

Original Article



Vitamin D Status of Breastfed Filipino Infants Aged Less Than 6 Months in an Urban Community

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ABSTRACT

Purpose: This study aimed to determine the serum 25-hydroxy-vitamin D (25(OH)D) status of breastfed infants less than six months old and their mothers, and factors affecting the status.

Methods: This cross-sectional study was done on breastfed, term, Filipino infants less than six months old who were seen at local health centers and clinics in an urban area. The serum 25(OH)D levels of these infants and their mothers were determined, and their demographic data, nutritional status, sun exposure behavior, and maternal vitamin D intake were analyzed for correlation using regression models.

Results: Among the 131 infants, 101 (77%) had vitamin D deficiency (VDD), which was defined as having 25(OH)D levels <37.5 nmol/L, and 13 (10%) had vitamin D insufficiency (VDI), with levels >37.5–50 nmol/L. Conversely, maternal VDD with levels <50 nmol/L was seen in 31 (24%) mothers and maternal VDI with levels 50–75 nmol/L, in 63 (48%) mothers. Infant age and maternal 25(OH)D status were independent predictors of infant VDD. Infants less than three months old were found to have a six-time increased risk of infant VDD ($p=0.004$). Infants who had mothers with VDD had a six-time increased risk, whereas those with maternal VDI had a four-time increased risk of infant VDD ($p=0.049$ and $p=0.020$, respectively).

Conclusion: Both infant and maternal VDD and VDI were seen to be highly prevalent in this tropical, urban community. Young infants and maternal VDD/VDI independently increased the risk of infant VDD, whereas lack of sun exposure of the mothers increased the risk for maternal VDI.

Keywords: Vitamin D deficiency; Infant; Mothers; Breast feeding; Philippines

INTRODUCTION

There is growing concern regarding vitamin D deficiency (VDD) worldwide. Vitamin D plays a major role in maintaining calcium and phosphorus homeostasis and bone health [1-3]. Its deficiency in children causes growth retardation and rickets. It also plays a vital role in nonskeletal health, as vitamin D receptors are expressed on cells of the immune system, bone marrow, brain, colon, breast, and malignant cells [4]. Vitamin D can modulate the innate and adaptive immune responses, with multiple studies showing that vitamin D decreases the risk of atopic diseases, diabetes, respiratory infections, schizophrenia, depression, and certain cancers [1,2,5]. The current coronavirus disease 2019 pandemic reemphasizes the need for

Conflict of Interest

The authors have no financial conflicts of interest.

vitamin D intake, as guidelines in many countries recommend supplementation to decrease the risk and severity of this disease [6].

Lactating women and breastfeeding infants are among the high-risk groups for deficiency [3,7]. Breastmilk from a vitamin D-sufficient mother has a vitamin D content of 33–68 IU/L [8], which is only about 20% of the 200 IU/day recommended intake for 0–5 month-old infants [9]. As exclusive breastfeeding remains the standard of care for the first 6 months of life, supplementation with oral vitamin D is recommended in several countries such as the United States, Canada, Europe, and China, where sun exposure and seasonal variation limit the endogenous production of vitamin D [3,5,10-12].

In the Philippines, due to differences in geography, skin complexion, eating habits, and race, the same conclusions cannot be made. Local studies are limited and mostly focused on adult populations, with an incidence rate as high as 60% in one area [13]. In a local pediatric study by Arnaldo et al. [14], the prevalence of vitamin D insufficiency (VDI; with 25-hydroxy vitamin D [25(OH)D] levels <50 nmol/L) was reported to be as high as 41.2%, with 20.6% as VDD (below 37.5 nmol/L) among 97 high school children. This demonstrated that despite abundant sunlight exposure, VDD and VDI appear to be common.

The vitamin D status of Filipino breastfed infants has not been explored. National recommendations for vitamin D supplementation are lacking [15], probably because supporting evidence are unavailable. This study aimed to determine the serum vitamin D levels of healthy breastfed infants and their association with infant sun exposure and maternal vitamin D status in an urban community.

MATERIALS AND METHODS

This cross-sectional study was conducted among exclusively breastfed infants aged <6 months who were residents of an urban community and went to health centers and lying-in clinics for routine immunization and well-baby checkups. Mothers of infants who were exclusively breastfed and born full term (≥ 37 weeks of gestation) without any birth complications or medical conditions such as neurological, cardiac, endocrine, liver, gastrointestinal (i.e., celiac disease, malabsorption, gastrointestinal resection), or kidney diseases were invited to participate. Infants receiving partial formula milk supplementation, vitamin D supplementation (in multivitamin drop preparations), and medications such as anticonvulsants and glucocorticoids that interfere with vitamin D metabolism were excluded. Preterm infants were excluded due to their higher risk of VDD and routine multivitamin supplement administration [16].

The mothers of all eligible infants were invited to a short nutrition education lecture on breastfeeding and the importance of vitamin D conducted by the primary investigator during the immunization/well-baby visits. Convenience sampling was also performed. As VDD prevalence in this age group was not known in the Philippines, we used the reported prevalence of VDD <50 nmol/L of 90.4% among fully breastfed infants aged 1–6 months in South Korea by Choi et al. [17]. The sample size was computed at 131 to estimate the prevalence within 5% of the true value with a 95% confidence interval (CI).

Data collection procedure

Recruitment was conducted from November 2018 to February 2018, during the start of the dry season in the country, in urban communities in Quezon City, which was one of the most populated areas in Metro Manila. After enrollment of the subjects following the procurement of an informed consent, the infants underwent a general physical examination conducted by the primary investigator. Data on demographics, maternal vitamin D intake, and the use of sunscreens were collected. The visual assessment of skin color (using the medial aspect of the volar forearm) on the mother-infant pair based on the Fitzpatrick/von Luschan scale was performed by the primary investigator [18,19]. The mothers were instructed to fill out a pretested 1-week sun exposure questionnaire, which was adapted from Cobb [20] and Dawodu et al. [21] and with Filipino translation to assess the sun exposure behavior of the infants. The questionnaire recorded the duration and time of sun exposure and the exposed body surface area (BSA) while outdoors based on the type of clothing, considering the differences in the BSA of an infant and an adult. The sun exposure index was calculated by multiplying the percentage of BSA exposed with the hours of sun exposure per week [21,22]. This index has been shown to correlate with vitamin D status in adults and children [22] and has been used in the International Human Milk Research Collaborative [7,21].

The mothers were also asked to answer a 3-day food diary that included the amount of food and drink intake for two weekdays and one weekend of the week prior to serum vitamin D determination. Brands of the food and drink products were specified to check for vitamin D fortification. The vitamin D content of the food and drink products consumed was assessed, and the average daily intake of vitamin D was recorded.

A single blood sample of 2–3 mL was extracted from the mothers and infants a week after submission of the questionnaires. Blood samples were sent on the same day for analysis using a chemiluminescence immunoassay.

Assessment of outcomes

The primary outcome measure was the infant 25(OH)D level and VDD prevalence. Infant vitamin D status was classified as follows based on the definition by the American Academy of Pediatrics [16]: (a) vitamin D sufficiency, serum 25(OH)D levels >50 nmol/L; (b) VDI, serum 25(OH)D levels 37.5–50 nmol/L; (c) VDD, serum 25(OH)D levels <37.5 nmol/L; and (d) severe VDD, serum 25(OH)D levels ≤12 nmol/L.

Secondary outcomes were the risk factors for infant VDD, including maternal 25(OH)D levels, sex, infant age at determination, infant nutritional status, infant sun exposure, and skin color type. Maternal vitamin D status was defined as follows: (a) vitamin D sufficiency, serum 25(OH)D levels >75 nmol/L; (b) VDI, serum 25(OH)D levels 50–75 nmol/L; and (c) VDD, serum 25(OH)D levels <50 nmol/L [2]. Risk factors for maternal VDD were also assessed for association with maternal age, nutritional status, skin color type, sun exposure, maternal supplementation of vitamin D, dietary intake of vitamin D, and use of sunscreen.

Data processing and analysis

Data collected were checked for errors, collated, tabulated, and analyzed using descriptive and inferential statistics. Categorical data are expressed as frequencies, and numerical data as means, medians, and interquartile ranges have been used to express variability. Spearman's correlations were used to assess the correlation between infant and maternal vitamin D levels, sun indices, and vitamin D intake. To test associations among subjects'

profiles and infant and maternal serum 25(OH)D levels and deficiency, the chi-square test of independence with 2×2 Fischer exact test adjustment was performed. Nonnormally distributed continuous dependent variables were tested for association using Mann–Whitney tests for dichotomous independent risk factors and Kruskal–Wallis tests for categorical independent data. Multivariate logistic regression modeling, as well as odds ratio (OR) estimation at 95% CI, was used to determine independent predictors of infant and maternal VDD. Any associated *p*-value <0.05 alpha was considered statistically significant. Stata 13 (StataCorp, College Station, TX, USA) was used for data analysis.

Bioethical considerations

This study adhered to the ethical principles set out in the Declaration of Helsinki, World Health Organization guidelines, International Conference on Harmonization-Good Clinical Practice, and National Ethics Guidelines for Health Research. The study was approved by the Institutional Review Board of the Philippine Children's Medical Center (PCMC IRB-EC 2018-022). None of the subjects participated without written documentation of informed consent.

RESULTS

Subject characteristics

A total of 164 mother-infant pairs were enrolled in the study. Dropout rate was 25%. High dropouts were noted in 26 participants (19%) who did not return for blood extraction and 7 participants (6%) with rejected blood samples for analysis. A total of 131 mother-infant pairs were finally included in the study. A slightly higher number of female infants (52%) were noted compared to males, with the highest proportion under the 0–1 month age group (41%), vaginally delivered (91%), and normal nutritional status (80%) with Fitzgerald skin type IV (55%) (**Table 1**). The mean age of the infants was 1.2 months. The breastfeeding mothers' ages ranged from 16 years to 41 years, with a mean age of 26±6 years. More than half were of normal nutritional status (57%) with a mean body mass index (BMI) of 23.4±4 kg/m². Most mothers had Fitzgerald skin color types IV (42.6%) and V (43.4%). Approximately 12% of the mothers used sunscreen, and six had vitamin D supplementation ranging from 200 IU to 1,100 IU per day. The mean vitamin D intake from dietary sources was 53 IU/day, with zero intake from 18 mothers (14%) and highest at 376 IU/day. The infant sun index, 0.78, was slightly higher than the mother sun index of 0.45, expressed as medians.

Prevalence of vitamin D deficiency among infants and breastfeeding mothers

Table 2 shows the proportion of mothers and infants based on their levels of vitamin D. Among the 131 infants, majority (77%) had VDD. Of those with VDD, 19 (14%) were classified as having severe deficiency. Only 17 (13%) infants had normal vitamin D levels, while the remaining 13 (10%) were classified as having VDI. Conversely, mothers had a lower prevalence of VDD, seen in 31 (24%) of them, with one mother being severely deficient. However, almost half of the mothers had VDI (n=63, 48%). Only 37 mothers (28%) had normal 25(OH)D levels.

Variables associated with infant and maternal vitamin D deficiency

Univariate analysis revealed that infants' age, sun index, and maternal VDD were significantly associated with infant VDD (*p*=0.003, *p*=0.020, and *p*=0.008, respectively). Sex, mode of delivery, nutritional status, skin color (types I–III categorized as having a lighter complexion and types IV–VI as darker complexion) showed no statistically significant association. In

Table 1. Characteristics of the mothers and infants included in this study

Characteristic	Infant (n=131)	Mother (n=131)
Sex		n/a
Male	63 (48)	
Female	68 (52)	
Age	1.2±1.4 (0–5) mo	26±6 (16–41) yr
Age group (mo)		n/a
0-<1	54 (41.2)	
1-<2	40 (30.5)	
2-<3	14 (10.7)	
3-<4	7 (5.3)	
4-<5	10 (7.6)	
5-<6	6 (4.6)	
Mode of delivery		n/a
NSD	119 (91)	
CS	12 (9)	
Body mass index (kg/m ²)	n/a	23.4±4 (16.6–35.7)
Nutritional status		
Normal	105 (80.1)	75 (57.3)
Overweight	7 (5.34)	34 (26.0)
Obese	9 (6.9)	10 (7.6)
Wasting	6 (4.6)	12 (9.6)*
Severe wasting	4 (3.1)	0
Skin color type		
I	0	1 (0.7)
II	2 (1.5)	3 (2.3)
III	30 (22.9)	13 (10.0)
IV	72 (55.0)	56 (42.7)
V	27 (20.6)	56 (42.7)
VI	0	2 (1.6)
Sun index	0.78 (0.27–1.8 [0–16.12])	0.45 (0.09–0.9 [0–14.75])
Sunscreen use	n/a	16 (12)
Vitamin D supplementation	n/a	6 (5)
Dietary vitamin D content daily (IU)	n/a	53±68 (0–376)

Values are presented as number (%), mean±standard deviation (range), or median (interquartile range [range]).

NSD: normal spontaneous delivery, CS: cesarean section, n/a: not applicable.

*Underweight.

Table 2. Prevalence of vitamin D deficiency and insufficiency among infants and mothers

Vitamin D level classification	Infant (n=131)		Mother (n=131)	
	25(OH)D level definition	n (%)	25(OH)D level definition	n (%)
Deficient	<37.5 nmol/L	101 (77)	<50 nmol/L	31 (24)
Insufficient	37.5–50 nmol/L	13 (10)	50–75 nmol/L	63 (48)
Normal	>50 nmol/L	17 (13)	>75 nmol/L	37 (28)

25(OH)D: 25-hydroxy vitamin D.

contrast, none of the maternal factors were found to have statistically significant differences between those with and without maternal VDD. Daily vitamin D intake based on diet and supplementation was categorized as ≥200 IU/day and <200 IU/day, which was chosen because this is the recommended daily dietary intake for Filipino lactating women [9]. Only 14 mothers (11%) met this daily requirement. However, the difference between VDD and non-VDD mothers was not significant.

Independent predictors for infant and maternal vitamin D deficiency

Using multiple logistic regression, infants <3 months of age and maternal vitamin D levels were found to be independent predictors of infant VDD (Table 3).

Table 3. Predictors of infant vitamin D deficiency using multiple logistic regression

Infant and maternal variables	Odds ratio	p-value	95% confidence interval
Infant age <3 months	5.99	0.004*	1.75–20.54
Darker skin color type (III–VI)	1.71	0.348	0.56–5.28
Sun index	0.78	0.060	0.57–0.98
Maternal VDD	5.96	0.049*	1.004–35.382
Maternal VDI	3.84	0.020*	1.24–11.92

Overall model: LR chi squared=30.83, $p=0.0003$.

VDD: vitamin D deficiency, VDI: vitamin D insufficiency.

*Significant.

Table 4. Predictors of maternal vitamin D deficiency using multiple logistic regression

Maternal variables	Odds ratio	p-value	95% confidence interval
Age	1.03	0.442	0.95–1.11
Body mass index	0.96	0.518	0.85–1.08
Vitamin D <200 IU/day	1.0	0.971	0.99–1.00
Darker skin color type (III–VI)	1.14	0.844	0.30–4.30
Sun index of 0	5.50	0.002*	1.86–16.26

Overall model: LR chi squared=12.09, $p=0.0335$.

*Significant.

Infants who were <3 months old had six times increased risk of VDD compared with that in the older age group (OR, 5.9; 95% CI, 1.75–20.54; $p=0.004$). Maternal 25(OH)D levels ≤ 75 nmol/L, classified as VDI, increased the odds of infant VDD by almost four times (OR, 3.84; 95% CI, 1.24–11.92; $p=0.020$), and maternal 25(OH)D levels ≤ 50 nmol/L, classified as VDD, by six times (OR, 5.96; 95% CI, 1.004–35.382, $p=0.049$). It is important to note that although the infant sun index was significantly associated with infant VDD in the univariate analysis, it was not found to be a significant independent predictor of infant VDD in the multivariate model.

The maternal sun index was found to be an independent predictor of maternal VDD using multivariate regression models (**Table 4**). Maternal sun index of zero, or no sun exposure, increased the odds of maternal VDD by 450% compared to those with sun exposure (OR, 5.5; 95% CI, 0.57–0.98; $p=0.037$). Age, BMI, vitamin D intake below 20 IU/day, and a darker skin color were found to have no statistically significant association with maternal VDD.

DISCUSSION

Prior to this study, no other investigations on vitamin D status were performed in healthy Filipino breastfeeding infants. In the Philippines, being sun-abundant, VDD is not considered a high-risk disease. Despite this, it was found that VDD and VDI were highly prevalent among Filipino breastfed infants. This study showed that 87% of infants had 25(OH)D levels below the sufficiency range (<50 nmol/L), similar to South Korea's prevalence of 90.4% [17].

Breastfed infants are known to be vulnerable to VDD due to low vitamin D levels in human breastmilk and are dependent on maternal diet and factors affecting its endogenous synthesis [3,23,24]. In this study, several factors were found to be significant predictors of infant VDD, including infant age, sun index, and maternal vitamin D levels. Breastfed infants <3 months old had 8.5 times the risk of VDD than those in the older age group, which could be explained by the high maternal VDD and VDI in this study. Furthermore, previous studies have shown that even infants born to mothers with normal vitamin D will become deficient after 8 weeks if not supplemented with vitamin D, while those born of VDD mothers will quickly reach a state of deficiency [10,25].

A total of 80% to 90% of vitamin D comes from cutaneous sources [2]. The time of day and duration of sunlight exposure are determining factors of the amount of synthesized vitamin D [2]. UV-B radiation, which is responsible for cutaneous synthesis of vitamin D precursors, is prone to scatter with oblique rays earlier or later in the day, which in turn produces little vitamin D in the skin. Thus, the maximal production of vitamin D occurs at solar noon between 10 AM and 3 PM [1]. Although no standard recommendation regarding sun exposure for infants is applied locally, physicians and caretakers would generally recommend daily sun exposure to infants during the early hours of the morning with maximal body surface exposure for approximately 30 minutes. In this study, most infants had exposure times during the early morning (6 AM to 10 AM), as commonly practiced. A few infants who had zero sun exposure in the previous week were also noted. The sun index was computed to measure sun exposure based on the duration and area exposed [22]. The higher the value, the greater the sun exposure in both duration and BSA, which correlates with the 25(OH)D levels [7,20-22]. In this study, the infant sun index was found to be significantly associated with infant VDD in the univariate analysis; however, it was not a significant predictor. This disagreement could be attributed to one of the limitations in the subjective data collection of sun exposure behaviors, that is, recall bias and responder fatigue. Skin color, nutritional status, and sex were not significantly associated with infant VDD.

As demonstrated in studies from different countries [26-30], maternal vitamin D levels are a strong factor in the infants' vitamin D status. In this study, the risk of infant VDD increased almost four times if the mother had VDI and further increased the risk by six times if the mother had VDD. As mentioned, human milk contains insufficient amounts of vitamin D, but more so if the mother has deficient blood levels of vitamin D [7]. A study by Gellert et al. [31] in Germany revealed that most (62.3%) breastfeeding women had 25(OH)D levels below 50 nmol/L and that they had four times higher odds of having VDD than non-breastfeeding women. In this study, the prevalence of VDD and VDI among breastfeeding mothers was 24% and 48%, respectively, which constituted almost three-fourths of the mothers. In the multivariate regression models, a maternal sun index of zero was found to be a significant predictor. Specifically, mothers who had no sun exposure during the previous week had 5.5 times increased odds of maternal VDD. In the Human Milk Study cohort, the prevalence of VDD among breastfeeding mothers in three countries (Ohio, USA, China, and Mexico) ranged from 17% to 62% [7]. Maternal vitamin D intake, obesity, season, and sun exposure were factors associated with it. In this study, factors such as maternal age, BMI, obesity, darker skin color, use of sunscreen, and vitamin D intake (dietary and supplementation) of less than 200 IU/day had no significant association with maternal VDD. Based on the Philippine Dietary Reference Index, daily intake of at least 200 IU/day of vitamin D is recommended for Filipino lactating women [9]. Even though no statistical significance was found between this value and maternal VDD, the observation that only 14 mothers (11%) were able to reach this daily requirement was a striking finding. Dietary sources without supplementation contribute, on average, only around 10% of the body's vitamin D requirement, and if sun exposure is limited, additional sources should be employed for breastfeeding mothers.

This study was conducted in health centers and lying-in clinics in a populated urban community that cater to a lower socioeconomic class. As such, factors such as pollution, cloud cover, and nutritional factors may partly contribute to the high prevalence of VDD in this population.

The high prevalence of VDD and VDI among breastfeeding infants and their mothers was unexpected in a sun-abundant country. The clinical implications are its impact in the growing infants' bone health, growth, and development, as well as the protective effects on immunity and multiorgan functioning. VDD can be subclinical, as demonstrated in this study of apparently healthy, term, breastfed infants.

Although generalization could only be made to majority of Filipinos in urban communities, our results may encourage further study of vitamin D in different subsets of Filipino infants, including those who are on mixed feeding and formula milk. This study emphasizes the importance of the first 1,000 days wherein nutritional interventions are essential, including micronutrients such as vitamin D for both the mother and baby. Raising awareness on VDD in the country should include the availability of fortified foods and drinks and vitamin D infant drop supplements. The high prevalence of VDD in exclusively breastfed Filipino infants aged <6 months underscores the need for awareness among health providers and caretakers for vitamin D supplementation. Intensifying micronutrient supplementation in breastfeeding mothers and providing education on its implication to the mother and her infant's overall health is needed.

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