

Comparison of 25-hydroxyvitamin D and Calcium Levels between Preeclampsia and Normal Pregnant Women and Birth Outcomes

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Abstract

Introduction

The main aim of this study was to compare serum level of 25-hydroxyl vitamin D [25(OH) D] between women with preeclampsia and normal individuals and birth outcomes.

Material and Methods

This cross sectional study was conducted on 650 normal primigravida women in their first 24–28 weeks of pregnancy. After 3 months of follow-up, serum levels of 25(OH) D and calcium were measured in 38 preeclampsia women (case) and in 38 normal pregnant (control). In addition, APGAR scores and newborn parameters were evaluated in infants of two groups.

Results

Preeclampsia was shown in about 5.84% (38 women) of pregnancies. Comparison of 25(OH) D levels between two main groups showed no significant differences ($P > 0.05$). Also, the calcium level was lower in preeclampsia women than the normal women ($P < 0.05$). The results revealed a significant correlation between neonatal two groups with regards to gestational age, birth weight, birth length, head circumference and Apgar score in the first minute of birth.

Conclusion

Although the difference of 25(OH)D levels between preeclampsia and healthy women is not significant, lower levels of serum calcium were associated with preeclampsia. Also, the newborn parameters improved in the neonates of the control group in comparison to those of the preeclampsia group. This difference might have resulted from mothers' different health statuses.

Key Words: Calcium, 25-hydroxyvitamin D, Preeclampsia, Neonates.

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Introduction

Preeclampsia is a pregnancy-specific disorder described by hypertension and proteinuria (1). Although still not completely known, the pathophysiology of preeclampsia possibly involves abnormal placentation and angiogenesis (2). Vitamin D may play a role in extraskeletal health, implantation, and placental function during pregnancy potentially due to anti-inflammatory, angiogenic and immunomodulatory effects (3, 4). Additionally, vitamin D supplementation during pregnancy may prevent perinatal complications (5). Moreover, 25-hydroxyvitamin D (25(OH) D) regulates calcium balance and affects the musculoskeletal system (6).

Up to now, controversial results have been obtained concerning the relationship between vitamin D and preeclampsia. Some previous studies have shown a relationship between the reduced risk of preeclampsia and higher 25(OH) D levels in pregnancy (7-10). However, some recent findings have indicated no association between plasma 25(OH) D concentrations and preeclampsia (11, 12).

The results of the study by Oken et al. demonstrated an association between higher dietary intakes of vitamin D and increased risk of gestational hypertension during pregnancy (13).

Most studies have reported the role of vitamin D and calcium in the pathophysiology of preeclampsia based on the measurement of 25(OH) D without concurrent measurement of other important factors, such as calcium. Calcium supplementation may lower blood pressure through affecting the Parathyroid hormone (PTH) levels and renin-angiotensin system (14). Furthermore, calcium supplementation appears to have reducing effects on the risk of preeclampsia and preterm birth and to decrease the rare occurrence of the

complex outcome "death or serious morbidity" (15). The hypocalcaemia were associated with preeclampsia and reduce the incidence of preeclampsia, especially in populations at high risk of preeclampsia (16, 17). Nonetheless, some previous findings revealed that calcium supplementation caused no reduction in the risk or severity of preeclampsia (18). Also, one other study showed no differences between preeclampsia and normal pregnant women with respect to the mean total serum calcium levels (19).

Some studies shown maternal vitamin D deficiency can adversely affect the offspring in terms of delayed growth or bone ossification, abnormal enamel formation and alterations in calcium metabolism during the neonatal periods (20). Studies that reported on birth weight, infants of mothers with 25(OH) D concentrations less than 37.5 nmol/l during pregnancy had lower birth weight however, birth length and head circumference did not differ significantly (21). However, there has been little discussion about differences between the women with preeclampsia and those with normal pregnancy regarding serum 25(OH)D concentration with concurrent measurement of serum calcium concentration and neonatal outcomes. Therefore, the present study aims to investigate serum 25(OH) D and calcium levels among preeclampsia and normal pregnant women in the third trimester of their pregnancy and neonatal outcomes.

Materials and Methods

This nested case-control study was carried on 650 eligible primigravida women during June to March 2012, in obstetric clinics of Shiraz University of Medical Sciences, Shiraz- Southwest of Iran. According to previous similar studies, the sample size was calculated to be 38 normal pregnant women and 38 women with preeclampsia.

The inclusion criteria of the patients were: singleton pregnancy, gestational age between 24 and 28 weeks based on the first trimester ultrasound exam, no history of chronic hypertension, not using any type of multivitamins, having lived in Shiraz during the previous 2 years, and no history of recognized internal diseases, such as kidney disease, diabetes, thyroid, etc., during the pregnancy.

All the participants signed written informed consents to participate in the study and completed the study questionnaires for obtaining their demographic data, method of contraception, and history of cigarette or tobacco consumption. Anthropometric parameters, including weight, height, and Body Mass Index (BMI) (weight/height²) were also evaluated. Maternal blood pressure (BP) was using a single standard mercury sphygmomanometer while the patients were seated and resting. Hypertension was defined as systolic BP ≥ 140 mmHg and/or diastolic BP ≥ 90 mmHg on two occasions within at least 6 h apart. It should be mentioned that none of the participants had high blood pressure at the beginning of the study.

Sample Collection and Analysis

In this study, 5 ml blood samples were collected from the participants by venous puncture early in the morning after an overnight fasting. Then, the serum was separated within 1 hour of sampling by centrifugation and stored at -70°C until analysis. The serum samples were assessed for 25(OH) D using a double antibody chemiluminescence assay (DiaSorin Inc., Stillwater, MN, USA). Two levels of control provided by the manufacturer were run in each assay. Afterwards, the obtained sera were analyzed for calcium using advanced clinical chemistry analytical system, cobas INTEGRA (Roche, Germany).

All the participants were followed every 2 weeks from the 24th until the 36th weeks, each week up to delivery, and until 2 days after the delivery.

The preeclampsia group participants included the patients with the two following clinical risk factors: BP equal to or greater than 90/140 mm Hg on two occasions with an interval of 6 h measured with a single standard machine by one of the researchers, and proteinuria equal to or greater than 300 mg in 24 h urine that is equal to or greater than +1 in the urine-stick test (23). The women who were unable or unwilling to cooperate, experienced any internal diseases at each stage of sampling, and had preterm delivery before the second phase of the test were excluded from the study. Finally, 38 subjects were excluded from the study.

For each preeclamptic patient, five matched maternal and gestational age pregnant women were selected from the healthy pregnant women in the first turn of blood sampling. Then, the second stage of sampling was performed in the preeclampsia and healthy individuals. In summary, serum 25(OH) D was measured at 28-32 weeks of gestation with twice sampling. Then, the mean of the two values was computed to obtain more stable estimates of 25(OH) D and calcium levels.

The study participants were followed-up to 48 h after delivery. One out of the five healthy matched pregnant women who had not developed the signs of preeclampsia was randomly selected and considered as a control case. Finally, in the second stage, a total of 38 women (in each group) and their infants were included in the analysis. Several parameters including: gestational age, weight, length, head circumference, and APGAR scores, were assessed for all the newborns in the first minute of birth. In addition, 3 cutoffs of 25(OH) D were used in our analysis to identify vitamin D status: <12 , $12-20$, and ≥ 20 ng/ml (24). In this study, all the statistical analyses were

performed using the SPSS statistical software (SPSS Inc, Chicago, IL, USA), version 19 and P-value<0.05 was considered to be statistically significant. The three pre-specified cut offs of 25(OH) D concentration representing vitamin D deficiency (<12 ng/ml), vitamin D insufficiency (12–20 ng/ml), and vitamin D sufficiency (\geq 20 ng/ml) were used to split the data. The values were expressed as mean \pm standard deviation (SD). Independent samples T-test and Chi-square test were used to compare the quantitative variables between the patients and controls groups. Besides, binary logistic regression was used to calculate the odds ratios.

Results

This study was conducted on 650 pregnant primigravida women. Preeclampsia was detected in about 5.84% of pregnancies (38 individuals). At baseline, no significant differences were found between the preeclampsia and control group regarding maternal age, anthropometric indices, and family history of preeclampsia, diabetes, and hypertension (22) (Table. 1). The study results also indicated no significant difference between the two groups concerning the mean maternal serum 25(OH) D levels (15 \pm 5.1 vs. 12.7 \pm 5.4 ng/ml) (P=0.06). However, a significant

difference between the two groups with respect to the mean maternal serum calcium levels was observed (8.9 \pm 0.6 vs. 9.3 \pm 0.6 mg/dl) (P=0.011). Nevertheless, no significant difference was found among the three groups of 25(OH) D (deficient, insufficient and sufficient) in the pregnant women with preeclampsia regarding calcium concentration (Table. 2). The risk of preeclampsia with a cut point for 25(OH) D levels less than 12 ng/ml versus higher showed a weak, and non-significant association (OR, 1.7; 95% CI, 0.66-4.4). However, no significant differences were found among the three groups of preeclampsia patients (deficient, insufficient and sufficient) regarding gestational age, birth weight, birth length, head circumference, and APGAR score in the first minute of birth (Table. 2). Furthermore, the study findings showed a significant difference between the women with normal pregnancy and those with preeclampsia concerning the mean time of delivery (39.5 \pm 1.2 vs.32.6 \pm 1.4 w). In the present study, all the neonates survived in both groups. Moreover, the results revealed a significant difference between the two groups with regards to gestational age, birth weight, birth length, head circumference, and Apgar score in the first minute of birth (Table. 3).

Table 1: Demographic and clinical characteristics of the patients and the control group

Variables	Control group Mean \pm SD	Preeclampsia Mean \pm SD	P-value
Maternal age, years	28.2 \pm 3.12	28.4 \pm 3.13	NS
Weight, kg	63.70 \pm 10.10	62.24 \pm 6.55	NS
Height, cm	161.7 \pm 3.5	161.2 \pm 2.2	NS
Body mass index, kg/m ²	23.9 \pm 3.33	24.95 \pm 2.76	NS
Systolic blood pressure, mm Hg	105.44 \pm 18.15	156.52 \pm 17.2	<0.0001
Diastolic blood pressure, mm Hg	74.07 \pm 11.03	96.11 \pm 12.19	<0.0001
Gestation in the first sampling, weeks	24.52 \pm 1.23	25.4 \pm 1.34	NS
Gestation at delivery, weeks	39.5 \pm 1.2	32.6 \pm 1.4	<0.0001
Cigarette smoker, n (%)	1 (2.63)a	1 (2.63) a	NS
Family history of preeclampsia, n (%)	-	-	-
Family history of hypertension, n (%)	2 (5.26) a	1 (2.63) a	NS
Family history of diabetes, n (%)	4 (10.52) a	3 (7.89) a	NS
Oral contraception	3 (7.89) a	1 (2.63) a	NS

NS: Not significant; a Number (%)

Table 2: Comparison of neonatal characteristics and calcium concentrations in the preeclampsia women according to the three 25(OH) D groups

Newborn parameters	Deficient	Insufficient	Sufficient	P-value
First min APGAR (n)	7±0.6	6.8±0.9	6.7±1.5	NS
Birth weight (g)	2350±216	2411±239	2475±146	NS
Birth length (cm)	47.5±3.4	48.6±3	50.8±3	NS
Birth head circumference (cm)	33.8±1.2	34.3±1.1	35.1±1	NS
Gestational age, weeks	32.2±0.8	32.9±1.7	33.2±0.7	NS
Calcium(mg/dl)	8.8±0.6	9±0.6	8.7±0.9	NS

NS: Not significant.

Table 3: Characteristics of the neonates in the case and control groups

Newborn parameters	Case group Mean± SD	Control group Mean± SD	P-value
First min APGAR (n)	6.89±0.89	9±0.67	<0.0001
Birth weight (g)	2400±223	2770±300	<0.0001
Birth length (cm)	48.57±3.24	50.95±2.63	<0.0001
Birth head circumference (cm)	34.28±1.19	35.55±0.99	<0.0001

Discussion

Preeclampsia is the third common cause of maternal mortality in the world and the risk of infantile mortality in preeclampsia is 4 times higher than that in normal pregnancies (25).

Evidences have shown that the overall worldwide incidence of preeclampsia was 4-8%, corresponding to our study results (26, 27). However, this incidence rate has been reported to be up to 15% in some studies, especially those conducted in developing countries (28). Although many researchers have been done to understand the etiopathogenesis behind this vascular dysfunction, no exact theory regarding its mechanism has been established yet.

There are several mechanisms by which vitamin D could potentially prevent or at least delay the progression to preeclampsia, such as defective control of effector T cells (29), having a role in effectiveness of calcitriol (30, 31), cardio-protective effect through influences on the renin-angiotensin-aldosterone system (32), and direct effects on the arterial wall by

calcitriol (33). The findings of the present study indicated no association between serum 25(OH) D concentrations and hypertensive conditions of pregnancy, such as preeclampsia, in the third trimester. Similarly, Burriset et al. (34) in their cohort study found no relationships between 25(OH) D levels at 27.9 weeks of gestation and risk of preeclampsia. Wetta et al. (35) measured serum 25(OH) D concentrations in 89 women with preeclampsia and 177 controls between the 15th and 21st weeks of gestation, and revealed no significant differences between the two groups (68 vs. 71 nmol/L, P=0.92). Consistently, Yu et al. (36) investigated 90 cases that developed early (before 34 weeks of gestation) and late preeclampsia (after 34 weeks of gestation) and 1000 controls and found no difference in the median serum 25(OH) D level (P=0.14 and P=0.23 comparing early and late preeclampsia cases to controls, respectively).

Our findings were in contrast to those of some other studies. For instance, Bodnar et al. (8) demonstrated that the women who

had developed preeclampsia were almost 2.5 times more likely to be vitamin D deficient in early pregnancy. Also, in the study by Haugen et al. (37), the women who had a higher total vitamin D intake; i.e., 15–20 µg/day, had a lower rate of preeclampsia compared to those with <5 µg/day intake. In a recent research on 3,6282 non-pregnant, postmenopausal women who were randomized to supplementation with 1000 mg calcium, 10 µg (400 IU) vitamin D, or placebo, no difference was observed in systolic and diastolic BP and development of hypertension over a median of 7 years of follow-up(38). Our findings showed a weak, and non-significant association (OR, 1.7; 95% CI, 0.66-4.4) for the risk of preeclampsia compared between serum 25(OH) D levels less than 12 ng/ml versus higher levels. The comparable result by Aghajafari et al. (21) showed a weak, non-significant association for the risk of preeclampsia with a cut point for 25(OH) D levels less than 75 nmol/l versus higher (adjusted OR, 1.51; 95% CI, 0.89-2.57). In contrast to our results, Wei et al. (39) and Tabesh et al. (11) reported that women with below 50 nmol/l 25(OH) D had higher odds of preeclampsia compared to those with higher 25(OH) D levels.

This discrepancy might be due to measuring serum 25(OH) D at different weeks of gestation (36, 40), multiplicity of taking vitamin D supplements, no preconception vitamin use (37, 40), different nutritional habits, ethnicity, smoking (40), seasonal variations (21,40), and focusing on severe preeclampsia which has been more consistently linked to vitamin D status (7, 10, 33, 42). Thus, further comprehensive evaluations should be performed on the role of vitamin D in prevention and treatment of hypertensive disorders.

Several studies have demonstrated that changes in the concentration of minerals, like calcium, might have a role as

physiopathology of preeclampsia (43-46). On the other hand, some other studies have not shown any association between the serum concentrations of minerals and incidence of preeclampsia (47).

Our study results showed a significant decrease in the preeclampsia group participants' serum levels of calcium compared to the healthy pregnant individuals, which is compatible with the findings of other studies (43-46, 49). This might be due to increased fetal demand, inadequate dietary intake, or hemodilution (48). Lambe et al. (48) revealed a significant negative correlation between serum calcium and systolic and diastolic BP, suggesting a strong relationship between calcium deficiency and risk of preeclampsia.

Decreased serum calcium could increase the parathyroid hormone levels that can cause shift of calcium intracellularly and increase the vascular smooth muscle contraction (45, 49). Low serum calcium level can also cause 1, 25-dihydroxy cholecalciferol response, eventually increasing the BP (50). Ayman and Abdrabo (17) and Lambe et al. (48) recommend that assessment of serum calcium could be considered as a factor playing a role in the etiopathogenesis of preeclampsia and as an index for predicting the disease.

Although our results revealed lower serum calcium levels in the preeclampsia group in comparison to the control group, this calcium level was not lesser than normal ranges. Thus, we accepted the role of calcium in the etiopathogenesis of the disease, but not as an index for diagnosis of preeclampsia.

In the present study, the newborn parameters, including gestational age, birth weight, birth length, head circumference, and first minute APGAR scores, improved in the neonates of the control group in comparison to those of the preeclampsia

group. This difference might have resulted from mothers' different health statuses. However, the difference cannot be attributed to calcium or other elements because this disease is associated with complex factors.

One of the limitations of our study was the small sample size and the small number of women with preeclampsia compared to other studies. A limitation of our study was lack of information about what dose from sources of vitamin D and calcium intake by cases and controls. Yet, the main limitation of this study was that the researchers did not measure the remaining factors that could regulate calcium homeostasis, such as vitamin D binding protein and calcitropic hormone levels.

Conclusion

In conclusion, the findings of the present study indicated no significant association between 25(OH) D levels obtained at 30 weeks of gestation and preeclampsia. Also, the results did not support the hypothesis that low serum 25(OH) D level was associated with hypertensive conditions of pregnancy, such as preeclampsia. In addition, no significant relationship was found between below 12 ng/mL 25(OH) D levels as deficient conditions and increased risk of preeclampsia. This suggests that the role of low vitamin D level is marginal compared to the other risk factors of preeclampsia, such as calcium. Similar results were also obtained by Carnevale et al. (51) in a study on the relationship between vitamin D and carotid intima-media thickness. The findings of the current study showed that lower levels of serum calcium were associated with preeclampsia, but this calcium level was not lesser than normal ranges. Thus, it cannot be used as an index for diagnosis of preeclampsia.

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Conflict of Interest: None.

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