

# The effect of place of residence and lifestyle on vitamin D deficiency in pregnancy: Comparison of eastern and western parts of Turkey

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## Abstract

**Objective:** The aim of this study was to determine the prevalence and the predictive factors of vitamin D deficiency in pregnancy and the compliance with “The National Vitamin D Support Program” at Turkey’s easternmost and westernmost provinces.

**Material and Methods:** Lifestyles of women at 24-28 weeks of pregnancy were assessed using a questionnaire form, and serum 25-hydroxyvitamin D3 (25(OH)D3) levels were measured.

**Results:** Vitamin D deficiency ( $\leq 20$  ng/mL) in pregnant women had a prevalence of 27.8% in İzmir and 76.3% in Erzurum. The compliance of “The National Vitamin D Support Program” was 8% in İzmir and 32.6% in Erzurum. Clothing style, fish consumption, seaside holiday duration, and 1200 IU/day vitamin D replacement had an effect on 25(OH)D3 levels in pregnant subjects in İzmir, whereas only holiday duration and 1200 IU/day vitamin D replacement affected 25(OH)D3 levels in Erzurum. However, when a threshold for 25(OH)D3 level was considered  $\geq 32$  ng/mL, lifestyles did not affect 25(OH)D3 level.

**Conclusion:** The effect of lifestyle on 25(OH)D3 level in pregnancy is limited, especially in cold regions. We recommended increasing the compliance with “The National Vitamin D Support Program” at the follow-up of all pregnant women, irrespective of region and season.

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**Key words:** Pregnancy, vitamin D deficiency, vitamin D replacement, lifestyle, place of residence

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## Introduction

Vitamin D is a cholesterol derivative steroid hormone that, unlike other vitamins, can be synthesized in the human body. Apart from its known role in calcium metabolism, it has important additional roles in many cellular events by virtue of its autocrine and paracrine effects. It leads to anti-inflammatory and anti-infective responses and regulates cellular proliferation, differentiation, and insulin synthesis (1, 2). Recent studies have shown that vitamin D has an important role in both healthy pregnancy processes and long-term health of offspring. Recent evidence has suggested that vitamin D has an association with multifactorial diseases of pregnancy, such as bacterial vaginosis, preterm birth, gestational diabetes, and preeclampsia (3-5). In addition, some epidemiologic studies reported that there might be a relationship between allergic diseases, asthma, diabetes mellitus type 1, schizophrenia, and autism and maternal vitamin D deficiency (6-8).

The prevalence of vitamin D deficiency in pregnancy is high all over the world. Classically, conditions, such as a low socioeconomic level, being of African or Latin American descent, obesity, dark skin color, wearing covering clothing, living in northern latitudes, and taking no vitamin D supplements, are known to be associated with vitamin D deficiency. However, high vitamin D deficiency rates have recently been detected in people not usually considered to be ‘at risk’: with a lighter skin color, have a holiday for a long time, have a higher socioeconomic level, living from sun-drenched, torrid zones, and those believed to have adequate sun exposure (9-11).

The best indicator of vitamin D status is serum 25-hydroxyvitamin D3 (25(OH)D3) concentration, because it reflects both dietary intake from vitamin D and cutaneous synthesis of vitamin D. However, there is no absolute consensus as to what a normal range for 25(OH)D3 in pregnancy should be. Most authors agree that severe vitamin D deficiency should be defined by a 25(OH)D3 concentration  $\leq 10$  ng/mL ( $\leq 25$



nmol/L) and mild vitamin D deficiency by a 25(OH)D<sub>3</sub> concentration 10-20 ng/mL (25-50 nmol/L); but, recent evidence suggests that the optimal serum 25(OH)D<sub>3</sub> levels may be even higher than >32 ng/mL (>80 nmol/L) (4).

Routine screening for vitamin D deficiency in pregnancy is not considered a cost-effective option. However, many health organizations recommend vitamin D supplementation during pregnancy. But, these issues, such as if vitamin D replacement will be given to all pregnant women or only to high-risk pregnant women, regional and seasonal differences, and the effective and safe dose of vitamin D in pregnancy, are still not clear, and further research is needed concerning these issues.

In the present study, we aimed to a) examine the prevalence of vitamin D deficiency in pregnancy at two extreme geographical points of Turkey; b) examine the effect of different geographical locations in the same latitudes and different lifestyles in the same ethnic origin to vitamin D deficiency prevalence; c) examine the compliance with "The National Vitamin D Support Program for Pregnancy;" and d) inform on future screening and/ or supplementation strategies.

## Material and Methods

İzmir and Erzurum are very distinct provinces of Turkey with respect to both geographical and climatic conditions and social life. They are located in similar latitudes (39.55 North and 38.25 North). İzmir lies on the westernmost point of Turkey (27.0 East). It is located on the Aegean Sea coast and has an altitude of 2 m. According to the Turkish Directorate of Meteorology, the average temperature in the study period was 22.6°C daily. Uncovered clothing is common among women. Thanks to its coastal location, rates of sea and fish consumption are high. Erzurum, on the other hand, is located at the easternmost point of Turkey (41.1 East). The city lies in mountainous terrain with an altitude of 1893 m. The average temperature in the study period was 12.3°C. Among women, Islamic covered clothing style and domestic life are common. Local cuisine typically does not contain seafood.

The present study was approved by the local ethical committee at Şifa University, written consent was obtained to participate, and the procedures followed were in accordance with the Helsinki Declaration of 1975 (revised in 2008). The study data were collected from Erzurum Şifa Hospital and Şifa University, Bornova Health Research and Application Hospital, between June 2012 and October 2012.

Vitamin D status was defined by serum levels of 25(OH)D<sub>3</sub> as follows: severe vitamin D deficiency, 25(OH)D<sub>3</sub> ≤10 ng/mL (25 nmol/L); mild vitamin D deficiency, 25(OH)D<sub>3</sub> of 10-20 ng/mL (25-50 nmol/L); normal ≥20 ng/mL (50 nmol/L), and optimal status, 25(OH)D<sub>3</sub> ≥32 ng/mL (80 nmol/L).

## Collection of the Study Data

Voluntary women at 24-28 weeks of pregnancy attending routine antenatal review or glucose challenge tests were the source population. Subjects with diabetes mellitus, hypertension, chronic liver and renal disease, rheumatic disease, gastrointestinal diseases with malabsorption, and other chronic

diseases, as well as subjects on chronic medical therapy were excluded. In total 687 participants, 387 participants in İzmir Bornova Health Research and Application Hospital and 245 participants in Erzurum Şifa Hospital; were founded suitable for study criteria in this period. Eligible women were questioned via a questionnaire form about their lifestyle, and blood samples were taken for 25(OH)D<sub>3</sub> levels.

The questionnaire form offered to the participants included questions about annual income, educational level; parity; smoking; the frequency of exposure to sunlight; clothing style; using sunscreen; seaside holiday duration within the last 6 months; status of fish, milk, egg, and vitamin D-enriched food consumption; and status of vitamin D and multivitamin use. Occupational status of the pregnant women was also assessed. The frequency of exposure to sunlight was questioned in terms of number of days of exposure in a week, not less than 30 minutes. Covered clothing style was defined as clothing covering all body parts except for the hands and face. Consumption of a half-liter milk or milk product was considered adequate. Fruit juice, margarines, and breakfast brittles are fortified with vitamin D and widely used in Turkey. However vitamin D-fortified milk is not used in our country. Regular use of at least one of these products was questioned under the title of vitamin D-enriched food consumption.

Skin color of the participants was classified according to the Fitzpatrick Skin Color Scale. Starting from 1 point up to 6, a score was given to each subject.

Body mass index (BMI, weight (kg)/height (m)<sup>2</sup>) was calculated by measuring the height and body weight simultaneously with blood collection.

25(OH)D<sub>3</sub> level may be affected by lifestyles, personal characteristics, and living place. We aimed that to create study groups with similar personal characteristics in both provinces to better understand the opportunity of lifestyles to affect vitamin D deficiency. For this reason, the study groups was defined as follows: a) skin color <4 according to Fitzpatrick Skin Color Scale, b) BMI 20-30 kg/m<sup>2</sup>, c) parity ≤3, d) educational level ≥8 year, e) annual income ≥\$4500, f) Caucasian ethnicity, and f) age 18-40. Afterwards, the study groups were created according to the questionnaires of participants. As a result, 208 pregnant were included in the study. Figure 1 represents a flowchart of the study design.

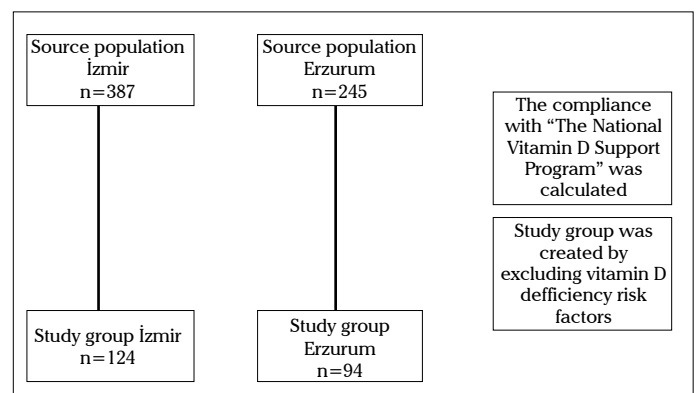


Figure 1. Flow chart of the study design

### Collection of Blood Samples

Blood samples collected in Erzurum were sent to İzmir at -20°C under protection from sunlight. All laboratory analyses were performed in a single laboratory. 25(OH)D<sub>3</sub> level was analyzed by ELISA (EUROIMMUN, D-23560 Lübeck, Seekamp 31, Germany) method.

### Statistical Analysis

Statistical analysis was performed by using the SPSS (15.0) for Windows (SPSS Inc., Chicago, IL, USA). Categorical variables were analyzed by forming a crosstable for inter-group differences, and  $\chi^2$  analysis was performed. For 25(OH)D<sub>3</sub> level, t-test for comparison of 2 groups was used. Variables within a group were analyzed using ANOVA test. A significant ANOVA test result was further analyzed with Bonferroni test when the variance was homogenous and Dunnett T3 test when it was not. A 2-sided analysis of variance was done for 25(OH)D<sub>3</sub> by considering the province and the variables. A logistic regression analysis was performed for predictor factors of vitamin D deficiency, with 2 separate analyses being performed by assuming threshold values of 20 and 32. A p value less than 0.05 was considered statistically significant.

### Results

In total, 387 participants in Bornova Health Research and Application Hospital and 245 participants in Erzurum Sifa Hospital were accepted for study. Usage of vitamin D in pregnancy in İzmir and Erzurum and compliance with "The National Vitamin D Support Program for Pregnancy" were calculated. Afterwards, study groups were created according to the personal characteristics of the participants. As a result, 124 pregnant women in İzmir and 94 pregnant women in Erzurum were included in the study.

The mean age was  $28.4 \pm 4.5$  in the İzmir study group and  $29.1 \pm 5.1$  in the Erzurum study group, the mean gestational week was  $25.2 \pm 3.1$  in the İzmir study group and  $26.1 \pm 5.4$  in the Erzurum study group, and BMI averaged  $27.1 \pm 3.5$  in İzmir and  $26.6 \pm 3.3$  in Erzurum, with no significant difference between the groups.

**Table 1. Vitamin D usage in İzmir and Erzurum**

Regular vitamin D replacement*	1200 IU/day (n, %)	400 IU/day (n, %)	No vitamin D replacement (n, %)
İzmir (n=387)	31 (8%)	325 (83.9%)	31 (8%)
Erzurum (n=245)	80 (32.6%)	157 (64%)	8 (3.2%)

\*Regular vitamin D replacement:  $\geq 3$  day/week

**Table 2. 25(OH)D<sub>3</sub> levels in İzmir and Erzurum**

25(OH)D <sub>3</sub> level (ng/mL)	Severe deficiency ( $\leq 10$ ng/mL)	Mild deficiency (10-20 ng/mL)	Normal level ( $\geq 20$ ng/mL)	Optimal level ( $\geq 32$ ng/mL)	Average level ( $\pm$ SD)	p value
İzmir (n, %)	14 (11.2)	29 (23.3)	81 (65.3)	38 (34)	38 (3.6)	<0.001
Erzurum (n, %)	16 (17)	55 (58.5)	23 (24.4)	1 (1)	16 (5.8)	

SD: standard deviation. 25(OH)D<sub>3</sub>: 25-hydroxyvitamin D<sub>3</sub>

### Compliance with "The National Vitamin D Support Program"

We found that 8% of pregnant women in İzmir and 32.6% of pregnant women in Erzurum took 1200 IU/day vitamin D, the recommended dose of The National Program, and 83.9% of pregnant women in İzmir and 64% of pregnant women in Erzurum used 400 IU/day vitamin D (multivitamin). Furthermore, we observed that 8% of pregnant women in İzmir and 3.2% of pregnant women in Erzurum did not take vitamin D supplementation. Vitamin D usage in İzmir and Erzurum is given in Table 1.

### Serum 25(OH)D<sub>3</sub> Levels by Provinces

25(OH)D<sub>3</sub> levels by provinces are given in Table 2. Severe vitamin D deficiency was present in 11.2% of the İzmir group and 17% of the Erzurum group. Mild vitamin D deficiency was present in 23.3% of the İzmir group and 58.5% of the Erzurum group. Normal vitamin D level rate ( $\geq 20$  ng/mL) was 65.3% in İzmir and 24.4% in Erzurum. However, 34% of pregnant subjects from İzmir and only 1% of those from Erzurum had optimal 25(OH)D<sub>3</sub> levels ( $\geq 32$  ng/mL). Average 25(OH)D<sub>3</sub> level was 38 ng/mL ( $\pm 3.6$  standard deviation (SD)) in İzmir and 16 ng/mL ( $\pm 5.8$  SD) in Erzurum.

### Factors Associated with Serum 25(OH)D<sub>3</sub> Level

Effects of lifestyle factors on 25(OH)D<sub>3</sub> level were separately assessed for İzmir and Erzurum. While uncovered clothing, fish consumption, a longer seaside holiday duration, and 1200 IU/day vitamin D replacement significantly increased 25(OH)D<sub>3</sub> level in pregnant subjects living in İzmir, long holiday duration and 1200 IU/day vitamin D replacement affected 25(OH)D<sub>3</sub> in the Erzurum group. The effects of lifestyle factors on local 25(OH)D<sub>3</sub> levels are summarized in Table 3.

Assuming the threshold level as 20 ng/mL, logistic regression analysis showed that covered clothing style by 2.9-fold, holiday duration less than 1 week by 23.5-fold, vitamin D replacement less than 3 days a week and less than 1200 IU/day by 6.2-fold, consuming fish less than once a week by 1.6-fold, and living in Erzurum by 38.3-fold were increased the risk of vitamin D deficiency. A threshold of 32 ng/mL, on the other hand, made living in Erzurum the only effective factor that increased vitamin D deficiency risk by 33.5-fold. The results of the multivariate logistic regression analysis are summarized in Tables 4 and 5.

### Discussion

Data from eastern and western parts of Turkey showed that even in summer and fall, the prevalence of vitamin D deficiency in pregnancy is high in our country, although the vitamin D defi-

**Table 3. Associations between lifestyle and 25(OH)D<sub>3</sub> level**

Predictor factors 25(OH)D <sub>3</sub> level	25(OH)D <sub>3</sub> level İzmir (ng/mL)	p value	25(OH)D <sub>3</sub> level Erzurum (ng/mL)	p value
State of employment				
Employed	22	0.08	15.8	0.9
Unemployed	26.5		16.2	
Clothing style		0.03		0.4
Covered clothing	24.5		15	
Uncovered clothing	31.6		17.9	
Smoking		0.9		0.7
Smokers	29.2		18.4	
Non-smokers	28.1		17.3	
Seaside holiday duration				
<1 week/last 6 month	15.4	<0.01	13.8	<0.01
≥1 week/last 6 month	31.8		20.8	
Sun exposure		0.5		0.9
≥3 day/week	27.1		16.5	
<3 day/week	28.2		15	
Using sunscreen		0.2		0.6
Sometimes	30		18.4	
Never	26.9		15.5	
Fish consumption		0.04		0.1
≥1 day/week	30.3		17.9	
More rare	23.2		15.8	
Egg consumption		0.5		0.4
≥3 day/week	27.1		15.7	
<3 day/week	27.5		16	
Vitamin D-enriched food consumption		0.6		0.1
≥3 day/week	27.2		14.8	
<3 day/week	29.3		14.6	
Milk consumption		0.4		0.5
≥3 day/week	29.4		15.8	
<3 day/week	25.3		17.1	
1200 IU/day Vitamin D supplement		0.04		0.03
≥3 day/week	35.7		21.9	
<3 day/week	27.7		14.1	
400 IU/day Vitamin D supplement		0.07		0.7
≥3 day/week	29.7		16.9	
<3 day/week	24.1		13.7	

Statistical evaluation was made with ANOVA. P<0.05 statistical significance.  
25(OH)D<sub>3</sub>: 25-hydroxyvitamin D3

ciency prevalence was significantly lower in İzmir (76.3% versus 27.8%). It would not be surprising to see much lower levels in winter. Hence, a study by Halicioglu et al. (12) that examined winter and spring levels of 25(OH)D<sub>3</sub> in 256 pregnant women living in İzmir reported a vitamin D deficiency (≤20 mg/mL)

prevalence of 90.3% and a normal level (≥30 mg/mL) in only 0.4%. Other studies from Turkey have reported that pregnancy-associated vitamin D deficiency remains a commonly seen problem (13-17). However, to our knowledge, the present study is the only study that has compared the difference in vitamin D

**Table 4. Significant predictive factors for vitamin D deficiency**

Significant predictive factors for vitamin D deficiency (threshold $\geq 20$ ng/mL)	Odds ratio	95% CI	p value
Living place (Living in İzmir versus living in Erzurum)	38.3	1.1-1276	0.04
Clothing style (uncovered versus covered clothing)	2.9	1.1-7.9	<0.02
Seaside holiday duration ( $\geq 1$ week/ last 6 month/ versus <1 week/last 6 month)	23.5	7.0-78.1	<0.001
1200 IU/day Vitamin D supplement ( $\geq 3$ day/week versus consuming less than)	6.2	1.2-4.1	<0.02
Fish consumption ( $\geq 1$ day/week versus consuming less than)	1.6	1.6-4.1	<0.03
Multivariate logistic regression was made. The threshold was considered $\geq 20$ ng/mL			

**Table 5. Significant predictive factors for vitamin D deficiency**

Significant predictive factors for vitamin D deficiency (the threshold $\geq 32$ ng/mL)	Odds ratio	95% CI	p value
Living place (Living in İzmir versus living in Erzurum)	33.5	3.8-296	0.02
Multivariate logistic regression was made. The threshold was considered $\geq 32$ ng/mL			

deficiency prevalence in pregnancy between regions of Turkey. Behavioral factors and personal characteristics can affect 25(OH)D<sub>3</sub> level (9). We aimed to create study groups with similar personal characteristics in both provinces to better understand the lifestyle effects on vitamin D deficiency. In our study, we observed that clothing style, seaside holiday duration, consuming fish, and 1200 IU/day vitamin D supplement affected 25(OH)D<sub>3</sub> level.

The body surface area required for ideal 25(OH)D<sub>3</sub> synthesis is not entirely known. In a study by Perampalam et al. (9), it was found that the critical body surface area required for sustaining an adequate 25(OH)D<sub>3</sub> level was >27% and that the body surface area exposed to sunlight was the main behavioral factor related to 25(OH)D<sub>3</sub> level. In Islamic clothing (hands and face uncovered), 8% of the total body surface area remains uncovered.

Among the dietary sources, the only ones that seem to affect 25(OH)D<sub>3</sub> synthesis are fish. However, 56% of the pregnant women in İzmir and 82% of those residing in Erzurum consumed fish less than once a week. Seafood is also a good source of omega-3 fatty acids, and it contains high-quality proteins (18). Promoting fish consumption in pregnant women may

be reasonable with respect to 25(OH)D<sub>3</sub> levels. In our study, we did not detect a relationship between vitamin D-enriched foods and 25(OH)D<sub>3</sub> levels. This situation can be explained by the limited vitamin D-reinforced food in Turkey (15), whereas in a study by Charatcharoenwithaya et al. (19), it was found that drinking vitamin-fortified milk affected 25(OH)D<sub>3</sub> levels in pregnancy.

Surprisingly, although we found that seaside holiday duration affected 25(OH)D<sub>3</sub> level in both İzmir and Erzurum, we did not detect any relationship between sunlight exposure duration and 25(OH)D<sub>3</sub> levels. This situation can be explained by factors, such as exposure to sunlight at times other than noon, covering clothing style, and use of sunscreen. Using sunscreen prevents vitamin D synthesis, disallowing ultraviolet B (UVB) rays to penetrate the skin. Our study may not have had the ability to detect effects of using sunscreen on vitamin D levels, since the rates of sunscreen use were as low as 11% and 20% in the groups. Additionally, since UVB rays have a short wavelength, 25(OH)D<sub>3</sub> synthesis reaches the top at noon, when the sunlight hits the earth at a perpendicular angle. Even 30 minutes of sunbathing without using sunscreen at noon may lead to synthesis of 25(OH)D<sub>3</sub> at an amount of 15-20,000 IU (20, 21). Therefore, having adequate sun exposure may not mean having adequate 25(OH)D<sub>3</sub> levels.

In our study, we found that a vitamin D dose of 400 IU/day in multivitamins did not affect 25(OH)D<sub>3</sub> level. On the other hand, 25(OH)D<sub>3</sub> levels increased in women using a dose of 1200 IU/day. There is no consensus with regard to vitamin D replacement to sustain a normal 25(OH)D<sub>3</sub> level for healthy pregnancy. The National Health Institute recommends 400 IU/day, and the World Health Organization recommends 200 IU/day vitamin D supplementation for pregnant women (22). Recent studies have shown that the official vitamin D dosing recommendations are far from meeting the demands of pregnancy, and 25(OH)D<sub>3</sub> levels may be more effectively boosted with doses as high as 1600, 2000, and 4000 IU/day, without increasing side effects (23, 24). Wagner et al. (25) reported that serum 25(OH)D<sub>3</sub> levels did not change significantly until after vitamin D supplementation exceeded 1000 IU/day. The dose that leads to vitamin D intoxication is not precisely known; however, vitamin D intoxication can be considered an extremely rare and exaggerated condition. The National Health Institution has reported that 4000 IU/day is the upper safe limit for pregnant women (22). No side effects have been reported in volunteer non-pregnant women at doses of 10,000 IU/day (26). It seems that the recommended supplementation doses will increase as the number of randomized controlled studies on this subject increases.

In Turkey, until May 2011, a health policy on vitamin D prophylaxis for pregnant women did not exist; thus, the vitamin D supplements prescribed for pregnant women were limited to low doses (200-400 IU), amounts generally included in commercial multivitamin preparations. Since 2011, the Turkish Ministry of Health has recommended vitamin D supplementation to all pregnant women at a dose of 1200 IU/day starting from 12 weeks of pregnancy (27). However, 8% of the İzmir group and 32.6% of the Erzurum group were taking vitamin D at recommended doses in our study groups. Our study found that the



compliance “The National Vitamin D Support Program” is poor. In our study, we found that the threshold was 32 ng/mL instead of 20 ng/mL for normal levels of 25(OH)D<sub>3</sub>, the living place was the only effective factor for vitamin D deficiency, and lifestyle did not affect 25(OH)D<sub>3</sub> level. Furthermore, only one-third of pregnant women living İzmir had optimal 25(OH)D<sub>3</sub> levels, even in summer and fall. For this reason, it seems reasonable to taking all pregnant subjects irrespective of region and season “The National Vitamin D Support Program.” In our study, the average 25(OH)D<sub>3</sub> level of pregnant women who took 1200 IU/day vitamin D in Erzurum was 21.9 ng/mL. It is suggested that a dose of 1200 IU/day vitamin D during the winter months in Erzurum may not be sufficient. The dose of vitamin D may have to be increased, or pregnant women living in cold regions may be screened for vitamin D levels.

There are some limitations of our study. One of them is that the study could not reflect seasonal differences, because the study was only conducted in the summer and fall, and the other is that we created study groups with similar personal characteristics in both provinces to better understand the lifestyle effects on vitamin D deficiency. Consequently, the study groups were small.

In conclusion, prevalence of vitamin D deficiency in pregnancy is high in Turkey even, in the sunny season. Behavioral factors are more effective on 25(OH)D<sub>3</sub> levels in pregnancy in sun-drenched regions. Living in high-altitude cold regions seems to be the most powerful risk factor for vitamin D deficiency. Even in sun-drenched regions, only one-third of pregnant subjects had ideal 25(OH)D<sub>3</sub> levels. Vitamin D supplementation is a cheap, safe, and effective means to fight vitamin D deficiency. It is recommended to increase compliance with “The National Vitamin D Support Program” at follow-up of all pregnant women, irrespective of region and season. The reasons of poor compliance with this program should be investigated. For more precise recommendations, more randomized, controlled studies on vitamin D deficiency in pregnancy are needed.

**Ethics Committee Approval:** Ethics committee approval was received for this study from the ethics committee of Şifa University.

**Informed Consent:** Written informed consent was obtained from patients who participated in this study.

**Peer-review:** Externally peer-reviewed.

**Author contributions:** Concept - E.B.G., S.G.; Design - E.B.G., S.G.; Supervision - M.K., S.G.; Resource - E.B.G., G.A.T., S.T.; Materials - S.T., A.G., G.C.; Data Collection&/or Processing - E.B.G., A.G., G.C.; Analysis&/or Interpretation - M.G.; Literature Search - M.G.; Writing - E.B.G., S.G.; Critical Reviews - M.K., S.G.

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