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*Observational Study*

**Prevalence of vitamin D deficiency in exclusively breastfed infants at charoenkrung pracharak hospital**

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**Abstract**

BACKGROUND

Vitamin D deficiency is a common problem in exclusively breastfed infants, with supplementation recommended by various international medical organizations. However, in Thailand, no advice for routine vitamin D supplementation is available. Thus, this study investigated the prevalence of vitamin D deficiency and its associated factors in exclusively breastfed infants in Bangkok, Thailand.

AIM

This study investigated the prevalence of vitamin D deficiency and its associated factors in exclusively breastfed infants in Bangkok, Thailand.

METHODS

This descriptive observational cross-sectional study assessed 109 4-month-old infants at Charoenkrung Pracharak Hospital from May 2020 to April 2021. The 25-OH vitamin D level of the infants was measured using an electrochemiluminescence binding assay. Vitamin D deficiency was defined as 25-OH level <20 ng/mL, with vitamin D insufficiency 20–30 ng/mL. The sun index and maternal vitamin D supplementation

data were collected and analyzed using the independent *t*-test, univariate logistic regression, and multivariate logistic regression to identify the associated factors.

## RESULTS

The prevalences of vitamin D deficiency and vitamin D insufficiency were 35.78% and 33.03%, respectively with mean serum 25-OH vitamin D levels in these two groups  $14.37 \pm 3.36$  and  $24.44 \pm 3.29$  ng/mL. Multivariate logistic regression showed that the main factors associated with vitamin D status were maternal vitamin D supplementation and birth weight, with crude odds ratios 0.26 (0.08–0.82) and 0.08 (0.01–0.45), respectively. The sun index showed no correlation with the 25-OH vitamin D level in exclusively breastfed infants ( $r = -0.002$ ,  $p = 0.984$ ).

## CONCLUSION

Two-thirds of healthy exclusively breastfed infants had hypovitaminosis D. Vitamin D supplementation prevented this condition and was recommended for both lactating women and their babies.

## INTRODUCTION

Vitamin D<sub>3</sub> is produced in human skin, whereas vitamin D<sub>2</sub> is produced in plants and fungi from ergosterol.<sup>(1)</sup> This is subsequently metabolized by 25-hydroxylase in the liver and converted to 25-OH vitamin D, its storage form. 25-OH vitamin D is then catalyzed by 1-hydroxylase in the kidneys and converted to its hormonally active form, 1,25-dihydroxyvitamin D.<sup>(2)</sup> 25-OH vitamin D and other metabolites play a crucial role in maintaining calcium and phosphate homeostasis. Active vitamin D stimulates calcium absorption in the intestines and directly influences bones and growth plates as well as extraskeletal organs.<sup>(2, 3)</sup> Vitamin D receptors and vitamin D metabolic enzymes are broadly expressed in the body. Several studies have shown that vitamin D has an effect on various organs and the immune system. Vitamin D deficiency causes osteopenia and

rickets in infants and is associated with nonskeletal diseases such as allergies and autoimmune diseases, diabetes, cardiovascular disease, and cancer.<sup>(3-5)</sup> Lack of exposure to ultraviolet light is a risk factor for vitamin D deficiency, which is defined as 25-OH vitamin D levels of <20 ng/mL.<sup>(4)</sup> However, several sunshine-rich countries, including Thailand, continue to report vitamin D deficiency cases despite the presence of abundant sunshine. Inadequate consumption of dairy products and low calcium intake are also causes of vitamin D deficiency.<sup>(5)</sup> Therefore, adequate ultraviolet light exposure and consumption of vitamin D-rich foods are crucial factors in maintaining normal vitamin D levels. Rickets, the most prevalent disease resulting from vitamin D deficiency, frequently occurs in children less than 2 years old. In 3-month-old infants, vitamin D is supplied through transplacental crossing, with the peak prevalence of rickets occurring in children aged 3–18 months.<sup>(5,6)</sup> Exclusively breastfed infants develop vitamin D deficiency rickets owing to inadequate sun exposure and low vitamin D content in breast milk (approximately 20 IU/Liter). The American Academy of Pediatrics has recommended that all breastfed infants should also be supplemented with 400 IU/day of vitamin D.<sup>(5,7)</sup>

In Thailand, routine vitamin D supplementation guidelines for lactating mothers or infants have not been established, while in South East Asia, the amount of sun exposure required to maintain normal vitamin D levels in infants has not been quantified. A literature search recommended that infants wearing only a diaper should be exposed to sunlight for up to 30 minutes per week, while those fully clothed with no hat should be exposed to sunlight for up to 2 h per week.<sup>(7)</sup>

This study investigated the prevalence of vitamin D deficiency and its associated factors in exclusively breastfed infants in Bangkok.

## **MATERIALS AND METHODS**

This descriptive observational cross-sectional study was conducted at Charoenkrung Pracharak Hospital, a tertiary and breastfeeding-friendly hospital in Bangkok, from May 2020 to April 2021. During this period, 510 4-month-old infants visited the well-

baby clinic in Charoenkrung Pracharak Hospital. The study was approved by the Bangkok Metropolitan Administration Human Research Ethics Committee (S008h/63) on April 20, 2020.

The sample size of the study was calculated using the equation below:

The expected proportion was estimated at 93.3% to give the largest sample size with a 5% degree of precision. The alpha error was accepted at 0.05, and the calculated sample size was 97, with 109 chosen after allowing for a 10% dropout rate.

A 10% value of the degree of precision was chosen due to financial and time limitations. Four-month-old infants who were exclusively breastfed at the well-baby clinic in Charoenkrung Pracharak Hospital were included in the study. All the infants were born at 37–42 wk of gestation, weighed 2,500–4,000 g, and were of Thai descent. Infants who had received vitamin D supplementation and medications, had started using complementary foods, had been previously admitted to a neonatal intensive care unit, or those with other medical conditions (renal, gastrointestinal, hepatobiliary, genetic, and dermatological conditions) were excluded from the study.

After obtaining written informed consent from the mothers, demographic data including gestational age, birth weight, and length were recorded. Maternal demographic data, body mass index (BMI), education level, status of vitamin D supplementation (defined as the mother taking a Obimin-AZ tablet containing 400 IU of vitamin D once daily after delivery until the date of enrollment), and family income were recorded. Vitamin D levels in the breast milk were not measured. The sun index record form was created to calculate the sun index, using hours of sun exposure per week and percentage body surface area exposed to sunlight (full sun is not needed). Directions describing seven consecutive days of sun exposure were provided to the mothers, and the accuracy of the sun index record forms completed by the enrolled mother-infant pairs was validated. All the mothers completed the sun index record form correctly without any errors, ensuring that the case record forms were valid. The

completed sun index record forms were collected, and the sun index was calculated using the following formula:

Sun index = hours of sun exposure per week × fraction of body surface area exposed to sunlight.

Blood samples were drawn once *via* venipuncture from the infants to measure the 25-OH vitamin D levels on the day of visiting the well-baby clinic. Other blood chemistry measurements were not assessed. The 25-OH vitamin D serum levels were measured using an electrochemiluminescence binding assay (cobas®; CE number 05894913 190). Vitamin D deficiency was defined as 25-OH vitamin D levels of <20 ng/mL, with vitamin D insufficiency defined as 25-OH vitamin D levels between 20 and 30 ng/mL, and vitamin D sufficiency defined as 25-OH vitamin D levels of ≥30 ng/mL. Hypovitaminosis D was defined as the combination of vitamin D deficiency and vitamin D insufficiency[Peter1] .<sup>(4)</sup> The data were computerized and analyzed using SPSS version 26 (SPSS, IBM Corp., Armonk, NY, USA). Normally distributed data were presented as means ± standard deviation. A univariate analysis was performed to compare and define the differences between the groups, while associations between the significant factors for vitamin D deficiency and other factors including sun index and vitamin D supplementation in mothers were analyzed using multivariate regression. The Chi-square test was used for categorical data, with analysis of variance for quantitative normally distributed data, and the Kruskal-Wallis test for non-normally distributed data. The p-value cutoff was set at <0.05.

## **RESULTS**

The flow diagram (Figure 1) shows demographic data for the 109 mother-infant pairs enrolled in the study. Incomplete case record forms were identified in five mothers and these were removed, with the remaining 104 maternal case record forms analyzed. Blood samples were drawn from 109 infants to measure 25-OH vitamin D levels, and a follow-up was conducted to collect the sun index record forms for analysis.

As shown in Table 1, the prevalences of vitamin D deficiency and insufficiency were 35.78% and 33.03%, respectively. The infants were categorized into three groups: vitamin D deficiency ( $n = 39$ ), vitamin D insufficiency ( $n = 36$ ), and vitamin D sufficiency ( $n = 34$ ). Among infants in the vitamin D deficiency group, 27 were male, and their mean weight and length were  $6,956.92 \pm 762.92$  g and  $62.28 \pm 1.81$  cm, respectively. Neonatal data showed that mean gestational age, birth weight, and birth length were  $38.62 \pm 1.09$  wk,  $3,110.51 \pm 338.45$  g, and  $51.38 \pm 2.09$  cm, respectively. The demographic data of the infants were not significantly different between the groups, except that the mean birth weight of infants in the vitamin D insufficiency group was slightly lower than for infants in the other two groups ( $P = 0.008$ ).

Infants in the vitamin D deficiency group were studied in the summer, monsoon, and winter seasons, with 11 (28.20%), 15 (38.46%), and 13 (33.33%) showing vitamin D deficiency, respectively. The sun index score for the vitamin D deficiency group was 1.44-10.95 hours/week and slightly higher than the vitamin D insufficiency and sufficiency groups. For example, the sun index in the vitamin D sufficiency group was 1.70-9.04 h/week. The mean 25-OH vitamin D levels in the vitamin D deficiency, vitamin D insufficiency, and vitamin D sufficiency groups were  $14.37 \pm 3.36$ ,  $24.44 \pm 3.29$ , and  $40.33 \pm 8.69$  ng/mL, respectively.

The demographic data of mothers in the vitamin D deficiency group for mean age, weight, and height were  $29.56 \pm 7.18$  years,  $60.03 \pm 11.61$  kg, and  $158.24 \pm 5.73$  cm, respectively. The mean BMI among mothers in the vitamin D deficiency group was  $23.95 \pm 4.4$ , with nine mothers classified as obese (23.07%). Maternal education level and vitamin D status were not significantly different between the groups ( $P = 0.678$  and  $P = 0.709$ , respectively). Overall, 42 out of 104 mothers reported that they received Obimin-AZ, and no significant differences were observed between the groups.

Univariate logistic regression identified male sex and vitamin D supplementation as two factors that were significantly associated with vitamin D status (Table 2), with crude odds ratios (ORs) 2.52 (95% confidence interval [CI]: 1.1-5.76) and 0.36 (95%CI: 0.15-0.85), respectively. No significant differences were recorded in sun index scores



and birth weights between the groups, with crude ORs 1.12 (95%CI: 0.89-1.39) and 0.46 (95%CI: 0.15-1.4), respectively. Multiple logistic regression analysis revealed that birth weight and vitamin D supplementation in mothers were significantly associated with vitamin D status, with adjusted ORs 0.08 (95%CI: 0.01-0.45) and 0.26 (95%CI 0.08-0.82), respectively. Vitamin D levels were not significantly correlated with the sun index (Pearson  $R$ -square = -0.002 [ $p$  = 0.984]) (Figure 2).

## DISCUSSION

A high prevalence of hypovitaminosis D was recorded in 4-month-old infants whose vitamin D status was slightly confounded by transplacental vitamin D. An age of 2 months is generally considered appropriate for investigating vitamin D levels in infants as they are no longer dependent on their mothers for vitamin D supply.<sup>(8)</sup> In this study, vitamin D intake from complementary foods did not interfere with the analyses because the infants were exclusively breastfed. We found that two-thirds of healthy exclusively breastfed infants had hypovitaminosis D, concurring with a previous study conducted in Thailand, which reported the incidence of 25-OH vitamin D levels of <20 ng/mL at 56.8%.<sup>(9)</sup> Our findings were also consistent with other studies, which found a high prevalence of vitamin D deficiency and insufficiency among exclusively breastfed infants in high-latitude and sunshine-rich countries.<sup>(9-14)</sup> Notably, the prevalence of vitamin D deficiency reported in this study in Thailand was lower than reported in India (83%),<sup>(12)</sup> Korea (90.4%),<sup>(15)</sup> Brazil (80.5%),<sup>(11)</sup> and the UAE (82%).<sup>(16)</sup> These differences can be attributed to multiple factors including the latitude of the country, air pollution, season, infant age, and study design. Numerous studies from different countries have reported that breast milk has low vitamin D content,<sup>(13, 17)</sup> with several international organizations, including the American Academy of Pediatrics, recommending routine vitamin D supplementation in exclusively breastfed infants.<sup>(18)</sup> A crucial source of vitamin D in humans is the ultraviolet radiation-dependent cutaneous synthesis of cholecalciferol.<sup>(19)</sup> with the positive effect of sun exposure on vitamin D status previously described.<sup>(20)</sup> Several studies have shown a lower



prevalence of vitamin D deficiency in summer, along with relatively large seasonal fluctuations in circulating vitamin D levels.<sup>(19-21)</sup> This study is the first report on sun index values in infants in Thailand. Sun exposure can be represented by the sun index, which is calculated from the exposed body surface area and the duration of sun exposure in hours per week. Sun index was expected to be a protective factor; however, the average sun index was low in all participants, and not significantly associated with vitamin D level ( $r = -.002$ ,  $P = 0.984$ ). Sun index was also inversely related to vitamin D status. Low sun exposure was reported in infants living in Bangkok because parents avoided taking their children outside due to air pollution. Our study subjects were 4-month-old infants who spent most of their daytime indoors. Some parents believed that their babies were too young to be exposed to sunlight, and this belief resulted in inadequate sun exposure, which is the major natural source of vitamin D production. Our results showed that seasons were not associated with vitamin D status. Approximately 40% of the infants in the vitamin D deficiency group were studied during the summer season, and their characteristics were similar to those examined during the winter and monsoon seasons. Despite the presence of abundant sunshine throughout the year in South East Asian countries, including Thailand, various studies showed that more than 30% of children and adolescents had vitamin D insufficiency.<sup>(22)</sup> By contrast, an Indonesian study reported that although Indonesia is located in similar latitudes to other South East Asian countries, the prevalence of vitamin D deficiency was only 16.7%. The authors described a routine cultural practice among Indonesian mothers that involved exposing their newborns (without any cover) directly to the morning sun. This practice may have contributed to cutaneous vitamin D production in these infants.<sup>(23)</sup> Lactating mothers have increased nutritional demands, including vitamin D delivered from breast milk to their infants. As previously mentioned, vitamin D content in breast milk is low, and lactating women with vitamin D deficiency are more likely to have deficient vitamin D levels in breast milk.<sup>(24)</sup> Therefore, these mothers should receive supplemental vitamin D to prevent vitamin D deficiency in their infants.<sup>(25)</sup> Several studies reported a significant correlation between maternal and cord

blood vitamin D levels.<sup>(6, 10, 26)</sup>, with maternal vitamin D levels correlating with vitamin D levels in breast milk.<sup>(17)</sup> Our findings were similar to previous studies.<sup>(27, 28)</sup> In this study, mothers received vitamin D supplementation through Obimin-AZ tablets, which contained 400 IU of vitamin D. An amount of 400 IU/day of vitamin D showed a more protective effect against vitamin D deficiency in mothers than 6,400 IU/day of vitamin D.<sup>(24)</sup> Despite the lower dosage of vitamin D supplementation in mothers in this study, the occurrence of hypovitaminosis D decreased in infants. Therefore, efforts should be made to identify an optimal dosage of vitamin D supplementation for lactating women. Female infants showed lower mean serum 25-OH vitamin D levels than male infants.<sup>(15)</sup> In this study, male sex was found to be associated with poor vitamin D status compared with female sex. However, sex does not influence vitamin D metabolism, and male sex cannot be a clinically significant factor. The birth weight of infants in [Peter1] <sup>1</sup> the vitamin D insufficiency group was lower than in the vitamin D deficiency and vitamin D sufficiency <sup>2</sup> groups and was also associated with hypovitaminosis D. Several studies have shown a correlation between low birth weight and vitamin D deficiency, especially in preterm infants.<sup>(29-31)</sup> Our study showed that the lowest mean birth weight was reported in the vitamin D insufficiency group. Therefore, other factors should be examined in future studies. We believe that <sup>3</sup> this is the first study to determine the prevalence of vitamin D deficiency in exclusively breastfed infants in Thailand without the effects of transplacental vitamin D or vitamin D from complementary foods. This is also the first study in Thailand to evaluate the sun index, which represents sun exposure. Low vitamin D content in breast milk and inadequate sun exposure in infants are significant risk factors for hypovitaminosis D. This study presented new data from Thailand that can be used to develop guidelines for mothers of infants. Supplementation of vitamin D with a dosage of at least 400 IU/day for infants since birth has also been recommended by the American Academy of Pediatrics. Our findings concur with the Institute of Medicine that vitamin D supplementation should be provided for lactating women.<sup>(4, 32)</sup>

This study had certain limitations. First, the vitamin D status of mothers and vitamin D levels in their milk were not measured. Therefore, vitamin D deficiency in mothers was not identified and the correlation between serum vitamin D levels and breast milk was not evaluated. Further studies should explore the association between these factors. Second, the sun index record form was difficult to understand and complete correctly for some mothers, resulting in incomplete reports with missing data. Following enrollment, the mothers were made aware that higher sun exposure resulted in increased vitamin D levels. Due to this knowledge and resulting behavioral changes, the sun index results could be artificially high, leading to bias. Additional studies, including a pilot for the sun index record form, should be conducted. Third, all the infants included in this study lived in Bangkok as an urban location, and the study was conducted in only one hospital. Therefore, the results cannot be considered representative of all Thai babies. Moreover, the air pollution in Bangkok is worse than in other provinces. and parents tend to avoid outside sun exposure for their children. A volunteer bias may also be present as a result of the sample population involved in the study, further limiting the generalizability of the results. Further studies that consider these limitations should be conducted to establish national guidelines.

## **CONCLUSION**

Our results showed a high prevalence of vitamin D deficiency in exclusively breastfed infants. A 400 IU daily supplement of vitamin D in mothers was found to ameliorate vitamin D deficiency in infants. Sun exposure in infants was inadequate to maintain vitamin D levels and was not correlated with vitamin D status. Thai medical organizations should establish guidelines for routine vitamin D supplementation in exclusively breastfed infants.

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