Journal of
Babol University of Medical Sciences
JBUMS
e-ISSN: 2251-7170 p-ISSN: 1561-4107

The Effect of Vitamin D Deficiency on Pregnancy Outcomes

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Article Type
Research Paper

Received:
Dec 28th 2021
Revised:
Mar 12nd 2022
Accepted:
May 14th 2022

ABSTRACT

Background and Objective: Supplying vitamin D during pregnancy is necessary for fetal calcium homeostasis and bone growth and its mineralization, and its deficiency can lead to pregnancy complications. The present study was conducted to investigate the effect of vitamin D level on pregnancy outcomes in pregnant women.

Methods: This cross-sectional study was conducted among 450 pregnant women referring to Ayatollah Rouhani Hospital in Babol. Mothers' information was collected using a questionnaire. Blood samples were collected from people to measure the serum 25 hydroxyvitamin D_3 level by ELISA method using the EUROIMMUN kit. Patients were divided into two groups (vitamin D level< 25 and vitamin D level> 25) and were compared.

Findings: 247 people (54.8%) had vitamin D level< 25 and 203 people (44.8%) had vitamin D level> 25. People with lower vitamin D levels had significantly more gestational hypertension (p=0.03) and premature rupture of membranes (PROM) (p=0.04) compared to people with higher vitamin D levels. The variables of labor pain, diabetes, fetal growth restriction and prolonged pregnancy did not show significant differences in people with high and low levels of vitamin D. Pregnancy complications including pre-eclampsia, preterm delivery, placental abruption and fetal growth restriction did not show any significant difference in people with high and low levels of vitamin D. In the evaluation of neonatal outcomes, infant weight, infant gender, fifth minute Apgar, head circumference and admission to the neonatal intensive care unit, no significant differences were found.

Conclusion: The results of the study showed that some pregnancy complications such as gestational hypertension and premature rupture of membranes (PROM) in pregnant women are related to vitamin D deficiency. Therefore, it is necessary to design plans to check the vitamin D level of mothers before pregnancy and correct it during pregnancy.

Keywords: *Vitamin D, Pregnancy, Newborn, Gestational Hypertension, Premature Rupture of Membranes (PROM).*

Cite this article: Habibi S, Javadian M, Ghorbani H, Khafri S, Abedi Samakoosh M, Yazdani Sh. The Effect of Vitamin D Deficiency on Pregnancy Outcomes. *Journal of Babol University of Medical Sciences*. 2022; 24(1): 453-62.

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Publisher: Babol University of Medical Sciences

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[DOR: 20.1001.1.15614107.1401.24.1.54.0]
[DOI: 10.22088/jbums.24.1.453]

Introduction

Maternal 25 hydroxyvitamin D deficiency is a major health problem, and approximately two-thirds of pregnant women in the United States have suboptimal vitamin D status (1). That's because pregnancy may increase the need for vitamins (2). On the other hand, it seems that vitamin D has multifaceted effects on pregnancy, which are beyond the specified activities of calcium and bone metabolism (3). Low levels of vitamin D during pregnancy are associated with various health problems and outcomes from egg implantation to adulthood diseases (4). It is now accepted that maternal vitamin D deficiency affects maternal and fetal calcium homeostasis and it is expected to affect bone development in the fetus as well (5).

Today, it has become clear that even in developed countries, vitamin D deficiency is more common than it was thought to be (6). This deficiency in pregnant women is not limited to a specific racial group or region and is seen with a wide prevalence all over the world (7, 8). Serum levels of 25 hydroxyvitamin D less than 25 ng/ml during pregnancy is reported in 17-18% of Caucasian women (9), 61% of women in New Zealand (10), 32-42% of Indian women (11), 59-84% of Kuwaiti women (12), 84% of Iranian women (13) and 75% of pregnant women in the United Arab Emirates (14). Such a widespread deficiency will undoubtedly have harmful effects on the health of pregnant women and their babies (15). Vitamin D deficiency during pregnancy is associated with preeclampsia, gestational diabetes, bacterial vaginosis, rickets, decreased bone density, asthma, and schizophrenia. The relationship between maternal vitamin D status and fetal growth, which is often determined by measuring infant birth weight and low birth weight, has been investigated by studies with multiple outcomes (1).

A study in 2021 reported higher rate of abortion in women with vitamin D deficiency, and the level of vitamin D in women of reproductive age has been introduced as one of the health indicators (16).

In pregnant women receiving vitamin D supplements, the risk of pre-eclampsia, gestational diabetes and low birth weight decreased, and the risk of premature birth decreased in the group receiving vitamin D supplements to a very small extent. In addition, in regard to maternal side effects, the risk of severe bleeding after delivery was also decreased (17, 18).

Due to the fact that in our region, no study has been conducted to determine the level of vitamin D in pregnant women and its relationship with pregnancy outcomes, this study was conducted to evaluate the effect of vitamin D deficiency on pregnancy outcomes in pregnant women referred to Ayatollah Rouhani Hospital in Babol.

Methods

After approval by the ethics committee of Babol University of Medical Sciences with code IR.MUBABOL.HRI.REC.1397.266, this cross-sectional study was conducted on 450 pregnant women with a gestational age of 24 weeks or more who referred to Ayatollah Rouhani Hospital for termination of pregnancy. According to 5% prevalence of fetal growth restriction in healthy people and default doubling of outcomes of vitamin D deficiency, vitamin D outcomes in alpha level equal to 0.05 and 80% balance, sample size in healthy and vitamin D deficiency groups was determined as 450 people.

Pregnant mothers of 24-40 weeks, singleton pregnancy and age of 18-35 years whose serum levels of 25 hydroxyvitamin D were examined during hospitalization were included in the study if they were willing to participate in the study. Pregnant women with a history of pre-pregnancy diabetes, dyslipidemia, chronic hypertension, untreated thyroid disease, parathyroid, kidney disease, the presence of a history of fetal abnormalities, twin pregnancy, or taking drugs that cause hyperglycemia (corticosteroids and thyroid

[DOR: 20.1001.1.15614107.1401.24.1.54.0]
[DOI: 10.22088/jbums.24.1.453]

hormones) and being unwilling to continue participation were excluded from the study. Demographic and fertility characteristics including age, BMI, level of education, prenatal visit status, family income level, fish consumption, gestational age, number of pregnancies, weight gain during pregnancy, serum level of 25 hydroxyvitamin D in the first trimester of pregnancy were recorded. Then the blood sample was prepared during the delivery of the mother and in a non-fasting state. A list containing questions related to the history and problems during pregnancy such as gestational diabetes, cases of insulin treatment, gestational hypertension, preeclampsia and eclampsia, bleeding during pregnancy, premature birth, placental abruption, placenta previa was completed with the help of the mother's prenatal record. Maternal and neonatal complications during and after delivery, such as type of delivery, age of termination of pregnancy, cause of termination of pregnancy (spontaneous, medical), weight of the baby, Apgar score of the baby (first and fifth minutes), bleeding after delivery were completed.

In order to evaluate the serum level of 25 hydroxyvitamin D, 3 cc blood sample was collected from the subject in a non-fasting state and sent to the laboratory of Ayatollah Rouhani Hospital in Babol. After centrifugation, the serum was kept at a temperature below 20 degrees until the analysis. The samples were taken out only on the day of analysis to evaluate the relevant analyte level and were read with EUROIMMUN kits and by automatic ELISA reader (AdvoCare Company). In this method, the measurement is designed competitively and with the help of monoclonal antibody. The respective wells are covered by the monoclonal antibody that is against the 25 hydroxyvitamin D molecule. Then, the standard solution and the patient's sample were coated with antibody and were adjacent to the extraction buffer at the bottom of the wells, and incubation of 25 hydroxyvitamin D bound to biotin was added to the wells to compete with serum 25 hydroxyvitamin D. Then, streptavidin bound to HRP enzyme was attached to Vitamin D Biotin. Rinsing was done and then the dye solution containing O_2H_2 and chromogen was poured into the well and incubated. After incubation, a blue color appeared and was measured colorimetrically, which was inversely proportional to 25 (OH) 4ddD concentration of the samples. The information and data obtained from the two groups were analyzed by SPSS 22 statistical software and X^2 statistical test, and $p<0.05$ was considered significant.

Results

In this cross-sectional study that was conducted on 450 pregnant women with a gestational age of 24 weeks or more who referred to Ayatollah Rouhani Hospital for termination of pregnancy, the mean age of mothers with vitamin D levels less than 25 was 28.8±4.8 years, and with vitamin D level greater than and equal to 25, it was 28.4±4.8 years (Table 1).

In the examination of obstetric records of pregnant mothers, among people with vitamin D level less than 25, 40.9% had one pregnancy and 59.1% had two pregnancies, while among people with vitamin D level more than 25, 52% had one pregnancy and 48% had two or more pregnancies (p=0.01). The highest rate of pregnancy over 20 weeks in people with vitamin D level less than 25 was related to the first pregnancy (46.2%), and the lowest value was related to two pregnancies over 20 weeks (16.2%). However, in people with vitamin D level above 25, these values were 59.4% and 11.4%, and this difference was statistically significant (p=0.01). In variables of abortion (p=0.09), number of visits (p=0.43), gestational age (p=0.44), history of infertility (p=0.54), infertility treatment (p=0.25) and the method of termination of pregnancy (p=0.23) did not show any significant difference (Table 2).

Disease history including gestational diabetes, hypothyroidism and other diseases did not show any significant difference in people with vitamin level higher or lower than 25. People with high vitamin D

[DOR: 20.1001.1.15614107.1401.24.1.54.0]
[DOI: 10.22088/jbums.24.1.453]

levels had significantly better history of vitamin D consumption (p<0.001). Moreover, vitamin D level did not show a significant relationship with hemoglobin level (p=0.97).

The reason for referral and hospitalization of the mothers in the two studied groups were compared, and gestational hypertension and premature rupture of membranes were significantly higher in the two groups with vitamin D deficiency. The reduction of fetal movements was more in two groups with normal vitamin D and above 25 (Table 3).

In the evaluation of pregnancy complications, the variables of preeclampsia (p=0.71), preterm delivery (p=0.17), premature rupture of membranes (p=0.14), placental abruption (p=0.41) and intrauterine growth restriction (p=0.55) did not show significant differences in people with high and low levels of vitamin D.

In the examination of neonatal outcomes, infant weight (p=0.06), infant gender (p=0.29), fifth minute Apgar (p=0.73), head circumference (p=0.22) and hospitalization in the intensive care unit (p=0.91) did not show a significant difference in people with high and low levels of vitamin D (Table 4).

Table 1. Evaluation of the demographic characteristics of the studied subjects based on vitamin D level

Group / Variable	Vitamin D levels		p-value*
	Less than 25 Number(%)	Greater than and equal to 25 Number(%)	
Mother's job			
Housewife	223(90.3)	176(87.1)	0.29
Employed	24(9.7)	26(12.9)	
Mother's education			
High school	74(30.0)	49(24.3)	
Diploma	113(45.7)	96(47.5)	0.35
University education	60(24.3)	57(28.2)	
Father's job			
Self-employed	195(78.9)	162(80.2)	
Employee	25(10.1)	28(13.9)	0.10
Worker and farmer	27(10.9)	12(5.9)	
Pregnancy			
Intended	204(82.6)	175(86.6)	0.24
Unintended	43(17.4)	27(13.4)	
Income			
Low	82(32.2)	49(24.3)	
Medium	132(53.4)	131(64.9)	0.04
High	33(13.4)	22(10.9)	
Fish consumption			
Yes	91(36.8)	50(24.8)	0.006
No	156(63.2)	152(75.2)	
Mother's age (Mean±SD)	28.8±4.8	28.4±4.8	0.87
BMI (Mean±SD)	29.5±4.5	29.6±4.8	0.74

*Chi-square test

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Table 2. Examination of the obstetric records of the study subjects based on vitamin D level

Group / Variable	Vitamin D levels		p-value
	Less than 25 Number(%)	Greater than and equal to 25 Number (%)	
Number of pregnancies			
One	101(40.9)	105(52)	0.01
More than two	146(59.1)	97(48)	
Pregnancy over 20 weeks			
Zero	114(46.2)	120(59.4)	
One	93(37.7)	59(29.2)	0.01
Greater than and equal to 2	40(16.2)	23(11.4)	
Abortion			
Yes	180(72.9)	161(79.7)	0.09
No	67(27.1)	41(20.3)	
Number of visits			
Non-routine	197(79.8)	167(82.7)	0.43
Routine	50(20.2)	35(17.3)	
Visit			
Midwife	31(12.6)	30(14.9)	0.47
Doctor	216(87.4)	172(85.1)	
Gestational age (Mean±SD)	37.45±2.85	37.65±2.47	0.44
History of infertility			
No	230(93.1)	185(91.6)	0.54
Yes	17(6.9)	17(8.4)	
Infertility treatment			
No	234(94.7)	186(92.1)	0.25
Yes	13(5.3)	16(7.9)	
Termination method			
Natural	121(52.6)	111(58.4)	0.23
Cesarean section	109(47.4)	79(41.6)	

Table 3. Investigating the reason for referral of pregnant mothers during admission based on vitamin D level

Group / Variable	Vitamin D levels		p-value
	Less than 25 Number(%)	Greater than and equal to 25 Number(%)	
Delivery pain			
No	228(92.3)	190(94.1)	0.46
Yes	19(7.7)	12(5.9)	
Decreased movement			
No	234(94.7)	175(86.6)	0.003
Yes	13(5.3)	27(13.4)	
PROM			
No	145(58.7)	137(67.8)	0.04
Yes	102(41.3)	65(32.2)	
Diabetes			
No	226(91.5)	185(91.6)	0.97
Yes	21(8.5)	17(8.4)	
Gestational hypertension			
No	210(85)	185(91.6)	0.03
Yes	37(15)	17(8.4)	
Intrauterine growth restriction			
No	232(93.9)	189(93.6)	0.87
Yes	15(6.1)	13(6.4)	
Post-term pregnancy			
No	238(96.4)	192(95)	0.49
Yes	9(3.6)	10(5)	

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[DOI: 10.22088/jbums.24.1.453]

Table 4. Examining the neonatal outcomes of the studied subjects based on vitamin D level

Group / Variable	Vitamin D levels		p-value
	Less than 25 Number(%) or Mean±SD	Greater than and equal to 25 Number (%) or Mean±SD	
Baby's weight (grams)	3081.57±611.71	3183.93±548.77	0.06
Gender of the baby			0.29
Boy	122(56.7)	90(51.4)	
Girl	93(43.3)	85(48.6)	
Fifth minute Apgar	9.84±0.57	9.82±0.59	0.73
Head circumference	34.10±2.02	34.32±1.81	0.22
Neonatal intensive care unit			0.91
Yes	9(3.6)	7(3.5)	
No	238(96.4)	195(96.5)	

Discussion

The results of the present study showed that people with lower vitamin D levels had significantly higher rate of gestational hypertension and premature rupture of membranes than people with high vitamin D levels. The history of disease such as diabetes did not show any significant difference in people with vitamin levels more than 25 and less than 25. Furthermore, the level of vitamin D did not show a significant relationship with the level of hemoglobin. According to the results, the variables of labor pain, diabetes, fetal growth restriction and prolonged pregnancy did not show significant differences in people with high and low levels of vitamin D. Moreover, in people with vitamin D levels greater than or equal to 25, the amount of income, use of sunscreen, and fish consumption were higher than those with vitamin D levels less than 25.

In the evaluation of pregnancy complications, the variables of preeclampsia, preterm delivery, premature rupture of membranes, placental abruption, and fetal growth restriction did not show significant differences in people with high and low levels of vitamin D. In the evaluation of neonatal outcomes, infant weight, infant gender, fifth minute Apgar, head circumference and admission to the neonatal intensive care unit, no significant difference was found in people with high and low levels of vitamin D.

The study of Rodriguez et al. is consistent with the present study. Nevertheless, there was no significant relationship between vitamin D level and head circumference and cesarean delivery in our study. In their cohort study, Rodriguez et al. reported that adequate vitamin D levels in pregnancy were associated with a reduced risk of cesarean delivery. They found no evidence of an association between maternal vitamin D levels in pregnancy with gestational diabetes and preterm birth. They also found that babies born to mothers with higher levels of vitamin D during pregnancy had smaller head circumferences. Moreover, no other neonatal prognoses at birth were associated with vitamin D levels (19). The decrease in the strength of the pelvic muscle structure and the ability of the mother to push out the fetus is one of the important issues. Therefore, vitamin D deficiency is related to muscle mass and strength, and since the calcium serum level, which is regulated by vitamin D, plays an important role in smooth muscle function at the beginning of labor, this hypothesis is strengthened. However, one of the reasons for the lack of difference between the two groups regarding cesarean delivery is that in our study, there were not enough people with severe vitamin D deficiency for a fair judgment.

[DOR: 20.1001.1.15614107.1401.24.1.54.0]
[DOI: 10.22088/jbums.24.1.453]

The study of Gernand et al. is in line with our study. Gernand et al. showed that maternal vitamin D status before 26 weeks was not associated with the risk of prolonged labor, instrumental delivery, and birth weight (20).

Eggemoen et al. conducted a longitudinal study aimed at investigating vitamin D levels during pregnancy. The result of this study is consistent with our study. In this study, fetal anthropometric indicators such as birth weight, head circumference and abdominal circumference were measured. The results showed that there is no relationship between the fetal anthropometric indices and the mother's vitamin D level (21).

The study of Miliku et al. was not consistent with our study. Miliku et al. showed that low levels of maternal vitamin D are associated with fetal growth restriction and increased risk of preterm birth and small for gestational age at birth (22). One of the reasons for the contradiction between our study and this study was that birth weight alone was evaluated as the main outcome related to fetal growth restriction, and this is associated with severe limitations. That's because birth weight does not provide information about fetal growth and longitudinal development or fetal growth patterns or fetal body proportions.

The results of the study by Khaing et al. showed that calcium supplementation can reduce the risk of preeclampsia. Vitamin D supplementation can also be useful (17). The result of this study is not consistent with our study. In our study, no statistical difference was observed between mother's vitamin D level and pregnancy complications such as preeclampsia. However, this issue was based on evidence from a limited number of studies that were conducted in a short period of time, so larger studies are needed to determine the usefulness of vitamin D supplementation alone or in combination with calcium to reduce the risk of preeclampsia.

The study of Palacios et al. is not consistent with our study. Palacios et al. conducted a systematic review regarding the effect of vitamin D supplementation on women during pregnancy. The results showed that the risk of preeclampsia, gestational diabetes, and birth weight may decrease in pregnant women receiving vitamin D supplements. The risk of premature birth in the group receiving vitamin D supplementation showed a very small decrease. In relation to maternal side effects, it reduces the risk of severe postpartum bleeding. No cases of hypercalcemia were observed (18).

According to evaluations, it can be concluded that people with lower levels of vitamin D significantly have more gestational hypertension and premature rupture of membranes than people with high levels of vitamin D. History of disease, including diabetes, hypothyroidism and other diseases, and hemoglobin level did not show significant difference in people with vitamin levels more than 25 and less than 25. Furthermore, labor pain, diabetes, fetal growth restriction and prolonged pregnancy did not show significant differences in people with high and low levels of vitamin D. In the evaluation of pregnancy complications and neonatal outcomes, there was no significant difference between people with high and low levels of vitamin D. In people with higher levels of vitamin D, the amount of income, use of sunscreen, and consumption of vitamin D supplements were higher than in people with lower levels of vitamin D.

Lack of access to the initial level of vitamin D due to the lack of a vitamin D measurement kit and the small number of patients with severe vitamin D deficiency, which can be used to better evaluate complications, were among the limitations of this study.

[DOR: 20.1001.1.15614107.1401.24.1.54.0]
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Acknowledgment

We hereby acknowledge the research and technology department of Babol University of Medical Sciences as well as the Research Development Unit of Ayatollah Rouhani Hospital.

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[DOI: 10.22088/jbums.24.1.453]

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