

# Association of Maternal Serum Vitamin D Level with Risk of Pregnancy-Related Complications and Neonatal Anthropometric Measures: A Prospective Observational Study

## Abstract

**Background:** Body of evidence is increasing about the importance of Vitamin D (VD) for normal development of the fetus and for maternal health. As limited data are available regarding the association between maternal VD level and pregnancy-related complications and neonatal anthropometric measures, the present study aimed to evaluate the neonatal anthropometric measures including weight, high, and head circumference and pregnancy-related complications such as preeclampsia, blood pressure, gestational diabetes mellitus, and nausea and vomiting in pregnancy with 25(OH)VD level. **Methods:** The current prospective observational study was conducted among 812 Iranian pregnant women during the first trimester in Isfahan, Iran. Needed data were collected using validated questionnaires and biochemical examinations. **Results:** Overall, this study demonstrated an inverse significant association between VD level and chance of having low-weight infant in the adjusted model (odds ratio [OR]: 0.03, 95% confidence interval [CI]: 0.004–0.26,  $P < 0.001$ ) in the first VD assessment. The same results were obtained in the second VD assessment (OR: 0.08, 95% CI: 0.01–0.40,  $P < 0.01$ ). However, such associations were not seen about other neonatal measures and pregnancy-related complications. **Conclusions:** We found that low maternal VD level might be associated with risk of low-weight infant. Such findings could be considered to implement informative interventional programs to control newborn adverse outcomes. Further studies are required to confirm these findings.

**Keywords:** Maternal Vitamin D, neonatal anthropometric measures, pregnancy complications

## Introduction

Over the past three decades, it has become clear that the role of Vitamin D (VD) goes beyond the calcium homeostasis and bone health.<sup>[1-3]</sup> VD is involved in a wide range of physiological processes including the regulation of cell proliferation, differentiation, apoptosis and immune response, modification of the inflammatory pathways, and maintenance of genome stability function.<sup>[4-7]</sup>

Body of evidence is increasing about the importance of VD for normal development of the fetus and for maternal health.<sup>[8]</sup> During the early weeks of pregnancy, 1,25(OH)<sub>2</sub>D increases 2–3-fold despite minimal increased calcium demands during that time of gestation.<sup>[8]</sup> Furthermore, increased expression of VD receptor and its metabolic enzymes are reported at the placenta and decidua, indicating the potential role of

this nutrient on maternal and offspring outcomes.<sup>[4]</sup>

However, VD deficiency during pregnancy is prevalent among populations across the world.<sup>[8,9]</sup> VD deficient or insufficient, is common among those with avoidance of sun exposure to prevent melanoma, the use of sunscreen which blocks VD synthesis, traditional costumes that prevent people to expose themselves to sunlight and low dietary intake.<sup>[10,11]</sup>

VD deficiency may cause pregnancy-related complications such as preeclampsia and gestational diabetes leading to health threat for the mother and her infant.<sup>[4,8,9,12]</sup>

Furthermore, evidence links mentioned deficiency with adverse birth outcomes.<sup>[8,9]</sup> Neonatal anthropometric measures, including body weight (BW), head circumference (HC), and height (H), are widely assessed as parameters of impaired fetal growth, intrauterine environment, and

Hossein Hajianfar<sup>1,5</sup>,  
Ahmad  
Esmailzadeh<sup>1,2</sup>,  
Avat Feizi<sup>3</sup>,  
Zahra Shahshahan<sup>4</sup>,  
Leila Azadbakht<sup>1,2</sup>

<sup>1</sup>Food Security Research Center, Isfahan University of Medical Sciences, Isfahan, Iran, <sup>2</sup>Department of Community Nutrition, School of Nutritional Sciences and Dietetics, Tehran University of Medical Sciences, Tehran, Iran, <sup>3</sup>Department of Biostatistics and Epidemiology, School of Health, Isfahan University of Medical Sciences, Isfahan, Iran, <sup>4</sup>Department of Gynecology, School of Medicine Science, Isfahan University of Medical Sciences, Isfahan, Iran, <sup>5</sup>Food Safety Research Center (salt), Semnan University of Medical Sciences, Semnan, Iran

## Address for correspondence:

Dr. Leila Azadbakht,  
Department of Community  
Nutrition, School of Nutrition  
and Food Science, Isfahan  
University of Medical Sciences,  
Isfahan, Iran.  
E-mail: azadbakhtleila@gmail.  
com

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maternal nutrition.<sup>[13]</sup> Restriction fetal growth is traumatic and one of the most important global public health problems. It provides a foundation on which chronic disease may develop in offspring throughout their life.<sup>[14]</sup>

Several studies have reported a positive association between maternal VD levels in pregnancy and some neonatal outcomes<sup>[1]</sup> although results are inconsistent.<sup>[2,5]</sup> Such results were obtained about association of maternal VD status with pregnancy-related complications risk.<sup>[15]</sup> However, the effect of maternal VD deficiency on pregnancy-related complications and neonatal anthropometric measures is not clear.

As limited data are available regarding the association between maternal VD level and pregnancy-related complications and neonatal anthropometric measures in Middle Eastern countries where VD deficiency is prevalent,<sup>[16]</sup> the present study aimed to evaluate the neonatal anthropometric measures including weight, high, and HC and pregnancy-related complications such as preeclampsia, blood pressure (BP), gestational diabetes mellitus (GDM), and nausea and vomiting in pregnancy (NVP) with 25(OH) VD level. Such findings could be considered to implement informative interventional programs to control maternal and newborn adverse outcomes and develop practical policies to improve the diet quality among pregnant women.

## Methods

### Study design and participants

The current prospective observational study was conducted among 812 Iranian pregnant women during the first trimester to assess the relationship between 25(OH) VD level and neonatal anthropometric measures, including weight, high, and HC and pregnancy-related complications such as preeclampsia, BP, NVP, and GDM in Isfahan, Iran.

Eligible criteria included pregnant women during the 8–16 weeks of pregnancy without any medical condition, use of medications, and following a specific diet. Exclusion criteria were avoiding of follow-up and using of VD supplements during the study. The study population consisted of 812 pregnant women selected by the multistage cluster random sampling method. Sample size for this study was calculated statistics formula. Hence, according to this formula ( $Z_{1-\alpha/2} = 1.96$ ,  $Z_{1-\beta} = 1.68$ ,  $d^2 = R/6 = 100/6$ , and  $d = 3$ ), 400 pregnant women were required for the study.

Informed consent was obtained from all participants. This study was approved by the research council (research project number: 193053) and ethics committee (research ethics number: IR.MUI.REC 193053).

### Data collection

#### *Assessment of Vitamin D level*

At 8–16 weeks, maternal 25(OH)D level was analyzed by enzyme-linked immunosorbent assay method at

the laboratory. The method measures total 25(OH)D (both 25(OH)D and D3). The second assessment was at 32–34 weeks. VD deficiency was defined conservatively as  $<10$  ng/mL, insufficiency as 10–29 ng/mL, and sufficiency as  $>30$  ng/mL.<sup>[17]</sup>

#### *Assessment of pregnancy-related complications*

Abnormal glucose homeostasis including fasting plasma glucose concentration  $>95$  mg/dL and 1-h plasma glucose after eating 50 g of glucose  $>140$  mg/dL at 24–28 gestational weeks was defined as GDM,<sup>[18]</sup> which assessed during the second visit at 24–28 weeks.

Preeclampsia is diagnosed in the presence of hypertension associated with proteinuria. Hypertension is defined as a BP of at least 140 mmHg (systolic) or at least 90 mmHg (diastolic) on at least two occasions and at least 4–6 h apart after the 20<sup>th</sup> week of gestation in women known to be normotensive beforehand. Proteinuria is also defined as a protein concentration of 300 mg/L or more in 24-h urine sample.<sup>[19]</sup>

BP was measured twice after the participants sat for 15 min during the first visit, at 8–16 weeks. A BP larger than systolic BP (SBP)  $\geq 140$  mmHg or diastolic BP (DBP)  $\geq 90$  mmHg during or after the 20<sup>th</sup> week of amenorrhea was considered as gestational hypertension.<sup>[20]</sup>

To assess NVP, participants were asked whether they had NVP during the first visit. Participants answered yes or no.

#### *Assessment of growth measures of the newborn*

Neonatal anthropometric measurements were performed by specially trained study personnel. All measurements including BW, HC, and height were recorded twice at birth and calculated as the mean of two measurements. Neonatal anthropometric measures were categorized according to the WHO standards and were as follows: low birth weight defines as the birth weight under 2500 g and normal birth weight also determines the birth weight from 2500 g to 3900, low height is the height less than 47 cm and normal height determines the height between (47-55) cm, low HC is the HC below 33 cm and normal HC is also the HC from 33 cm to 37 cm.<sup>[21]</sup>

#### *Assessment of other variables*

For the mothers, data were collected on the anthropometric measurements as well as demographic and clinical information.

To assess participants' information on age and gender, marital status, physical activity, educational, and socioeconomic levels, we used a standard self-reported questionnaire. Participants' weight was measured using a balanced scale digital scale to the nearest 100 g, in light clothing and barefoot. Height was measured with a tape measure while the participants were in a standing position. Body mass index (BMI), defined as weight in kilograms divided by height in meters squared, was calculated.<sup>[22]</sup>

Furthermore, energy and nutrient intakes were assessed by validated and reliable 117-item semi-quantitative food frequency questionnaire during the first visit, at 8–16 weeks. The validity and reliability of the questionnaire was previously confirmed.<sup>[23]</sup> For each item, participants were asked about the portion sizes and frequency of consumption in the previous year on a daily, weekly, or monthly basis. Portion sizes of consumed foods were converted to grams from household measures. We included supplements to total intake of specific nutrients. All nutrient values were energy adjusted using the residuals method. Then, nutrient and energy intakes were computed using Nutritionist IV software (version 7.0; N-Squared Computing, Salem, OR), which was designed for Iranian foods.

### Statistical analysis

Data were statistically described in terms of mean  $\pm$  standard deviation and percentages when appropriate. The results of Kolmogorov–Smirnov and Q-Q plot test and histogram showed that the distributions of quantitative variables were normal. Comparison of categorical variables between the study participants was done using Chi-square test. For comparing continuous variables, one-way analysis of variance was performed. Analysis of covariance was used for assessment of adjusted intakes of foods and nutrients across categories of VD levels including deficient, insufficient, and sufficient. Adjustments for energy intake, age and BMI, physical activity, and socioeconomic levels were considered in the adjusted model. All models were done by treating the deficient category of VD as the reference.  $P < 0.05$  was considered statistically significant. All statistical calculations were done using computer programs SPSS (Statistical Package for the Social Science; SPSS Inc., Chicago, IL, USA) version 19 for Microsoft Windows.

### Results

Pregnant women ( $n = 812$ ), in their first trimester, consented to participate in this study. The overall characteristics of the study population across different categories of VD levels including deficient, insufficient, and sufficient are presented in Table 1.

Women with the sufficient levels of VD were more likely to be in the category of high economic level in both the first and the second VD assessment. In addition, they were significantly more likely to have low physical activity in the second VD assessment.

Crude and adjusted odds ratio (OR) and 95% confidence interval (CI) for ORs resulted from analysis, which neonatal anthropometric measures and pregnancy-related complications were as dependent variables and VD levels were as independent variables in different models, were given in Table 2. In all fitted models, the VD deficiency level was defined as the reference category.

The comparison of neonatal weight in different categories of VD levels showed VD sufficient levels which were related to neonatal weight in crude and adjusted model.

Women with VD sufficient level were less likely to having low-weight infant compared with those in the deficient level in crude (OR: 0.03, 95% CI: 0.004–0.23,  $P < 0.001$ ) and in the adjusted model (OR: 0.03, 95% CI: 0.004–0.26,  $P < 0.001$ ) in the first VD assessment. The same results were obtained in the second VD assessment in crude (OR: 0.09, 95% CI: 0.01–0.4,  $P < 0.006$ ) and in the adjusted model (OR: 0.08, 95% CI: 0.01–0.40,  $P < 0.01$ ).

However, VD sufficient level was not related height and HC of infants in all crude and adjusted models.

Finding about pregnancy-related complications showed that women in the different VD categories had not higher chance of having diabetes, intrauterine growth restriction, preeclampsia, SBP, DBP, BP, and NVP in the first and second assessment of VD.

### Discussion

This study is one of the largest studies evaluating the association of maternal VD level during the first-trimester period of pregnancy with pregnancy-related complications and neonatal anthropometric measures. This study demonstrated an inverse significant association between VD level and chance of having low-weight infant. However, such associations were not seen about other neonatal measures and pregnancy-related complications.

The most important mechanism which may explain the association between 25(OH)D level and mentioned measures has been described such that VD regulates several genes throughout the body, as much as 5% of the human genome.<sup>[24]</sup> Earlier meta-analysis study has link VD deficiency with an increased risk of preeclampsia and GDM.<sup>[25]</sup> Furthermore, a significant correlation between VD with NVP was observed in early pregnancy.<sup>[26]</sup>

However, in a systematic review of observational studies, Harvey *et al.* identified inconsistent findings about the effect of maternal serum 25(OH)D concentration during pregnancy on gestational hypertension risk.<sup>[27]</sup>

About neonatal measures, consistent with this study, Leffelaar *et al.* showed that women with deficient VD levels had infants with lower birth weights compared with women with adequate VD levels.<sup>[28]</sup> Inconsistently, others found no independent relation between maternal VD levels and any of the neonatal anthropometric measures.<sup>[5]</sup>

We cannot rule out that the biological effect of VD could differ between the populations. The most important points that need to be considered are the genotypes coding for the VD receptor, VD binding protein, and regulatory enzymes which may all differ due to genetic background and be potential effect modifiers. Hence, it is more likely that this

**Table 1: General characteristics of the study participants across different categories of Vitamin D in the first and third semester in pregnancy**

	Vitamin D first				Vitamin D second			
	Deficiency	Insufficient	Sufficient	P	Deficiency	Insufficient	Sufficient	P
BMI								
Normal	79 (20)	223 (58)	77 (20)	<0.001	6 (21)	210 (73)	70 (24)	<0.001
Overweight	75 (24)	185 (59)	50 (16)		13 (5)	178 (72)	53 (21)	
Work mother								
Housekeeper	134 (24)	319 (59.1)	87 (16)	0.001	17 (3)	328 (75)	88 (20)	0.02
Employer	16 (14)	65 (59.1)	29 (26)		1 (1)	42 (60)	26 (37)	
Teacher	4 (11)	20 (58)	10 (29)		1 (4)	15 (62)	8 (33)	
Work husband								
Employer	47 (21)	113 (52)	54 (25)	0.001	4 (2)	102 (67)	45 (29)	0.002
Other	74 (19)	239 (64)	59 (15)		12 (4.1)	21 (71)	71 (24)	
Worker	32 (32)	55 (55)	12 (12)		2 (2)	75 (90)	6 (7)	
Education								
Diploma	72 (24)	181 (62)	36 (12)	0.03	11 (4)	188 (79)	37 (15)	0.001
Graduate	82 (20)	225 (56)	90 (22)		8 (2)	198 (67)	86 (29)	
Husband education								
Diploma	89 (25)	220 (61)	47 (13)	0.001	14 (4)	222 (79)	45 (16)	<0.001
Graduate	65 (19)	184 (55)	80 (24)		5 (2)	162 (66)	78 (31)	
Socioeconomic								
Low	11 (23)	32 (69)	3 (6)	0.02	3 (7)	34 (87)	2 (5)	0.003
Moderate	69 (27)	145 (57.1)	40 (15)		4 (2.1)	152 (78)	38 (19)	
High	65 (19)	193 (58)	73 (22)		8 (3)	171 (67)	73 (28)	
Delivery								
Cesarean	47 (19)	147 (60)	47 (19)		7 (4)	119 (68)	49 (28)	<0.001
Normal	107 (23)	261 (58)	79 (17)		12 (3)	269 (75)	73 (20)	
IUGR								
No	144 (22)	387 (59)	120 (18)	<0.001	18 (3)	360 (73)	115 (23)	<0.001
Yes	9 (27)	17 (51)	7 (21)		1 (3)	25 (78)	6 (18)	
Early delivery								
No	151 (22)	394 (58)	125 (18)	<0.001	19 (3)	377 (73)	118 (22)	<0.001
Yes	3 (20)	10 (66)	2 (13)		0	9 (75)	3 (25)	
Stillbirth								
No	153 (22)	404 (59)	125 (18)	<0.001	19 (3)	384 (73)	123 (23)	<0.001
Yes	1 (14)	4 (57)	2 (28)		0%	4 (100)	0	
Abortion								
No	123 (22)	326 (59)	98 (17)	<0.001	13 (3.1)	304 (72)	104 (24)	<0.001
Yes	31 (21)	82 (57)	29 (20)		6 (5)	84 (77.1)	19 (17)	
Activity								
Very low	2 (25)	4 (50)	2 (25)	0.01	0	3 (75)	1 (25)	<0.001
Low	88 (22)	216 (54)	93 (23)		12 (3)	217 (68)	88 (27)	
Moderate	52 (24.1)	143 (66)	21 (9)		6 (3)	134 (78)	30 (17)	
High	10 (17)	38 (64)	10 (17)		1 (3)	29 (87)	3 (9)	

BMI=Body mass index, IUGR=Intrauterine growth restriction

difference in results between studies is a result of residual genetic confounding.<sup>[29]</sup> We cannot also overlook the other confounding factors including the seasonal variation on VD level<sup>[30]</sup> as the study extended over a whole year. We also attribute this disparity in the results to the effects of maternal VD levels before placentation on maternal and newborn health outcomes.<sup>[31]</sup> Furthermore, assessment measurements error in results increase the likelihood of misclassification which contributes to the discrepancy among our results.

Another potential drawback should be also considered. Lack of funding to allow for more frequent examination to the whole study is an issue in the analyses. Hence, we did not identify a trend of VD level during pregnancy rather than a consistent level. In addition, women at higher risk of pregnancy-related complications were not excluded from the present study. Therefore, these potential limitations might be reasons why we failed to report significant associations between maternal VD



**Table 2: The comparison of weight of neonatal, height of neonatal, head circumference of neonatal, and complication of pregnancy in different levels of Vitamin D in the first and third semester of pregnancy**

	Vitamin D first				Vitamin D second			
	Deficiency	Insufficient	Sufficient	P	Deficiency	Insufficient	Sufficient	P
BMI								
Normal	79 (20)	223 (58)	77 (20)	<0.001	6 (21)	210 (73)	70 (24)	<0.001
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Graduate	82 (20)	225 (56)	90 (22)		8 (2)	198 (67)	86 (29)	
Husband education								
Diploma	89 (25)	220 (61)	47 (13)	0.001	14 (4)	222 (79)	45 (16)	<0.001
Graduate	65 (19)	184 (55)	80 (24)		5 (2)	162 (66)	78 (31)	
Socioeconomic								
Low	11 (23)	32 (69)	3 (6)	0.02	3 (7)	34 (87)	2 (5)	0.003
Moderate	69 (27)	145 (57.1)	40 (15)		4 (2.1)	152 (78)	38 (19)	
High	65 (19)	193 (58)	73 (22)		8 (3)	171 (67)	73 (28)	
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Yes	1 (14)	4 (57)	2 (28)		0	4 (100)	0	
Abortion								
No	123 (22)	326 (59)	98 (17)	<0.001	13 (3.1)	304 (72)	104 (24)	<0.001
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Moderate	52 (24.1)	143 (66)	21 (9)		6 (3)	134 (78)	30 (17)	
High	10 (17)	38 (64)	10 (17)		1 (3)	29 (87)	3 (9)	

Resulted from ANCOVA. Adjusted Energy intake, age, physical activity, and socioeconomic levels. BMI=Body mass index, IUGR=Intrauterine growth restriction, ANCOVA=Analysis of covariance

level and pregnancy-related complications and neonatal anthropometric measures.

However, the main strengths of the current study include its multidisciplinary scope including epidemiology, statistics, pediatrics, maternity, and VD. Another positive point is that few studies have assessed the maternal VD levels on gestational complications and newborn measures.

## Conclusions

In summary, evaluations carried out in the present study indicate an inverse significant association between maternal VD level and chance of having low-weight infant. Such findings could be considered to implement informative interventional programs to control newborn weight. Further studies are required to confirm these findings.

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## Conflicts of interest

There are no conflicts of interest.

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