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Original Article

Is 400 IU per day of Vitamin-D given to healthy well-nourished mothers antenatally enough to prevent neonatal Vitamin-D deficiency?

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ABSTRACT

Background: The incidence of Vitamin D deficiency (VDD) among newborns is very high in India, this is usually secondary to maternal deficiency. Currently Vitamin D at the dose of 400–600 IU/day is being prescribed during pregnancy for prevention. There is paucity of data regarding efficacy of current recommendations in preventing neonatal hypovitaminosis D.

Our aim was to determine if good nutritional status of the mother and regular calcium and Vitamin D supplementation during pregnancy in the currently recommended doses help prevent or reduce VDD in neonates.

Methods: Cord blood Serum 25(OH)D levels were studied in 342 neonates born to 323 healthy mothers at a tertiary care center in Mumbai. All the mothers were on regular antenatal follow up and received Vitamin D and calcium supplements as per current recommendations.

Results: Mean umbilical cord blood 25(OH)D level was 12.088 ng/ml (SD 3.349, range 31.1–5.1). Out of 342 neonates, 335 (97.95%) had Vitamin D deficiency [cord blood 25(OH)D level <20 ng/ml] 1.75% had cord blood 25(OH)D level between 21 and 29 ng/ml in the insufficiency range and only 0.29% of the subjects had 'normal' cord blood 25(OH)D levels of >30 ng/ml. However, none of the neonates in the study were found to have severe VDD (<5 ng/ml).

Conclusion: Antenatal supplementation of well nourished, socioeconomically better off mothers in our population with 400 IU/day of Vitamin D3 is ineffective in preventing VDD in neonates.

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Introduction

Vitamin D is a fat soluble vitamin which plays an essential role in calcium and phosphorus homeostasis and skeletal growth. One of the most surprising revelations in recent decades was that deficiency of Vitamin D is very widespread.¹ More surprising is the fact that though the vitamin can be photosynthesized by humans, deficiency is widespread even in tropical countries where there is abundant sunlight.² Some Indian studies have reported a prevalence of 80–90% deficiency, cutting across ethnic, socioeconomic, gender and age barriers.³

Women are more prone to Vitamin D deficiency (VDD), and this tends to intensify during pregnancy.⁴ VDD during pregnancy is associated with elevated risk of preeclampsia, increased cesarean sections, intra uterine growth retardation, premature labor and gestational diabetes, all of which impact the fetal outcome adversely.⁵ The developing fetus is completely dependent on the mother for vitamin D so a newborn, who inherits VDD from a deficient mother, is at risk of hypocalcemia, seizures, infantile rickets, wheeze associated illness, diabetes and possible autism in early life.^{6–8}

Studies from the Indian subcontinent have documented VDD in cord blood of neonates.^{9–11} Most of the subjects in these studies were from lower socio-economic groups, where poor nutrition and poor antenatal calcium and Vitamin D supplementation in the mothers may have confounded the study outcomes. Very few studies have addressed the prevalence of VDD in neonates born to healthy well-nourished Indian mothers who received calcium and Vitamin D in recommended doses antenatally. Given the lack of literature in this aspect, it is important to know if good nutritional status of the mother and regular calcium and Vitamin D supplementation during pregnancy in the currently recommended doses help prevent or reduce VDD and this study attempts to provide such data.

Material and methods

This study was conducted between July 2014 and June 2015 at a tertiary care teaching hospital located in Mumbai (latitude 19.07° North) and study subjects were neonates born to pregnant women between the ages of 18 and 45 years. The hospital caters to the families from all parts of the country, belonging to the middle socioeconomic group and residing in adjoining urban areas. All mothers were literate; none of them followed purdah, hijab etc. which would have affected biosynthesis of Vitamin D. Approval of the Institutional Ethics Committee was obtained.

Detailed maternal history was obtained and examination performed. Maternal exclusion criteria were underweight or overweight as per standard charts, chronic renal or liver disease, treatment with antiepileptic drugs or any chronic medication, anti-tubercular treatment any time during pregnancy. Mothers absent from Mumbai for more than eight weeks during the present pregnancy were also excluded. All mothers were on regular antenatal follow up and received supplementation with Calcium 1000 mg and Vitamin D3 400 IU

per day from second trimester till delivery. This was in keeping with the Institute of Medicine of National Academies, US (IOM) guidelines.¹² Since commercial preparations have 200 IU per tablet, 400 IU instead of the recommended 600 IU was given.

All infants including those born of multiple gestations were included and were examined at birth. Infants with obvious congenital anomalies and severe birth asphyxia were excluded from the study. Gestational age in weeks at the time of birth based on best obstetrical estimate using the date of last menstrual period and confirmed by clinical assessment using New Ballard's Scores if in doubt. Birth weight, crown-heel length, and head circumference recorded according to standard practices. Detailed head to toe and systemic examination was carried out. Cord blood from the placental end was collected for each neonate after clamping the cord and serum was stored at –80 °C until testing. The 25(OH)D levels were estimated on a Stratec SR300, RIA analyzer (STRATEC Biomedical Systems AG, Germany), using 25(OH)D Iodine-125 RIA kit following manufacturer's instructions after proper standardization. The cut-off levels for Vitamin D deficiency, insufficiency and sufficiency were <20 ng/ml, 21–29 ng/ml and >30 ng/ml respectively, as per Endocrine Society's Practice Guidelines of 2011.¹³

Analysis of data was done using Statistical Package for Social Sciences software (SPSS; version 20). Continuous variables were analyzed using Student's *t* test. Proportions were compared using Chi-Square test. Correlations were studied using Spearman's Correlation coefficient. A *p* value of <0.05 was taken to be statistically significant and all *p* values were two-tailed.

Results

A total of 323 pregnant women were eligible and enrolled for the study. Including 19 pairs of twins, a total of 342 newborns were delivered. Half the neonates were delivered vaginally (*n* = 171); rest by Cesarean section. Twenty-eight (8.18%) neonates were born following in vitro fertilization, the remaining 314 were conceived spontaneously. There were 184 (53.8%) males and 158 females, 279 (81.58%) were term neonates and 63 preterm; 273 (79.82%) had normal birth weight (NBW) while 69 were low birth weight (LBW).

Mean umbilical cord blood 25(OH)D level was 12.088 ng/ml (SD 3.349, range 31.1–5.1). Out of 342 neonates, 335 (97.95%) had VDD [cord blood 25(OH)D level <20 ng/ml.] 1.75% had cord blood 25(OH)D level between 21–29 ng/ml in the insufficiency range and only 0.29% of the subjects had 'normal' cord blood 25(OH)D levels of >30 ng/ml.

Full term infants had a cord blood 25(OH)D level of 12.075 ng/ml (SD 3.468, range 31.1–5.1 ng/ml), 97.4% were Vitamin D deficient. Preterm infants had a mean cord blood 25(OH)D level of 12.146 ng/ml (SD 2.793, range 19.1–7.9), all preterm infants had VDD. However, this difference in cord blood 25(OH)D levels between full term and preterm infants was not statistically significant (*p* = 0.8628).

The mean cord blood 25(OH)D levels of Low Birth Weight infants (<2.5 kg) was found to be less than those with normal birth weight (2.5–4 kg), 11.855 ng/ml (SD 2.908, range 19.1–7.6 ng/ml) former versus 12.147 ng/ml (SD 3.455, range 31.5–5.1 ng/ml)

Table 1 – Umbilical Cord Blood 25(OH)D levels by maternal variables.

Maternal characteristic's	(n)	Cord Blood 25(OH)D levels (ng/ml)	p value (for paired values)
Maternal age			
18–30 years	286	12.016 (3.203, 26.9–5.1)	$p = 0.4422$
>30 years	56	12.457 (4.028, 31.1–7.6)	
Gravida			
Primigravida	140	12.183 (3.27, 26.9–7.6)	$p = 0.662$
Multigravida	202	12.023 (3.41, 31.1–5.1)	
Mode of delivery			
Spontaneous	171	12.217 (3.828, 31.1–5.1)	$p = 0.4787$
LSCS	171	11.959 (2.425, 23.4–7.1)	
Mode of conception			
Spontaneous	314	12.175 (3.409, 31.1–5.1)	$p = 0.04125$
IVF – ET	28	11.118 (2.425, 16.3–7.9)	

Table 2 – Cord Blood Serum 25(OH)D levels by neonatal variables.

Neonatal characteristic's	(n)	Cord Blood 25(OH)D levels (ng/ml)	p value (for paired values)
All	342	12.088 (3.349, 31.1–5.1)	
Sex			
Male	184	11.923 (2.877, 21.1–7.0)	$p = 0.337$
Female	158	12.28 (3.828, 31.1–5.1)	
Period of gestation			
Term	279	12.075 (3.468, 31.1–5.1)	$p = 0.8628$
Preterm	63	12.146 (2.793, 19.1–7.9)	
Birth weight			
NBW	273	12.123 (3.489, 31.1–5.1)	$p = 0.475$
LBW	69	11.855 (2.908, 19.1–7.6)	
Singleton/twins			
Singleton	304	12.174 (3.432, 31.1–5.1)	$p = 0.0978$
Twins	38	11.405 (2.538, 16.9–7.9)	

in the latter. However this difference too, was not statistically significant.

There was no significant difference in the mean cord blood 25(OH)D levels by sex or mode of delivery, in singleton versus twins, or between infants born to mothers of different parities. Maternal age too, did not significantly affect neonatal cord 25(OH)D levels.

The mean cord blood 25(OH)D level of infants conceived by IVF were found to be significantly lower than those conceived spontaneously ($p = 0.040125$). The mean cord blood 25(OH)D level of IVF conceived infants was 11.118 ng/ml (SD 2.425, range 16.3–7.9 ng/ml) while in the latter it was 12.174 ng/ml (SD 3.409, range 31.1–5.1 ng/ml).

The differences in cord blood levels of 25(OH)D by maternal variables is depicted in Table 1 while the same by neonatal variables is shown in Table 2.

Discussion

This study looks at the cord blood levels of Vitamin D in neonates born to healthy mothers who had received calcium and Vitamin D supplementation during pregnancy. The mean cord blood Vitamin D level in this study was found to be 12.088 ng/ml (SD 3.349, range 31.1–5.1) with 97.75% of the studied neonates having levels in the deficient range. However, none of the neonates in the study were found to have severe VDD (<5 ng/ml).

Sachan et al. in a study done in 2002 with 207 subjects had reported a mean cord blood 25(OH)D level of 8.4 ng/ml (SD 5.7), which is much lower than that found in this study. The subjects of Sachan's study were predominantly from the lower socioeconomic class with poor maternal nutrition and no history of antenatal calcium and Vitamin D supplementation.⁹

In two recent studies of 2015, Naik et al. reported a mean cord blood 25(OH)D level of 11.36 ng/ml (SD 4.75, range 3.9–24.9 ng/ml) from a sample size of 50¹⁰ and Kumar et al. reported a mean cord blood 25(OH)D level of 12.8 ng/ml (SD 8.5) from 106 samples of cord blood.¹¹ The nutritional status of the mothers or history of antenatal Vitamin D supplementation was not commented upon in either of these studies. Since maternal VDD can result in fetal VDD, vital maternal data from these two studies is lacking. Moreover, each of these two studies had much smaller number of neonates unlike the present study.

Nandal et al. in their study published in 2016, reported a mean cord blood 25(OH)D of 11.35 ng/ml in infants born to 60 unsupplemented mothers.¹⁴ Sablok et al. in a study done in 2012 at Delhi reported a cord blood 25(OH)D levels of 43.11 ± 81.32 nmol/L (17.2 ± 32.4 ng/ml) in neonates born to mothers who did not receive any supplements.¹⁵ This study, unlike these studies, included only healthy mothers who received antenatal calcium and Vitamin D supplementation.

The comparison of the neonatal cord blood values of Vitamin D in this study with previous studies, where pregnant

mothers did not receive calcium and Vitamin D supplements reveals only marginal differences in mean cord blood 25(OH)D levels and nearly identical prevalence of VDD. The selection of healthy mothers who received calcium and Vitamin D supplements did not influence the cord blood levels of Vitamin D in neonates except to offer some protection against severe VDD. One possible reason could be in the Vitamin D status of the mothers antenatally – a variable which this study did not check for.

During pregnancy there is many fold increase in the 1,25(OH)₂D primarily due to production from the placenta and the maternal kidney. This enhances calcium absorption from the gut to cater to the skeletal development of the growing fetus. To ensure adequate levels of 1,25(OH)₂D in the pregnant mother it is recommended that the level of 25(OH)D is maintained above 30 ng/ml. The RDA for Vitamin D advised by Endocrinological Society Guidelines to maintain 25(OH)D at these levels during pregnancy is 1500–2000 IU/day.¹³ However an earlier IOM recommendation which came out in 2011 recommends a RDA for Vitamin D in the range of 400–600 IU/day in pregnant women without VDD.¹² Curiously, the World Health Organization recommendations of 2012 do not recommend routine Vitamin D supplementation in pregnancy, and only 200 IU/day in case of VDD which is clearly contrary to other recommendations.¹⁶ The RDA for Vitamin D recommended by ICMR is 400 IU/day for all categories of patients.¹⁷ A check of commercially available calcium and Vitamin D preparations in the Indian market show most preparations to contain between 100 and 200 IU of Vitamin D per 500 mg of calcium. In actual practice, this translates to an intake of 500–1000 mg Calcium and 200–400 IU Vitamin D3 per day with current antenatal supplementation protocols in our country.¹⁸

Most studies from South Asia report a very high incidence (70–100%) of VDD in women of child bearing age.³ It follows that a large majority of women are Vitamin D deficient right at the beginning of pregnancy. The recommended dose of Vitamin D3 for treatment of VDD ranges from daily dose of 4000–10,000 IU per day or 60,000 IU weekly for 6–8 weeks, in addition to RDA. Which implies, to achieve an acceptable level of 25(OH)D to prevent neonatal VDD, these women need to be exhibited high doses of Vitamin D3. In this scenario, clearly the 200–400 IU/day which they presently receive is grossly inadequate to treat VDD in pregnant mothers. This could be the reason why most neonates in this study were found to be having VDD in spite of their mothers receiving antenatal calcium and Vitamin D3 supplementation as per the current recommendations.

Treating VDD in pregnant mothers would require Vitamin D assay in early pregnancy, an option which is costly and not easily available. Exhibiting high doses of Vitamin D to presumptively treat VDD would carry the risk of Hypervitaminosis D which is unacceptable. At this juncture, it is important to note that both IOM as well as Endocrinological Society Guidelines state that the safe upper limit of RDA for Vitamin D in pregnancy and lactation is 4000 IU/day.^{12,13} Based on these recommendations its prudent to assume that a daily supplementation of Vitamin D at the dose of 2000–4000 IU/day will be safe in pregnancy. Though at these doses of antenatal supplementation, it is unlikely that VDD in neonates will be totally eliminated, is likely to substantially decrease neonatal VDD.

In a randomized controlled trial from Unites States of America, Hollis et al. in 350 subjects exhibited 400, 2000 and 4000 IU of Vitamin D to three equally sized maternal groups from 12 to 16 weeks of gestation till birth. Mean neonatal 25(OH)D levels were significantly different by treatment group: 45.5 ± 25.3 nmol/L (18.2 ± 10.1 ng/ml) in control group, 57.0 ± 24.5 nmol/L (22.8 ± 9.8 ng/ml) in 2000 IU group and 66.3 ± 25.8 nmol/L (26.5 ± 10.3 ng/ml) in 4000 IU group. What is notable is there was not even a single instance of hypervitaminosis.¹⁹ In an Indian RCT done in 2016 with a sample size of 87, Mir et al. exhibited antenatal Vitamin D3 to two groups of pregnant women. The first group received 1000 IU/day or 30,000 IU/month and the second group received 2000 IU/day or 60,000 IU per month. The post-supplementation levels of 25(OH)D were higher in the second group (42.86 ± 12.83 ng/ml) than in the first group (36.96 ± 10.56 ng/ml). No major adverse effects were noted following supplementation in these doses.²⁰ In an Iranian RCT carried out in 2015 Mojibian et al. divided 500 pregnant women into two groups: the first group received 400 IU/day and the second group got 50,000 IU every two weeks. The mean cord blood level of 25(OH)D of neonates born to mothers in the second group was significantly higher than neonates born to mothers in the first group (37.9 ± 18 ng/ml versus 29.7 ± 19 ng/ml, respectively). No adverse effects were reported.²¹

Both the IOM as well as Endocrinological Society Guidelines recommend antenatal supplementation at higher doses with 4000 IU/day being the safe upper limit for administering Vitamin D3 during pregnancy and lactation.^{12,13} There are many studies demonstrating the safety and efficacy of antenatal supplementation with Vitamin D3 in the doses ranging from 2000 to 4000 IU per day to 50,000 IU every two to four weeks starting from 12 to 16 weeks till birth.^{19–21}

There is an urgent need to review the recommendations for antenatal supplementation with Vitamin D in our country to reduce VDD in neonates. To select the doses of antenatal supplementation most suitable for our population, a larger study that looks at baseline maternal Vitamin D levels, evaluates the effect of antenatal Vitamin D supplementation at different doses on prevalence of VDD in neonates born to such mothers is an urgent necessity.

The strengths of this study include the large sample size and the fact that variations in maternal Vitamin D levels due to socio-economic and nutritional differences at baseline were eliminated. The drawback of this study include the fact that maternal Vitamin D levels were not estimated and it was presumed that in the absence of malnutrition and with recommended antenatal calcium and Vitamin D supplementation, VDD in neonates would be significantly reduced, if not eliminated totally.

In conclusion, this study brings out the fact that antenatal supplementation of well nourished, socio-economically better off mothers in our population with 400 IU/day of Vitamin D3 is ineffective in preventing VDD in neonates.

Conflicts of interest

The authors have none to declare.

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