am–1 pm) was calculated, the trend remained the same in all the zones (Table 3).

Change in serum 25(OH)D levels following exposure in winter

The mean baseline serum 25(OH)D for the intervention group was $6.3 \pm 4.6 \text{ ng/mL}$ with no significant difference between boys and girls ($6.4 \pm 4.7 \text{ ng/mL}$ vs $5.9 \pm 4.0 \text{ ng/mL}$; $P=0.5$). An overall decrease in serum 25(OH)D from 6.3 ± 4.6 to $5.1 \pm 2.7 \text{ ng/mL}$ ($P<0.001$) following sun exposure was primarily because of a significant decrease in girls (girls $-1.7 \pm 3.8 \text{ ng/mL}$ vs boys $+0.1 \pm 1.7 \text{ ng/mL}$, $P=0.006$). Table 4 illustrates the changes in biochemical parameters following sun exposure in the study group.

Fig. 3 Monthly mean UVB Index at different geographic locations



Vitamin D deficiency (serum 25(OH)D $< 20 \text{ ng/mL}$) was observed in 95.6 % (172/180) and secondary hyperparathyroidism (PTH $\geq 65 \text{ pg/mL}$) in 49.2 % (88/179) of study subjects.

Discussion

Vitamin D deficiency is considered a global problem affecting nearly 1 billion people worldwide [10]. It is also a major public health problem in India, needing urgent attention, as it leads to rickets in children and osteomalacia in adults besides being implicated in several systemic illnesses such as diabetes mellitus, autoimmune diseases, cutaneous disorders, and malignancies [1–6]. Hence, maintaining adequate levels of circulating 25(OH)D is crucial for good health.

Dietary measures and supplementation strategies are unlikely to be effective public health measures in India as majority of Indians are vegetarians with a maximum intake of 1.5–2.8 μg of vitamin D daily, and a very small proportion of them consume vitamin D supplements regularly [1, 6, 8]. Exposure to sunlight and food fortification are the only other two ways of dealing with this health issue. Unfortunately, there are still no uniform regulations regarding fortification of food with vitamin D, and therefore, sun exposure may be the only practical solution to this major public health problem at this stage [8].

Solar UVB irradiation is critical for conversion of 7-DHC to previtamin D3. The quantity of UVB irradiation reaching the earth's surface is affected by season (rotation of the earth around the sun), time of the day, and the solar zenith angle. There is paucity of literature with regard to the influence of season, latitude, altitude, and time of the day on the cutaneous production of vitamin D [11–13]. The present study aims to fill this gap partially by evaluating the UVB irradiation at different latitudes during different seasons and time of the day in India. Furthermore, we studied the impact of

Table 2 Monthly mean UVB index ($\mu\text{W}/\text{cm}^2$) at different geographic locations

Zones	January	February	March	April	May	June	July	August	September	October	November	December	Whole Year
North (2 centers)	1.4±0.5 (N=55)	2.0±0.9 (N=47)	3.7±1.2 (N=55)	5.3±1.2 (N=52)	5.9±1.2 (N=56)	7.6±2.0 (N=46)	6.8±2.7 (N=48)	6.8±2.0 (N=54)	5.9±2.3 (N=51)	5.5±2.5 (N=49)	2.3±0.8 (N=52)	1.5±0.5 (N=55)	4.5±2.7 (N=620)
North-East (1 center)	1.0±0.3 (N=31)	1.3±0.3 (N=28)	1.9±0.7 (N=31)	2.5±0.7 (N=30)	2.5±1.1 (N=31)	2.6±1.1 (N=30)	3.5±1.3 (N=31)	3.0±1.3 (N=31)	2.4±1.4 (N=30)	2.3±0.6 (N=31)	1.5±0.7 (N=30)	1.0±0.3 (N=31)	2.1±1.2 (N=365)
South (2 centers)	3.7±1.1 (N=62)	4.9±1.3 (N=55)	5.3±1.3 (N=59)	4.8±0.9 (N=59)	4.7±1.4 (N=62)	4.6±1.4 (N=60)	3.9±1.5 (N=61)	4.6±1.9 (N=62)	4.8±1.8 (N=60)	3.9±2.0 (N=62)	3.0±1.6 (N=59)	2.7±1.3 (N=62)	4.2±1.7 (N=723)
West (2 centers)	3.4±1.2 (N=62)	4.6±1.2 (N=56)	5.3±1.0 (N=62)	5.2±0.8 (N=60)	5.3±1.2 (N=62)	4.5±1.8 (N=60)	3.0±2.2 (N=62)	3.8±2.0 (N=62)	4.6±1.9 (N=60)	4.4±1.6 (N=62)	3.6±0.9 (N=60)	3.0±0.9 (N=62)	4.2±1.7 (N=730)
All Zones (7 centers)	2.6±1.5 (N=210)	3.5±1.8 (N=186)	4.4±1.7 (N=207)	4.7±1.3 (N=201)	4.9±1.7 (N=211)	5.0±2.3 (N=196)	4.2±2.5 (N=202)	4.7±2.3 (N=209)	4.7±2.2 (N=201)	4.2±2.1 (N=204)	2.8±1.3 (N=201)	2.2±1.2 (N=210)	4.0±2.1 (N=2438)

Table 3 Seasonal mean UVB Index ($\mu\text{W}/\text{cm}^2$) at different geographic locations

Zones	Winter overall (10 am–3 pm)	Winter peak hour (11 am–1 pm)	Summer overall (10 am–3 pm)	Summer peak hour (11 am–1 pm)	Rainy/autumn overall (10 am–3 pm)	Rainy/autumn peak hr (11 am–1 pm)	P value
North (2 centers)	1.8±0.8 (N=209)	2.3±0.9 (N=208)	5.5±2.0 (N=209)	6.3±2.1 (N=208)	6.3±2.4 (N=202)	7.1±2.6 (N=199)	<0.001
North-east (1 center)	1.2±0.5 (N=120)	1.6±0.7 (N=120)	2.4±1.0 (N=122)	3.1±1.4 (N=122)	2.8±1.3 (N=123)	3.6±1.7 (N=123)	<0.001
South (2 centers)	3.5±1.5 (N=238)	4.2±1.9 (N=237)	4.9±1.3 (N=240)	5.9±1.6 (N=238)	4.3±1.8 (N=245)	5.1±2.2 (N=242)	<0.001
West (2 centers)	3.6±1.2 (N=240)	4.4±1.5 (N=240)	5.1±1.3 (N=244)	6.1±1.7 (N=244)	3.9±2.0 (N=246)	4.9±2.6 (N=246)	<0.001
P value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	

Table 4 Change in biochemical parameters among school children with sun exposure during winter

Biochemical parameter	Number of children	Pre exposure	Post exposure	<i>P</i>
25(OH)D (ng/mL)	180	6.3±4.6	5.1±2.7	<0.001
PTH (pg/mL)	179	82.1±73.2	77.6±68.6	0.20
Calcium (mg/dl)	190	10.2±0.6	10.0±0.7	0.004
Phosphate (mg/dl)	190	4.1±0.7	4.1±0.6	0.74
ALP (IU/mL)	191	197.5±93.7	214.7±111.0	0.002

sunlight exposure on vitamin D synthesis during winter season.

We observed that UVBI peaked between 11 am and 1 pm in all the zones of the country. Harinarayan et al. reported similar observation in their in vitro study using an ampoule model consisting of 7-DHC at Tirupati in southern India (latitude 13.40° N and longitude 77.2° E) where maximal formation of vitamin D₃ and its photoproducts was between 11 am and 2 pm. They also observed that 28.9 % of 7-DHC was converted to vitamin D₃ at 1 pm with the solar zenith angle of 5° when compared to only 1.3 % conversion of 7-DHC at 9 am when the solar zenith angle was 67° [14]. Terushkin et al. from Miami and Boston also reported highest UVB irradiance between 12 noon and 1 pm, both during summer and winter months [15]. Our observations, together with these studies, suggest that the best time of the day for maximal vitamin D synthesis is between 11 am and 1 pm.

The maximum UVBI was recorded during summer (March to June) and monsoon/autumn (July to October) months followed by winter months (November to February). Similarly, maximum UVB irradiation was observed during summer months (March to June) in our earlier study from Delhi [8]. The fact that maximum UVBI was recorded during both sunny and cloudy days of monsoon/autumn season followed by summer and the least during winter suggests that atmospheric pollution could be responsible for preventing UVB rays from reaching the earth's surface. Similar observation was recorded in an earlier report where the authors noted a significantly lower haze score from a heavily polluted zone of Delhi as compared to a lesser-polluted zone in the periphery of Delhi [16].

The overall trend of UVBI in the four zones evaluated showed that there was a significant increase in the UVBI in the north zone, from March through October followed by a steep decline from November to January. Interestingly, the northeast zone showed the lowest UVBI throughout the year when compared with the other zones without any significant increase in summer.

The lower UVBI in northeast may also be due to the least number of sunny days observed during the study period. However, the south zone showed a constant UVBI throughout the year except in the months of July and August, where there was a slight dip probably due to cloudy and rainy weather as reported. These differences recorded in the UVBI in different zones could possibly be due to differences in altitude, latitude, solar zenith angle, ozone layer, atmospheric pollution, and aerosol layers in these cities [13]. However, this study provides UVBI data (season and zone wise, monthly and hourly) of different zones in India for the first time.

In our earlier study, we have shown a significant increase of 3.4 to 4.9 ng/mL of serum 25(OH)D following 4 weeks of sun exposure with 15–30 % BSA during summer months [8]. In contrast, a significant decline in the serum 25(OH)D levels was observed following 4 weeks of sun exposure of ~10 % BSA during winter months. This was because of very low UVBI recorded during the study period in winter ($2.0 \pm 0.9 \mu\text{W}/\text{Cm}^2$) as compared to that in summer ($5.7 \pm 3.2 \mu\text{W}/\text{Cm}^2$). In winter, with increase in solar zenith angle, the stratospheric ozone absorbs much of the UVB irradiation, and hence, less UVB rays reach the earth's surface. Therefore, any exposure to sunlight during winter may not result in any significant increase in the serum 25(OH)D levels. This is consistent with the observations made by Webb et al. [17] Hence, it would be appropriate to suggest vitamin D supplementation for maintenance of adequate serum 25(OH)D levels during winter months.

The limitations of the study include:

- Impact of atmospheric pollution on UVB rays reaching the earth's surface in different zones could not be studied.
- Measurement of UVBI between 8 and 10 am were not recorded because of administrative reasons.

In conclusion, this communication provides data on UVBI and its seasonal and regional variations in India. The least UVBI recorded during winter months corroborating with no increase in the serum 25(OH)D levels following 4 weeks of sun exposure suggests that vitamin D supplementation is vital during winter months.

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Compliance with ethical standards

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Conflicts of interest None.

References

1. Marwaha RK, Goswami R (2010) Vitamin D deficiency and its health consequences in India. In: Holick MF (ed) *Vitamin D: physiology, molecular biology and clinical applications*, 2nd edn. Springer, New York, pp 529–542
2. Marwaha RK, Tandon N, Reddy D et al (2005) Vitamin D and bone mineral density status of healthy school children in northern India. *Am J Clin Nutr* 82:477–482
3. Puri S, Marwaha RK, Agarwal N et al (2008) Vitamin D status of apparently healthy schoolgirls from two different socioeconomic strata in Delhi: relation to nutrition and lifestyle. *Br J Nutr* 99: 876–882
4. Chouhan K, Sethuraman G, Gupta N et al (2012) Vitamin D deficiency and rickets in children and adolescents with ichthyosiform erythroderma in type IV and V skin. *Br J Dermatol* 166:608–615
5. Sethuraman G, Sreenivas V, Yenamandra VK et al (2015) Threshold levels of 25-hydroxyvitamin D and parathyroid hormone for impaired bone health in children with congenital ichthyosis and type IV and V skin. *Br J Dermatol* 172:208–214
6. Londhey V (2011) Vitamin D, deficiency : Indian scenario. *JAPI* 59:695–696
7. Schmalwieser AW, Schauburger G, Grant WB, et al (2006) A first approach in measuring, modeling, and forecasting the vitamin D effective UV radiation. *Proc. SPIE* 6362, Remote sensing of clouds and the atmosphere XI, 63622C. doi:10.1117/12.689735
8. Marwaha RK, Sreenivas V, Talwar D et al (2015) Impact of solar ultraviolet B radiation (290–320 nm) on vitamin D synthesis in children with type IV and V skin. *Br J Dermatol* 173:604–606
9. Lindgren J, Gehrman WH, Ferguson GW et al (2008) Measuring effective vitamin D₃—producing ultraviolet b radiation using solartech’s Solarmeter® 6.4 handheld, UVB radiometer. *Bull Chicago Herp Soc* 43:57–62
10. Wahl DA, Cooper C, Ebeling PR et al (2012) Global representation of vitamin D status in healthy populations. *Arch Osteoporos* 7:155–172
11. Holick MF, Vitamin D (2007) Deficiency. *New Eng J of Med* 357: 266–281
12. Ginde AA, Liu MC, Camargo CA Jr (2009) Demographic differences and trends of vitamin D insufficiency in the US population, 1988–2004. *Arch Intern Med* 169:626–632
13. Norman AW (1998) Sunlight, season, skin pigmentation, vitamin D, and 25-hydroxyvitamin D: integral components of the vitamin D endocrine system. *Am J Clin Nutr* 67:1108–1110
14. Harinarayan CV, Holick MF, Prasad UV et al (2013) Vitamin D status and sun exposure in India. *Dermato Endocrinol* 5:130–141
15. Terushkin V, Bender A, Psaty EL (2010) Estimated equivalency of vitamin D production from natural sun exposure versus oral vitamin D supplementation across seasons at two US latitudes. *J Am Acad Dermatol* 62:929, e1-9
16. Agarwal KS, Mughal MZ, Upadhyay P, Berry JL, Mawer EB, Puliyl JM (2002) The impact of atmospheric pollution on vitamin D status of infants and toddlers in Delhi, India. *Arch Dis Child* 87: 111–113
17. Webb AR, Kline L, Holick MF (1988) Influence of season and latitude on the cutaneous synthesis of vitamin D₃: exposure to winter sunlight in Boston and Edmonton will not promote vitamin D₃ synthesis in human skin. *J Clin Endocrinol Metab* 67:373–378