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VITAMIN D SUPPLEMENTATION IN PREGNANT IRANIAN WOMEN: EFFECTS ON MATERNAL AND NEONATAL VITAMIN D AND PARATHYROID HORMONE STATUS

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Abstract

Context. Vitamin D is essential for skeletal and nonskeletal health and prolonged deficiency results in infantile rickets and adult osteomalacia. The aim of this study is to determine 25(OH) VitD and iPTH status in pregnancy and to evaluate the effects of monthly 100.000 IU dose of vitamin D supplementation.

Materials and Methods. In a double blind trial of vitamin D supplementation in pregnant Iranian women, vitamin D3 (cholecalciferol , 100/00 IU/month) was administered to 25 women and placebo to 25 controls during the last trimester. The two groups had similar distributions of maternal age, height, gravity, weight and age of gestation. Hydroxycholcalciferol and iPTH were measured in mothers at 27 weeks and at delivery. Cord blood was used to assess the same parameters.

Results. Comparing the data final maternal 25 - hydroxyvitamin D levels were significantly higher in the supplemented group *versus* control group (61.45±30 ng/mL *versus* 29.4±16 ng/mL); $P \leq 0.001$.Cord 25 - hydroxyvitamin D levels were significantly higher in supplementation group in comparison to control group (52 ± 40.5 ng/mL*versus* 36±21.3 ng/mL); $P<0.005$.

Conclusion. Administration of 100/000 IU/monthly of vitamin D3 in the last trimester significantly increased 25(OH) VitD to high normal concentration. However, even with supplementation, only of mother and of newborn had serum 25(OH) VitD greater than 30 ng/mL a small percentage of women and babies were vitamin D sufficient. According to data of study we propose 100/000 IU monthly is safe for pregnant women.

Key words: Cholecalciferol, vitamin D3 , iPTH , pregnancy, cord blood.

INTRODUCTION

The two sources of vitamin D in the body are the skin and the diet.

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However, majority of vitamin D comes from sunlight exposure and the main cause of vitamin D deficiency is lack of sunlight exposure (1). Most foodstuffs contain little Vitamin D, hence adequate Vitamin D store depends on sun exposure or external sources. Vitamin D deficiency is seen with high frequency in Iranians despite ample sun exposure which may be due to traditional clothing that prevent people especially women from receiving adequate exposure to sunshine (2-4). Ghazi *et al.* reported seasonal variation in serum 25(OH) VitD concentration in a large Tehranian population, and values for the women were almost half of the men and the nadir of serum levels seen in December and February, was 12±13 and 14 ±14ng/mL in women, and 28±16 and 28±18mg/mL in men, respectively. (5). Several studies carried out during last two decades have shown a high prevalence of vitamin D deficiency in tropical countries such as China, Turkey, India and Saudi Arabia and it was estimated its prevalence as ranging between 30% and 93% (6-7). Due to widespread Vitamin D deficiency, pregnant women of the communities also suffer from deficiency during pregnancy. This is important as hypovitaminosis D during pregnancy is related to development of myopathy and pathologic fractures in mothers (8) and neonatal tetany and rickets in infants (9). It may also be associated with secondary hyperparathyroidism in neonates (10). Some preliminary data revealed that moderate to severe Vitamin D deficiency during pregnancy has significant effects on maternal weight gain and fetal growth (11).

At present there is not enough evidence to evaluate the effectiveness of vitamin D in pregnancy and, therefore, vitamin D supplementation is not routinely offered to all pregnant women. A clinical trial in Iran showed that monthly administration of 50.000IU vitamin D3 in high schoolchildren significantly increased serum 25(OH) D3 without causing vitamin D toxicity (12). Based on the results of that study we decided to evaluate the effects of monthly 100.000 IU cholecalciferol in the third trimester on serum 25(OH) VitD and iPTH in a group of pregnant Iranian women and their offspring.

SUBJECTS AND METHODS

Fifty pregnant women, scheduled to deliver at Mahdieh Hospital in Tehran, were recruited for this study, which took place between 2009 and 2010. Women with pre-existing sarcoidosis, renal and hepatic dysfunction and tuberculosis were excluded from the study. Maternal blood samples at 27 - 28 weeks gestation were taken to measure 25(OH) Vit D and iPTH. One more sample was taken from the mothers at the time of delivery and cord blood samples were taken at birth. Cord blood was obtained while the placenta was still in situ. The cord was cut with a sterile blade and blood was allowed to drop into tubes. Blood was allowed to clot, centrifuged and the serum samples were stored at -80°C until analysis.

Each participant gave written consent and completed a life style questionnaire, which included details

regarding the maternal age, weight, parity and any use of recreational drugs. We also asked about clothing style and clinical features suggestive of vitamin D deficiency. Most of them had been clothed and covered in thick dark dresses and veils during the pregnancies. Each patient was randomly allocated to the treatment (oral vitamin D3 100,000- IU, monthly, 3 times) or the control (placebo) group, the preparation to be taken monthly until term. All of the procedures described were reviewed and approved by the ethical committee of the Research Institute for Endocrine Sciences and the study was registered by the Iranian Registry of Clinical Trials Review Board on 19/09/2011, Registration ID IRCT201104306335N1.

Biochemical measurements

Serum 25 (OH) D concentrations were measured by EIA using the 25(OH) Vit D kit (Immune diagnostic system Ltd, Bolden, UK). Serum levels of 25(OH) Vit D <30 ng/mL were considered to reflect vitamin D insufficiency. Intact parathyroid hormone levels were determined by EI MA (Immune diagnostic system Ltd, Bolden, UK). Serum levels of 0.8-3.9 pmol/L were considered normal range. Inter-assay coefficients of variations were 4.4% for iPTH and 3.6% for 25(OH) Vit D.

Statistical analysis

Data are expressed as mean±SD, except for data that were not normally distributed, in which case median values, ranges and percentages (%) are reported. Group means and medians were compared using the student's t test and the Man-Whitney test. Pearson correlation coefficient was used to examine the association between maternal and infant characteristics and the prevalence of vitamin D deficiency. Paired t-test and simple linear regression were used to compare the mother's and infant's serum 25 (OH) D and iPTH concentrations.

RESULTS

Maternal and infant characteristics according to each group are shown in Table 1. There were no significant differences in the base line characteristics across the two groups.

Table 2 illustrates the plasma concentration of 25(OH) VitD, iPTH and also in cord blood in the treated and control groups during the study. There were no significant differences between the two groups at 28 weeks. The final maternal 25(OH) VitD levels at delivery were significantly higher in the treated group in comparison to the control group. (61.45±30 ng/mL *versus* 29.4±16 ng/mL) P≤0.001. Cord 25(OH) VitD levels were significantly higher in the supplemental groups in comparison to controls. (52 ± 40.5 ng/mL *versus* 26±21.3 ng/mL) P<0.005. We found a higher prevalence of serum 25(OH) VitD <30 ng/mL in newborn infants of control group mothers (52%) compared with the treatment group (24%). There was no significant difference in maternal serum iPTH between the two groups (2.56±1.3pmol/L versus 3.37±1.4pmol/L), and we find a significant difference between maternal and cord blood iPTH concentration in the two groups. We noticed an inverse relationship between serum 25(OH) VitD and serum iPTH levels in the treated group. Mean parathyroid hormone concentration (2.18 pmol/L) was

Table 1. Sociodemographic data and clinical factors for subjects as a function of study group

Maternal and infant factors		Treatment group (n=25)	Control group (n=25)
Maternal	Age (yr)	26.6±4.7	26±6.2
	Weight (kg)	72±10	70±9
	Systolic blood pressure (mmHg)	110(107-120)	110(110-120)
	Diastolic blood pressure (mmHg)	70(70)	70(60-70)
	Gravidity 1 (%)	58.3%	60%
	Gravidity 2(%)	41.7%	40%
	Parity 1 (%)	88%	84%
	Parity ≥ 2(%)	12%	16%
	No abortion (%)	95%	88%
Infant			
	Birth weight (g)	3293±334	3248±320
	Height Baby (cm)	51 (50-51)	50 (49-51)

Data are means ± SD, medians (interquartile range), or percent.

Table 2. Change in biochemical variables in treated and placebo groups

	Groups	28wk	At term	Cord
25(OH)D(ng/mL)	Treated	33.5±21.4	61.45±30*	52±40.5†
	Control	38.3±23.2	29.4±16*	26±21.3†
iPTH(pmol/L)	Treated	2.98±1.17	2.56±1.3	1.18±0.95
	Control	3.39±2.04	3.37±1.4	1.54±1.25

Maternal and cord plasma biochemical results in two groups of pregnant Iranian women and their infants. *p<0.0001, †p<0.005.

significantly lower among women with serum 25(OH) VitD level >30 ng /mL, p<0.003. (Data not shown).

DISCUSSION

This is the first controlled trial of vitamin D supplementation in Iranian women during pregnancy. Initial biochemical assessment at 28 weeks gestation showed 60% of pregnant Iranian women have plasma 25(OH) VitD concentrations below 30 ng/dL. According to our data plasma concentrations of 25(OH) VitD were lower than 30ng/mL in 60% of mothers and 52% of offspring. Maghbooli *et al* reported 66.8% of 552 pregnant women had serum 25(OH) VitD levels less than

14 ng/mL. (13) Prevalences of vitamin D deficiency in the various study groups have been widely reported to range between 26-84% (5-14-15). Ainy *et al.* reported 60% of Iranian women in the first trimester had either severe or moderate vitamin D deficiency (16). Several other studies from developing and developed nations across the world have reported that the prevalence of hypovitaminosis D [25(OH) VitD, 10 ng/mL] during pregnancy ranged from 18 to 84 (17-18-19-20). Marvaha *et al.* found that 96.3 % of pregnant women had Hypovitaminosis D [25(OH) VitD < 20 ng/ml] and a strong positive correlation was observed between 25(OH) VitD levels of mother-infant pairs (21).

Vitamin D deficiency as documented is becoming a major problem during pregnancy although data on newborn infants are limited. One report, however, found a high prevalence of vitamin D deficiency in newborn infants and pregnant women from India, a country with abundant sun light (22). In our study, mean cord blood 25(OH) VitD level in control group in comparison to the treated group was almost half of the values (26.19±21.3 ng/mL *versus* 52 ± 40.5 ng/mL). Few studies have reported the consequences of maternal and neonatal vitamin D deficiency for fetal growth and bone development (12-23-20). Mother-offspring cohort studies have shown that maternal vitamin D insufficiency has a detrimental effect on bone mineral mass at birth and in childhood (20 - 24). Mahon *et al.* demonstrated that maternal vitamin D insufficiency can influence fetal femoral development as early as 19 weeks gestation (25). Zeghoud *et al.* found neonatal 25(OH)D concentrations below 12 ng/mL to be associated with elevated PTH, and they proposed this level of concentration as the cutoff for diagnosing hypovitaminosis D in the newborn (26). In our study, 52% of our newborns (in control group) have 25(OH) D concentrations less than 30 ng/mL that can predispose them to neonatal hypocalcemia and infantile rickets and to the attendant morbidity.

Controversy still exists regarding the effects of pregnancy on circulating maternal parathyroid hormone levels and cut-off value as a screen test. There is now considerable data indicating that plasma levels of parathyroid hormone increase with poor vitamin D status. It has been shown that vitamin D deficiency and secondary hyperparathyroidism are more common in the non-Caucasian ethnic groups (27). In our study, the lowest serum iPTH levels were observed in the group with a serum 25(OH) VitD level of more than 30 ng/mL, where as the highest serum iPTH was observed in the group with a serum 25(OH) VitD level <30 ng/mL, an association which was more significant in groups of pregnant women receiving vitamin D supplements (P<0.003) (11). In accordance with Haney's finding study results suggest that secondary hyperparathyroidism could be prevented in most of individuals by increasing serum 25(OH) VitD to at least 48ng/mL (28).

Several other studies have assessed the effects of large intermittent doses of vitamin D. Adams administered vitamin D2 500.000 IU over five weeks to deficient patients, resulting in a mean increase in 25(OH) VitD of 24ng/mL (29) and Prsybelski gave 600.000 IU of D 2

over 4 weeks with an increase of 18.8ng/mL (30). The present study demonstrates the efficacy of monthly 100.000 IU vit D3 during the last trimester of pregnancy. The neonates of these mothers have a substantially improved nutritional vitamin D status, because of the transfer of vitamin D into the placenta. But, one of the major limitations of our study was inability to measure serum calcium concentration due to use of EDTA in the collection tubes.

Further studies are needed to provide more conclusive evidence of the long-term consequences of fetal and neonatal vitamin D deficiency. The lack of evidence so far has led to contradictory recommendations on vitamin D supplementation during pregnancy in many countries. In the Netherlands the health council advises that pregnant women at risk should receive vitamin D supplements; however, this is not common practices (31-32).

In conclusion, our results provide substantial evidence in favor of supplementing vitamin D intake in all pregnant women and this could be administered monthly as 100.000 units Vit D. Further research is needed to determine the optimum timing and dosing of vitamin D supplementation in pregnancy.

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Conflict of interest.

The authors declare that there is no conflict of interest.

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