

## The Relationship of Vitamin D and Calcium level with Preeclampsia Severity: A Case- control Study

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### Abstract

#### Background

Vitamin D deficiency is associated with physiologic changes that are similar to pathogenesis of preeclampsia. Although association of vitamin D and preeclampsia has been studied previously, their results are not consistent. The aim of this study was to investigate the relationship of serum vitamin D and calcium with preeclampsia severity.

**Materials and Methods:** This case- control study was conducted in 75 healthy pregnant women and 74 pregnant women with preeclampsia (46 mild preeclampsia and 28 severe preeclampsia) in Qazvin, Iran in 2015. Serum vitamin D, calcium, and albumin were measured; corrected calcium was also calculated. Hypocalcemia and vitamin D deficiency were compared between the groups. Logistic regression analysis was used to study the independent association of hypocalcemia and hypovitaminosis D with preeclampsia.

#### Results

Mean serum vitamin D level was  $27.7 \pm 15.3$ ,  $22.9 \pm 15.9$ , and  $27.6 \pm 16.6$  in normal, mild preeclampsia, and severe preeclampsia groups ( $P > 0.05$ ); also vitamin D deficiency was not different between the groups. Hypocalcemia in severe preeclampsia group was more frequent than normal group (25.9% vs. 6.6%,  $P: 0.017$ ). Hypocalcemia was associated with severe preeclampsia after adjustment for age, parity, and calcium supplement consumption (OR: 6.7, 95% CI: 1.45-30.79;  $P: 0.015$ ).

#### Conclusion

There was not any association between vitamin D deficiency and preeclampsia in the present study, however low corrected serum calcium was associated with about six times increased risk of severe preeclampsia. More studies are needed to determine the role of hypocalcemia and vitamin D in preeclampsia.

**Key Words:** Hypocalcemia, Pre-eclampsia, Pregnancy, Vitamin D deficiency.

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## 1- INTRODUCTION

Preeclampsia is a major complication that occurs in 3-7% of pregnancies (1). In a meta-analysis, the overall prevalence of preeclampsia was 5% in Iran and the prevalence of preeclampsia was increased from 4% (95% CI: 3%-5%) during 1996 to 2005 to 7% (95% CI: 4%-9%) during 2010 to 2013 (2). Reduced placental perfusion occurs in the early stages of preeclampsia. This change cause cytokines and other inflammatory materials release that affect different organs and results in a triad including hypertension, proteinuria, and edema (3). Vitamin D deficiency is associated with physiologic changes such as altered endothelium function and increased cytokine levels (4) that are similar to pathogenesis of preeclampsia. Vitamin D is also a major suppressor of rennin synthesis. Vitamin D deficiency can increase rennin secretion that causes increased blood pressure (5). Vitamin D improves tissue perfusion through decrease in vascular smooth muscle hyperplasia, decrease in insulin resistance, and improvement in endothelial function (6).

In addition, vitamin D modulates immune system and regulates macrophages function and cytokines release (4). Placental 1- $\alpha$  hydroxylase convert vitamin D to the active form (7). There are Vitamin D receptors in placenta that justify the autocrine role of vitamin D in regulation of placental functions (8). Vitamin D deficiency is prevalent among pregnant women especially in developing countries. The prevalence of vitamin D deficiency is about 80% in Iran (9). Although, association of vitamin D and preeclampsia has been studied previously, their results are not consistent. In some studies preeclampsia has been associated with greater frequency of vitamin D deficiency, while no association has been reported in other studies. Surprisingly, in one study, pregnancy hypertension was associated with higher vitamin D level (10). The aim

of this study was to investigate the association of serum vitamin D and calcium with preeclampsia severity in pregnant women.

## 2- MATERIALS AND METHODS

### 2-1. Study design and population

This case control study was conducted in 75 healthy pregnant women and 74 pregnant women with preeclampsia who referred to the Kowsar Hospital affiliated to Qazvin University of Medical Sciences, Iran, in 2015.

### 2-2. Methods

Pregnant women with diagnosis of late preeclampsia were considered as case group (n=74), from March to May 2015. Healthy pregnant women, referred for delivery were considered as control group (n=75), and were matched for age, gestational age, parity, season, and place of residence. Pregnant women with preeclampsia were classified as mild and severe preeclampsia.

Mild preeclampsia was defined as 140/90 < Blood pressure < 160/110 in both measurements and proteinuria after 20 weeks of gestation. Proteinuria was defined as protein  $\geq$  300 mg in 24 hours urine sample.

Severe preeclampsia was identified when at least one of the following conditions were met: Blood pressure  $\geq$  160/110, 2 grams proteinuria in 24 hours urine sample, more than 2 + protein in urine dipstick, creatinine (Cr) > 1.2 mg/dl without previous history of Cr rise, platelets fall below 100,000 per microliter of blood, microangiopathic hemolysis, and increased serum transaminase (11).

### 2-3. Measuring tools

Data were collected through a questionnaire including demographics variables, gestational age, history of vitamin D and calcium supplement

consumption, season of sampling, and body mass index (BMI). All anthropometric indices were measured by a gynecologist. The height was measured in barefoot standing position using a wall mounted stadiometer Seca nearest 1 mm. The weight was also measured using Seca scale (Vogel and Halke, Hamburg, Germany), nearest 100 grams. The BMI was calculated as weight (kilograms) per height (meters) squared. Blood pressure (BP) was measured twice in a seated position using a mercury sphygmomanometer.

#### 2-4. Laboratory measurements

During admission period, 5ml venous blood sample was collected to measure serum vitamin D, calcium, and albumin. Fasting Urine calcium/creatinine ratio was calculated. All samples were analyzed in the Kowsar hospital laboratory.

Vitamin D was measured by ELISA method using MAN Co kit. Inter-assay and intra-assay coefficient of variations (CVs), were 1.9% and 1.1%, respectively. Vitamin D deficiency was defined as 25 (OH) D  $\leq$  50 nmol/l, and severe vitamin D deficiency was defined as 25 (OH) D  $\leq$  25 nmol/l (12).

Calcium was measured by calorimetric method using Pars Azmoon kit. Inter-assay and intra-assay CVs, were 2.7% and 1.4%, respectively. Albumin was measured by calorimetric method using Zistchem kit. Inter-assay and intra-assay CV, were 1.4% and 1.1%, respectively. Creatinine was measured by calorimetric method using Pars Azmoon kit.

Corrected calcium was calculated by formula: serum calcium (mg/dL) + 0.8 \* [4.0 – serum albumin (g/dL)]. Based on the reference range of calcium in Pars Azmoon kit, using Arsenazo reagent, hypocalcemia was defined as corrected calcium less than 8.3mg/dl.

#### 2.5-Ethical consideration

The study protocol was confirmed in the ethics committee of Qazvin University of Medical Sciences (ID number: 28/20/8285). The participants gave their written informed consent.

#### 2-6. Inclusion and exclusion criteria

The inclusion criterion was diagnosis of late preeclampsia. The exclusion criteria were diabetes mellitus, multiple pregnancy, major fetal anomaly, history of chronic hypertension, underline diseases including cardiovascular diseases, liver diseases, thyroid disorders, and chronic renal insufficiency, known thrombophilia, and using anticonvulsant drugs.

#### 2-7. Data Analyses

Kolmogorov Smirnov test was used to examine the normality of variables. Data were described as mean  $\pm$  standard deviation (SD) for quantitative variables or as frequency for categorical variables. ANOVA was used for analysis of normally distributed continuous variables and non-normally distributed variables were compared by Kruskal Wallis.

Categorical variables were analyzed using Chi-square test. The independent association of hypocalcemia and vitamin D levels with preeclampsia was studied using logistic regression analysis. P-value less than 0.05 were considered as significant.

### 3- RESULTS

The present study was conducted in 75 healthy pregnant women and 74 pregnant women with preeclampsia (46 (62.16 %) with mild preeclampsia and 28 (37.84 %) with severe preeclampsia). Baseline characteristics of the study subjects are shown in **Table.1**.

Age, body mass index, parity, and calcium supplement consumption was not different between the groups ( $P>0.05$ ). All study participants received multivitamin tablets including 400 IU vitamin D according to the national prenatal care protocol. Clinical

and biochemical characteristics of the study subjects are shown in **Table.2**. Mean serum vitamin D level was not significantly different between the groups. 85% of the study subjects had vitamin D insufficiency. The frequency of vitamin D insufficiency and vitamin D deficiency was not different between the groups. Based on corrected serum calcium level,

25.9% of the pregnant women with severe preeclampsia had hypocalcemia that was significantly higher than normal group (P=0.017). In multinomial logistic regression analysis, hypocalcemia was associated with severe preeclampsia after adjustment for age, parity, gestational age, BMI, and calcium supplement consumption (**Table. 3**).

**Table-1:** Baseline characteristics of the study subjects

Variables	Total (n=149)
Age (year) [mean ± SD]	28.5±5.9
Body mass index (Kg/m <sup>2</sup> ) [mean ± SD]	29.4±5.1
Parity (number) [mean ± SD]	2.2±1.2
Gestational age (weeks) [mean ± SD]	37.9±3.6
Place of Residence	
Rural	34.9%
urban	65.1%
Sampling time	
March	14.8%
April	44.3%
May	40.9%
Calcium supplement	20%

SD: Standard deviation.

**Table-2:** Clinical and biochemical characteristics of the study subjects

Variables	Normal (n=75)	Preeclampsia (n=74)	P-value <sup>†</sup>	Mild Preeclampsia (n=46)	Severe Preeclampsia (n=28)	P-value <sup>††</sup>
25(OH)D <sup>a</sup> nmol/l	27.7±15.3	24.7±16.2	0.239	22.9±15.9	27.6±16.6	0.22
25(OH)D<25nmol/l <sup>b</sup>	52	61.3	0.249	64.6	55.5	0.386
25(OH)D<50nmol/l <sup>b</sup>	86.6	92	0.290	91.6	92.6	0.567
Corrected calcium <sup>a</sup>	8.9±0.4	8.7±0.4	0.182	8.8±0.3	8.7±0.4	0.21
Hypocalcemia <sup>b</sup>	6.6	14.6	0.113	8.3	25.9	0.017*
Urine Calcium to creatinine ratio <sup>a</sup>	0.22±0.1	0.21±0.1	0.626	0.22±0.08	0.18±0.08	0.465
Systolic blood pressure <sup>a</sup> (mmHg)	112.6±9.1	151.3±13.3	0.005	144.1±8.8	164.3±9.7	0.001**

Diastolic blood pressure <sup>a</sup> (mmHg)	71.6±7.1	97.0±13.3	0.002	91.1±8.4	107.4±13.5	0.001**
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<sup>a</sup> Data are presented as mean ± SD; <sup>b</sup> Data are presented as percent; † Comparison of normal group and preeclampsia group; †† Comparison of normal group and preeclampsia subgroups; \*Difference was related to normal group and severe preeclampsia subgroup; \*\*Difference was related to normal group and both preeclampsia subgroups.

**Table-3:** Multinomial logistic regression for vitamin D deficiency and hypocalcemia as predictors of mild and severe preeclampsia

Variables	Odds ratio*	95% CI	P-value
<b>Mild preeclampsia</b>			
Hypovitaminosis D	1.86	0.43-7.98	0.404
Hypocalcemia	1.70	0.34-8.4	0.515
<b>Sever preeclampsia</b>			
Hypovitaminosis D	1.97	0.35-10.9	0.436
hypocalcemia	6.68	1.45-30.79	0.015

\* Adjusted for age, gestational age, BMI, and calcium supplement consumption.

#### 4- DISCUSSION

In the present study, mean vitamin D level was not different between the preeclampsia and normal groups. After adjustment for age, gestational age, BMI, and supplement consumption, the difference of mean vitamin D level was not significant between the groups, too. The corrected hypocalcemia in pregnant women with severe preeclampsia was more frequent than other two groups. After adjustment for age, parity, gestational age, BMI, and calcium supplement consumption, hypocalcemia was associated with six times increased risk of severe preeclampsia.

In recent years, the role of vitamin D deficiency as a precipitating factor of preeclampsia has been studied with regards to common pathophysiologic mechanisms of vitamin D deficiency and preeclampsia (changes in vascular endothelium and renin–angiotensin system (RAS), and increase in inflammatory mediators), and presence of vitamin D receptors on placenta. Despite the higher

prevalence of vitamin D deficiency in patients with preeclampsia in some studies, the results are not consistent. Previous studies are different regarding to their design. These studies can be categorized as nested case control studies and case control studies. In the nested case control studies, mostly the relationship of vitamin D level in 3-6 months of pregnancy and occurrence of preeclampsia in the last weeks of pregnancy has been investigated. Some of these studies reported higher rate of vitamin D deficiency in preeclampsia group compared to normal group (13-15).

But, the cut off point for definition of vitamin D deficiency was different from 30 nmol/L (15) to 50 nmol/L (13). However, the relationship of vitamin D level in the first trimester of pregnancy and preeclampsia has not been confirmed in other nested case control studies. In a study in Shiraz- Iran, the difference of vitamin D level was not significant between preeclampsia and healthy women while serum calcium level in preeclampsia women was lower than the normal women (16). Other studies conducted in UK,

Spain, Poland, and USA failed to find a significant association between vitamin D and preeclampsia (17-21). The results of case control studies are also inconsistent. Some studies have reported lower vitamin D level in preeclampsia group in the time of preeclampsia occurrence (22-24), but in another study such difference has not been found (25). In a meta-analysis of published studies until 2012 by Tabesh et al., vitamin D level < 50 ng/ml was associated with increased risk of preeclampsia, but in subgroup analysis, the difference was only significant in studies of American population (26).

Pathophysiologic processes ended to preeclampsia are originated in the first half of pregnancy. Therefore, nested case control studies can find more precisely association of any contributing factors with preeclampsia. But the present study had a case control design. The second reason could be attributed to the season of sampling. In the present study, sample selection was performed in late winter and early spring. So, the first trimester of the pregnancy which is critical for pathophysiology of preeclampsia was mainly in the sunny months, and vitamin D level were possibly higher in these months. Ethnicity can be other reason for such differences. As mentioned above, in meta-analysis of well-designed studies by Tabesh et al., lower vitamin D level in women with preeclampsia was only detected in American population (26). It seems that the relationship of vitamin D level and preeclampsia is not simple and other related hormones and minerals may play roles in the occurrence of preeclampsia that results in differences of mentioned studies.

Placenta convert 25(OH) D to 1, 25 (OH) D by placental 1 $\alpha$  hydroxylase. This activated form of vitamin D has paracrine function. In addition, 1, 25 (OH) D and calcium are major modulators of parathormone (PTH). However, few

studies have focused on the complex association of serum 1, 25 (OH) D, calcium, and PTH with preeclampsia. In the present study, the prevalence of hypocalcemia in patients with severe preeclampsia was significantly higher than other groups. The relationship of serum calcium with preeclampsia have been investigated in limited studies. In two studies, serum calcium in patients with preeclampsia was lower than normal subjects (27, 28).

The cause and effect association of hypocalcemia and preeclampsia has not been established. Hypocalcemia can be considered the result of preeclampsia as a critical illness. On the other hand, complications of hypocalcemia e.g. secondary hyperparathyroidism may play a role in the etiology of preeclampsia (29).

Few studies have investigated the association of PTH and preeclampsia. In Seely et al. study, vitamin D level was similar in preeclampsia and normal group; but PTH level in preeclampsia was about two times higher than normal group while 1, 25 (OH) D in preeclampsia was lower than normal group (30). PTH level had negative correlation with serum and urine calcium. In a cohort study on 1141 pregnant women by Scholl et al., preeclampsia was more prevalent in cases that had higher PTH level simultaneously (31). These results indicate the potential role of PTH in pathogenesis of preeclampsia.

#### **4-1. Limitations of the study**

The present study had some limitations including the time of blood sampling that was at preeclampsia occurrence while pathogenesis of preeclampsia begins from weeks to months prior to the preeclampsia.

#### **5- CONCLUSION**

In summary, there was not any relationship between vitamin D deficiency and preeclampsia in the present study, but

low corrected serum calcium was associated with about six times increased risk of severe preeclampsia. More studies are needed to determine the role of hypocalcemia and vitamin D in preeclampsia.

**6- CONFLICT OF INTEREST:** None.

### 7- ACKNOWLEDGMENTS

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