Vitamin D Status of Exclusively Breastfed 4-Month-Old Infants Supplemented During Different Seasons

WHAT'S KNOWN ON THIS SUBJECT: Despite numerous preventive strategies including prophylaxis with 400 IU/day of vitamin D in recent years, the deficiency of vitamin D in infants is still a global health problem.

WHAT THIS STUDY ADDS: This study reveals that the risk of vitamin D deficiency is high in exclusively breastfed infants, especially in winter, despite vitamin D supplementation. Therefore, it is suggested that an adjustment of vitamin D dosage for seasonal variation might be necessary.

abstract

OBJECTIVE: To examine the vitamin D status of 4-month-old exclusively breastfed infants supplemented with 400 IU daily of vitamin D and to determine whether there was any seasonal variation in serum 25-hydroxyvitamin D (25(OH)D) levels of infants.

METHODS: In this cross-sectional study, serum calcium, phosphorus, alkaline phosphatase, parathyroid hormone, and 25(OH)D levels of 143 exclusively breastfed 4-month-old infants supplemented daily with 400 IU of vitamin D were measured in a temperate latitude, Izmir, Turkey, between May 2008 and April 2009. A questionnaire on demographic characteristics of infants and mothers, vitamin D supplementation after birth, mothers’ multivitamin supplementation, dressing habits, and consumption of dairy products during pregnancy was used.

RESULTS: Vitamin D deficiency (=50 nmol/L) and insufficiency (51–74 nmol/L) were determined in 40 (28%) and 55 (38.5%) infants, respectively. During winter days, serum 25(OH)D levels were <20 ng/mL in 45.4% of infants and <10 ng/mL in 10.6% of infants. Season of blood sampling, compliance of vitamin D supplementation, maternal education level, and consumption of dairy products were highly predictive of serum 25(OH)D levels in multiple linear regression analysis ($P < .05$). The use of the Pearson correlation test found a statistically significant negative correlation between 25(OH)D and parathyroid hormone levels ($r = −0.419$, $P < .001$).

CONCLUSIONS: Despite supplementation with 400 IU of vitamin D daily, the rate of vitamin D deficiency was worryingly high in 4-month-old exclusively breastfed infants living in Izmir, Turkey. So, additional studies are needed to clarify optimal amount of vitamin D supplementation to the infants, especially during winter days. Pediatrics 2012;130:e921–e927
Vitamin D is essential for bone development. It has also potential health benefits in persons of all ages in reducing the risk of autoimmune diseases, common cancer, and cardiovascular disease.\(^1,2\) The main sources of vitamin D are the synthesis in the skin after cutaneous exposure to sunlight and dietary intake, but its availability naturally in the diet is very limited. So, the major foods such as milk, orange juice, cereals, or breads are generally fortified with vitamin D in many developed countries to prevent vitamin D deficiency.\(^3\) Despite numerous preventive strategies in recent years, vitamin D deficiency is still a global health problem both in the developed and developing countries.\(^4\) The affecting factors for vitamin D status are skin pigmentation, dressing style owing to religious or traditional causes, and use of sunscreen or lifestyle choices limiting exposure to sunshine, degree of latitude, season, the extent of air pollution, and maternal deficiency.\(^1,5,6\)

Vitamin D content of breast milk is low (20–60 IU/L), even in vitamin D–replete mothers.\(^7,8\) Therefore, exclusively breastfed infants, especially when the exposure to sunlight is limited or supplementation with vitamin D is insufficient, are at risk for vitamin D deficiency.\(^8,9,10\) Daily vitamin D supplementation is considered to be the most appropriate way to prevent vitamin D deficiency and its clinical manifestations such as rickets, growth failure, lethargy, or irritability.\(^11,12\)

Guidelines for vitamin D supplementation in the first year of life differ from country to country and have been modified several times during the past decade.\(^13\) The American Academy of Pediatrics recommends a daily intake of 400 IU vitamin D for all infants, children, and adults.\(^9\) However, some studies showed that vitamin D deficiency may develop even in the supplemented infants.\(^9,14–18\) The Ministry of Health of Turkey also recommends daily 400 IU of vitamin D supplementation during the first year of life.\(^19\) Maternal vitamin D deficiency has been identified in 46% to 80% in different regions of Turkey despite the sunny climate.\(^10,20,21\) In a previous study, we determined vitamin D deficiency in all newborns of maternal vitamin D–deficient mothers.\(^21\) It seems that the infants born with inadequate storage of vitamin D may not maintain effective serum levels of 25-hydroxyvitamin D (25 (OH)D), especially during winter seasons despite supplementation. In addition, Turkish traditional practices such as keeping infants indoors for the first 6 weeks of life contribute to the development of vitamin D deficiency. Therefore, in the current study, we aimed to investigate year-round vitamin D status of 4-month-old exclusively breastfed infants supplemented with 400 IU vitamin D daily.

**METHODS**

**Study Design**

This study was conducted between May 2008 and April 2009 at Tepecik Teaching and Research Hospital and Ege University Medical School Well-Child Care Clinics in Izmir, Turkey. The climate of the study region, which is located at latitude 38.25°N, is temperate and sunny most of the year.\(^22\) The study population included infants who were routinely followed up in well-child clinics, and who met the study criteria. The infants were healthy, term, exclusively breastfed 4-month-old infants supplemented with 400 IU vitamin D daily within 15 days after birth. All infants had been provided with free vitamin D solution (Cholecalciferol, Devit-3 oral solution, Deva Drug Factory, 4. Levent, Istanbul, Turkey) that contains 133 IU vitamin D in 1 drop.

The study was approved by the hospital’s institutional review board. The mothers of the infants were informed about the study and signed an informed consent form. Mothers were asked individually by a single researcher to answer a questionnaire that contained sections about demographic characteristics of infants and mothers, vitamin D supplementation of infants after birth, mothers’ multivitamin supplementation, dressing habits, and consumption of dairy product during pregnancy.

Socioeconomic status of the study participants was evaluated according to our national poverty level criteria.\(^23\) The subsistence wage according to national poverty criteria is currently 305 US dollars equivalent Turkish Liras per month. Family incomes below this sum were accepted as a low income. Monthly income, which was between the subsistence wages and up to threefold of the subsistence wage, was accepted as middle-high income. The patient profile of the hospital, in general, consists of the low socioeconomic group.

Educational status of each mother was grouped according to the duration of education as follows: 0 to 8 years (iliterate and primary school graduate) and 9 and more (high school and university graduate). Covered dressing style was defined as covering head and arms, but not hands and face with clothes, whereas the uncovered dressing style was defined as head and arms uncovered.

The mothers were asked about the frequency of milk and dairy product consumption during pregnancy. The consumption of dairy products daily or 4 to 5 days in a week of at least 200 mL milk or its equivalent dairy products such as yoghurt and cheese was regarded as “sufficient” milk consumption, whereas consumption of dairy products 3 times a week or less was accepted as “insufficient” milk consumption.\(^24\) Multivitamin supplementation of mothers in pregnancy with recommended minimum standard dose (a pill containing 100–200 IU vitamin D) was noted.
The use of vitamin D for the infants and the regularity of the use were questioned. Daily intake of 400 IU vitamin D was defined as “regular use,” whereas intake of 6 times a week or less was defined as “irregular use.” Infants with vitamin D use <3 times a week were excluded from the study. The infants with premature delivery, who were small for gestational age, who had intrauterine growth retardation or chronic diseases, and who were given formula alone or complementary food in addition to breast milk were not included in the study. Infants with incomplete questionnaires and inappropriate blood samples were excluded.

Sample Collection and Laboratory Analysis

Blood specimens for vitamin D (25(OH)D), calcium (Ca), phosphorus (P), alkaline phosphatase (ALP), and parathyroid hormone (PTH) were withdrawn from the infants during routine follow-up at their fourth month. Dates of blood sampling were recorded. Summer days were defined as May 1 to September 30, and winter days were defined as October 1 to April 30 according to the latitude of the geographic region of the study.

The blood samples obtained from the infants were collected in red-top tubes and centrifuged at 4000 rpm for 10 minutes. The sera were extracted and stored at −30°C until the analysis day. Serum calcium, phosphorus, and ALP were measured spectrophotometrically. Total 25(OH)D levels in the sera were measured by chemiluminescence immunoassay method (DiaSorin Inc, Stillwater, MN). This assay uses an antibody that is able to detect both forms of 25-OH vitamin D (D2 and D3). The manufacturer gave the analytical measuring range as 4 to 150 ng/mL (9.96–373.5 nmol/L). The intra-assay precision was 2.8% to 8.1%, and the interassay precision was 7.3% to 17.5%. Intact PTH levels were measured by an electrochemiluminescence immunoassay “ECLIA” method (Cobas autoanalyzer, Roche Diagnostic GmbH, Mannheim, Germany). This assay uses a sandwich test principle in which a biotinylated monoclonal antibody reacts with the N-terminal fragment (1–37), and a monoclonal antibody labeled with a ruthenium complex reacts with the C-terminal fragment (38–84). The antibodies used in this assay are reactive with epitopes in the amino acid regions 26 to 32 and 37 to 42. The manufacturer gave the measuring range as 1.20 to 5000 pg/mL (or 0.127–530 pmol/L). The intra-assay precision was 1.9% to 4.1%, and interassay precision was 2.6% to 6.2%.

Data Analysis

Serum 25(OH)D levels of all infants were measured. However, serum calcium, phosphorus, ALP, and PTH levels were tested in 141, 140, 140, and 118 infants, respectively. Serum 25(OH)D levels <50 nmol/L were defined as “deficient,” between 51 and 74 nmol/L as “insufficient,” and 75 nmol/L and above as “sufficient.” Vitamin D deficiency was also classified as:

- Mild vitamin D deficiency: Serum 25(OH)D level of 10 to 20 ng/mL (25–50 nmol/L)
- Moderate/severe vitamin D deficiency: Serum 25(OH)D level of <10 ng/mL (<25 nmol/L)

Because the 2010 Institute of Medicine report set the lowest normal value and the definition of deficiency at <20 ng/mL, we compared 2 groups of children with serum 25(OH)D levels of ≤20 ng/mL (<50 nmol/L) and >20 ng/mL (>50 nmol/L) with respect to different independent characteristics.

Statistical analysis was performed with the SPSS for Windows (version 15.0). The χ² test was applied for univariate analysis. The continuous variables were analyzed by using Student t test. The Pearson correlation was used to analyze the statistical relationship between 25(OH)D and calcium, phosphorus, ALP, and PTH levels. A multiple linear regression method was used to predict serum 25(OH)D levels from potential independent variables. A P value of <.05 was considered statistically significant.

RESULTS

A total of 202 mothers were interviewed. Nineteen (9.4%) mothers refused to participate in the study. Excluded cases and reasons of exclusion are as follows: 28 (13.8%) infants because of the use of vitamin D <3 times a week, 7 (3.5%) infants with an incomplete questionnaire, 5 (2.5%) infants with an inappropriate blood sample. The study was performed with the remaining 143 infants who met the study criteria (Fig 1). Of 143 exclusively breastfed infants supplemented with daily 400 IU of vitamin D, 113 (79%) infants received vitamin D regularly. Mean serum 25(OH)D levels were 75.2 ± 27.3 nmol/L and 42.1 ± 19.7 nmol/L in infants with regular and irregular use of vitamin D, respectively (P < .001). All infants were evaluated to assess the factors affecting vitamin D status (Table 1).

Vitamin D deficiency and insufficiency were determined in 40 (28%) and 55 (38.5%) infants, respectively. Vitamin D was sufficient in only 48 (33.6%) infants. During winter days, serum 25(OH)D levels were <50 nmol/L in 45.4% of infants and <25 nmol/L in 10.6% of infants. The rate of sufficient 25(OH)D level was 54.5% during summer days, whereas it was as low as 9.1% during winter days (Fig 2). There was a statistically significant relationship between the 25(OH)D levels of the infants and season of blood sampling, regular use of vitamin D, mothers’ dressing style, education level, consumption of dairy products, and multivitamin use during...
gestation. Of 40 vitamin D–deficient infants, routine follow-up time of 30 (75%) infants coincided with winter days. Of 48 vitamin D–sufficient infants, blood sampling of 42 (87.5%) infants was performed on summer days (Table 2).

The Pearson correlation test found a statistically significant negative correlation between 25(OH)D and PTH levels ($r = -0.419, P < .001$) (Table 3, Fig 3). In multiple regression analysis, season of blood sampling, regular vitamin D supplementation, maternal education level, and consumption of dairy products were independent determinants of 25(OH)D levels (Table 4).

**DISCUSSION**

The rates of vitamin D deficiency and insufficiency were high, especially during winter days in exclusively breastfed 4-month-old infants from a temperate region of Turkey who had received daily 400 IU vitamin D supplementation in our study group. Maternal features such as dressing style, education level and diet, and seasonal differences were strongly associated with 25(OH)D levels in the study group in which the rate of regular use of vitamin D was 79%.

The Ministry of Health of Turkey has provided daily 400 IU of vitamin D supplementation free of charge during the first year of life since 2005. This state-financed step seems to be a major initiative toward eliminating nutritional rickets in Turkey.27 However, high rates of maternal vitamin D deficiency predispose the infants to the risk of rickets or vitamin D deficiency during early infancy.10,19–21,28 Low sunshine exposure due to spending more time indoors and covered dressing habits, low intake of dairy products or vitamin D, and low education level predispose mothers and their breastfed infants to vitamin D deficiency.5,7,20,21 In our study, those maternal factors were correlated also with infants’ vitamin D status.

Maternal influences on vitamin D status in the exclusively breastfed neonate is more pronounced during the first 2 months of life, but, thereafter, infant vitamin D status is more directly affected by sunshine exposure and vitamin D supplementation.5,29,30 Therefore, it is suggested that factors such as adequacy of sunlight exposure of mothers and infants, diet and vitamin supplementation, and skin pigmentation should be considered as well as seasonal differences. In the present study, all infants were standardized with respect to onset and dosage of vitamin D prophylaxis, age, and nutritional characteristics. Seasonal variation in the vitamin D status of the infants was the main result of our study.

Regulation of calcium and phosphorus levels by vitamin D and PTH is critical for proper bone development and mineralization. Intestinal calcium absorption is maximal at the vitamin D–sufficient state, whereas, in a vitamin D–deficient state, it can decrease to as low as 10% to 15% and stimulates PTH secretion and ALP activity. A normal level of 25(OH)D prevents excess production of...
PTH and contributes to normal bone homeostasis. There is no absolute consensus as to what a normal range for 25(OH)D should be in infants. However, it is reported that the PTH levels began to plateau at their nadir when 25(OH)D levels are between 30 and 40 ng/mL (75–100 nmol/L). Although we found a negative correlation \((r = -0.419)\) between serum 25(OH)D and PTH levels in our study population, we could not observe a convincing PTH plateau because of the limited number of children with serum 25(OH)D level >100 nmol/L.

Many reports about vitamin D deficiency in all age groups have been published in recent years. However, study groups and demographic features of the population were heterogeneous in most of the childhood studies. Conflicting results were reported in the studies investigating the vitamin D deficiency and seasonal impact. In a randomized study from New Zealand, seasonal effect on vitamin D deficiency was evident during both winter and spring time in urban children with different feeding habits aged 6 to 23 months. In healthy breastfed infants living in Iowa, vitamin D deficiency was more severe, mainly in winter time, but not exclusively, than reported previously for the United States. On the contrary, 2 studies from Israel and India suggested that vitamin D deficiency is not related to the seasonal differences in children at different age groups. Conflicting results were reported from studies in Turkey. Mutlu et al found that vitamin D deficiency and insufficiency was determined in 12% of children aged 3 to 18 months supplemented with 400 IU vitamin D in the spring and summer time. In a similar study from Turkey, although supplementation with 400 IU/day vitamin D seems to be favorable, it is suggested that at least 600 IU/day vitamin D should be encouraged.

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The results from our study suggest that the dose of vitamin D supplements may be adjusted based on seasonal differences in exclusively breastfed infants. One of the limitations of our study is that maternal 25(OH)D levels were not studied. Lee et al found that a majority

### TABLE 2 Distribution of Some Independent Characteristics According to Vitamin D Status

| Characteristic | Serum 25(OH)D Level | \(P\)  \\
<table>
<thead>
<tr>
<th></th>
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<tbody>
<tr>
<td>Gender</td>
<td>(\leq 50) nmol/L, (n = 40, n (%))</td>
<td>(&gt;50) nmol/L, (n = 103, n (%))</td>
</tr>
<tr>
<td>Male</td>
<td>14 (35)</td>
<td>50 (48.5)</td>
</tr>
<tr>
<td>Female</td>
<td>26 (65)</td>
<td>53 (51.5)</td>
</tr>
<tr>
<td>Vitamin D Supplementation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Regular</td>
<td>19 (47.5)</td>
<td>94 (81.3)</td>
</tr>
<tr>
<td>Irregular</td>
<td>21 (52.5)</td>
<td>9 (8.7)</td>
</tr>
<tr>
<td>The season of blood sampling</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Summer days</td>
<td>10 (25)</td>
<td>67 (65)</td>
</tr>
<tr>
<td>Winter days</td>
<td>30 (75)</td>
<td>36 (35)</td>
</tr>
<tr>
<td>Mothers’ dressing habits</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Uncovered</td>
<td>12 (30)</td>
<td>57 (55.3)</td>
</tr>
<tr>
<td>Covered</td>
<td>28 (70)</td>
<td>46 (44.7)</td>
</tr>
<tr>
<td>Multivitamin supplementation during pregnancy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>26 (65)</td>
<td>80 (77.7)</td>
</tr>
<tr>
<td>No</td>
<td>14 (35)</td>
<td>23 (22.3)</td>
</tr>
<tr>
<td>Consumption of milk/dairy products during pregnancy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sufficient</td>
<td>14 (35)</td>
<td>66 (64.1)</td>
</tr>
<tr>
<td>Insufficient</td>
<td>28 (65)</td>
<td>37 (35.9)</td>
</tr>
<tr>
<td>Monthly income</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(&lt;305) $</td>
<td>25 (62.5)</td>
<td>67 (65)</td>
</tr>
<tr>
<td>(305–915) $</td>
<td>15 (37.5)</td>
<td>36 (35)</td>
</tr>
<tr>
<td>Maternal education</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(\leq 8) y</td>
<td>39 (97.5)</td>
<td>80 (77.7)</td>
</tr>
<tr>
<td>(\geq 9) y</td>
<td>1 (2.5)</td>
<td>23 (22.3)</td>
</tr>
</tbody>
</table>

### TABLE 3 The Correlation of Serum 25(OH)D Levels With Serum PTH, ALP, Calcium, and Phosphorus Levels

| Correlation Coefficients | \(r\) | \(P\)  \\
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>PTH (pg/mL)</td>
<td>(-0.419)*</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>ALP (U/L)</td>
<td>(-0.081)</td>
<td>.338</td>
</tr>
<tr>
<td>Ca (mg/dL)</td>
<td>0.124</td>
<td>.144</td>
</tr>
<tr>
<td>P (mg/dL)</td>
<td>0.152</td>
<td>.074</td>
</tr>
</tbody>
</table>

*Correlation is significant at the .01 level (2-tailed). Ca, calcium; P, phosphorus.

### FIGURE 2
Vitamin D status of 143 infants according to seasons.
of mother-infant pairs from a high-risk population were vitamin D deficient in the immediate postpartum period, with mean levels of plasma 25(OH)D in the deficient range (<30 nmol/L). Similarly, in another study, we found that the strong correlation between maternal and newborn plasma 25(OH)D levels offers further evidence that newborn plasma 25(OH)D levels depend on maternal plasma 25(OH)D levels. The study population comprised the infants who had been followed up routinely at well-child out-patient clinic since birth and whose mothers had been instructed about the use of vitamin D. The evaluation of the compliance to the use of vitamin D was based on the mothers’ responses in this cross-sectional study. Because our study population was restricted to infants living in Izmir, which is temperate and sunny for most of the year, our results may not be generalizable to the entire population in Turkey. However, more prevalent vitamin D deficiency can be expected in the eastern part of Turkey.

**CONCLUSIONS**

Despite daily supplementation with 400 IU of vitamin D, the rate of vitamin D deficiency was high in 4-month-old exclusively breastfed infants living in Izmir, Turkey, especially on winter days. So, further studies showing functional outcomes of vitamin D deficiency are needed to clarify optimal amount of vitamin D supplementation to the infants, especially on winter days.

**REFERENCES**

of three intermittent doses (15, 5, or 2.5 mg) on 25-hydroxyvitamin D concentrations. Am J Clin Nutr. 1984;60(9):393–396
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