Vitamin D deficiency prevalence among healthy term infants at the age of 4–6 months: A cross-sectional observational study from tertiary care hospital setting

Uma Devi Karuru[1], Bhavneet Bharti[2], Sanjay Verma[2], Naresh Sachdeva[3]

From [1]Junior Resident, [2]Professor, Department of Pediatrics, Advanced Pediatrics Centre, Postgraduate Institute of Medical Education and Research, [3]Professor, Department of Endocrinology, Postgraduate Institute of Medical Education and Research, Chandigarh, India

ABSTRACT

Background: Role of Vitamin D has expanded from bone health to a myriad of physiological and pathological conditions in humans. Although guidelines recommend supplementation of Vitamin D in infants, evidence for healthy term infants at 4–6 months is limited in Indian settings. **Objectives:** The objectives of the study were to estimate the prevalence of Vitamin D deficiency and its risk factor in healthy term infants at 4–6 months of age before initiation of complementary feeding. **Materials and Methods:** Four–six-month-old infants ($n = 93$) were enrolled. The healthy term infants >37 weeks, birth weight >2.5 kg with no diagnosed medical disorders in the mother or baby, and not on Vitamin D supplementation were included. Levels of calcium, phosphorus, alkaline phosphatase, parathormone, and Vitamin D were also assessed. **Results:** The prevalence of Vitamin D deficiency (25OHD ≤ 20 ng/ml) and insufficiency (20–30 ng/ml) was 76.3% and 15.05%, respectively, with a mean (SD) 25(OH) D level of 13.42 ± 10.08 ng/ml and median level of 11.58 ng/ml. On multivariate analysis, rural children continued to have lower odds of severe Vitamin D deficiency (≤ 5 ng/ml) as compared to urban children (OR 0.18; 95% CI: 0.03–0.92) ($P = 0.04$). In addition, odds of severe Vitamin D deficiency (≤5 ng/ml) increased significantly by 20% for everyone unit increase in socioeconomic score by Kuppuswamy (modified) (OR 1.20; 95% CI: 1.04–1.40, $P = 0.01$). **Conclusions:** High level of Vitamin D insufficiency in our study among apparently healthy infants with uncomplicated antenatal and neonatal period clearly supports current guidelines for daily supplementation of Vitamin D for infants and children for optimizing their Vitamin D levels and preventing complications.

Key words: 25(OH)D, Deficiency, Infant, Prevalence, Vitamin D

Vitamin D has harnessed vast attention in medical research as well as clinical practice for the past few decades [1-3]. Role of Vitamin D has indeed expanded from bone health to a myriad of physiological as well as pathological conditions in humans. Classic childhood signature statement of Vitamin D deficiency (VDD) is a disorder called rickets which disproportionately targets growing bones [4-7]. Vitamin D deficiency with a resurgence of rickets is increasingly being reported in infants and toddlers from various parts of the world, especially from temperate regions and among African-American and Indian children [2-5]. A series of studies from different parts of our country have also pointed towards widespread VDD in Asian Indians of all age groups including neonates, toddlers, schoolchildren, pregnant women, and adults [8-11]. Most studies published from India showing wide ranging VDD (75–90%) had mainly focused on pregnant women, newborn babies, schoolchildren, and adolescents [12-14].

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Although Indian Academy of Pediatrics “Guideline for Vitamin D and Calcium in Children” Committee has recommended daily Vitamin D supplementation of 400 IU for infants beyond neonatal period [15], studies evaluating Vitamin D levels of infants before the initiation of complementary feeding are limited [16]. In a study by Seth *et al*., Vitamin D levels of exclusively breastfed infants and their mothers (n=180) were studied, but the age of the group of 4–6 months infants was less than one-fourth of the total study cohort and they did not study infants on breast milk substitutes [16]. In this backdrop, our study aimed to estimate prevalence of Vitamin D deficiency in healthy infants at 4–6 months of age (a period immediately before initiation of complementary feeding) attending tertiary care center for routine OPD consultations. In addition, we explored the demographic, anthropometric, and feeding related factors associated with Vitamin D deficiency in these young infants.

MATERIALS AND METHODS

We carried out a cross-sectional survey in pediatric outpatient department of a tertiary care North Indian hospital between April

Correspondence to: Bhavneet Bharti, Department of Pediatrics, Advanced Pediatrics Centre, Postgraduate Institute of Medical Education and Research, Chandigarh - 160 012, India. E-mail: bhavneetsahul@gmail.com

and June 2013. The study was approved by the Institutional Ethics Committee. All eligible infants (*n* = 93) in the age range of 4–6 months were enrolled consecutively after taking written informed consent from their caregivers. Parents or caregivers of infants reporting to the outpatient department for their 3rd DPT vaccination were contacted and advised to report for nutritional assessment as well as their serum Vitamin D levels between 4 and 6 months of age and their visits were also coupled to nutritional counseling regarding complementary feeding.

A preformed structured questionnaire was used to elicit sociodemographic and clinical information. Majority of enrolled children belonged to Chandigarh tricity (include adjoining districts of Panchkula, Haryana and Mohali, Punjab). For classification of Vitamin D levels, we used criteria given by the US Endocrine Society and also endorsed by American Academy of Pediatrics. The sample size of 90 infants was calculated for an anticipated prevalence of Vitamin D deficiency of 75% to fall within 7.5% of the true prevalence with 95% confidence.

The inclusion criteria were as follows: Healthy term infants >37 weeks, birth weight more than 2.5 kg with no diagnosed medical disorders in the mother or baby. Infants receiving Vitamin D supplementation or formula feeds at the time of enrolment were excluded. Routinely well-calibrated scales (Seca Digital Baby Scale Model no. 334) were used to record infant's birth weight and length that measure weight to within 5 g and length to 2 mm, respectively.

Level of total Vitamin D [Vitamin D_3 25(OH) and Vitamin D_2 25(OH)] was assessed using electrochemiluminescence assay (ECLIA) on Elecsys 2010 analyzer using specific kits (Roche Diagnostics, Germany). The serum 25(OH)D levels were examined, which is the major circulating form with a half-life of 2–3 weeks, thus proposed to be the most reliable indicator of Vitamin D adequacy as it reflects both cutaneous synthesis and absorption from dietary sources. The sensitivity of the assay was 1.5 ng/ml with an intra-assay CV of 5.6% at 15.9 ng/ml and 11.6% at 58.9 ng/ml. The interassay CV at these two levels was 9 and 12%, respectively.

Quantitative variables were reported as measures of central tendency and dispersion while qualitative variables were described in proportions. Univariate analyses included comparison of quantitative outcomes using independent samples t-test and one-way ANOVA, whereas comparison of categorical variables was carried out using Chi-square test. Spearman correlation coefficients were also estimated between Vitamin D levels and other continuous covariates. Factors associated with infants' serum 25(OH) D concentration were further subjected to both univariate and multivariate regression analyses. All analyses were two tailed and level of significance was taken as 5%. Data analysis was done by Stata statistical software (version 12, IC).

RESULTS

Healthy term infants in the age group of 4–6 months were consecutively enrolled when they reported to outpatient department. The sociodemographic characteristics of the enrolled infants and their parents are described in Table 1.

More than half of the mothers 55 (59%) enrolled, belonged to Hindu middle-income families and resided in urban plains. All the mothers habitually wore clothing that allowed exposure of head, face, and arms and did not practice "purdah" or used sunscreens. Out of 93 mothers enrolled, 51 (55%) of them were primigravidas. During their antenatal period, 88 (94.6%) mothers reported calcium and Vitamin D supplementation during their pregnancy containing 500 mg of calcium and 250 IU of Vitamin D3, once or twice per day in pregnancy. This was continued postnatally for varying periods of time. Approximately two-thirds (64.5%) of the mothers exclusively breastfed while the remaining gave breast milk substitutes such as cow or buffalo milk 1–4 times/day. No mother was giving formula milk fortified with Vitamin D. Various dietary habits and lifestyle characteristics are also described in Table 1.

As the study included healthy infants, none of the enrolled infants had frank features of rickets. However, frontal bossing was observed in 81% (*n* = 76) and Harrison's sulcus in 27% (*n* = 25). Mean calcium and phosphate concentrations were normal in infants. However, the mean concentration of ALP was markedly raised above the upper limits of normal in study infants [Table 2].

The prevalence rates of Vitamin D deficiency and insufficiency in infants were 76.3% and 15.05%, respectively, with mean (SD) level of 13.42 ± 10.08 ng/ml. The median 25(OH) D level among these infants was 11.58 ng/ml. The distribution of serum 25(OH) D levels in study infants is shown in Fig. 1. The prevalence of Vitamin D deficiency in current literature is marked by variability of underlying cutoffs. Table 2 describes prevalence of Vitamin D inadequacy based on different cutoffs used in literature. In addition, prevalence rates of hypocalcemia and hyperparathyroidism using cutoffs established in our institute are also shown. Notably, severe Vitamin D deficiency characterized by 25(OH) D level of < 5 ng/ml marked one-quarter of our total study participants.

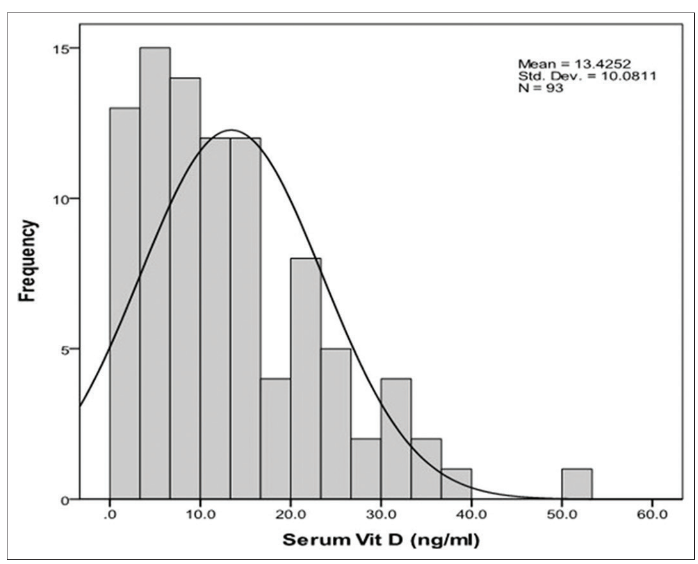

Figure 1: Distribution of serum 25(OH) Vitamin D levels of the study participants

Table 1: Sociodemographic and lifestyle characteristics of the study cohort (n=93)

Category	n (%)
Mean age ± SD (months)	4.52±0.71
Sex – Male:female	64 (68.8):29 (31.2)
Residence – Rural:urban	37 (39.8):56 (60.2)
Place of living – Hilly areas:plains	8 (8.6):85 (91.4)
Religion – Hindu/Muslims/Sikh	55 (59.1)/02 (2.2)/36 (38.7)
Family type – Nuclear:combined	15 (16.1):78 (83.9)
Socioeconomic category*: Upper/ Middle/Lower	16 (17.2)/73 (78.5)/4 (4.3)
Income (Mean ± SD)(in rupees)	20107.53±17612.8
Father's education	
Profession/graduate or post-graduate	39 (41.9)
Intermediate	29 (31.2)
High school/middle school	20 (21.5)
Mother's education	
Illiterate	05 (5.4)
Profession/graduate or postgraduate	43 (46.9)
Intermediate	27 (29)
Primary to high school	17 (18.3)
Illiterate	6 (6.5)
Father's occupation	
Profession	
Semi-profession	8 (8.6)
Clerical/shop/business/farmer	19 (20.4)
Skilled	48 (51.6)
Unskilled worker	18 (19.4)
Mother's occupation	
Profession/semi-profession	8 (8.6)
Shop/business	1 (1.1)
Housewife	84 (90.3)
Maternal age (years; Mean ± SD)	27.72±4.256
Maternal BMI (kg/m^2)	23.73±4.58
Does sunlight reaches at your home – Yes	92 (98.9)
Skin color – Dark:wheatish	8 (8.6):85 (91.4)
Maternal diet – Vegetarian:non-vegetarian	66 (79):27 (21)
Maternal milk intake – Everyday	**77** (82.8)
Sometimes	5 (5.4)
Rarely	11 (11.8)
Mother spent time in sunlight/day <3 h:> 3 h	64 (68.9):29 (31.2)
Baby exposed to sunlight/day <1 h:>1 h	70 (75.3):23 (24.8)
Maternal use of sunscreens	
Yes	8 (8.6)
No	85 (91.4)
Exclusive breastfeeding	
Yes	60 (64.5)
No	33 (35.5)
Calcium intake during pregnancy	
Yes	88 (94.6)
No	5 (5.4)

*As per Modified Kuppuswamy classification

Using a liberal cutoff of > 30 ng/ml as normal, only 8.6% of study infants qualified as Vitamin D sufficient [Table 2].

Table 2: Clinical and biochemical characteristics of the enrolled infants

Parameter	Mean ± SD	95%CI
Quantitative outcomes		
Current weight (kg)	6.39±1.09	6.14–6.63
Wt for age (Z score)	−0.93±1.23	−1.28–−0.67
Wt for length (Z score)	0.23±1.52	−0.82–0.54
Length for age (Z score)	−1.52±1.24	−1.77–−1.26
Head circumference for age (Z score)	−0.89±1.24	−1.15–0.64
Serum calcium (mg/dl)	9.65±0.93	9.46–9.84
Serum phosphorus (mg/dl)	5.70±1.03	5.47–5.92
Serum ALP (U/L)	428.79±184.74	390.53–467.05
Serum 25OH D (ng/ml)	13.42±10.08	11.34–15.50
Serum PTH (pg/ml)	66.74±86.25	48.25–85.24
Categorical outcomes		
Vitamin D levels	n (%)	95%CI
≤ 5 ng/ml	23 (24.7)	15.8–33.7
≤10 ng/ml	42 (45.2)	35.2–55.5
≤12 ng/ml	48 (51.6)	41.3–61.7
≤20 ng/ml	71 (76.3)	67.5–85.1
≤30 ng/ml	85 (91.4)	83.5–95.7
15–20 ng/ml	9 (9.68)	3.56–15.8
20–30 ng/ml	14 (15.05)	7.65–22.46
>20 ng/ml	22 (23.66)	14.86–32.46
>30 ng/ml	8 (8.6)	2.80–14.40
Hypocalcemia (<9 mg/dl)	15 (16.13)	8.51–23.74
Raised ALP (>420 U/l)	36 (39.13)	28.97–49.29
Hyperparathyroidism (>46 pg/ml)	30 (34.88)	24.60–45.16

Comparison of 25 (OH) Vitamin D levels was also carried out after stratifying study cohort into various demographic and known risk factors [Table 3].

Socioeconomic score was also inversely correlated with serum Vitamin D levels using Spearman's correlation coefficient ($r = -0.23$) and it was statistically significant ($P = 0.03$). We also dichotomized serum PTH levels into normal (≤46 pg/ml) versus raised (>46 pg/ml) and compared them with binary variable of Vitamin D deficiency (≤20 ng/ml vs. 20 ng/ml). Out of 65 subjects with low Vitamin D levels (≤20 ng/ml), 43% ($n = 28$) of infants showed secondary hyperparathyroidism (with serum PTH > 46 pg/ml), whereas in Vitamin D non-deficient group, only 9.52% (2/9) showed secondary hyperthyroidism. This difference was statistically significant ($P = 0.005$). The odds of secondary hyperparathyroidism were 7.19 higher in Vitamin D deficiency group (≤20 ng/ml) (OR 7.19; 95% CI: 1.50–67.49) which was statistically significant ($P = 0.005$).

A multivariate logistic regression was carried out to find out independent predictive abilities of various risk factors (significant on univariate analysis) in predicting severe Vitamin D deficiency. A model with factors of residential background, SES score, religion, education, and occupation of mother was statistically significant (Pseudo R-square 24.44%) [Table 4].

Table 3: Univariate comparison of Vitamin D levels stratified by various demographic and risk factors

Variable	n (%)	Mean±SD 25(OH)D (ng/ml)	P-value
Rural	37 (39.8)	17.65±10.92	<0.01
Urban	56 (60.2)	10.63±8.48	
Mean difference (95%CI)		−7.02 (−11.03–−3.01)	
Hilly	8 (8.6)	14.01±5.64	0.31
Plains	85 (91.4)	13.37±10.42	
Mean difference (95%CI)		−0.64 (−0.68–−8.09)	
Birth weight <3 kg	51 (54.84)	13.57±9.10	0.56
>3 kg	42 (45.16)	13.25±11.27	
Mean difference (95%CI)		−0.33(−3.87–4.52)	
Religion			0.02
Hindu	55 (59.14)	11.44±9.53	
Sikhs+Muslim (36+2)	38 (40.86)	16.30±10.28	
Mean difference (95%CI)		−4.87 (−8.99–−0.74)	
Sunlight exposure <1 h	70 (75.26)	13.31±10.04	0.85
>1 h	23 (24.73)	13.77±10.41	
Mean difference (95%CI)		−0.46 (−5.29–4.38)	
Mother's milk intake – Daily	77 (82.8)	13.83±10.51	0.40
Sometimes	16 (17.2)	11.48±07.65	
Mean difference (95%CI)		2.34 (−3.16–7.86)	
Mothers working status			0.09
Working	12 (12.9)	8.78±5.25	
Non-working	81 (87.1)	14.11±10.46	
Mean difference (95%CI)		−5.33 (−11.46–0.80)	
Maternal education			0.06
≤6th class	22 (23.7)	16.92±10.50	
>6th class	71 (76.3)	12.34±9.77	
Mean difference (95%CI)		4.58(−0.24–9.40)	
Gender			0.84
Boy	64 (68.8)	13.56±10.22	
Girl	29 (31.2)	13.12±9.93	
Mean difference (95% CI)		0.44 (−4.06–4.95)	
Exclusive breast fed	60 (64.5)	13.34±10.08	0.91
Mixed feed	33 (35.5)	13.57±10.24	
Mean difference (95%CI)		−0.23 (−4.59–4.14)	

Table 4: Multivariate logistic regression analysis for the outcome of severe Vitamin D deficiency (≤5 ng/ml)

Predictor	Reference group	Odds ratio	95% CI for OR	P-value
Background				
Rural	Urban	0.18	0.03 to 0.92	0.04
Kuppuswamy score		1.20	1.04-1.40	0.01
Religion				
Non-Hindu (Sikhs mainly)	Hindu	0.31	0.08-1.18	0.09
Mother education				
>6th class	≤6th class	1.34	0.22 to 8.06	0.74
Mother occupation				
Homemaker	Working	3.31	0.51 to 21.57	0.21

Rural children continued to have lower odds of Vitamin D deficiency as compared to urban children (OR 0.18; 95% CI: 0.03–0.92) ($P = 0.04$). There was a statistically significant relationship between severe Vitamin D deficiency and socioeconomic status measured on a continuous scale by Kuppuswamy (modified) (OR 1.20; 95% CI: 1.04–1.40, $P = 0.01$). For everyone unit increase in modified Kuppuswamy socioeconomic score, odds of sever Vitamin D deficiency (≤ 5 ng/ml) increased by 20%.

DISCUSSION

Vitamin D deficiency offers a paradoxical niche in countries like India where there is abundance of sunlight due to its tropical location and favorable latitudes promoting Vitamin D production on one side and sociocultural practices of dressing, covering of exposed body parts, poor dietary intake, and dark-colored skin inhibiting Vitamin D production on the other hand.

In our study of 93 infants between the age of 4 and 6 months, we found the prevalence of Vitamin D deficiency (<20 ng/ml) as 76.6% and Vitamin D insufficiency (20–30 ng/ml) as 15%. This is consistent with studies from Mediterranean, Middle East, and South Asian countries [6,9,17-19]. In the Indian context, a study by Jain *et al*. among healthy term breastfed infants aged 2.5 to 3.5 months old showed 66.7% prevalence of Vitamin D deficiency (<15 ng/ml) [8]. This finding closely mirrored our study results where serum Vitamin D levels <15 ng/ml were also seen in 65.6% of infants. Another study from Chandigarh by Angurana *et al*. involving children 3 months–12 years of age showed that 40.25% of children were Vitamin D deficient (<20 ng/ml) which was lower than our reported prevalence [20]. In contrast, two studies from Delhi reported lower prevalence of Vitamin D deficiency than our study [21,22]. A study by Ray *et al*. enrolled 72 infants aged 9–10 months who were term healthy and breastfed till 6 months. Vitamin D deficiency (<20 ng/ml) was observed only in 34.7% of infants [21]. Similarly, a study by Chowdhury *et al*. among 6–30 months old infants from New Delhi, the prevalence of Vitamin D deficiency (<10 ng/ml) was seen in 34.5% of cases which is again lower than the 45.2% observed in our study [22].

The prevalence of Vitamin D deficiency in current literature is marked by variability of underlying cutoff values and analytical methods used for the assessment of Vitamin D levels making it difficult to make direct comparisons between studies [10,23,24]. Two concentrations of 25 (OH) D are most commonly used to define Vitamin D insufficiency which are <30 or <20 ng/ml. Revised Institute of Medicine (IOM) guidelines in 2011 proposed that Vitamin D concentrations of ≥20 ng/mL cover the requirement of 97.5th percentile of the population [10,20]. In addition, US Endocrine Society Clinical Practice Guidelines determined Vitamin D deficiency as 25(OH) D below 20 ng/ml and insufficiency as 21–29 ng/ml [14]. Using the cutoff points defined by US Endocrine Society's guidelines, we found that overall prevalence of Vitamin D deficiency (<20 ng/ml) in study infants between the age of 4 and 6 months was 76.6% and Vitamin D insufficiency (20–30 ng/ml) was 15%.

We studied various other clinical and biochemical correlates of Vitamin D deficiency in our study cohort. Infants from rural background had significantly better Vitamin D adequacy as compared to urban born infants and this observation is well supported in literature where rural background has been shown as a protective factor for higher Vitamin D levels as compared to urban residential settings [11,19]. However, similar to a study by Kapil *et al*., there was no significant difference in mean serum 25(OH) D levels between hilly and plain regions among our study participants [25].

American Academy of Pediatrics already recommends supplementation of the exclusively breast fed infants with 400 I U daily within a few days of birth [2]. Only infants receiving human milk substitute as infant formula receive adequate Vitamin D if they consume at least 1 L a day. This, indeed, is possible beyond first month of life as the existing infant formulas in the market provide 400IU/L. Indeed, infants on human milk substitutes in our study cohort (35.5% $n = 33$) received only unfortified animal milk (cow and buffalo milk) and there was no difference in their mean Vitamin D levels. This finding emphasizes the need for Vitamin D supplementation even among infants on cow/buffalo milk which lack the fortification of formula milks.

Importantly, the high magnitude of Vitamin D deficiency shown in our cohort of healthy term infants echoes the need for formulating a policy of Vitamin D supplementation to achieve substantial progress toward improving infant nutritional Vitamin D status [15]. Nevertheless, the study has few limitations which include failure to calibrate Vitamin D levels of infants with their maternal values. Seasonal influence was not considered, as all our samples were drawn only during summer months, that is, April–June. However, in a study by Ray *et al*., among 9–10 months old infants, seasonal prevalence of Vitamin D deficiency showed no significant difference. Furthermore, we did not evaluate bone mineral content or quantify sunlight exposure in terms of sun index. No questionnaires were used for outdoor activity of mothers or their infants. Skin color too was not measured using the cosmetic Color ruler which has 16 points ranging from 1 (lightest) to 16 (darkest) which could have allowed more objectivity into the study of factors responsible for hypovitaminosis D.

CONCLUSIONS

Our cross-sectional study evaluated Vitamin D level adequacy of apparently healthy babies, aged 4–6 months born at term. The age group of 4–6 months was specifically targeted so as to identify subsequent complementary feeding preparedness in terms of micronutrient supplementation in Indian setting. High level of Vitamin D insufficiency in our study among apparently healthy infants with uncomplicated antenatal and neonatal period clearly supports current guidelines for daily supplementation of Vitamin D for infants and children for optimizing their Vitamin D levels and preventing complications.

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