Original Article

Vitamin D Status in Infants during the First 9 Months of Age and its Effect on Growth and Other Biochemical Markers: A Prospective Cohort Study

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Abstract

Background: Despite food fortification policies in many countries and recommendations for Vitamin D supplementation of at-risk groups, Vitamin D deficiency (VDD) and infantile rickets remain major public health challenges in many developed and developing countries. **Materials and Methods:** Ninety-six participants at birth were enrolled and followed up until 9 months of age. Serum 25OHD was estimated in cord blood at birth and at 14 ± 1 weeks of life. Seventy-seven participants were followed up at 9 months for estimation of serum 25OHD, parathyroid hormone (PTH), alkaline phosphatase (ALP), calcium, and phosphorus. VDD was defined as serum 25OHD <15 ng/mL as per USIOM guidelines. **Results:** Serum 25OHD levels at 9 months of age (15.78 ± 8.97 ng/mL) were significantly increased in comparison to the level of 3 months of age (14.04 ± 7.10 ng/mL) and at birth (8.94 ± 2.24 ng/mL). At birth, all the participants (77) were deficient in 25OHD levels. It was found that 16/94 (17%) and 19/77 (24.7%) participants at 3 and 9 months of age, respectively, became Vitamin D sufficient without any Vitamin D supplementation. There was a significant inverse correlation between serum 25OHD and PTH concentration ($r = -0.522$, $P < 0.001$), serum 25OHD and ALP ($r = -0.501$, $P < 0.001$). It was found that reduction in serum Vitamin D level to <10.25 ng/mL results in a surge of serum PTH. **Conclusion:** VDD is common from birth to 9 months of age but incidence decreases spontaneously even without supplementation. Furthermore, a large number of babies may be falsely labeled as Vitamin D deficient with currently followed cutoffs. Hence a new cutoff for VDD needs to be established for neonates and infants.

Keywords: *Infants, parathyroid hormone, serum 25OHD, Vitamin D*

Introduction

Vitamin D deficiency (VDD) in the neonatal and pediatric age groups is being increasingly documented worldwide including the Indian subcontinent.[1-4] Such prevalence of VDD is unexpected in a tropical country such as India, where there is the abundant overhead sun for most of the year and infants are capable of producing all the Vitamin D they need in their skin during casual exposure to sunlight.[1] Researchers have attempted to categorize serum 25OHD concentrations as sufficient, insufficient, or deficient based on functional outcomes related to bone health. However, the scientific community has not reached consensus on the cutoff levels of Vitamin D to define its insufficiency. The cutoff used in USIOM classification was recommended on the basis of available evidence that serum parathyroid hormone (PTH) values decrease to a nadir at different levels of serum 25OHD, ranging from 15 to 50 ng/mL. On this basis, they suggested that the VDD should be defined as level <15 ng/mL. Although the USIOM guidelines for VDD are predominantly for adults, they have been extrapolated for the paediatric population as well. However, this classification can potentially lead to overdiagnosis of VDD and can unduly label the children or infants as Vitamin D deficient. The demarcation line between sufficient and insufficient Vitamin D status is even more ill-defined in the paediatric age group. Although many studies have documented a high prevalence of VDD at birth, there is no data on prospective follow-up of Vitamin D levels from birth to infancy. Hence, a new classification might be desirable to define Vitamin D insufficiency and deficiency in infant and paediatric age groups. Hence, we planned this prospective cohort study in term

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newborn babies delivered in our hospital, intending to study the incidence of VDD at birth in our geographical area and follow them till 9–12 months without supplementation of age to see its natural course in the 1st year of life without routine Vitamin D supplementation. We also wanted to determine the level of Vitamin D, which triggers the physiological PTH axis of the body to differentiate truly deficient from insufficient or sufficient Vitamin D status and use them to formulate a new classification for VDD.

Materials and Methods

This study was initiated after clearance from the institutional ethical committee as a prospective cohort study. Participants were full-term babies born in SVBP Hospital and they were followed at 14 ± 1 weeks and 9 months of age. An informed, written consent was obtained from parents of eligible mothers before delivery. The patient's records were kept confidential and anonymous. Babies whose parents opted to withdraw consent during the study continued to receive standard of care as per the hospital practice. As per our national program recommendation, no Vitamin D supplementation was given to the babies in the study period. As per protocol, babies were followed up at 14 ± 1 weeks and 9 months of postnatal age. We made telephonic contacts to all parents and reminded them about routine immunization of baby at 14 ± 1 weeks of age, 9 months of age and visit to the primary investigator in the hospital. We assessed the feeding pattern of the infants and enquired about any illness to build a rapport with parents and gain their confidence. Serum sample of cord blood of term neonates was collected in dark-colored vials for storage at −20°C. The second sample was collected at 14 ± 1 weeks of postnatal age on follow-up. Both samples were analyzed simultaneously for Vitamin D levels using chemiluminescence immunoassays (fully-automated, chemiluminescence liaison 25OHD assay system, DiaSorin Corporation, USA). The data of Vitamin D level at birth and 14 ± 1 weeks of age were kept confidential with the laboratory and not disclosed. The third sample was collected at 9 months of age on follow-up along with clinical and anthropometrical evaluation. The sample taken at 9 months was analyzed for Vitamin D and intact PTH level by using chemiluminescence immunoassays (fully-automated chemiluminescence, Architect I 1000 TM SR, a new chemiluminescent immunoassay analyzer, Abbott Laboratories, USA).

VDD was defined as serum 25OHD <15 ng/mL, severe VDD as 25OHD <5 ng/mL, and insufficiency as 25OHD 15–20 ng/mL, as per recommendations of Misra *et al*.[5] PTH was said to be raised significantly if its level was >45 pg/mL.[3,6]

Statistical analysis

Data were entered in the microsoft excel worksheet and analyzed using R Core Team (2013) (R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria). Variables were described by mean (± standard deviation [SD]) and median (interquartile range). We used nonparametric Spearman's correlation for comparison of analyses, value of $P < 0.05$ was considered statistically significant.

Results

We enrolled 96 babies at birth, of which two mothers refused for further participation in the study, and thus, 94 infants were available for follow-up at 14 ± 1 weeks and 77 infants at 9 months of age. At the final follow-up, the infants were 9.08 ± 0.1 months of age. Baseline and feeding characteristics are mentioned in Table 1. All babies were Vitamin D deficient at birth with a mean (SD) of 8.94 ± 2.2. Severe VDD was found in two infants at birth. Although the increase in mean Vitamin D level from birth to 3 months was statistically significant, the increase from 3 months to 9 months was not statistically significant [Table 2]. At 14 ± 1 weeks of age, it was found that 16 (17%) participants became Vitamin D sufficient, 78 (83%) remained insufficient with none of them in the severely deficient group without receiving any Vitamin D supplementation. On further follow-up at 9 months of age, we found that 19 (24.7%) participants became sufficient and 42 (54.5%) participants remained deficient with none of them in the severely deficient group [Table 3]. We

Table 1: Baseline characteristics of the study population

Parameters	Values
Age (months)*	9.08±0.1
Birth weight (g)*	2839±229
Sex (male), *n* (%)**	53 (55.2)
Weight at 9 months (g)*	8524±967
Overall duration of breast feeding (months)*	6.81±2.5
Exclusive breast feeding, *n* (%)**	50 (64.93)
Age of weaning (months)*	5.97±1.1

*Data are expressed as mean±SD, **Data are expressed as *n*=*n* (%). SD: Standard deviation

Table 2: Outcome at 9 months of age

Variables	Values
Serum parathyroid hormone (pg/ml)#	36.3 (31.7-48.1)
Serum ionic calcium (mmol/L)*	1.1±0.1
Serum phosphorous (mg/dl)*	4.55±0.2
Serum ALP (IU/L)#	166.1 (96.3-256.6)
Hemoglobin (gm/dl)*	10.02±1.6
Anthropometrical indices*	
Weight (g)	8524±961.3
Length (cm)	71.4±2.4
Head circumference (cm)	43.9±1.2
Mid arm circumference (cm)	13.6±0.8

*Data are expressed as mean±SD, #Data are expressed as median (IQR). SD: Standard deviation, IQR: Inter-quartile range, ALP: Alkaline phosphatase

also found that at 9 months of age, serum PTH levels increased with a decrease in serum Vitamin D level and demonstrated significant inverse correlation (r = −0.52; P = 0.0001) [Figure 1]. Receiver operating characteristic (or ROC) curve demonstrates that at different Vitamin D levels, there will be different sensitivity and specificity to predict a surge in PTH level [Figure 2]. The ROC curve also demonstrated that the reduction in serum Vitamin D level to below 10.25 ng/mL results in a surge in serum PTH.

Discussion

The study demonstrated that there was a high prevalence of VDD from birth to 9 months of age. At birth, almost all our babies were deficient in Vitamin D. However, on follow-up at 3 months, even without any Vitamin D supplementation, a significant number of babies (27%) spontaneously changed from deficient to insufficient or sufficient state. The mean serum level of 25OHD in subjects was 14.04 ± 7.1 ng/mL at 3 months and 15.78 ± 8.9 ng/mL at 9 months of age, which is well below the widely accepted institute of medicine cutoff value for Vitamin D sufficiency (20 ng/mL). The proportion of babies with serum 25OHD levels in insufficiency (<20 ng/mL) range was 80% at 3 months of age and 75.3% at 9 months of age, which is much higher than the prevalence reported in infants in studies from the United States.[1,7] However, the prevalence is comparable to 80%-84% prevalence reported in newborn and infants from other countries such as Greece, UAE, and Pakistan[8-10] and other Indian studies.[11-13]

We measured serum parathyroid levels simultaneously in the participants at 9 months of age to diagnose secondary hyperparathyroidism and subclinical rickets. Elevated PTH has been defined as a serum concentration >45 pg/mL based on the study by Souberbielle *et al*.[6] and used in other studies as well.[3] As it was found in our study, several studies have also reported nonlinear inverse correlation between Vitamin D levels and abnormally increased PTH.[3,6,14-17] This cutoff of serum Vitamin D level below 20 ng/mL was originally described for the adult population. Hence, it can lead to the overestimation of VDD in the pediatric population. We observed that when Vitamin D level was between 15–20 ng/mL, the majority of babies in our study had PTH levels <45 pg/ml and serum ionic calcium was >1.1 mmol/L. It signifies that in this range of Vitamin D levels, the body does not perceive physiologic VDD and negative feedback mechanisms are not activated. USIOM has recommended that PTH values decrease to a nadir at different levels of serum 25OHD ranging from 15 to 50 ng/mL. On this basis, the USIOM suggested that the VDD should be defined

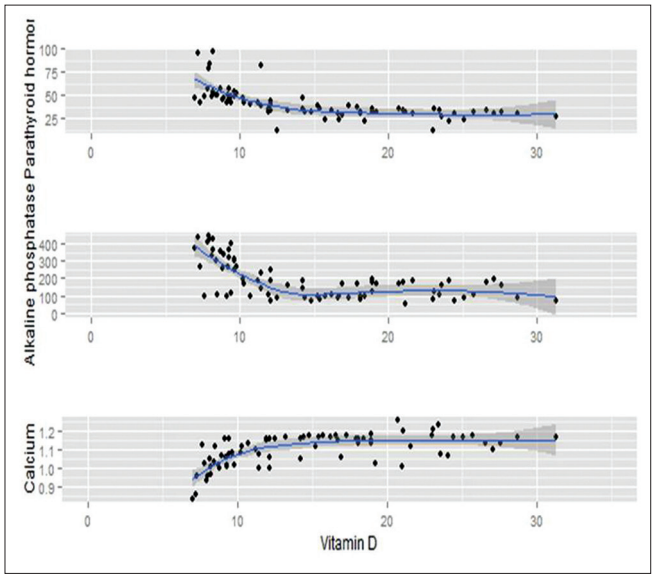

Figure 1: Grammar of graphics plot showing relation between Vitamin D with paraathyroid hormone, Alkaline phosphatase and ionic calcium

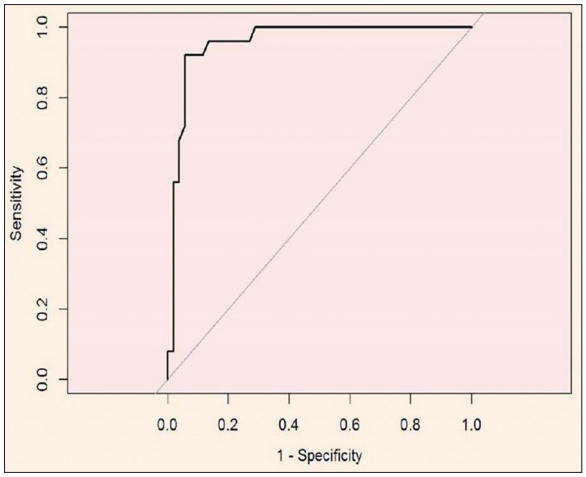

Figure 2: Receiver operating characteristic curve to predict high parathyroid hormone level from Vitamin D level at 9 months

Table 3: Vitamin D deficiency status frequency at different ages

Variables	Birth (n=96), n (%)	3 months (n=94), n (%)	9 months (n=77), n (%)
Total deficient	96 (100)	78 (83.0)	58 (75.3)
Not sufficient (ng/mL)			
Severe deficient (<5)	2 (2.1)	0	0
Deficient (<15)	94 (97.9)	70 (74.5)	42 (54.5)
Insufficient (≥15-20)	0	8 (8.5)	16 (20.8)
Sufficient (≥20 ng/mL)	0	16 (17.0)	19 (24.7)
Vitamin D level (ng/mL)[#]	8.93 (7.8-10.2)	10.9 (9.5-16.7)	14.2 (9.4-9.2)

as serum 25OHD level <15 ng/mL. Studies have also demonstrated that the calcium absorption reaches near maximum between 25OHD levels of 8–20 ng/mL, and reported that at 25OHD level of 20 ng/mL, 97.5% of the general population are assured bone health while 16 ng/mL ensured bone health in approximately half the population. On this basis, they suggested that the Vitamin D sufficiency should be defined as level ≥20 ng/mL. Using the USIOM classification of Vitamin D sufficiency, a large number of babies in our study populations had their Vitamin D level below the sufficient level (<20 ng/mL) but in the range of 15–20 ng/mL, without stimulation of negative parathyroid feedback axis or decrease in ionic calcium. It is widely perceived that significant V should be considered only at a level below which either clinical or biochemical changes are observed in the body. Hence, the infants with Vitamin D levels in the range of 15–20 ng/mL should not be considered insufficient. Therefore, we realize the need of a different cutoff for the pediatric age group.

We propose to lower the cutoff of sufficient Vitamin D from 20 to 15 ng/mL in infancy because infants with serum Vitamin D level in the range of 15–20 ng/mL neither had any increase in PTH nor decrease in ionic calcium. We further propose to subdivide USIOM group of 5–15 ng/mL into two groups. Groups with Vitamin D level between 10–15 ng/mL should be called insufficient Vitamin D group because, though there was a decrease in ionic calcium, the serum PTH remained below the cutoff range in majority (82%) of the population. The other group with Vitamin D in the range of 5–10 ng/mL should be called as Vitamin D deficient group, as in this group, ionic calcium further decreased, and this was accompanied by a significant increase in serum PTH levels. This finding gets more strength when we compared the frequency of high PTH (>45 pg/ml) and high alkaline phosphatase (ALP) (>200 IU/L) between these two classifications. The study demonstrated that the frequency of high PTH (88%) and ALP (84%) is significantly higher in group with 25OHD <10 ng/mL compared to USIOM group cutoff of 25OHD <15 ng/mL where high PTH and ALP was observed in only about 60% and 55%, respectively. Hence, babies with VDD as per USIOM classification who have normal PTH and ALP may not actually be suffering from significant VDD. Thus, we recommend that the cutoff value for VDD should be 10 ng/mL, insufficiency between 10 and 15 ng/mL and sufficiency above the level of 15 ng/mL to avoid the unnecessary label of VDD in otherwise healthy infants. It is important to note that almost 21% of our cohort was in the category of 15–20 ng/mL, which would have been labeled insufficient by USIOM but will not be insufficient as per our classification. Considering the vast populations of India in this age group, treating extra 21% of infants will confer a financial burden of almost 120 crores approximate of the Indian rupee. In another study by Haugen *et al*. in a healthy population of lactating mothers and their infants, maternal Vitamin D status and infant age were important predictors of infant Vitamin D status, and season and maternal age were predictors of maternal Vitamin D status. Although a significant correlation between maternal and infant 25(OH)D concentrations and a high prevalence of Vitamin D insufficiency among the mothers, the prevalence of Vitamin D insufficiency was low among the infants.[18]

Cutoff of Vitamin D other than that recommended by USIOM has been suggested by several other researchers as well. In another study, it was also demonstrated that raising serum 25OHD from 16 ng/mL to 35 ng/mL by oral Vitamin D supplements resulted in 22% decrease in serum PTH. Randomized controlled trials have compared higher versus lower doses of infant Vitamin D supplementation, but no studies have compared infant Vitamin D supplementation to placebo and eliminated external sources of Vitamin D to fully quantify its effect on Vitamin D status.[19] These observations are compatible with the findings of Jesudason *et al*. that levels of 25OHD of 15–20 ng/mL by[20] are required to minimize bone resorption and therefore bone loss in postmenopausal women in Australia. Never the less, because the inverse relationship between serum PTH and 25OHD is not linear, the critical threshold is, in fact, difficult to determine. In a cross-sectional study in term born neonates by Sai *et al*.[4] it was shown that neonates with Vitamin D levels >12 ng/mL had no hypocalcemia and PTH levels were within the normal adult range. However, those with Vitamin D levels <12 ng/mL had a significantly lower mean serum calcium and raised PTH levels. Similarly, Docio *et al*.[21] in a randomized control trial on 21 children demonstrated that if serum 25OHD was >10–12 ng/mL, the administration of an exogenous supplement of 25OHD was not followed by a significant decrease in serum PTH. However, when basal 25OHD levels were higher than 20 ng/mL, the supplement did not induce changes in either serum PTH or 1, 25 $(OH)_2D$, a proof for this being the desirable 25OHD levels.[22,23] On this basis, they suggested that the level between Vitamin D deficient and insufficient states maybe somewhere between 12 and 20 ng/mL. The present study also demonstrated that a decrease in 25OHD concentrations <10.25 ng/mL, led to increase in PTH concentrations beyond its cutoff value of 46 pg/mL and significant decrease in mean ionic calcium level a good marker of bone resorption. Further, it was observed that only infants with Vitamin D levels <10 ng/mL had significantly lower weight, but the cause-effect relationship is difficult to establish. However, there was no difference in length or head circumference irrespective of Vitamin D levels.

To the best of our knowledge, this is the first prospective cohort study from India which was planned to observe the natural course of Vitamin D level in supplemented infants over the 1st year of life and to (study the surrogate markers of Vitamin D insufficiency) determine adequate level of Vitamin D in infants essential for normal bone

homeostasis. However, we enrolled subjects only from the northern part of India and we did not quantify exposure to sunlight and effect of the season in Vitamin D level. Hence further studies with a subject population from a larger geographical area are needed, and hence that the data may be generalizable to the whole of population.

Conclusion

In India, however, there are no government guidelines for Vitamin D supplements in infancy. Furthermore, there is no standard cutoff of Vitamin D level customized for the Indian population. Whether universal deficiency of Vitamin D at birth is actually a pathological deficiency or just a physiological level needs to be evaluated further.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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