

Vitamin D status in the first-trimester: effects of Vitamin D deficiency on pregnancy outcomes.

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Abstract

Objective: To assess serum levels of 25-hydroxyvitamin D [25(OH)D] in the first trimester and to determine the factors affecting deficiency levels and its association with pregnancy outcomes.

Methods: Serum 25(OH)D concentrations were measured at 11-14 weeks' gestation in 229 singleton pregnancies using liquid chromatography-tandem mass spectrometry.

Results: The median serum 25(OH)D concentration was 10.8 ng/mL and 45.9% of women had severe vitamin D deficiency with concentrations of <10 ng/mL. Logistic regression analysis revealed that covered dressing style, lack of multivitamin intake, season of blood sampling (November-April) were factors associated with 25(OH)D deficiency. There was a negative correlation between 25(OH)D levels and gestational age at sampling. Low 25(OH)D levels were not associated with adverse pregnancy outcomes. Higher rate of cesarean section (CS) was noted in women with 25(OH)D \geq 10 ng/mL compared to those with 25(OH)D < 10mg/ml ($p= 0.01$).

Conclusion: A high prevalence of vitamin D deficiency was observed in early pregnancy which was related to dress code, use of multi-vitamins and season at sampling. Low 25(OH)D levels were not related with adverse pregnancy outcomes. Women with severe vitamin D deficiency were more likely to deliver vaginally.

Keywords: 25-hydroxyvitamin D; pregnancy outcome; vitamin D deficiency

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Introduction

The prevalence of vitamin D deficiency has increased and continues to be a major public health problem in many countries¹. High prevalence of vitamin D deficiency has been observed among pregnant women even in sun-rich areas². The main source of vitamin D is endogenous synthesis in the skin following exposure to sunlight, though a small percentage comes from dietary sources and supplement intake³. Vitamin D level is influenced by many factors such as skin pigmentation, use

of sunscreen, ethnicity, dress code,, geographic latitude, seasonal variation and use of prenatal vitamins^{4,5,6,7}.

Several studies reported the relationship between maternal vitamin D deficiency and adverse maternal and fetal outcomes including gestational diabetes, preeclampsia, preterm labor, low birth weight, and cesarean section^{8,9}.

Determination of vitamin D status in the first trimester provides an opportunity for early detection and prophylaxis of vitamin D deficiency that may help to reduce adverse pregnancy outcomes. The aim of this study was to assess the first trimester serum levels of 25-hydroxyvitamin D [25(OH)D] and the factors affecting the low levels in pregnant women. The association between vitamin D status and obstetric-neonatal outcomes were also evaluated.

Materials and methods

A total of 229 consecutive pregnant women who attended for their first antenatal visit (11-14 weeks of preg-

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nancy) at the outpatient clinic of Obstetrics and Gynecology, Bezmialem Vakif University, Istanbul, Turkey between December 2012 and July 2014 were studied. This study was approved by the Ethics Committee of our hospital. All participants were informed about the purpose of the study and requested to participate after giving written consent. They were asked to complete a questionnaire that included the characteristics of their pregnancy and information about the factors associated with vitamin D status. Non-fasting blood samples were taken and analyzed for 25(OH)D levels. The pregnancies were followed up until the end of their last trimester and the pregnancy outcomes were recorded in our database. At the end of the study, the data of pregnancy and neonatal outcomes were obtained from medical records and reviewed cautiously.

Women with a history of thyroid, parathyroid or adrenal disease, hepatic or renal failure, metabolic bone diseases and those taking medications that might affect vitamin D metabolism were excluded from the study. Multiple pregnancies were also excluded. None of the women in this study had previously received vitamin D supplementation.

Data on maternal characteristics included age, parity, blood pressure, pre-pregnancy weight, height, date of last menstrual period, smoking status (yes or no), education level, gestational age at blood sampling, type of clothing, consumption of milk and dairy products, use of multivitamin supplements, sunlight exposure (between 10 AM and 3 PM) and season of blood collection. Covered dressing style was defined as wearing dresses which cover body completely excluding hands and face whereas uncovered dressing style was wearing dresses exposing body to more sun light in a permissive manner. Consuming dairy products of at least 200 mL of milk or other milk products including cheese, butter, yoghurt 4-7 days/week was regarded as 'sufficient' milk consumption, whereas intake of dairy products three times a week or less was defined as 'insufficient' consumption¹⁰. The season for blood collection was dichotomized into winter (November-April) and summer (May-October).

Obstetric and neonatal data including gestational age, mode of delivery, and frequency of pregnancy-related complications, birth weight, Apgar scores, neonatal gender and presence of congenital anomalies were also recorded. The gestational age at delivery was determined by menstrual dating, while the ultrasound esti-

mates of gestational age were used in women that had missed or uncertain dates. The estimated date of delivery was determined by last menstrual period or by early ultrasound. Gestational hypertension was defined as a blood pressure of 140/90 mmHg or higher that develops after 20 weeks of gestation. Proteinuria was defined as a urine dipstick test $\geq 2+$, or more than 300 mg of protein in a 24-hour urine collection. Pre-eclampsia was defined as gestational hypertension with proteinuria¹¹. The diagnosis of gestational diabetes mellitus (GDM) was performed using a 2-step approach which involves an initial screening by measuring the plasma glucose 1 hour after a 50-g oral glucose load. If the plasma glucose level was 135 mg/dL or higher, it was followed by a 3-hour 100-g oral glucose tolerance test to establish the diagnosis of GDM¹². We defined preterm delivery as live births between 21 weeks and 36 weeks 6 days of gestation¹³. Stillbirth was defined as fetal death occurring after 20 weeks of gestation¹⁴. Infants were considered to be small-for gestational age (SGA) if the birth weight was less than the 10 th percentile for their gestational age¹⁵.

Measurement of 25(OH)D₃ and 25(OH)D₂ in human serum was made by using liquid chromatography-tandem mass spectrometry (LC-MS/MS) with an Agilent 1200 Infinity liquid chromatography (Agilent Technologies) coupled to an Agilent Technologies 6460 LC-MS/MS. The summation of serum 25(OH)D₂ and 25(OH)D₃ was used to reflect total serum 25(OH)D concentrations. The inter-assay and intra-assay coefficients of variation of total serum 25(OH)D level were 6.84% and 2.21%, respectively. The lower and upper limits of detection were 4.0 and 200 ng/mL, respectively. Although there is no consensus regarding the optimal 25(OH)D level, vitamin D status was categorized into groups as serum 25(OH)D levels <10 ng/mL, between 10–19 ng/mL, 20–29 ng/mL and >30 ng/mL, indicating severe, moderate and mild vitamin D deficiency and desirable reference limit, respectively¹⁶.

Data was presented as mean \pm standard deviation (SD) and median (minimum to maximum), and as percentages for categorical variables. The presence of a normal distribution for each variable was tested by the Shapiro Wilks test. Continuous variables were compared between groups by independent samples t tests for normally distributed variables and the Mann-Whitney U test for non-normally distributed variables. Pearson chi-square, Yates corrected chi-squared, and Fisher's exact tests were performed for categorical variables.

Spearman's rho correlation coefficient was used to identify the relationship between 25(OH)D and several continuous and numeric outcome variables: gestational age at serum collection, BMI, birth weight, Apgar score, gestational age at delivery. A logistic regression model was used to determine the factors associated with low levels of vitamin D. All data was analyzed using SPSS (SPSS, Inc., Chicago, IL, USA) and p values <0.05 were considered statistically significant.

Results

A total of 286 women were invited to participate in this study. Fifty-seven of them were excluded from the analysis. Reasons for exclusion were planned to be

followed-up elsewhere (n=15), decline to participate (n=5), lost to follow up (n=27), insufficient serum available for analysis (n=7), miscarriage (n=2), and termination of pregnancy because of Down's syndrome (n=1). After exclusion of the women mentioned above, 229 women were eligible for follow-up at the end of their pregnancy.

Characteristics of the study population are shown in Table 1. The median serum 25(OH)D concentration was 13 ± 9.4 ng/mL at 11-14 weeks of gestation, only 4.4% of the pregnancies had adequate serum 25(OH)D levels (>30 ng/mL). The prevalence of severe, moderate, and mild vitamin D deficiency was 45.9% (n=105), 36.2% (n=83), and 13.5% (n=31), respectively.

Table 1. Characteristics of the study population

Age (years)	29.49 ± 4.879
Parity	
Nulliparous, n (%)	81 (35.5)
Parous, n (%)	147 (64.5)
BMI (kg/m ²)	25.3 ± 4.5
Education, n (%)	
0-5 years	75(35.2)
6-8 years	38 (17.8)
≥9 years	100 (46.9)
Smoking, n (%)	15 (6.6)
Gestational age at serum collection (week)	13.2 (11-14)
Season of blood sampling, n (%)	
Winter (November-April)	116 (51.1)
Summer (May-October)	111 (48.9)
Mean systolic BP at trial entry (mmHg)	100 (80-165)
Mean diastolic BP at trial entry (mmHg)	70 (50-100)
Maternal multivitamin use, n (%)	
Yes	118 (63.1)
No	69 (36.9)
Consumption of dairy products, n (%)	
Sufficient	97 (49.7)
Insufficient	98 (50.3)
Dressing style, n (%)	
Covered	117 (61.3)
Uncovered	74 (38.7)
Exposure to sun, n (%)	
Yes	89 (46.4)
No	103 (53.6)

Data are shown as mean ± SD, median (minimum-maximum) or n (%).

BMI, body mass index; BP, blood pressure; 25(OH)D, 25-hydroxyvitamin D

Table 2 shows the baseline characteristics, and the obstetric-neonatal outcomes of the participants accord-

ing to vitamin D status. The subjects were divided into two groups using 25(OH)D < 10 ng/mL as a cut off

limit to identify severe deficiency; 105 (46%) women had 25(OH)D <10 ng/mL (group 1), while 124 (54%) women had 25(OH)D ≥ 10 ng/mL (group 2). We also compared the groups and found no significant differ-

ences between the groups concerning maternal age, BMI values, parity, education level, smoking, systolic and diastolic blood pressures at the baseline study visit and gestational age at serum collection.

Table 2. Association of serum 25-hydroxyvitamin D [25(OH)D] with pregnancy outcomes

	Serum 25 OH concentration (ng/mL)		p
	< 10 ng/mL (n=105)	= 10 ng/mL (n=124)	
Age (years) ^a	28.85 ± 4.99	30.03 ± 4.73	0.067
Parity ^b			
Nulliparous, n (%)	39 (37.1)	42 (34.1)	0.63
Parous, n (%)	66 (62.9)	81 (65.9)	
BMI (kg/m ²) ^a	25.60 ± 4.8	25.03 ± 4.21	0.35
Education, n (%) ^b			
0-5 years	41 (42.7)	34 (29.1)	0.10
6-8 years	14 (14.6)	24 (20.5)	
=9 years	41 (42.7)	59 (50.4)	
Smoking, n (%) ^c	6 (5.7)	9 (7.4)	0.81
Gestational age at serum collection (week) ^d	13.4 (11-14)	13.2 (11-14)	0.06
Gestational age at delivery (week) ^d	39 (34-41)	39 (24-41)	0.56
Season of blood sampling, n (%) ^b			
Winter (November-April)	71 (68.3)	45 (36.6)	< 0.0001
Summer (May-October)	33 (31.7)	78 (63.4)	
Mean systolic BP at trial entry (mmHg) ^d	100 (80-165)	100 (80-140)	0.84
Mean diastolic BP at trial entry (mmHg) ^d	70 (50-95)	70 (50-100)	0.59
Maternal multivitamin use, n (%) ^b			
Yes	49 (53.8)	69 (71.9)	0.011
No	42 (46.2)	27 (28.1)	
Consumption of dairy products, n (%) ^b			
Sufficient	37(39.4)	60(59.4)	0.005
Insufficient	57(60.6)	41(40.6)	
Dressing style, n (%) ^c			
Covered	75 (79.8)	42 (43.3)	<0.001
Uncovered	19 (20.2)	55 (56.7)	
Exposure to sun, n (%) ^b			
Yes	42 (44.7)	47 (48)	0.64
No	52 (55.3)	51 (52)	
Mode of delivery, n (%) ^c			
Vaginal delivery	63 (53.8)	54 (46.2)	0.018
Primary caesarean section	17 (32.7)	35 (67.3)	
Gestational diabetes, n (%) ^c	10 (9.5)	10 (8.1)	0.87
Hypertensive states, n (%)			
Pre-eclampsia ^e	2 (1.9)	2 (1.6)	1
Gestational hypertension ^c	5 (4.8)	6 (4.8)	1
Preterm birth, n (%) ^c	5 (4.8)	7 (5.6)	0.99
Small for gestational age, n (%) ^c	7 (6.7)	6 (4.8)	0.75
Intrauterine fetal death, n (%) ^e	1 (1)	1 (1)	1
Congenital malformation, n (%) ^c	2 (1.9)	1 (0.8)	0.88
Birth weight (g) ^a	3245 ± 496	3272 ± 440	0.66
Apgar score ^d			
1 min	8 (6-9)	8 (6-9)	0.36
5 min	9 (8-10)	9 (8-10)	0.22
Infant gender (female), n (%) ^b	51 (51)	50 (44.6)	0.35

Data are shown as mean ± SD, median (minimum-maximum) or n (%). ^at-test; ^b Pearson chi-squared test; ^c Yates-corrected chi squared test; ^dMann-Whitney U test; ^eFisher's exact test
BMI, body mass index; BP, blood pressure

Vitamin D status was found to be significantly higher in women who used multi-vitamins during pregnancy and who had higher consumption of dairy products compared to women who did not use multi-vitamins and had lower consumption of dairy products respectively. Pregnant women whose blood samples were drawn in summer months had significantly higher levels of 25(OH)D than those examined in winter months. Women with covered dressing style had significant-

ly lower levels of 25(OH)D than that of women who were dressed in uncovered style. Binary logistic regression analysis determined that covered dressing style, absence of receiving multivitamin supplements, season of blood sampling (November-April) were factors related to severe vitamin D deficiency, while other variables such as BMI, parity, smoking, consumption of dairy products and sun exposure were not significantly related to 25(OH)D levels (Table 3).

Table 3. Logistic regression analysis of factors associated with serum 25(OH)D status

Variables	OR ^a	95 % CI	p-value
Dressing style			
Covered	Ref		
Uncovered	5.1	2.26-11	<0.0001
Maternal multivitamin use			
No	Ref		
Yes	2.69	1.28-5.66	0.009
Season of blood sampling			
Winter	Ref		
Summer	5	2.32-10.7	<0.0001

OR, odds ratio; CI, confidence interval; Ref, reference category.

There were no differences in the frequency of adverse obstetric and neonatal outcomes in women with 25(OH)D concentrations <10 ng/mL compared to those with concentrations ≥10 ng/mL (Table 2).

The mode of delivery was vaginal in 118 cases (51.5%), and caesarean delivery in 111 cases (48.5%) of which 52 (30.7%) had primary caesareans. The current analysis was limited to women who delivered vaginally and who delivered by primary caesarean. A significantly higher primary caesarean section rate was noted in women with 25(OH)D concentration ≥10 ng/mL compared to those with 25(OH)D < 10 (67.3% vs 32.7%, p= 0.018).

There were no significant correlations between the 25(OH)D levels and numerical outcome variables including gestational age at delivery, birth weight and Apgar scores while there was a weak negative correlation between 25(OH)D levels and gestational age at sampling (Spearman's rho=-0.161, p=0.015). The correlation analysis revealed that 25(OH)D levels at 11th week of gestation was higher than those at 14th week of gestation.

Discussion

The findings of this study indicate that the higher prevalence of severe vitamin D deficiency (45.8%) in preg-

nant women is likely to be a result of less exposure to sunlight due to their dress code and lack of multivitamin usage. Higher rates of severe vitamin D deficiency were previously reported in the literature from Turkey^{10,17}. Those prior studies have shown that covered dress style may be an important factor affecting the prevalence of vitamin D deficiency in Muslim populations due to the religious and cultural reasons^{10,18}.

The prevalence of low vitamin D levels was greater in cases which were sampled in winter, in women who had limited access to sunlight, those living in countries at higher latitudes and those with higher skin pigmentation^{16,18-20}. In a study by Perez et al.,²¹ it was observed that only 35.9% of pregnant women in the first trimester had adequate serum 25(OH)D levels (≥30 ng/mL) and non-Caucasian ethnicity, season at sampling (autumn/winter) and nulliparity were found to be associated with 25(OH)D deficiency. The season at the time of serum sampling was also found to be important similar to previous studies which reported lower levels of vitamin D in the winter compared with summer season^{19,21}.

A study measuring serum 25(OH)D levels of 79 pregnant women in the third trimester in our country found considerably a high rate of vitamin D deficiency in pregnant women during the winter season and it showed

also the relationship between multivitamin supplementation, good socioeconomic status and high levels of vitamin D¹⁷.

We found no significant association between low 25(OH)D levels in the first trimester and adverse pregnancy outcomes which is in agreement with other reports^{9,13,22}. Bodnar et al.²³ reported a five-fold increased risk of pre-eclampsia in women with 25(OH)D <15 ng/mL at <22 weeks of pregnancy. On the contrary, Shand et al.²⁴ evaluated vitamin D status (between 10 and 20 week of gestation) in a group of women at high risk of pre-eclampsia and found no association between vitamin D deficiency and risk of pre-eclampsia. Wei et al.¹¹ reported that 25(OH)D < 20 ng/mL at late mid-trimester (24-26 weeks of gestation) but not early pregnancy (12-18 weeks of gestation) was associated with increased risk of pre-eclampsia. In contrast to our study, Parlea et al.²⁵ noted a two-fold increased risk of GDM in women with 25(OH)D <29.4 at 15-18 weeks of gestation.

Similar to our findings, Thorp et al.²⁶ reported no relation between recurrent preterm birth and low vitamin D levels (<20 ng/mL) in women at 16-22 week of gestation in a case control study of women with prior preterm birth. Although we found no correlation between the first trimester serum 25(OH)D levels and the neonatal gestational age or weight, a recent study demonstrated that first trimester 25(OH)D concentration of less than 12 ng/mL was associated with increased risk of low birth weight and SGA infants¹⁸. A study including 152 nulliparous women in Turkey showed that 44.6% of pregnant women have vitamin D deficiency and maternal vitamin D deficiency is associated with an increased risk of SGA²⁷. The variation in results between studies may also be explained by differences in cut-off points used, population characteristics, sample size and methods to measure 25(OH)D²².

An interesting finding in our study was that women with higher levels of vitamin D at first trimester had higher rate of caesarean section than those with severe vitamin D deficiency. It might be explained by the fact that women who deliver vaginally were more likely to have risk factors for vitamin D deficiency such as wearing covered dress styles and less use of multi-vitamins than women who had caesarean section. Furthermore,

the current study was focused primarily on the first trimester of pregnancy and we cannot rule out the possibility that differences in 25(OH)D concentrations become apparent only in the third trimester. A recent study with a large size of population revealed no association between the first trimester vitamin D status and the mode of delivery²⁸. Our findings are in contrast to those of two other studies, Merewood et al.⁹ found almost four-fold increase in the rate of primary caesarean section in women with 25(OH)D levels below 15 ng/mL at the time of the delivery, and Scholl et al.²⁹ reported increased risk for caesarean delivery for women with 25(OH)D levels below 12 ng/mL.

Strengths of the current study include its prospective design and the use of the gold-standard method for measuring 25(OH)D levels. The main limitation of this study, on the other hand, is the small sample size and the small number of women with adverse pregnancy outcome which limit making a definitive conclusion regarding the impact of vitamin D status on adverse pregnancy outcomes. In addition, we could not completely evaluate the underlying factors such as sunscreen use, skin pigmentation, socioeconomic status and lifestyle behaviour contributing to vitamin D status. The other limitation of our study is determining vitamin D status only in first trimester and the determination of vitamin D status in third trimester would be beneficial to indicate the possible interaction of vitamin D in pregnancy outcomes. In spite of the limitations, this study adds to the few that report on serum 25(OH)D levels in the first trimester and its relation to pregnancy outcomes.

Conclusion

We propose that high prevalence of severe vitamin D deficiency in the first-trimester among pregnant women can be related with dress code, use of multi-vitamins and season at sampling. Low 25(OH)D levels may not be associated with increased risk of adverse pregnancy outcomes. Moreover, it is unanticipated that women with lower levels of vitamin D are more likely to deliver vaginally. Yet, further research is required to determine the levels of vitamin D in early pregnancy and the impact of low 25(OH)D concentrations on adverse pregnancy outcome.

Declaration of interest

The authors have no conflict of interest.

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