Evaluation of Vitamin D Supplementation Doses during Pregnancy in a Population at High Risk for Deficiency

Gul Yesiltepe Mutlu a Elif Ozu a Sibel Kalaca c Aysegul Yuksel a Yuksel Pehlevan b Filiz Cizmecioglu a Sukru Hatun a

a Division of Pediatric Endocrinology and Diabetes, School of Medicine, Kocaeli University, and b Department of Gynecology, Kocaeli Maternity and Children Hospital, Kocaeli, and c Department of Public Health, School of Medicine, Marmara University, Istanbul, Turkey

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Vitamin D · Pregnancy · Dose

Abstract
Aim/Background: Vitamin D supplementation during pregnancy is a well-accepted recommendation worldwide; however, the debate about the correct dose is ongoing. We aimed to compare daily doses of 600, 1,200, and 2,000 IU in this randomized, controlled study. Methods: The study group consisted of 91 pregnant women aged 16–42 years admitted to Kocaeli Maternity and Children Hospital between April 2011 and April 2012. The participants were randomly divided into 3 groups. 600, 1,200, and 2,000 IU/day of vitamin D was supplemented to group 1 (control group, n = 31), group 2 (n = 31), and group 3 (n = 32), respectively. Serum calcium, 25-hydroxyvitamin D (25OHD), and the calcium/creatinine ratio in spot urine samples were measured in the follow-up period. The serum calcium and 25OHD levels of the mothers’ infants were measured as well. Results: The frequency of vitamin D sufficiency after supplementation was 80% in group 3 and it was significantly higher than in groups 1 (42%) and 2 (39%) (p = 0.03). The frequency of vitamin D sufficiency in the infants of the participants was 91% in group 3 and it was significantly higher than in groups 1 (36%) and 2 (52%) (p = 0.006). Conclusions: At least 2,000 IU/day of vitamin D is needed to ensure adequate vitamin D status in pregnancy and early infancy.

Introduction

Maternal vitamin D deficiency, defined as serum 25-hydroxyvitamin D (25OHD) < 20 ng/ml [1–3], is the most important risk factor for congenital/infantile rickets and neonatal hypocalcemia [4]. Recent studies have suggested that vitamin D deficiency in infancy may persist throughout life, potentially causing a reduction of bone mineral density and extraskeletal health problems such as a low birth weight, immunologic problems, eczema, and wheezing [5–7]. The risk factors for maternal vitamin D deficiency are: low availability of overhead sun, darker skin pigmentation, low amounts of outdoor activity, and excessive skin coverage by clothing. As a result, the prevalence of maternal vitamin D deficiency worldwide is variable and ranges from 18% in the UK [8] to 80% in Iran [9]. In Turkey, the prevalence of vitamin D deficiency during pregnancy varies between 27 and 94.8% [10–13].
Vitamin D serum levels have been shown to return to normal with sun exposure and dietary supplementation [4]. In 2011, the Turkish Ministry of Health implemented a vitamin D supplementation program that recommended 1,200 IU/day for women during the last 6 months of pregnancy. However, the optimal vitamin D supplementation dose during pregnancy is still a controversial issue. Finding the adequate dose of vitamin D supplementation is key in countries such as Turkey, where vitamin D deficiency is common. While the Institute of Medicine (IOM) recommends an intake of 600 IU/day [1], some researchers suggest an intake of 2,000 IU/day for severely deficient women [4]. Emerging evidence suggests that an intake of 4,000 IU/day may be necessary [14, 15]. We aimed to compare daily vitamin D₃ supplementation doses of 600, 1,200, and 2,000 IU during pregnancy in a randomized, nonblinded intervention study.

Methods

Study Group

This study was conducted among pregnant women at the Kocaeli Maternity and Children Hospital outpatient obstetric clinics between May 2011 and May 2012. Eligibility criteria included: being at least 16 years old, having a singleton pregnancy, and having no previously known calcium (Ca) metabolism or untreated thyroid disorders. The study protocol was approved by the Kocaeli University Ethics Committee. Written consent was obtained from all participants at the first visit.

Study Protocol

To achieve 80% power (α = 0.005), a minimum of 28 patients per group was required for an expected frequency of sufficient vitamin D status of 35% in the group taking a routine dose (600 IU/day) of vitamin D supplementation and 75% in the higher-dose groups after supplementation [16]. Women were selected from the outpatient clinic at any pregnancy stage between gestational weeks 13 and 32. Participants were recruited by an obstetrician and a pediatric endocrinologist. Gestational age was calculated based on the last menstrual period date.

Initial Study Visit

Baseline blood samples were obtained from women at their first study visit. Vitamin D supplementation was started regardless of the gestational age at enrollment, with the earliest at 13 weeks and the latest at 32 weeks of gestation. Women were randomized to 1 of 3 daily vitamin D₃ supplementation doses (600, 1,200, or 2,000 IU) using a simple randomization method. Daily vitamin D was administered in droplet form (50,000 units of cholecalciferol per 15 ml). The participants were given a schedule for keeping track of their daily vitamin D₃ dose. Additional multivitamins were not prescribed to the women. Blood samples were taken for baseline measurement of 25OHD, Ca, phosphorus (P), alkaline phosphatase (ALP), and parathyroid hormone (PTH) levels.

Follow-Up Period

Participants were evaluated 3 months after the initiation of vitamin D supplementation. The number of subjects who left the study is shown in figure 1. If a participant failed to take the prescribed vitamin D drops for at least 15 consecutive days, she was categorized as an irregular user. In the follow-up period, blood samples were taken for measurement of 25OHD, Ca, P, ALP, and PTH levels. Spot urine samples were taken for measurement of the urine Ca/creatinine ratio. The schedules of the participants for keeping track of their daily vitamin D₃ dose were checked.

The newborn birth weight was obtained from medical records. Venous blood samples were taken from newborns to measure serum 25OHD, Ca, P, ALP, and PTH levels.

Blood samples of pregnant women for baseline 25OHD measurement were taken between March and September 2012 [74% (n = 66), 22% (n = 21), 2% (n = 2), and 2% (n = 2) of the participants’ blood samples were taken in May, June, April, and July, respectively]. There was no significant difference with regard to the month of the year among the groups (p = 0.44).

Laboratory Measurements

Blood samples were frozen and stored at the Kocaeli University Medicine Faculty Hospital. Serum 25OHD levels were measured via the enzyme immunoassay method (IDS Immunodiagnostic Systems). Vitamin D sufficiency was defined as serum 25OHD >20 ng/ml, deficiency was defined as 25OHD 10–20 ng/ml, and severe deficiency was defined as 25OHD <10 ng/ml [1–3]. The inter- and intra-assay coefficients of variation were 6.7 and 8.7%, respectively.

Measurements of serum Ca, P, and ALP as well as urine Ca and creatinine levels were performed using Architect c and Aeroset System (Abbott) kits in a Beckman CX-9 autoanalyzer. The upper limit of normal for the spot urine Ca/creatinine ratio was defined as ≥0.8 in pregnancy [17]. Serum intact PTH was measured using a Roche Diagnostics E-17 Modular Analytics immunoanalyzer with its original kit. The intra-assay and total coefficients of variation were 2.8 and 3.4%, respectively. The normal limit of serum intact PTH levels was defined as 15–65 pg/ml according to the manufacturer’s recommendations. Levels higher than 65 pg/ml were defined as hyperparathyroidism.

Statistical Analyses

Statistical Package for Social Sciences (SPSS 16) software was used for statistical analyses. The mean, SD, and frequency distribution were used for descriptive statistics. Kruskal-Wallis and χ² tests were used for intergroup comparisons and Wilcoxon’s test was used for intragroup comparisons. p < 0.05 was considered statistically significant. Bonferroni’s correction was used to determine the significance level in multiple comparisons.

Results

Characteristics of the Study Participants

A total of 120 women were selected from the Kocaeli Maternity and Children Hospital outpatient obstetric clinics. After exclusions, 91 women were randomized to
receiving 1 of 3 vitamin D doses: 600 (n = 28; group 1), 1,200 (n = 31; group 2), or 2,000 IU (n = 32; group 3). The mean (range) age of the women was 29 ± 5.7 years (21.5–42.2) in group 1, 26.2 ± 4.5 years (19.5–36.7) in group 2, and 26.6 ± 4.8 years (16.1–35.5) in group 3. The groups were similar in terms of baseline 25OHD levels and sociodemographic characteristics such as age, gestational week, clothing style, and monthly income (table 1). The mean (range) duration of supplementation was similar in all groups [91.5 ± 13.4 days (63–120) in group 1, 87.1 ± 13.9 days (53–118) in group 2, and 88.8 ± 12.6 days (56–102) in group 3; p = 0.55.]

The mean serum 25OHD level at the first study visit was 10.4 ± 3.4 ng/ml (range 4.9–24.8; median 9.8), the mean serum Ca level was 8.9 ± 0.48 mg/dl (range 7.8–10.4; median 9), the mean P level was 3.2 ± 0.47 mg/dl (range 1.4–4.2; median 3.3), the mean ALP was 55.2 ± 16.4 IU/l (range 24–111; median 53), and the mean intact PTH level was 28.6 ± 15.5 pg/ml (range 6.4–76.5; median 25.9). Only 2% of the women had sufficient vitamin D status (25OHD >20 ng/ml); however, the secondary hyperparathyroidism frequency was only 2.2%.

Fifty-eight (63.7%) women consumed vitamin D regularly, 10 (11%) consumed it irregularly, and 14 (15.3%) were nonconsumers. Thus, 26.3% of the study group (n = 24) consumed vitamin D3 irregularly or never; 9.9% of the participants (n = 9) could not be contacted. Of the 9 participants who could not be contacted, 4 were in group 1, 3 were in group 2, and 2 were in group 3. The rate of regular users was 87.5% (n = 21), 71.4% (n = 20), and 56.7% (n = 17) in groups 1 (600 IU/day), 2 (1,200 IU/day), and 3 (2,000 IU/day), respectively. Thus, the rate of regular consumption of vitamin D3 was significantly lower in group 3 (p = 0.04).

**Effect of the Vitamin D Supplementation Dose on Serum 25OHD Levels**

We compared the baseline and supplemented serum 25OHD levels for each group using Wilcoxon’s test. Supplemented 25OHD levels were significantly higher than baseline 25OHD levels in all groups (p = 0.001; table 2).

We calculated differences between the pre- and post-supplementation 25OHD levels (Δ increment) of the groups using the Kruskal-Wallis test. There was no significant difference between groups 1 and 2 with regard to the Δ increment of 25OHD levels. However, in group 3 (2,000 IU/day) the Δ increment was significantly higher than in groups 1 and 2 (p = 0.02; table 2).

There was no statistically significant difference among the groups regarding baseline sufficient vitamin D level frequencies (p > 0.05). Among participants who consumed vitamin D regularly, we also analyzed the frequency of vitamin D sufficiency after supplementation in each
dose group. In group 1 (600 IU/day), 42.1% of the participants who consumed vitamin D regularly had sufficient vitamin D levels. The frequency of sufficient vitamin D levels was 38.9 and 80% in groups 2 (1,200 IU/day) and 3 (2,000 IU/day), respectively. The frequency of sufficient vitamin D status after supplementation was significantly higher in group 3 than in group 1 and group 2 (p = 0.03; table 2).

### Association between Maternal Serum 25OHD Levels and Neonatal Measurements

The mean birth weight of the babies whose mothers consumed vitamin D regularly was 3,375 ± 546 g (range 2,080–4,400; median 3,440), 3,399 ± 469 g (range 2,690–4,500; median 3,400), and 3,315 ± 351 g (range 2,800–4,050; median 3,250) in groups 1, 2, and 3, respectively. There was no significant difference in birth weight among the 3 groups (p = 0.8; table 3). There was no significant correlation between birth weight and maternal supplemented 25OHD levels (r = 0.01; p = 0.83).

The serum 25OHD levels of the babies whose mothers consumed vitamin D regularly (n = 51) were measured on day of life 20.7 ± 13.3 (range 2–50 days). There was a significant positive correlation between maternal and neonatal 25OHD levels (p = 0.001; r = 0.53). The frequency of the babies who received a maintenance dose (400 IU/day) of vitamin D3 from the 15th day was similar in each group (p > 0.05). The mean (range) duration of vitamin D supplementation administered to the infants in each group was similar [8.4 ± 10.6 days (0–31) in group 1, 11 ± 12 days (0–36) in group 2, and 8.7 ± 9 days (0–20) in group 3; p = 0.7].

The mean 25OHD levels of the babies were 18.8, 23.6, and 34 ng/ml for group 1 (600 IU/day), group 2 (1,200 IU/day), and group 3 (2,000 IU/day), respectively. The mean serum 25OHD level of the infants in group 3 was significantly higher than in the other 2 groups (p = 0.015). The serum 25OHD levels of the infants in each group are given in table 3.

### Side Effect Monitoring

The serum Ca levels and spot urine Ca/creatinine ratios of participants who consumed vitamin D regularly were evaluated during the third visit. The mean Ca levels of groups 1, 2, and 3 were 8.9 ± 0.48 mg/dl (range 7.9–10; median 9), 8.9 ± 0.33 mg/dl (range 8.5–10; median 8.9),
and 8.8 ± 0.67 mg/dl (range 8.1–9.5; median 8.9), respectively. There was no significant difference between the groups in terms of serum Ca levels (p = 0.5). None of the participants had hypocalcemia.

There was no significant difference between the groups in terms of spot urine Ca/creatinine ratios (p = 0.47) and none of the participants had hypercalciuria. The mean Ca/creatinine ratios of groups 1, 2, and 3 were 0.11 ± 0.12 (range 0.01–0.41; median 0.14), and 0.15 ± 0.17 (range 0.01–0.65; median 0.08), respectively.

**Discussion**

This randomized clinical study aimed to compare daily vitamin D₃ supplementation doses of 600, 1,200, and 2,000 IU during pregnancy. The results showed that, compared to groups 1 and 2, women assigned 2,000 IU/day of vitamin D₃ during pregnancy had the highest serum vitamin D levels and their newborns achieved the highest serum vitamin D levels. Vitamin D supplementation in pregnancy is a well-accepted recommendation worldwide. However, the debate on the correct supplementation dose is ongoing. The recommended daily allowance of the IOM was 200 IU/day for vitamin D₃ in pregnancy and the tolerable upper intake limit was 2,000 IU/day in 1997 [18]. In 2010 the IOM increased the recommended daily allowance from 200 to 600 IU/day and the upper intake limit from 2,000 to 4,000 IU/day [1]. However, previous studies have shown that daily supplementation with 200–600 IU vitamin D is inadequate to obtain sufficient vitamin D status, particularly in high-risk populations. Risk factors for hypovitaminosis D include a high latitude, excessive skin coverage by clothing, low amounts of outdoor activity, and darker skin pigmentation [19–21]. Despite the large amount of sunshine in Turkey (Kocaeli, where we conducted this study, has a latitude of 29° N), vitamin D insufficiency/deficiency is very common in this country and region. Recent studies have indicated that the prevalence of vitamin D deficiency in Turkey among reproductive-aged and pregnant women ranges from 46 to 80% [10–13, 22].

**Table 2. Effects of vitamin D supplementation on pregnant women**

<table>
<thead>
<tr>
<th></th>
<th>Group 1ᵃ (n = 28)</th>
<th>Group 2ᵇ (n = 31)</th>
<th>Group 3ᶜ (n = 32)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean baseline 25OHD level ± SD, ng/ml</td>
<td>9.7 ± 2.6 (5.1–14.6)</td>
<td>11.4 ± 4.1 (6.2–24.8)</td>
<td>9.8 ± 3.2 (4.9–17.4)</td>
<td>0.39ᵈ</td>
</tr>
<tr>
<td>Mean supplemented 25OHD level ± SD, ng/ml</td>
<td>17.1 ± 6.1 (6.1–26.7)</td>
<td>18.2 ± 9.7 (6.3–46)</td>
<td>24.7 ± 5.4 (17.3–38.9)</td>
<td>0.01ᵈ</td>
</tr>
<tr>
<td>Mean Δ increment in 25OHD level ± SD, ng/ml</td>
<td>7.4 ± 5.6 (5.4, to 4.9)</td>
<td>7.5 ± 9.9 (3.8, to 37.8)</td>
<td>14.5 ± 6.9 (5.5, –33.9)</td>
<td>0.02ᵈ</td>
</tr>
<tr>
<td>Prevalence of vitamin D sufficiency before supplementation, %</td>
<td>0</td>
<td>5</td>
<td>0</td>
<td>0.75ᵉ</td>
</tr>
<tr>
<td>Prevalence of vitamin D sufficiency (25OHD ≥20 ng/ml) after supplementation, %</td>
<td>42 (n = 8)</td>
<td>39 (n = 7)</td>
<td>80 (n = 12)</td>
<td>0.03ᵉ</td>
</tr>
</tbody>
</table>

Values in parentheses are ranges unless otherwise stated. Numbers in bold represent statistical significance. ᵃ Dose: 600 IU/day. ᵇ Dose: 1,200 IU/day. ᶜ Dose: 2,000 IU/day. ᵈ Kruskal-Wallis test. ᵉ χ² test. ᶠ Significant in relation to group 3.

**Table 3. The effects of vitamin D supplementation during pregnancy in infants**

<table>
<thead>
<tr>
<th></th>
<th>Group 1ᵃ (n = 19)</th>
<th>Group 2ᵇ (n = 20)</th>
<th>Group 3ᶜ (n = 12)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean serum 25OHD level ± SD, ng/ml</td>
<td>18.8 ± 9.9 (5.8–46)</td>
<td>23.6 ± 10 (6.6–43.4)</td>
<td>34 ± 19.1 (14.8–74)</td>
<td>0.015ᵈ</td>
</tr>
<tr>
<td>Prevalence of vitamin D sufficiency, %</td>
<td>36</td>
<td>52</td>
<td>92</td>
<td>0.02³</td>
</tr>
<tr>
<td>Mean birth weight ± SD, g</td>
<td>3,375 ± 546 (2,080–4,400)</td>
<td>3,399 ± 469 (2,690–4,500)</td>
<td>3,315 ± 351 (2,800–4,050)</td>
<td>0.8³</td>
</tr>
<tr>
<td>Mean serum Ca level ± SD, mg/dl</td>
<td>8.9 ± 0.5 (7.9–10)</td>
<td>8.9 ± 0.3 (8.5–10)</td>
<td>8.8 ± 0.7 (8.1–9.5)</td>
<td>0.5³</td>
</tr>
</tbody>
</table>

Values in parentheses are ranges unless otherwise stated. Numbers in bold represent statistical significance. ᵃ Dose: 600 IU/day. ᵇ Dose: 1,200 IU/day. ᶜ Dose: 2,000 IU/day. ³ Kruskal-Wallis test. ⁴ χ² test. ⁵ Significant in relation to group 3.
In our study, only 2.2% of the participants had sufficient vitamin D status at baseline and 51.6% of the participants had severe vitamin D deficiency. These results show that our region is at high risk for vitamin D deficiency.

When we compared the effect of the vitamin D dose on serum 25OHD levels, we saw that 2,000 IU vitamin D₃ provided a better increase in serum 25OHD levels than 600- and 1,200-IU doses. There was no significant difference between groups 1 and 2 in terms of 25OHD levels after supplementation; the small number of pregnant women in each subgroup could explain this. The frequency of vitamin D sufficiency among the participants and their babies was higher with the 2,000-IU dose than with the lower doses in our study. The relationship between maternal and neonatal vitamin D status is well established. It has been shown that the most important risk factor for low serum 25OHD levels in newborns is a maternal level of 25OHD <10 ng/ml (OR = 15.2, p = 0.002) [12]. A recent study found vitamin D levels <10 ng/ml in 27% of pregnant sera and 64% of cord blood [10]. Vitamin D supplementation is administered to neonates during the first days of life. However, it does not prevent neonatal hypocalcemia in cases of maternal-infant pair vitamin D deficiency [23]. In light of this data, it is essential to ensure maternal vitamin D sufficiency in order to obtain neonatal vitamin D sufficiency. Our results indicate that supplementation with 2,000 IU vitamin D₃ in pregnancy is a safe way to prevent vitamin D deficiency in early infancy.

Hollis et al. [14] found that 4,000 IU/day of vitamin D₃ supplementation resulted in a higher rate of vitamin D sufficiency in both mothers and infants than 400- and 2,000-IU daily doses. There is a close relationship between the dose of vitamin D supplementation and the cutoff level of serum 25OHD sufficiency. Hollis et al. [14] established the lowest 25OHD level for vitamin D sufficiency at 32 ng/ml, attainable via daily supplementation with 4,000 IU vitamin D₃ [14]. The divergence of opinion about the 25OHD level necessary for sufficiency has persisted in recent consensus reports. The US Endocrine Society accepts a 25OHD level of 32 ng/ml as the threshold value at which the PTH level starts to rise and suggests this value as the limit of sufficiency [24, 25]. The IOM asserts that PTH levels remain stable between 16 and 50 ng/ml 25OHD and therefore levels of 20 ng/ml are adequate for 97.5% of the population, so there is little or no additional benefit to be gained from serum levels above 20 ng/ml [26, 27]. Although the serum 25OHD levels of all of the participants in our study were lower than 32 ng/ml, only 2.2% had high PTH levels. Previous studies of our group have revealed a rare secondary hyperparathyroidism frequency despite the high incidence of vitamin D deficiency among adolescents in our study population [28, 29]. Similarly, Datta et al. [21] reported normal PTH levels in 80% of vitamin D-deficient pregnant women. This data affirms that a 25OHD level of 20 ng/ml can be accepted for sufficiency in pregnancy, too. However, at least 2,000 IU/day is needed to achieve the minimum 20 ng/ml level of 25OHD [26]. Obstetricians site possible side effects as the main drawback for vitamin D supplementation during pregnancy in our country, especially when considering the daily tolerable upper intake limit of the IOM for vitamin D₃ of 4,000 IU [1]. Hollis et al. [14] did not encounter any side effects in their study when they administered 4,000 IU/day of vitamin D₃ to pregnant women [14]. Recently, Dawodu et al. [15] also showed that vitamin D doses of 2,000 and 4,000 IU/day are safe in pregnancy [15]. Serum Ca, 25OHD, and urine Ca measurements are the primary screening tools for hypervitaminosis D. In our study, none of the participants had a 25OHD level higher than 100 ng/ml (the accepted upper level for hypervitaminosis D) and none had hypercalcemia or hypercalciuria. These results support the safety of vitamin D₃ doses of 2,000 IU/day.

Our study is the first report from Turkey, and from a region with high vitamin D deficiency rates, comparing vitamin D supplementation doses in pregnancy. However, it has some limitations. The loss of patients from the study group led to a decrease in sample size. Most of the pregnant women who left the study stated that they had difficulty counting the number of drops they consumed. Thus, the number of lost patients was significantly higher in the 2,000 IU/day group. This result indicates a risk of decreased compliance with higher vitamin D doses. In our opinion, use of the capsule form of cholecalciferol instead of the liquid form would increase compliance. Another limitation of this study is the long duration (postnatal days 2–50) of the period for measurement of infantile 25OHD levels. Nevertheless, the higher serum 25OHD levels of the infants in the 2,000 IU/d group support the correlation between the vitamin D status of the mother and that of the infant, even at postnatal week 12 [30, 31].

**Conclusions**

We found that 2,000 IU/day of vitamin D₃ provided a better increase in 25OHD levels than 600 IU/day and 1,200 IU/day doses. Similarly, the frequency of vitamin D sufficiency among pregnant women and their babies was higher with the 2,000 IU dose than with the lower doses in this study.
Supplementation with at least 2,000 IU vitamin D is needed to achieve sufficient serum vitamin D levels in both pregnancy and the early infancy period. Larger studies are required to determine whether 1,200 IU/day of vitamin D₃, which is recommended in our national vitamin D deficiency prevention program, is adequate or inadequate in pregnancy.

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