ORIGINAL RESEARCH PAPER

Obstetrics & Gynaecology

TO STUDY SERUM MARKERS OF VITAMIN D AND CALCIUM IN PREGNANT WOMEN AND IN THE CORD BLOOD

KEY WORDS: Parathyroid hormone, Cord blood, hypovitaminosis D, sunlight, Dietary calcium.

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ABSTRACT

VDD in pregnant women and their children is a major health problem, with potential adverse consequences for overall health. Prevention strategies should ensure the Vitamin D sufficiency in women during pregnancy and lactation. Evidence based interventions to improve maternal and fetal nutrition, such as for Vitamin D, are accompanied by a decrease of the impact on the health of their children. This study also points towards the fact that the high level of newborn's hypovitaminosis D may be correctable by correction of maternal hypovitaminosis D. Vitamin D supplementation campaigns which should cover pregnant women and the newborn to prevent maternal and perinatal vitamin D deficiency should be implemented especially in high risk areas.

INTRODUCTION:

Vitamin D deficiency during pregnancy has important implications for the newborn and infant. Mild hypovitaminosis D might induce secondary hyperparathyroidism that results in resorption of bone.2 According to Islamic Ideas, females after puberty (1) are expected to cover themselves except their face and hand in public3 and development of urbanization results in poor outdoor activity. (2) Dietary calcium deficiency has caused secondary vitamin D deficiency and calcium replenishment improved serum 25(OH) vit D concentrations. (3) Hypovitaminosis D during pregnancy has important consequences for the newborn, including fetal hypovitaminosis D, neonatal rickets and tetany, and infantile rickets.(4-5) As few data on serum 25(OH)D concentration and the prevalence of Hypovitaminosis D among pregnant women from Indian are present (6)

Dietary vitamin D intake is important in individuals where endogenous synthesis of Vitamin D in the skin is inadequate (7) A typical Indian diet lacks rich sources of vitamin D like cod liver oil. There is no provision for Vitamin D fortification of food items in India. In addition, an Indian diet mainly consists of phytate rich cereals which possibly impairs vitamin D absorption through intestine. Thus sunlight remains practically the only source of vitamin D for most Indians. In a population that already has a high prevalence of Vitamin D deficiency and poor dietary calcium intake, the problem is likely to worsen during pregnancy because of the active transplacental transport of calcium to the developing fetus. Hypovitaminosis D during pregnancy has important consequences for the newborn, including fetal hypovitaminosis D, neonatal rickets, tetany, Infantile rickets (8) and enamel hypoplasia of primary dentition. Rickets during infancy has been associated with high prevalence of lower respiratory tract infections the largest cause of infant mortality in India.

This study was undertaken to determine the prevalence of maternal and fetal Hypovitaminosis D among Indian women. To study serum bone mineral markers of Vitamin D and calcium metabolism in pregnant women and in the cord blood., to study post partum 3rd day serum calcium and paratharmone in newborns and to study the effect of Vitamin D supplementation during third trimester of pregnancy on maternal and neonatal biochemical indices.

MATERIAL AND METHODS:

The women attending the antenatal clinic were explained about the presumed beneficial effects of Vitamin D supplementation during pregnancy. Healthy pregnant women at 28 weeks gestation with single viable pregnancy were recruited. Vitamin-D supplementation: The study group women were supplemented with Vitamin D 60,000 IU / Month at monthly intervals during the third trimester. The women were advised to come for regular antenatal follow up at 2 weeks interval. All the recruited antenatal women were given the mobile number and advised to contact at the time of admission in the labour ward. In the study group one baseline sample collection was done at the time of recruitment and analysed for all the analytes. Mother blood was collected before delivery after admission into labourward by peripheral venipuncture. One part of blood was collected in tubes on ice for intact parathoramone assay. Sera was separated in a refrigerated centrifuge and stored at -50OC in separate aliquots for subsequent analysis. At the time of delivery cord blood was collected. After the delivery of the baby, cord clamped and baby separated, 10 ml of cord blood was collected from placental end of the cord while placenta is still insitu. Sera was separated and divided into 2 aliquots. One was used immediately for assessment of alkaline phosphatase activity and the other was stored at -20OC for subsequent assessment of 25 (OH) D levels.

STATISTICAL ANALYSIS:

Data was analysed using SPSS package version 11.5 and comparison of means was done by students t-test and Wilcoxan rank – sum test for parametric and non – parametric data respectively.

RESULTS:

Of the 350 women recruited into the study group 290 women had regular follow up but not had taken 3 doses of vitamin D. 210 women had regular antenatal checkups and taken 3 dose of vitamin D complete data was available for 113 of 210 women. Among the 300 women recruited into the control group 190 women had complete data. Significant difference was observed between cases and control in age, duration of sun exposure, supplemental calcium in mg / day and weight at term in kg ($P<0.005$) and no significant difference was observed in no. of pregnancies and height in cm. (Table- 1).

Serum 25(OH)D level at term in subjects supplemented with vitamin D was higher (18.6317.011 ng/ml) ($P<0.5$) and the PTH value in the supplemental group (18.9612.006) was low compared to unsupplemented group (24.21412.635) ($P<0.01$). Serum 25(OH)D level in cases, women who received 3 sachets of vitamin D of 60,00 IU each in last trimester was not different from their 25(OH)D at the time of recruitment (p NS) where as PTH was high at the time of recruitment (23.896112.107) ($P<0.005$). Other words 60,000 IU of vitamin D monthly in last trimester was not sufficient to elevate vitamin D status of the subjects. However it still had same physiological effect in terms of lowering the serum PTH. (Table- 2).

Neonatal calcium levels among babies with normal 25(OH) D (>10ng/ml); In the vitamin D supplemented group 41 babies (38.31%) had calcium level less than normal and 66 babies had calcium level 8.5mg/dl (61.68%). In the supplemented group 32 babies (29.09%) had calcium below normal and 78 babies (70.9%) had 8.5mg/dl. Prevalence of neonatal hypocalcemia among neonates with normal cord blood 25(OH)D (>10mg/ml) in the supplemented group was 38.31% and in the unsupplemented group it was 28.82%. Neonatal cord blood 25(OH)D was > 10mg/dl in 72 babies (67.28%)born to unsupplemented subjects and 38 babies (35.51%) had < 10ng/dl. In the supplemented group 52 babies (48.59%) had 25(OH)D > 10ng/dl and 55 babies (51.4%) had < 10 ng/dl. If the serum 25(OH)D was < 30ng/ml it is considered as hypovitaminosis D. The serum 25(OH) D level in cases at the time of recruitment was high (18.9612.006) compared to 25(OH)D level at term (18.639.021). Controls despite having high sun exposure had lower S. Vitamin D levels than cases at term. Neonatal S. PTH was found to be high in controls as compared to the subjects again high lighting the prevention of vitamin D deficiency by maternal vitamin D supplementation. However neonatal S calcium was found to be lower in cases rather than controls. However none of the babies born to either cases or controls had neonatal hypocalcemia. Effects of vitamin D supplementation on the nutrition of the newborn was evident by the fact that cases (recruited subjects supplemented with vitamin D) gave birth to babies with higher birth weight than controls (P<0.001). The largest anterior fontanelle diameter was higher in the babies born to unsupplemented women. This again high lights the role of maternal vitamin D nutrition in the mineralization of newborn babies. The head circumference and mid upper arm circumference of the babies born to subjects supplemented with vitamin D was higher compared to unsupplemented subjects (P<0.001). But there was not much difference between crown heel length of babies born to vitamin D supplemented subjects and unsupplemented subjects (P = NS). (Table- 3).

Prevalence of hypovitaminosis D [25(OH)D) <30ng/dl]: In the supplemented subjects 82 (76.63%) women had 25(OH)D < 30 ng/dl. In the unsupplemented group 96 (87.27%) women had 25 (OH) D< 30 ng/dl. (P = 1.00 Not Significant). In the neonates 58 babies (54.2%) in the supplemented group and 82 babies (74.54%) in the unsupplemented group were considered to have hypovitaminosis D.

DISCUSSION:

We expected to find a higher serum 25(OH) D concentration in vitamin D3 supplemented women in our study. However the results were contrary to expectation with supplemented and unsupplemented women having equally low mean serum concentrations and equally high prevalence of the vitamin D deficiency. The explanation could lie in the prolonged deficiency of dietary calcium intake among poorer parts of India, because of the expensive nature of milk and milk products. Dietary calcium deficiency has been shown to lead to secondary vitamin D deficiency in rats (6). Similar findings are also suggested in studies on humans. The higher intake of dietary calcium in the women in our study is likely to have been short lived and attributable to the social custom of providing extra milk to pregnant and locating women (9-10).

In the present study high prevalence of vitamin D deficiency serum in pregnant women is probably due to occupation dress code and duration of exposure to sunlight of the rural subjects, who are agricultural labourers working for about 8 hrs a day in sunlight. In the region where the study was conducted season has little impact on cutaneous synthesis of vitamin D. We found a positive association between maternal serum 25(OH) D at term, birth weight of the babies, mid upper arm circumference and head circumference. Cord blood albumin and 25 (OH) D were higher in the supplemented group and cord blood 25 (OH) D level was raised in the supplemented group compared to un supplemented group. Cord blood 25 (OH) D strongly correlated with maternal values, which is in keeping with reports in the literature. The cutoff of 10 ng 25(OH) D/ml is used for defining hypovitaminosis D in newborns. The corresponding 25(OH) D value in mothers was 30 ng/ml. Accordingly 76.34% women in the supplemented group and 87.13% in the unsupplemented group was considered to have hypovitaminosis D(11-12). Like previous studies the present study found that levels of 25(OH)D appear to be higher in maternal than cord blood. In the cord blood the 25(OH) D level in the supplemented group was higher compared to unsupplemented group. The circulating levels of 25(OH) D in the fetus appear to be maintained by renal regulation as well as placental synthesis. Serum 25 Hydroxy D level in cases, women who received 3 sachets of vitamin D of 60,000 IU each in last trimester was not different from their 25(OH)D at the time of recruitment (P NS). In other words 60,000 IU of vitamin D monthly in last trimester was not sufficient to elevate the vitamin D status of the subjects. However it still had some physiological effect in terms of lowering the serum PTH.

On the basis of our results we conclude that such recommendations perhaps also warranted for pregnant Indian women so that they remain healthy and provide adequate vitamin D to their fetus. The exact cause of or factors contributing to the occurrence of hypovitaminosis D in women in a tropical country remains to be elucidated in future studies.

CONCLUSION:

Both groups were comparable with duration of sun exposure and daily supplement of calcium during pregnancy though the duration of sun exposure was high in the control group their serum 25(OH) D level at term was low compared to cases. The serum 25(OH) D level of cases at term was not much increased when compared to their base line. Neonatal serum PTH in control group was high compared to cases, suggesting same physiological role of vitamin D. Vitamin D supplementation in pregnant women during third trimester at monthly intervals given orally has some effect though still not sufficient to normalize the mineral metabolism of mother. There was an increase in birth weight, mid upper arm circumference and head circumference of the babies of vitamin D supplemented mothers.

Vitamin D deficiency among pregnant women is common, preventable and treatable. The ambiguities between the definitions of ViD status, combined with a lack of consistency in recommendations related to incorporation of routine testing of 25(OH)D levels in the prenatal period, especially in women with risk factors for VDD, dose and gestational age for the start of ViD supplementation, universal cutoffs for normal ViD values, lack of education about the benefits of ViD and the need for adequate sunlight exposure represent important barriers to the advance of the implementation of ViD supplemental guides, in order to improve this important health problem in pregnant women and their children in the short term.

Table .1:Shown MSD of base line characteristics of patients and controls.

Parameters	Cases	Controls	P Value
Age (years)	20.301.84	21.552.56	P<0.001
Duration of sun exposure for 3 months(minutes)	60.1279.03	159.3699.01	P<0.001
Supplemental calcium (mg/day)	1000.00	274.28110.01	P<0.001
No. of pregnancies	1.420.51	1.530.69	P=NS
Height (cm)	153.674.13	153.213.71	P=NS
Weight at term(kg)	56.326.01	54.322.18	P=NS < 0.005

Table .2:Shown MSD of vitamin D in serum and cord blood PTH and calcium levels of patients and controls

Parameters	Cases	Controls	P Value
Serum 25(OH)D at term	18.639.021	14.0367.011	<0.05
Cord blood 25(OH)D	12.056.732	6.1322.631	<0.001
Maternal plasma PTH at term	18.9612.006	23.89612.107	<0.01
Neonate S. calcium on day3	8.131.66	9.181.76	<0.005
Neonate S. PTH	31.23023.080	42.31034.008	<0.01

Table .3:shown M SD of birth weight and circumference of patients and controls.

Parameters	Cases	Controls	P Value
Birth weight of baby (kg)	3.000.26	2.830.20	< 0.001
Crown heel length of baby (cm)	50.011.24	50.111.38	NS
Largest Anterior Fontanelle diameter (cm)	1.1640.09	1.1970.07	<0.05
Head circumference (cm)	32.760.71	32.440.85	<0.05
Mid upper arm circumference (cm)	9.130.16	8.980.15	<0.001

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