

Effect of Treatment with Vitamin D on Maternal and Neonatal Indices in Pregnant Women with Hypocalcemia: A Randomized Controlled Trial

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Abstract

Background

The impact of concomitant vitamin D deficiency and maternal hypocalcemia on fetal growth has is not clear. The aim of this study was to determine the effect of treatment with vitamin D on maternal and neonatal indices in pregnant women with hypocalcemia.

Materials and Methods

This clinical trial was conducted on 110 pregnant women (22-26 weeks of gestational age) with simultaneous mild hypocalcemia ($8 < \text{serum calcium} < 8.5 \text{ mg/dL}$) and vitamin D deficiency ($25 \text{ (OH) D} < 75 \text{ nmol/L}$). The study subjects were randomly allocated to intervention ($n=55$) and control ($n=55$) groups. In the control group, the subjects were given daily prenatal capsule until delivery. In the intervention group, the subjects were given 50,000 Units vitamin D weekly for eight weeks in addition to prenatal capsules until delivery similar to the control group. At delivery, maternal calcium and 25 (OH) D level and neonatal indices (weight, height, and head circumference) were measured and compared between the groups.

Results

At delivery, mean maternal vitamin D level was $97.5 \pm 23.4 \text{ nmol/L}$ in the intervention group and $48.9 \pm 17.2 \text{ nmol/L}$ in the control group, respectively ($P < 0.001$). Mean maternal calcium level in the intervention group was higher than the control group ($9.0 \pm 0.6 \text{ mg/dl}$ vs. $8.8 \pm 0.5 \text{ mg/dl}$) but the difference was not statistically significant ($P > 0.05$). Mean neonatal weight, height, and head circumference were not significantly different between the two groups ($P > 0.05$).

Conclusion

In pregnant women with mild hypocalcemia, treatment with vitamin D would not have effect on mean serum calcium and neonatal indices.

Key Words: Body height, Body weight, Hypocalcemia, Pregnancy, Vitamin D deficiency.

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1- INTRODUCTION

Vitamin D deficiency is a common problem worldwide (1-3). In Iran, vitamin D deficiency is highly prevalent. According to previous reports about 80% of Iranian people are vitamin deficient or insufficient (4). Despite widespread use of supplements during pregnancy, most of pregnant Iranian women (about 78%) are still have not sufficient vitamin D level (5). There is widespread distribution of vitamin D receptors in different tissues in human body and activated vitamin D regulates many human genomes (6). Vitamin D concentration is correlated with gene expression of placental amino-acid transporter that is responsible for numerous nutrients transport to the fetus (7). In recent decades many studies have focused on maternal and neonatal complications of vitamin D deficiency during pregnancy. Relationship of maternal vitamin D deficiency with gestational diabetes, preeclampsia, neonatal rickets, small for preterm delivery and neonatal anthropometric indices have been investigated (8-16).

Data about some of these complications such as gestational diabetes and preterm delivery are more robust, however effect of vitamin D deficiency with neonatal growth indices are inconsistent. Some studies have reported association of maternal vitamin D with at least one neonatal growth indices, but in other studies such associations have not been found. Significant heterogeneity has been found in meta-analysis performed for association of birth outcomes and maternal vitamin D level (17). Maternal serum calcium is another essential parameter that is transported by placental calcium transporter and affects fetal bone accretion. Vitamin D can modulate this transportation via its effect on maternal calcium level and regulation of placental calcium transporter genes (18). Hypocalcemia has been reported in 30% to

60% of pregnancies (19, 20). Although hypocalcemia in pregnancy has been attributed to hypoalbuminemia due to maternal serum dilution (21), other pathophysiological mechanism can contribute to the development of hypocalcemia. In animal studies, low ionized calcium has been reported in some pregnant pigs (22). Furthermore, high incidence of pregnancy induced hypocalcemia in Asian women has been reported (23). With regards to combined effects of maternal serum vitamin D and calcium level on fetal growth, this clinical trial was designed to determine the effect of treatment with vitamin D on maternal calcium and neonatal indices in pregnant women with hypocalcemia.

2- MATERIALS AND METHODS

2-1. Study design and population

This clinical trial was conducted on 110 pregnant women referred for prenatal care in 22-26 weeks of gestational age to the private gynecology and obstetrics clinic. This research was registered as clinical trial in Clinical trial.gov (code: NCT02021864).

2-2. Methods

Firstly, serum calcium and 25 (OH) D were measured for all pregnant women. Then women with simultaneous mild hypocalcemia and vitamin D deficiency were entered the study. Based on the reference range of calcium in Pars Azmoon kit, using Arsenazo reagent, mild hypocalcemia was defined as $8 < \text{serum calcium} < 8.5 \text{ mg/dL}$. Vitamin D deficiency was defined as $25 \text{ (OH) D} < 75 \text{ nmol/l}$ (24). The subjects were followed till delivery. The prenatal care was performed every two weeks in the beginning of the study and continued weekly in the third trimester of the pregnancy. Vitamin D and multivitamin consumption was monitored. After delivery, maternal calcium and 25 (OH) D were measured and neonatal

indices including weight, height, and head circumference were recorded.

2-3. Measuring tools

Data were collected through a questionnaire including demographics variables, gestational age, 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. After delivery, weight, height, and head circumference of the neonate were measured in labor room and were recorded in the birth documents. The values recorded in birth documents were applied in the present study.

2-4. Intervention

The study subjects were randomly allocated to intervention (n= 55) and control (n= 55) groups using a computer based method (random number generator). In the control group, the subjects were given daily prenatal capsule including 400 Units (U) vitamin D and 250 mg elemental calcium from single company until delivery. In the intervention group, the subjects were given 50,000 Units vitamin D weekly for eight weeks in addition to prenatal capsules until delivery similar to the control group.

2-5. Laboratory measurements

At delivery, a 5 ml venous blood sample was taken to measure calcium and 25 (OH) D in mothers. 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. Calcium was measured by calorimetric method using Pars Azmoon kit. Inter-assay and intra-assay CVs were 2.7% and 1.4%, respectively.

2-6. Ethical consideration

The study protocol was confirmed in the ethics committee of Qazvin University of Medical Sciences (ID number: 28/20/7933). Subjects gave their written informed consent.

2-7. Inclusion and exclusion criteria

The inclusion criterion was diagnosis of simultaneous mild hypocalcemia and vitamin D deficiency in 22-26 weeks of gestational. Exclusion criteria were premature rupture of membrane (PROM), preterm labor and history of parathyroid disorders, renal or liver diseases, osteomalacia, malnutrition, and epilepsy.

2-8. Data Analyses

Kolmogorov-Smirnov test was used to examine the normality of variables of interest. Quantitative variables were described as mean \pm standard deviation (SD) and categorical variables were described as percent. T-test was used to compare quantitative variables and Chi-square test was used to compare categorical variables. P-values less than 0.05 were considered as significant.

3- RESULTS

Fifty five pregnant women were evaluated in every study groups (intervention and control groups). Eleven women (20%) in the intervention group (three (5.45%) due to Premature rupture of membranes (PROM), and eight (14.55%) due to delivery in other places) and 14 women (25.45%) in the control group (two (3.63%) due to PROM and 12 (21.82%) due to delivery in other places) were excluded (**Figure.1**).

Baseline clinical and biochemical characteristics of the study subjects are shown in **Table.1**. Age, parity, gestational age, and serum calcium and 25 (OH) D levels were not different between the groups (P>0.05). Maternal mineral status and neonatal anthropometric indices after

the intervention are shown in **Table.2**. Mean weight, height, and head circumference were not significantly different between groups. Mean serum vitamin D level in the intervention group

was significantly higher than the control group after the intervention ($P<0.001$). Serum calcium level and the prevalence of hypocalcemia were not different between the groups after the intervention ($P>0.05$).

Table-1: Clinical and biochemical characteristics of the study subjects before intervention

Variables	Intervention group (n=44)	Control group (n=41)	P-value
Age (year)	28.6±4.5	29.0±4.8	0.981
Body mass index	28.3±3.7	26.7±3.2	0.559
Parity	1.8±1.1	1.7±0.8	0.725
Gestational age (week)	24.6±1.9	24.8±1.9	0.610
25(OH) D (nmol/L)	47.5±15.3	45.0±17.1	0.496
Calcium (mg/dl)	8.3±0.4	8.3±0.3	0.728

Data are presented as mean ± standard deviation.

Table-2: Maternal mineral status and neonatal anthropometric indices after the intervention

Variables	Intervention group (n=44)	Control group (n=41)	P-value
Neonatal			
Weight ^a (gr)	3080.0±426.2	3046.3±386.3	0.705
Height ^a (Cm)	48.6±1.8	49.0±1.9	0.296
Head circumference ^a (Cm)	34.5±1.2	34.4±1.4	0.714
Maternal			
25(OH) D ^a (nmol/L)	97.5±23.4	48.9±17.2	<0.001
Calcium ^a (mg/dl)	9.0±0.6	8.8±0.5	0.136
Hypocalcemia ^b	17	24	0.125

^aData are presented as mean± standard deviation; ^bData are presented as percent.

4- DISCUSSION

In the current study, after 8 weeks treatment with vitamin D₃, maternal vitamin D level raised effectively without side effects such as hypercalcemia. Calcium level was higher in the treatment group, but the difference was not significant. Neonatal height, weight or head circumference were not different between the intervention and control groups. Dietary sources of vitamin D are very limited; furthermore significant proportion of people does not receive vitamin D especially in seasons with

minimal sunshine. Intake of calcium is also less than recommended even in the developed countries (25). Vitamin D deficiency is a worldwide problem. Regarding to widespread distribution of vitamin D receptors throughout the body, vitamin D deficiency may have potentially various roles in pathophysiology of different diseases. Vitamin D regulates genes responsible for placenta amino-acids transporters (7). Amino-acids are essential for fetal soft tissues and bone matrix growth. So, vitamin D potentially can influence fetal growth (26). The

correlation of maternal vitamin D level with activity of placental calcium and amino-acid transporter has been reported. But the cut-off point of vitamin D level for maximum activity of this transporter has not been introduced (27). In recent years, the results of different meta-analysis studies have been published about the association of maternal vitamin D and fetal growth. Results of these meta-analyses are different. In the Cochrane meta-analysis on four clinical trials (17), it was a trend for higher length in neonates in the supplemented group (mean difference: 0.70, 95% CI: -0.02-1.43 cm) and head circumference (0.43, 95% CI: 0.03-0.83cm). In another meta-analysis performed based on two clinical trials (12), birth length and head circumference was not significantly different between the supplemented and control groups.

In a most recent meta-analysis (28), pooled analysis showed no significant difference in mean length of the supplemented and control groups. Not only the difference of birth weight was not significant, but also the neonatal weight decreased in the supplemented group in one of the studied trials. All of these meta-analyses pointed out significant heterogeneity between trials. Differences of results of meta-analyses can be attributed to the criteria of study selection and method of meta-analysis.

Baseline level of maternal 25(OH) D is an important source of heterogeneity. Regarding the vitamin D role in mineral metabolism and its effects on placental transporter (7), lower vitamin D level in study population can result in more significant results compared to higher baseline vitamin D level. For example in one study (29), height, weight, and head circumference of the neonates of pregnant women with severe vitamin D deficiency (25(OH) D < 25nmol/L) were lower than these indices in the neonates of pregnant women with higher vitamin D levels. In

another study, height of the neonates of mothers with 25(OH) D less than 25 nmol/L were only significantly lower than height of the neonates of mothers with 25(OH) D more than 50 nmol/L (30). On the other hand, in a study neonatal weight and height in two groups of pregnant women with 25(OH) D levels lower than and higher than 50 nmol/l were compared and no significant difference was found (31). In a previous study in Iran, mean maternal calcium and mean neonatal height, weight, and head circumference in the pregnant women who underwent treatment with vitamin D was higher than the control group and mean vitamin D level in pregnant women were 26 nmol/L (32). In the present study, mean vitamin D levels were 45 nmol/L that are considerably higher than Hashemipour et al. study in Iran (32). Attention to diagnosis and treatment of vitamin D deficiency in women who are planning for pregnancy are increased in recent years, and explain the higher vitamin D level in the present study compared to the previous study.

4-1. Limitations of the study

The present study had some limitations including lack of placebo administration as well as lack of serum ionized calcium measurement.

5- CONCLUSION

In conclusion, the results of the present study showed that treatment with vitamin D would not have effect on mean serum calcium and neonatal indices in cases of concomitant mild hypocalcemia and vitamin D deficiency in pregnancy. It is possible that the association of maternal serum vitamin D with neonatal indices as well as the effect of vitamin D on maternal calcium and fetal growth is dependent to the severity of vitamin D deficiency.

6- CONFLICT OF INTEREST: None.

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