RESEARCH PAPER

Seasonal Variation in Serum 25-hydroxy Vitamin D and its Association with Clinical Morbidity in Healthy Infants from Northern India

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Objective: To evaluate the seasonal change in serum 25-hydroxyvitamin D (25-OHD) level in healthy infants and to relate it to common childhood morbidities. **Methods:** 72 healthy breastfed infants residing in Delhi were enrolled at the end of summer and followed till the end of winter [mean (SD) duration 200 (10) d]. Serum 25-OHD was estimated at baseline and follow-up. Infants were monitored for common childhood diseases. **Results:** Mean (SD) serum 25-OHD level was lower at the end of winter (20.7 (8.02) ng/mL) than summer (22.9 (8.70) ng/mL) [mean difference (95% CI) –2.14 ng/mL (–3.36, –1.06), *P*<0.001). The seasonal distribution of children according to vitamin D status in summer and winter - Deficient (15.3%, 12.5%), Insufficient (19.4%, 30.6%) and Sufficient (65.3%, 56.9%), respectively was comparable *P*=0.17). The morbidity profile remained unaffected by change in vitamin D status from summer to winter. **Conclusions:** Seasonal changes in vitamin D levels do not have significant clinical effect or effect on overall vitamin D status in apparently healthy infants from North India. This may have implications for results of population surveys for vitamin D status, irrespective of the season when they are conducted.

Keywords: *Extra-skeletal, Hypovitaminosis D, Outcome, Risk, Summer, Winter*.

Sunlight is the main source of endogenous synthesis of vitamin D in the human body. Vitamin D deficiency remains a widely prevalent disorder in Northern India, a region which lies in the temperate belt of the earth and receives adequate sunlight [1]. Vitamin D levels are reported to be lower during winters [2], postulated due to increasing obliquity (Zenith angle) of sun rays reaching the earth's surface [3]. Seasonal variation may have important implications on prevalence of vitamin D deficiency and cutoffs used to define vitamin D deficiency across different latitudes [4]. People living at northern latitudes have lower vitamin D levels during winters with minimal sunlight exposure, thereby necessitating vitamin D fortification or supplementation during winters [5].

There has been renewed interest in the effect of environment on human health. Global warming is causing climatic changes around the world such that summers have become harsher (leading to less outdoor activity), and winters have become pleasant (allowing more time outdoors) thus affecting duration of exposure to sunlight in each of the seasons [6]. We hypothesized that the seasonal variation in vitamin D status may have declined to an insignificant level, especially in regions with abundant sunshine. The study was conducted to measure the seasonal variation in serum 25-OHD levels in a sample of infants attending immunization clinics of a tertiary-care hospital in Delhi. The secondary objective was to determine whether seasonal change in vitamin D nutriture has any implication for common childhood morbidities.

METHODS

This longitudinal observational study was conducted in the Departments of Pediatrics and Endocrinology at a tertiary-level hospital in Delhi (28.7ºN, 77.1ºE) from May 2016 till April 2017. The study was approved by the Institutional Ethics Committee. Written informed consent was obtained from the parents for participation.

Apparently healthy infants aged 9-10 months were enrolled at the end of peak summer season in Delhi (first two weeks of July) during their measles immunization visit. Infants were considered eligible if they had a birthweight more than 2.5 kg and were born at term gestation, were predominantly breastfed till 6 months of age, and whose families resided within 5 km of the hospital. Any infant with congenital malformations, history of seizures, clinical evidence of rickets, chronic systemic disorder, past hospitalization, or with history of receiving calcium or vitamin D supplements or mega dose (>60000 IU) in last six months was excluded. Infants receiving vitamin D-fortified milk or artificial formula were also excluded.

The relevant maternal details for any antenatal complication and intake of calcium or vitamin D supplements during antenatal period and lactation were recorded at the time of enrolment. The birth details and feeding history were also recorded. Family history of any skeletal disease was noted. Weight, length and head circumference of the infant were measured at baseline and interpreted as per WHO Growth Standards [7].

Baseline venous blood sample (2 mL) was obtained at enrolment; serum was separated by centrifugation and stored at -20°C. The infants were followed up monthly either during outpatient visits or telephonically for next 7 months till completion of winter season (first two weeks of February). During follow up period, any episodes of febrile illness, acute respiratory infection, diarrhea, seizure, or meningitis were recorded. Children were examined for any signs of rickets at baseline and during follow-up based on clinical examination and radiological findings on wrist *X*-ray. Morbidities were defined as follows: *Febrile illness*: Axillary temperature ≤38°C for >3 days [8]; *Diarrhea:* Passage of 3 or more loose or liquid stools per day (or more frequent passage than is normal for the individual) [9]; *Acute respiratory infection:* Fever with cough, with or without fast breathing [10]; *Meningitis:* Acute onset of fever (usually >38.0°C axillary), headache, and one of the following signs: neck stiffness, altered consciousness or other meningeal signs [11].

A second blood sample was collected at the end of 7 months which coincided with the end of winter season and Measles-Mumps-Rubella (MMR) vaccination between 15-18 months of age. Paired serum samples were analyzed for 25-OHD, calcium, phosphorus and alkaline phosphatase. Serum 25-OHD was estimated by Radio immunoassay technique (RIA) with kits manufactured by Beckman Coulter, USA (total imprecision ≥10.0% CV at > 15.0 ng/mL, and total standard deviation (SD) ≥1.5 ng/mL at ≥15.0 ng/mL). Vitamin D status for serum 25-OHD values was interpreted as per the following cut-offs: sufficient >20 ng/mL, insufficient 12-20 ng/mL, and deficient <12 ng/mL [12]. The vitamin D status based on absolute serum 25-OHD values was assessed separately during summer and winter for each infant.

Assuming a 25% drop in serum vitamin D levels during winter from summer levels as clinically significant and mean serum 25-OHD level in Indian infant during summer as 16.96 ng/mL [13] with power of study as 80% and one-tailed α error 0.05, the sample size was calculated as 67 (SD of change is 14 ng/mL). Assuming an attrition rate of 10%, total sample required was 75.

Statistical analyses: Seasonal changes in laboratory parameters were compared with paired t-test. McNemar-Bowker test was applied to compare the vitamin D status between summer and winter. The mean seasonal change in serum 25-OHD between vitamin D deficient, insufficient, and sufficient children was analyzed by Kruskal-Wallis test (for overall difference) followed by pair-wise comparison by Mann-Whitney test for differences between any two categories (with Bonferroni correction); since the change in serum 25-OHD was not normally distributed. For the secondary outcome variable, participants were stratified in three groups according to change in vitamin D status during winter from summer (improved, no change, and reduced). Kruskal-Wallis test was applied to compare the distribution of morbidity episodes among these three groups. P value less than 0.05 was considered as significant.

RESULTS

A total of 98 infants were approached for enrollment, out of which 75 infants (47 boys, mean age 9.3 months) were enrolled. Mean (SD) birthweight was 2.9 (0.25) kg and weight-for-age SDS was 0.59 (0.63). Mothers of 55 (73.3%) infants took antenatal calcium supplementation (500 mg tablet single daily dose; mean (SD) duration of 46.9 (26.3) day). All infants received home based complementary feeds without vitamin D fortification after six months of age. Of these, 72 were available at the end of follow up [mean (SD) duration, 200 (10) d]. History of sun exposure was present in 73 children and two children had no history of regular sun exposure. The duration of sun exposure ranged from 30 min to 3.5 hours per week without any significant seasonal variation (1.52 hr in winters and 1.5 hr in summer). Only 17 (22.7%) families had history of practices promoting sun exposure. Mean (SD) serum 25-OHD levels in summer and winter were 22.9 (8.70) and 20.7 (8.02) ng/mL, respectively [mean difference –2.14 (range -11 to +8 ng/mL) (95% CI: –3.36, –1.06); P<0.001] **(*Fig.* I).** The average drop was 9.34% in winter. Despite this, the distribution of children according to vitamin D status categories (deficient, insufficient, and sufficient) was comparable between summer and winter (P=0.168) (***Table* I**). The mean (SD) change in serum 25-OHD (from summer to winter) among vitamin D deficient, insufficient, and sufficient children was -0.25 (3.69), -0.08 (4.46) and 3.36 (4.6) ng/mL, respectively (P=0.005); among groups, difference was significant between deficient and sufficient children (P=0.03). There was no significant difference in sun exposure behavior between three groups.

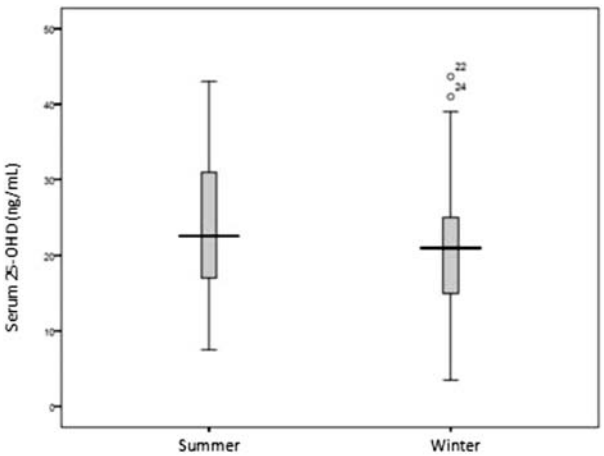

FIG. 1 *Box-Whisker plot of serum 25-OHD (ng/mL) during summer and winter season.*

Mean (SD) serum calcium level was comparable between summer and winter (9.2 (1.41) and 8.9 (1.51) mg/dL, respectively *P*=0.11). Similarly, mean (SD) serum alkaline phosphatase levels in summer and winter were comparable (291.6 (132.8) and 297.4 (145.7) IU, respectively; *P*=0.57). Mean (SD) serum phosphorus level in summer was significantly lower than winter (4.1 (1.21) and 4.7 (1.46) mg/dL, respectively; *P*=0.009).

During follow-up, 69/72 (96%) children had at least one episode of illness and 23 children (31.9%) were hospitalized. The morbidities included: febrile illness (57, 79.2%), acute respiratory infection (49, 68.1%), diarrhea (31, 43.1%), seizures (9,12.5%) and meningitis (2, 2.7%). Additional 10 children became vitamin D insufficient in winter from summer (***Table* I**). Total 18 children changed their vitamin D status categories; 7 improved while 11 shifted to a lower category. There was no significant difference in various morbidities and total morbidities median count among three groups according to change in vitamin D status category (improved, no change, and reduced) between summer and winter (***Table* II**). Serum 25-OHD across either did not have any influence on incidence of morbidity (data not shown).

TABLE I SEASONAL VARIATION IN VITAMIN D STATUS OF INFANTS

Vitamin D status		*Winter*			
		Deficient	*Insufficient*	*Sufficient*	*Total*
Summer	Deficient	8	3	0	11 (15.3%)
	Insufficient	1	9	4	14 (19.4%)
	Sufficient	0	10	37	47 (65.3%)
	Total	9 (12.5%)	22 (30.6%)	41 (56.9%)	72

Vitamin D status: deficient (<12ng/mL), insufficient (12-20 ng/mL),

Four children developed rickets and one developed hypocalcemic seizures during follow-up (mean serum 25-OHD 15.5, 18.0, 20.2 and 12 ng/mL, and 10.8 ng/mL, respectively).

DISCUSSION

We documented lower mean serum 25-OHD levels in winter than summer in healthy infants from northern India. However, this was not associated with any significant change in the overall vitamin D nutritional status of the population, especially among those with borderline or low serum 25-OHD levels. Further, there was no significant association between seasonal change in vitamin D levels with the overall occurrence of extraskeletal childhood morbidities

Higher vitamin D levels during summer have been reported among children and adults in temperate countries; the difference ranging from 5.4 to 15 ng/mL [2,14]. We, in an earlier study, documented a significant correlation of serum vitamin D levels with sunlight exposure in infants [15]. To the contrary, Jain, *et al* [6] did not find significant seasonal difference in infants' serum vitamin D levels between summer and winter. Observations of insignificant seasonal difference or higher winter serum 25-OHD values in young children have been similarly reported by other authors [16]. A longer sun exposure of children during winter than

TABLE II ASSOCIATION OF SEASONAL CHANGE IN VITAMIN D STATUS WITH COMMON MORBIDITIES

Morbidity	*Change in vitamin D status from summer to winter*			*P value*
	Improved (n=7)	*No change (n=54)*	*Reduced (n=11)*	
Febrile illness	1.0 (1.0-1.0)	1.0 (1.0-2.0)	1.0 (1.0-1.0)	0.332
Acute respiratory infection	1.0 (0.0-1.0)	1.0 (0.0-1.25)	1.0 (0.0-1.0)	0.942
Diarrhea	0.0 (0.0-1.0)	0.0 (0.0-1.0)	0.0 (0.0-1.0)	0.246
Total illnesses	1.0 (1.0-2.0)	2.0 (1.0-3.0)	2.0 (1.0-3.0)	0.5

Values represent median number (Inter-quartile range) of episodes of morbidity.

WHAT THIS STUDY ADDS

- There is a small seasonal variation in serum 25 OHD in Northern India which neither affects the overall vitamin D status of the infants nor relates to common childhood illnesses.

summer due to harsher summer temperatures was postulated to increase winter vitamin D levels [6]. The winter serum 25-OHD levels were only marginally lower than summer levels in the present study. It is possible that harsher summers in Delhi (maximum temperature 46ºC) resulted in avoidance of sunlight exposure by the participants leading to only a small difference in vitamin D levels between summer and winter despite Delhi lying in a temperate zone. This may also support the higher summer vitamin D seen in temperate countries which receive minimal sunlight during winters [2,14]. It was interesting to note that vitamin D deficient children showed little seasonal variation as compared to those with vitamin D sufficiency. It is probable that low serum 25-OHD levels in the former group did not reach statistical significance and that factors other than sunlight exposure maybe more important in maintaining vitamin D status, following a compromise in the body vitamin D status. It is possible that compensatory mechanisms such as increased gastrointestinal absorption of vitamin D are more active in vitamin-D deficient children, which prevent further reduction in vitamin D status of the body following a reduction in sunlight exposure that might have happened in winter.

We did not observe any association between seasonal variation in vitamin D nutriture and common extra-skeletal childhood morbidities, like earlier reports [17,18]. Observational studies have reported an inverse association between serum vitamin D levels and risk of childhood infections [19,20]. However, supplementation of vitamin D did not significantly improve disease outcomes in clinical trials [21].

Our study had some limitations like lack of an objective record of sun-exposure, lack of availability of maternal vitamin D levels, and non-availability of spectroscopy for vitamin D estimation. The sample size for subgroup analysis, for childhood morbidities based on vitamin D levels was also small.

To conclude, the present study reported a slightly lower winter level of serum vitamin D among Indian infants than in summer. The change was not associated with common extraskeletal morbidities, thereby lacking clinico-epidemiological relevance. These results have important implications when considering season as a confounding factor in population surveys of vitamin D status. As the vitamin D status categories do not change much during summer and winter, the surveys to estimate prevalence of vitamin D deficiency should remain valid, irrespective of season in which they are conducted. On the other hand, seasonality may be of importance in children having normal vitamin D status, and studies to estimate normal levels of vitamin D among healthy children should consider the season of data collection.

Contributors: PG,DS.AD,RD,DS,RKM, study was conceived; SVM: contributed to the study design and writing the proposal for research; RD: data collection was handled; AD, DS, SVM and PG. SVM: data collection was handled, supervised and also supervised the laboratory work"up of vitamin D status; RKM,PG: statistical analysis was carried; AD,RD,PG: literature search was conducted; RD,AD: initial draft of the manuscript was written; PG,DS,SVM,RKM: initial draft of the manuscript was written which was edited and refined by provided critical inputs to the draft manuscript. The manuscript was seen and approved by all authors.

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