



# ORIGINAL: Impact of Vitamin D Deficiency on Pregnancy Outcomes in Iranian Women: An Observational Study in 2020-2021

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## ABSTRACT

**Introduction:** Vitamin D deficiency in pregnancy can lead to pre-eclampsia, gestational diabetes and preterm delivery. Adequate vitamin D intake from supplements and sunlight is essential, but careful monitoring is required to avoid toxicity. Further research is needed to fully understand the effects of vitamin D on pregnancy.

**Material and Methods:** A study with a sample size of 73 individuals investigated the relationship between vitamin D levels and pregnancy outcomes. The study included 148 pregnant women with normal or low vitamin D levels. Previous research suggests a significant prevalence of vitamin D insufficiency in pregnant women. The effects of maternal vitamin D status on both the foetus and the mother were the focus of the study.

**Results:** The results of the current study showed that the mean age of the mothers in the normal vitamin D group was  $30.00 \pm 4.98$  years and in the vitamin D deficient group it was  $29.33 \pm 5.49$  years. The results show that there is no significant relationship between maternal age and vitamin D level. Also gravidity, parity, number of live births, gestational age and pre-eclampsia were not significantly associated with vitamin D deficiency. While preterm birth, gestational diabetes and preterm rupture of membranes were significantly more common in people with vitamin D deficiency.

**Conclusion:** Insufficient vitamin D levels are associated with adverse pregnancy outcomes. Insufficient levels increase the risk of preterm birth, pre-eclampsia, gestational diabetes, premature rupture of membranes, miscarriage and stillbirth. Maintaining optimal levels is essential. More research is needed on prevention strategies.

## Introduction

Vitamin D, a fat-soluble vitamin, is essential for human health, primarily by regulating calcium absorption and bone mineralisation. However, its influence extends beyond skeletal health to include immune function, hormone regulation and cell

growth (1-4). Vitamin D is primarily produced by skin exposure to sunlight and is converted in the liver and kidneys to its active form, calcitriol, which modulates gene expression (5). Vitamin D deficiency, which is prevalent worldwide, is associated with several health

problems, including rickets, osteomalacia and increased fracture risk (6-8). Dietary sources, such as fatty fish and fortified foods, are limited and supplementation is required for many individuals (9, 10). While excess vitamin D can lead to toxicity, appropriate supplementation is generally safe and beneficial (11, 12).

In addition to bone health, vitamin D is increasingly recognised for its role in reproductive health, with deficiency associated with infertility and pregnancy complications (13, 14). Optimal vitamin D status during pregnancy is essential for both maternal and fetal well-being. However, a balanced intake of vitamin D, calcium and other nutrients is essential to prevent adverse effects (12, 15). Vitamin D deficiency is associated with impaired immune function and increased susceptibility to infection (8, 16). During pregnancy, adequate vitamin D levels are critical for reducing the risk of complications such as pre-eclampsia, gestational diabetes, and preterm birth (16, 17). Public health interventions and supplementation strategies are needed to address vitamin D insufficiency (18). The timing and dose of vitamin D supplementation during pregnancy may have a significant impact on pregnancy outcomes. Research suggests an association between adequate vitamin D levels and a reduced risk of preterm birth and inflammation. However, more research is needed to fully understand the relationship between vitamin D supplementation and various pregnancy outcomes and to guide optimal supplementation recommendations (16,19). This study aims to investigate the association between maternal vitamin D status throughout pregnancy and a range of pregnancy outcomes, including preterm birth, gestational diabetes and pre-eclampsia. By elucidating these associations, we aim to improve our understanding of the role of vitamin D in maternal and fetal health and to inform strategies to optimise vitamin D status during pregnancy.

## Methods

### Study Design

This observational longitudinal study was

conducted at [Vali-e-Asr Hospital] in Qaemshahr between 2020 and 2021.

### Sample size calculation

Sample size calculation was performed using G\*Power 3.1 software. The parameters for the sample size calculation were as follows:

- Alpha (significance level) ( $\alpha$ ): 0.05
- Power ( $\beta$ ): 0.8
- Standard deviation (SD): Based on similar studies, an SD of 10 units was assumed for serum vitamin D levels.
- Effect size ( $\mu$ ): 0.5 units, representing the expected mean difference in serum vitamin D levels between groups.

Given the specified parameters, the required sample size for the study was estimated to be 73 participants. This sample size provides sufficient power to detect a medium effect with 80% power at a significance level of 0.05.

$$N = \frac{\left( Z_{1-\frac{\alpha}{2}} + Z_{1-\beta} \right)^2 (S_1^2 + S_2^2)}{(\mu_1 - \mu_2)^2} = \frac{(1.96 + 1.28)^2 (10^2 + 9.4^2)}{(16.2 - 11)^2} = 73$$

### Participants and Inclusion/ Exclusion Criteria

The present study included 148 pregnant women receiving care at Vali-e-Asr Hospital in Qaemshahr, Iran, between 2020 and 2021.

#### Inclusion criteria

- Age 17 to 44 years
- Singleton pregnancy
- No known chronic diseases
- Literacy in both reading and writing
- Willingness to participate in the study

#### Exclusion criteria

- Patient non-cooperation in providing information
- Lack of patient agreement for the continuation of the research endeavor
- Age below 17 or above 44 years
- Inadequate patient documentation

### Participant characteristics

The participants were divided into two groups based on serum vitamin D levels:

#### Normal vitamin D group (n=74):

These participants had serum vitamin D

levels of 20 ng/ml or higher.

#### Low vitamin D group (n=74):

These participants had serum vitamin D levels below 20 ng/ml.

#### Data Collection and Analysis

Informed consent was obtained from all participants prior to data collection. A structured questionnaire was administered to collect information on demographics, medical history, obstetric history, diet, and lifestyle. The questionnaire was developed based on previous research and was pilot tested on a small group of pregnant women to ensure its validity and reliability. Blood samples were collected from all participants at the first prenatal visit. The samples were analyzed for serum vitamin D levels using a standard radioimmunoassay (RIA) method.

The collected data were entered into SPSS software version 26 for statistical analysis. Descriptive statistics were used to summarize the demographic characteristics and serum vitamin D levels of the participants. Chi-square and Fisher's exact tests were used to compare the categorical variables between the two groups. Independent t-tests or Mann-Whitney U tests were used to compare the continuous variables between the two groups. To examine the association between serum vitamin D levels and pregnancy outcomes, multivariable logistic regression models were used. The models were adjusted for potential confounding variables, such as age, parity, and body mass index. A significance level of 0.05 was used for all statistical tests.

## Results

#### Data Distribution Assessment

Maternal age and body mass index (BMI) showed normal distribution patterns, whereas gestational age, gravidity and parity showed deviations from normality. There was a high prevalence of overweight or obesity (BMI > 29) in both the normal and low vitamin D groups. The Kolmogorov-Smirnov test confirmed the normal distribution of maternal age and BMI ( $p > 0.05$ ), while the remaining variables showed non-normal distributions (**Table 1**).

#### Group comparability

T-tests and Mann-Whitney U tests were used to assess the comparability of the study groups based on vitamin D status. As shown in **Table 2**, no significant differences in baseline characteristics, including number of pregnancies, deliveries, live births, and stillbirths, were found between the vitamin D deficient and vitamin D sufficient groups ( $p > 0.05$  for all comparisons). These results suggest that the two groups were comparable at baseline.

#### Investigation of the correlation between serum vitamin D levels and patient demographics

##### Association between gestational age, body mass index and vitamin D status

The present study investigated the possible associations between gestational age, body mass index (BMI), and serum vitamin D levels. The data presented in **Table 3** were subjected to statistical analysis to evaluate these associations. No statistically significant

**Table 1. Data normality check.**

Variable	Vitamin D level	Test statistic	Degrees of freedom	P-value
Maternal age	Deficient	0.990	73	P = 0.067
	Normal	1.050	71	P = 0.053
BMI	Deficient	0.060	73	P = 0.200
	Normal	0.076	71	P = 0.200
Live birth number	Deficient	2.590	73	P = 0.001
	Normal	2.270	71	P = 0.001
Stillbirth number	Deficient	2.880	73	P = 0.001
	Normal	2.970	71	P = 0.001

**Table 2. Comparison of Baseline Variables Between Normal and Deficient Vitamin D Levels.**

Variable	Mean (Normal Vitamin D)	SD (Normal Vitamin D)	Mean (Vitamin D Deficiency)	SD (Vitamin D Deficiency)	P-value
Age	30.05	5.02	29.28	5.36	0.369
BMI	29.54	5.74	30.05	5.02	0.471
Number of pregnancies	0.95	1.86	2.04	0.95	0.000
Number of deliveries	1.68	0.64	1.68	0.77	0.918
Number of live births	1.60	0.49	1.60	0.49	0.000
Number of stillbirths	0.45	0.45	0.45	0.45	0.072

\* T test \*\* Mann-Whitney

association was found between gestational age and serum vitamin D concentrations ( $p = 0.168$ ). In addition, no significant differences in gestational age were observed between women with vitamin D insufficiency and those with adequate vitamin D status. Regarding the relationship between BMI and vitamin D status, both groups characterised by either insufficient or normal vitamin D levels had a high prevalence of overweight or obesity ( $BMI > 29$ ). However, statistical analysis showed no significant association between BMI and serum vitamin D concentrations ( $p = 0.550$ ).

### Gravidity and parity in relation to vitamin D status

The study investigated the possible association between vitamin D status and reproductive history, specifically gravidity (number of pregnancies) and parity (number of live births). Participants were classified as deficient or sufficient based on their serum vitamin D levels. Chi-squared tests were used to examine the relationship between these

variables. Statistical analysis showed no significant association between gravidity and vitamin D status ( $p=0.191$ ). The distribution of gravidity was similar between the vitamin D deficient and sufficient groups, with a predominance of one or two pregnancies in both categories. Similarly, there was no significant association between parity and vitamin D status ( $p=0.191$ ). The frequency of parity values (1 and 2) was consistent in both vitamin D groups. These results suggest that gravidity and parity were not associated with vitamin D status in the study participants.

### Miscarriage history and vitamin D status

Participants were categorised according to their serum vitamin D levels: deficient or sufficient. Data on the number of previous miscarriages were collected for each participant. Chi-squared analysis was used to examine the relationship between miscarriage history and vitamin D status. Statistical analysis revealed a significant association between miscarriage frequency and serum vitamin D levels ( $p=0.014$ ).

**Table 3. Association between gestational age and BMI with serum vitamin D levels**

Variable	Level	Vitamin D deficiency		Normal level of Vitamin D		Total		P-value
		Number	Percent	Number	Percent	Number	Percent	
Gestational age	31-37	18	66.7	9	33.3	27	100	$P^*=0.168$
	38-39	36	48.0	39	52.0	75	100	
	40-42	18	43.9	23	56.1	41	100	
	19.8>	0	0.0	2	100.0	2	100	
BMI	19.8-26	15	55.6	12	44.4	27	100	$P^*=0.550$
	26.1-29	17	48.6	18	51.4	35	100	
	29<	39	47.0	44	53	83	100	

\* Chi-squared test \*\* Fisher's test

**Table 4. Investigating the relationship between the number of pregnancies and births and the serum level of vitamin D.**

Variable	Level	Vitamin D deficiency		Normal level of Vitamin D		Total		P-value
		Number	Percent	Number	Percent	Number	Percent	
Gravida	1	24	45.3	29	54.7	53	100	P*=0.191
	2	31	52.5	28	47.5	59	100	
	3	11	42.3	15	57.7	26	100	
	4 and more	8	80.0	2	20.0	10		
number of births	1	24	45.3	29	54.7	53	100	P*=0.191
	2	31	52.5	28	47.5	59	100	
	3	11	42.3	15	57.7	26	100	
	4 and more	8	80.0	2	20.0	10	100	

\*Chi-squared test

While about half of the participants in both groups reported no history of miscarriage, there were differences in those with a history of pregnancy loss. The vitamin D-sufficient group had a higher proportion of women with one previous miscarriage, whereas the vitamin D-deficient group had a higher proportion of women with two or more previous miscarriages (**Table 5**). These findings suggest a possible association between vitamin D status and the likelihood of recurrent miscarriage.

#### Birth outcomes and vitamin D status

No significant differences were observed in the number of live births between women

with sufficient and insufficient vitamin D status (**Table 6**). Both groups had similar distributions of mothers with one or no live births and those with two or three live births. While no association was found between vitamin D status and the occurrence of a single stillbirth, a significant difference emerged for women with two or more stillbirths ( $p = 0.035$ ). The vitamin D insufficiency group had a higher prevalence of women with multiple stillbirths than the vitamin D sufficiency group. These findings suggest that vitamin D deficiency may not affect the number of live births, but may be a potential risk factor for recurrent stillbirths.

**Table 5. Investigating the relationship between the number of miscarriages and the serum level of vitamin D.**

Number of abortions	Vitamin D deficiency		Normal level of Vitamin D		Total		P-value
	Number	Percent	Number	Percent	Number	Percent	
0	54	49.1	56	50.9	110	100	P*=0.14.0
1	9	36.0	16	64.0	25	100	
2	9	90.0	1	10.0	10	100	

\*Chi-squared test

**Table 6. Investigating the relationship between the number of live and dead babies and the serum level of vitamin D.**

Variable	Level	Vitamin D deficiency		Normal level of Vitamin D		Total		P-value
		Number	Percent	Number	Percent	Number	Percent	
Number of live babies	0 or 1	29	50.0	29	50.0	58	100	P*>0.999
	2 or 3	45	50.0	45	50.0	25	100	
The number of stillborn babies	0	50	47.2	56	52.8	106	100	P*=0.035
	1	15	51.7	14	48.3	29	100	
	2 or 3	9	90.0	1	10.0	10	100	

\*Chi-squared test



## Research Objectives

### Vitamin D Status and Birth Outcomes and pre-eclampsia

A significant association was identified between serum vitamin D levels and the occurrence of preterm and postterm births ( $p = 0.013$ ) (**Table 7**). Women with sufficient vitamin D levels were less likely to experience these adverse birth outcomes compared to those with vitamin D insufficiency. These findings suggest a potential protective effect of adequate vitamin D status against preterm and postterm birth.

While an association was observed between vitamin D insufficiency and the occurrence of pre-eclampsia, this relationship did not reach statistical significance (**Table 8**). Although the group with lower vitamin D levels exhibited a higher prevalence of pre-eclampsia, further research with a larger sample size is required to confirm or refute this potential association.

### The association of serum vitamin D levels with gestational diabetes, age, mode of delivery, and premature rupture of membranes

#### Vitamin D status and gestational diabetes

A significant association ( $p = 0.010$ ) was found between vitamin D status and the development of gestational diabetes (**Table 9**). Participants with lower vitamin D levels were more likely to develop gestational diabetes than those with adequate vitamin D levels. These findings suggest a possible association between vitamin D deficiency and an increased risk of gestational diabetes. Further research is needed to elucidate the underlying mechanisms and to explore potential preventive interventions.

#### Age and serum vitamin D levels

An analysis of serum vitamin D levels in different age groups was performed (**Table 10**). Although the 26-34 age group contained the largest proportion of both vitamin D deficient and vitamin D sufficient individuals, no significant association was found between age and serum vitamin D levels ( $p = 0.506$ ). Furthermore, there were no significant differences in the age distribution between the vitamin D insufficiency and vitamin D sufficiency groups. These results suggest that age may not be a primary determinant of serum vitamin D levels in this study population.

**Table 7. Investigation of the Relationship Between Timing of giving birth and Serum Vitamin D Levels.**

Obstetric Status		Vitamin D deficiency		Normal level of Vitamin D		Total		P-value
		Number	Percent	Number	Percent	Number	Percent	
Preterm giving Birth	Yes	18	75.0	6	25.0	24	100	$P^* = 0.013$
	No	56	45.2	68	54.8	124	100	
Postterm giving Birth	Yes	5	100	0.0	0.0	5	100	$P^* = 0.029$
	No	69	48.3	51.7	51.7	143	100	

\* Chi-squared test \*\* Fisher's test

**Table 8. Investigating the relationship between preeclampsia and vitamin D serum levels.**

Preeclampsia	Vitamin D deficiency		Normal level of Vitamin D		Total		P-value
	Number	Percent	Number	Percent	Number	Percent	
Yes	7	70.0	3	30.0	10	100	$P^* = 0.163$
No	67	48.6	71	51.4	138	100	

\*Fisher's test

**Table 9. Investigating the relationship between Gestational diabetes and serum vitamin D levels.**

Gestational Diabetes	Vitamin D deficiency		Normal level of Vitamin D		Total		P-value
	Number	Percent	Number	Percent	Number	Percent	
Yes	16	76.2	5	23.8	21	100	$P^* = 0.010$
No	58	45.7	69	54.3	127	100	

\*Chi-squared test

**Table 10. Investigating the relationship between age and vitamin D serum level.**

Age	Vitamin D deficiency		Normal level of Vitamin D		Total		P-value
	Number	Percent	Number	Percent	Number	Percent	
17-25	23	57.5	17	42.5	40	100	P*=506.0
26-34	36	46.2	42	53.8	78	100	
44-35	15	50.0	15	50.0	30	100	

\*Chi-squared test

**Mode of delivery and vitamin D status**

The relationship between mode of delivery (vaginal or caesarean) and serum vitamin D levels was examined. Although a higher rate of caesarean deliveries was observed in women with vitamin D deficiency compared with those with sufficient levels, this difference did not reach statistical significance ( $P = 0.739$ ) (**Table 11**). These results suggest that mode of delivery is unlikely to be a significant factor influencing serum vitamin D levels in this study population.

**Vitamin D status and preterm premature rupture of membranes (PROM)**

A significant association was found between vitamin D status and the occurrence of preterm premature rupture of membranes (PROM) ( $p = 0.006$ ) (**Table 12**). Women with low vitamin D levels were more likely to have PROM than women with adequate vitamin D levels. These findings suggest a possible link between vitamin D deficiency and an increased risk of PROM.

**Table 11. Investigation of the Association between Delivery Mode of Membranes and Serum Vitamin D Levels.**

Premature Rupture of Membranes	Vitamin D deficiency		Normal level of Vitamin D		Total		P-value
	Number	Percent	Number	Percent	Number	Percent	
Yes	13	81.3	3	18.8	16	100	P*=0.006
No	58	45.0	71	55.0	129	100	

\*Chi-squared test

**Table 12. Investigation of the Association between Premature Rupture of Membranes and Serum Vitamin D Levels**

Delivery Mode	Vitamin D deficiency		Normal level of Vitamin D		Total		P-value
	Number	Percent	Number	Percent	Number	Percent	
Normal	30	48.4	32	51.6	62	100	P*=0/739
Cesarean section	44	51.2	42	48.8	86	100	

\*Fisher's test

**Discussion**

This study investigated the association between serum vitamin D levels and various pregnancy outcomes. A notable finding was the association between vitamin D insufficiency and an increased risk of preterm birth (20). This is consistent with previous research showing lower vitamin D concentrations in mothers of preterm babies compared with full-term babies (21). The potential influence of vitamin D on inflammatory and immune responses suggests a possible biological mechanism underlying this association. However, the

literature is inconsistent, with some studies reporting no association between maternal vitamin D and preterm birth (22, 23).

Our analysis showed a higher prevalence of pre-eclampsia in the vitamin D-deficient group, although this did not reach statistical significance. Existing research on this topic is inconclusive, with some studies reporting lower vitamin D levels in women with pre-eclampsia (24-27) and others finding no association (23). Small sample sizes may contribute to these discrepancies. Our results support an association between vitamin D deficiency and gestational diabetes, as evidenced by a higher prevalence of gestational diabetes in the deficient group.

This is consistent with previous studies showing increased vitamin D deficiency in women with gestational diabetes compared with healthy controls (28, 29). This study found no significant association between gestational age or mode of delivery and vitamin D levels. This contradicts some studies that have found an association between lower vitamin D levels and earlier gestational age (30, 31) or higher caesarean section rates. However, other studies have not reported such associations (32,33). A notable finding was the higher prevalence of preterm premature rupture of membranes (PROM) in the vitamin D-deficient group, which contradicts some studies showing no association (34). Further research is needed to clarify this potential association. Vitamin D deficiency is a growing public health problem, with high prevalence rates reported in various populations (35, 17). Emerging evidence suggests a potential role for vitamin D in the prevention of pregnancy complications, including gestational diabetes, pre-eclampsia, preterm birth and fetal growth abnormalities (12). Our study contributes to this ongoing research and highlights the need for further investigation to clarify the complex relationship between vitamin D and pregnancy outcomes. Given the potential benefits of vitamin D for maternal and fetal health, maintaining adequate levels during pregnancy may be crucial to improving outcomes.

### Conclusion

This study investigated the association between vitamin D status and several pregnancy outcomes. Although no significant associations were found between vitamin D levels and factors such as gravidity, parity, live births, gestational age, mode of delivery, or pre-eclampsia, several important findings emerged. Vitamin D insufficiency was significantly associated with an increased risk of miscarriage, stillbirth, preterm birth and gestational diabetes. These findings suggest a potential protective role for adequate

vitamin D levels during pregnancy. Further research is needed to elucidate the underlying mechanisms linking vitamin D deficiency to these adverse pregnancy outcomes. In addition, randomized controlled trials are needed to assess the efficacy of vitamin D supplementation in preventing these complications.

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### Conflicts of interest

The authors declare no conflict of interest.

### Authors' contributions

All authors were involved in the conception and design, analysis and interpretation of the data, drafting of the manuscript and revising it critically for intellectual content, approved the final version for submission, and agreed to be accountable for all aspects of the work.

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