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A hospital based study of prevalence of hypovitaminosis D among pregnant women & their new-borns attending govt. maternity hospital, Tirupati

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Abstract
Present study was designed to know the prevalence of hypovitaminosis D, and the effect of vitamin D supplementation during third trimester of pregnancy among pregnant women and their new-borns attending Govt. Maternity Hospital, Tirupati. Of the 382 women who were recruited for the study and received vitamin D supplementation, 107 women had complete data. Out of 214 women recruited for control group 110 women had complete data. Both groups are compared for serum 25(OH) D at term, cord blood 25 (OH)D maternal plasma PTH at term, Maternal calcium on day 3 and Anthropometric measurements of the new-borns. The main outcome measured was prevalence of hypovitaminosis D among pregnant women and their new-borns.

1. Both groups were comparable with age, no. of pregnancies, height and weight at term in kg.
2. Both groups were comparable with duration of sun exposure and daily supplement of calcium during pregnancy [1].
3. 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.
4. The serum 25(OH) D level of cases at term was not much increased when compared to their base line.
5. Neonatal serum PTH in control group was high compared to cases, suggesting some physiological role of vitamin D [1].
6. Birth weight, head circumference, and mid upper arm circumference was higher in babies of vitamin D supplemented group and anterior fontanelle diameter was greater in babies of unsupplemented group [2].
7. The prevalence of hypovitaminosis D among pregnant women in supplemented group was 76.63% and in unsupplemented group was 87.27% and in the new-borns it is 54.2% and 75.54 % respectively.

Keywords: Hypo-vitaminosis D, pregnancy, new-borns, 25(OH) D, Birth weight, head circumference. anterior fontanelle

Introduction

Aim: To conduct a study among pregnant women to determine the prevalence of hypo-vitaminosis D and the effect of Vitamin D supplementation during third trimester of pregnancy.

Objectives:

1. To study serum bone mineral markers of Vitamin D and calcium metabolism in pregnant women and in the cord blood.
2. To study post partum 3rd day serum calcium and paratharmone in new-borns.
3. To study the effect of Vitamin D supplementation during third trimester of pregnancy on maternal and neonatal biochemical indices [3].
4. To study the effect of vitamin D supplementation on anthrapometric measurements of new-borns of the subjects.

Subjects and Methods: The study was conducted in the government maternity Hospital, Tirupati and Sri Venkateswara Institute of Medical Sciences, Tirupati from March 2008 – August 2009 and 2016 to 2017.

Government Maternity Hospital, Tirupati is a tertiary care hospital. Sri Venkateswara Institute of Medical Sciences is a tertiary care centre,

Study Design: Open label intervention trial.

Subjects

1. The women attending the antenatal clinic were explained about the presumed beneficial effects of Vitamin D supplementation during pregnancy.
2. Healthy pregnant women at 24 – 28 weeks gestation with single viable pregnancy were recruited.
3. Exclusion Criteria: Evidence suggestive of chronic renal disease. Evidence suggestive of chronic liver disease [4].
4. 382 pregnant women attending the antenatal clinic with 26-28 weeks gestation with single viable pregnancy were recruited into the study group.
5. 214 women who are admitted to the labour ward directly were recruited into the control group.
6. Written and informed consent was obtained from all the subjects.
7. Detailed history and examination were performed with special regard to current and post pregnancies and labour socioeconomics status and clinical features suggestive of osteomalacia, [5] (eg : proximal muscle weakness, bone pain, tenderness of fractures) or past rickets.
8. Chronic systemic illness that can alter calcium metabolism [5].
9. All the recruited antenatal women were supplemented with 1000 mg of calcium lactate and advised to take daily till term [6].
10. Vitamin D 60,000 IU / Month at monthly intervals during the third trimester [7].

Sample Collection

1. In the study group one baseline sample collection was done
2. Mother blood was collected before delivery for parathoramone assay.
3. At the time of delivery 10 ml of cord blood was collected. neonates.
4. Anthrapometric measurements of the newborn was taken with in 6 hrs after delivery for birth weight, crown heel length, Head Circumference, mid upper arm circumference and largest diameter of anterior fontanelle.
5. The women were kept in the hospital till 3rd day and their neonates 3rd post partum day blood sample was collected for serum paratharmone and the other for serum calcium and albumin.

Calcium

Corrected calcium = observed calcium + [0.8(4-albumin(g/dl)]

(mg/dl) (mg/dl)

Serum 25 (OH) Vitamin D

Serum 25 (OH) D was assayed using a two step procedure, with use of Diasorin I 125 RIA kit (Still water, USA). First step involves a rapid extraction of 25 (OH) D and other hydroxylated metabolites from serum with acetonitrite. Following extraction the assay is performed using an equilibrium RIA procedure.

PTH: Serum PTH assay was done in samples of all subjects and some newborns using standard IRMA kit. This assay is a two site immuno radiometric assay assessing only intact PTH molecule by using two different polyclonal antibodies directed to non – overlapping N- terminal PTH fragments.

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. analysis was done to determine

1. Percentage of women who were supplemented with vitamin D and those were not supplemented, with elevated heat labile SAP (>90 IU/L)
2. Percentage of Women who were supplemented with Vitamin D and those were not supplemented with low 25 (OH) D (<30 ng/ml). 3. Cord blood 25(OH) D levels in neonates born to both supplemented and unsupplemented subjects. 4. Neonatal serum calcium and PTH levels in neonates born to both supplemented and unsupplemented subjects. 5. Prevalence of neonatal hypocalcemia and hypovitaminosis D.

Comparison of Means Was Done In the Following Groups

1. Serum 25(OH)D and PTH before and after supplementation in the supplemented subjects.
2. Serum 25 (OH)D and PTH at term, among the supplemented and the unsupplemented women.
3. Neonatal calcium levels among newborns with
   a) Normal 25 (OH) D (> 10ng/ml) and
   b) Low 25(OH) D (<10ng/ml) levels.
4. Neonatal PTH and cord blood 25 (OH) D in neonates with and without hypocalcemia.
5. Birth weight, crown heel length and head circumference of newborns among subjects with
   a) Low serum 25(OH) D and
   b) Normal serum 25 (OH) D levels

Prevalence Was Compared In the Following Groups

Prevalence of hypovitminosis D (25(OH)D <30ng/ml) a) Supplemented subjects and b) Unsupplemented subjects

Correlations Was Done Among Following Parameters

	Cases	Controls	P Value
Serum 25(OH)D at term	18.971±9.035	15.038±7.083	<0.05
Cord blood 25(OH)D	12.391±6.883	6.850±2.908	<0.001
Maternal plasma PTH at term	19.531±12.208	24.214±12.635	<0.01
Neonate S. calcium on day3	8.92±1.71	9.67±1.8	<0.005
Neonate S. PTH	31.950±23.145	42.790±34.173	<0.01
Parameter 1	Parameter 2	R value	P value
Maternal serum 25 (OH) D	Cord Blood 25 (OH) D	0.48	<0.001

Sun exposure in minutes for 3 months	25 (OH) D levels at Term	0.222	<0.05
Maternal plasma PTH at base line	Maternal serum 25 (OH) D at base line	-0.155	NS
Maternal Plasma PTH at term	Maternal serum 25 (OH) D at aterm	-0.328	<0.001
Cord blood 25 (OH)D	Neonatal PTH	-0.108	NS
Cord blood 25 (OH)D	Neonatal serum calcium	-0.06	NS
Neonatal PTH	Neonatal serum calcium	0.05	NS

Results: Of the 382 women recruited into the study group 296 women had regular follow up but not had taken 3 doses of vitamin D. 184 women had regular antenatal check-ups and taken 3 doses of vitamin D complete data was available for 107 of 184 women. Among the 214 women recruited into the control group 110 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. Serum 25(OH)D level at term in subjects supplemented with vitamin D was higher (18.971±7.083 ng/ml) (P<0.5) and the PTH value in the supplemental group (19.531±12.208) was low compared to unsupplemented group (24.214±12.635) (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 (24.2411±12.208) (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. 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 (19.53±12.208) compared to 25(OH)D level at term (18.97±9.035). 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 [8]. However none of the babies born to either cases or controls had neonatal hypocalcaemia. Effects of vitamin D supplementation on the nutrition of the new-born was evident by the fact that cases (recruited subjects supplemented with vitamin D) gave birth to babies with higher birth weight than controls (3.000±0.264 Vs 2.834±0.202) (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). 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: The most important finding in our study is unexpectedly high prevalence of hypovitaminosis D among pregnant women [9]. Hypovitmainosis D and osteomalacia among pregnant South Asian women have been widely reported [1]. We expected to find a higher serum 25(OH)D concentration in vitamin D_3 supplemented women in our study [10]. 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. 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 cut off 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 76.63% women in the supplemented group and 87.27% in the unsupplemented group was considered to have hypovitaminosis D. 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.

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. Unexpectedly we found a positive correlation between mid upper arm circumference, largest anterior fontanelle diameter and cord blood 25(OH)D, maternal 25(OH)D and PTH. We have shown that low maternal 25 (OH)D in case pregnancy is associated with increased anterior fontanelle diameter decreased mid upper arm circumference. Vitamin D deficiency among pregnant woman is preventable and treatable but it is increasingly reported from around the world, especially among women

with dark skin living at higher latitudes and among women who rarely expose their skin to sunlight.

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.

Conclusion: 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.

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