Oral Supplementation of Parturient Mothers with Vitamin D and Its Effect on 25OHD Status of Exclusively Breastfed Infants at 6 Months of Age: A Double-Blind Randomized Placebo Controlled Trial

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Abstract

Background: Exclusively breastfed infants are at increased risk of vitamin D deficiency and many lactating mothers have been found deficient in 25OHD stores.

Objective: To compare serum vitamin D levels in exclusively breastfed infants at 6 months of age with or without oral supplementation of 600,000 IU of vitamin D3 to mothers in early postpartum period.

Methods: Exclusively breastfeeding term parturient mothers were randomized 24–48 hours following delivery to receive either 600,000 IU of vitamin D3 (Cholecalciferol) over 10 days in a dose of 60,000 IU/day or placebo. 25OHD levels were measured by Radio Immuno Assay method at recruitment and after 6 months in all mothers and their infants. Urinary calcium and creatinine ratio was measured to monitor adverse effects of vitamin D3 in both mothers and infants at 14 weeks and 6 months of age. X-ray of both wrists in anteroposterior view and serum alkaline phosphatase of infants were done in both groups at 6 months of age to look for evidence of rickets.

Results: Maternal profile was similar in intervention (A) and control (B) groups. Mothers' serum 25OHD levels at recruitment were also similar being 16.2 ± 9.3 ng/mL in group A and 14.1 ± 7.1 ng/mL in group B. After 6 months, 25OHD levels were 40.3 ± 21.6 and 22.9 ± 20.1 ng/mL in group A and group B mothers ($p \leq 0.00$), respectively. The serum 25OHD levels in cord blood were 9.9 ± 5.7 and 8.9 ± 5.1 ng/mL, respectively, in infants born to mothers in intervention and control groups ($p = 0.433$). At 6 months of age, the serum 25OHD levels significantly ($p < 0.00$) raised to 29.1 ± 14.6 ng/mL in infants of group A compared to those of group B (15.7 ± 17.7 ng/mL). Four infants developed radiological rickets at 6 months of age, two infants each in intervention group and study group. As against 10 infants in the control group (16.94%), no infant in the study group had biochemical rickets. Urinary calcium and creatinine ratio in mothers and infants at 14 weeks and 6 months of age in both intervention and study group was within normal limits, indicating there was no adverse effects of oral administration of 600,000 IU of vitamin D3.

Conclusion: Serum 25OHD levels of exclusively breastfed infants significantly rise at 6 months of age when their mothers are orally supplemented with 60,000 IU of vitamin D3 daily for 10 days in the early postpartum period in comparison to infants of vitamin D3 unsupplemented mothers.

Keywords: infants, 25OHD, exclusive breastfeeding, rickets, postpartum period

Introduction

E XCLUSIVE BREASTFEEDING IS recommended up to 6 months of age for optimal growth and development, and beneficial effects on child survival. Doubts, however, have been raised and infallibility of breastfeeding as a perfect natural food for the infant has been questioned, based on naive scientific interpretations, or sometimes due to commercial interests of the infant milk substitute manufacturers. Vitamin D is one such micronutrient that has been claimed to be deficient in exclusively breastfed infants and recommendations have been made to supplement vitamin D to

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all newborn infants since birth.[1] World Health Organization, however, has no such guidelines for vitamin D supplementation for exclusively breastfed infants.

It has been estimated that breast milk of a vitamin D-replete mother contains between 20 and 60 IU/L of vitamin D, which may not be sufficient for a growing infant solely on breastfeeding.[2–4,5] Several studies from developed and developing countries have reported vitamin D deficiency in large number of pregnant and lactating mothers.[6–10] Aggarwal and colleagues from our institution found that 70% lactating women were deficient in vitamin D at 10 weeks postpartum and 44% of their infants had severe vitamin D deficiency (25OHD ≤11 ng/mL) at 6 months of age.[8] Rothberg et al. after supplementing mothers with vitamin D during lactation have reported that maternal vitamin D intake directly affected the vitamin D concentration in the breast milk and 25OHD status of the infant.[7] Wagner and colleagues could also establish that vitamin D3 supplementation of mothers with 6400 IU/day significantly improved the 25OHD status in both the lactating women and their breastfeeding infants, and the effect was comparable to oral vitamin D3 supplementation of 300 IU/day to infants.[11]

This study was conducted to compare serum 25OHD levels in infants and mothers, and incidence of rickets in infants at 6 months of age with or without oral supplementation of 6,00,000 IU vitamin D3 to mothers in the early postpartum period.

Materials and Methods

This randomized double-blind placebo-controlled trial was carried out from March 2013 to April 2014. The study protocol was approved by the Institutional Ethics Committee-Human Research (IEC-HR). The study was registered with the Indian Council of Medical Research registry trial. The number of subjects was based on a study entitled ''Vitamin D status of term exclusively breastfed infants and their mothers from India.''[8] According to the study 44.33% of exclusively breastfed infants were found to have 25OHD levels <11 ng/mL at 6 months of age. Assuming that 50% of exclusively breastfed infants (22%) will have serum 25OHD levels <11 ng/mL at 6 months of age if their mothers are supplemented with 600,000 IU of vitamin D in the early postpartum period and considering power of study as 80% and α error of 5%, then 56 mother-infant pairs each in the intervention and control group would achieve the desired objective of comparing serum 25OHD levels in infants at 6 months of age. Assuming an attrition of 15% mother-infant pairs over a follow-up period of 6 months, 65 mother-infant pairs were recruited in each group.

All consecutive mothers, with spontaneous term (37–41 completed weeks) healthy pregnancy and single live fetus, admitted in the labor, were approached and purpose of the study was explained. Mothers delivering term appropriate-for-gestational-age (AGA) infant and willing for exclusive breastfeeding and regular follow-up were recruited after obtaining written informed consent. Mothers who have received vitamin D within last 3 months were excluded. However, routine supplementation of tablet calcium (elemental calcium 500 mg; vitamin D3 125 IU) according to postnatal protocol was continued. An infant was designated as AGA if birth weight was between 10th and 90th centile for the gestational age.[12] As a hospital policy, breastfeeding was initiated within 1 hour after normal delivery and within 2 hours following lower section cesarean section. No prelacteal feeds were given by any mother. Infants were thoroughly examined and gestational age was determined by Naegli's rule[12] and Modified Ballard' Scoring.[13] If there was disparity of more than 2 weeks, gestational age estimated by later method was taken into account. Infants born with congenital malformations, suspicion of chromosomal anomalies and endocrine disorders, or suffered from perinatal asphyxia, hypocalcemia, hypoglycemia, respiratory distress, or sepsis in the neonatal period were excluded. All mothers were counseled to practice exclusive breastfeeding by a trained person; however, predominantly breastfed infants were also included in the study if mothers occasionally gave few sips of water or juice on follow-up; it was assumed that occasional sips of water or fruit juice will not affect 25OHD levels. None of the infant was excluded on account of mixed feeding.

Randomization and blinding

Block randomization of mother-infant pairs was done into two groups (13 blocks of 10 patients each) by computer-generated randomization table to receive either drug (Cap Vitanova® containing 60,000 IU vitamin D3) or placebo, which was an inert sugar. The drug and placebo were coded in the randomization sequence as A or B and numbered 1 to 130 by a third person not directly involved in the study. The key was kept by the same person. The allocation of the drug or placebo was done by opaque envelop concealment technique. The key was opened in two steps. First, sequence A or B was provided for serial numbers. After the analysis was completed by the biostatistician, the key was decoded as ''A'' for drug and ''B'' for placebo.

Administration of drug or placebo. Ten capsules containing either vitamin D3 (vitanova) or placebo were given to each mother on the day of parturition. She was advised to take one capsule daily in the morning after breakfast with water for 10 days. In the hospital, mothers ingested capsules under supervision. On discharge, the mother was counseled to take the capsule regularly and report to follow-up after 1 week with the empty blister strip. All mothers consumed capsules containing vitamin D3 or a placebo as advised. A note sheet was given to each mother to record sunlight exposure after explaining how much body part to be exposed to sunlight and to bring the note sheet at each follow-up.

Follow-up

Each mother and baby was followed up at 6 (+1), 10 (+1), and 14 (+1) weeks and 6 months (+2 weeks) of age. At each visit, the mother was counseled to practice exclusive breastfeeding. Duration of sunlight exposure was inquired and recorded. Anthropometry of the infants, including weight, length, head circumference, chest circumference, upper and lower segment ratio, and area of the anterior and posterior fontanels, was measured as per standard methods. Infants were immunized as per National Immunization Schedule at each visit. Development assessment, including motor milestones, and language was done at 6 months and mothers were counseled to initiate complementary feeding along with breastfeeding. Mothers were asked to bring urine

samples of their own and their infants in a clean container at 14 weeks and 6 months of follow-up.

Investigations

Two milliliters of maternal and cord blood was collected in a plain vial at recruitment and at 6 months of age for estimation of 25OHD. Serum was separated by centrifugation and stored at −20°C. One milliliter blood of the mother and infant was collected at 6 months in a separate vial for estimation of serum calcium, phosphate, and alkaline phosphatase levels.

Urinary calcium and creatinine ratio was done in the urine samples of the mother and infant at 14 weeks and 6 months. At 6 months of age, digital X-ray anteroposterior view of both the wrist joints of the infant was taken to look for evidence of radiological rickets.

25OHD levels were measured by Radio Immuno Assay (using Diasorin kits). Hypovitaminosis D was defined as Serum 25OHD level <20 ng/mL.[14] Severe vitamin D deficiency was qualified when serum 25OHD level was <11 ng/mL.[14] Serum and urine calcium levels were estimated by ortho-cresolphthalein complex method, serum phosphate by ammonium molybdate+sulfuric acid phosphorus phosphomolybdic complex method, and alkaline phosphatase by kinetic method recommended by International Federation of Clinical Chemistry (IFCC).[15] Urine creatinine was done by Jaffe's kinetic method.[16] Urinary calcium and creatinine ratio <2 was taken normal.[17] Infant was diagnosed as having biochemical rickets if serum alkaline phosphate level was >420 IU[17] (Fig. 1).

Statistics

The data were analyzed by Software SPSS 20. Paired Student's t test was done to compare vitamin D levels within the group and unpaired student's t test was employed to compare intergroup 25OHD levels at birth and after 6 months. Qualitative data were compared by Chi-square test. Multivariable logistic regression was used to find relationship between maternal and infant serum 25OHD levels at birth and after vitamin D3 supplementation after controlling potential confounders.

Results

Maternal characteristics

The mean age of the mothers in the study and control groups was 24.32 ± 2.65 and 24.51 ± 3.56 years, respectively ($p = 0.769$). The parity, weight, height, and body mass index (BMI) of mothers belonging to two groups were also comparable. The dietary and dressing habits of mothers were similar in two groups. The mode of delivery, socioeconomic status, and education of mothers in study and control groups were similar. The average daily time spent under the sunlight by lactating mothers was 32.7 ± 8.6 min in the study group and was comparable to average time of 31.5 ± 6.6 min spent in sunlight by mothers in the control group (Table 1).

Infant characteristics

The mean birth weight of the babies in study (2.95 ± 0.35 kg) and control groups (2.95 ± 0.28 kg) was similar ($p = 0.135$). The male ($n = 35$ versus 31) and female ($n = 21$ versus 28) ratio was 1.66:1 and 1.11:1 in the study and control group, respectively. There was no difference in the weight gain till 6 months of age in both the groups. The size of the anterior fontanel was similar in two groups at all times.

Serum vitamin D levels of mothers and infants at baseline

The serum 25OHD levels in the cord blood were 9.9 ± 5.7 ng/mL (interquartile range 4.9–13.3 ng/mL) in study group and 8.9 ± 5.1 ng/mL (interquartile range 5.3–12.1 ng/mL) in the control group and were comparable ($p = 0.433$). There was large variation in the cord blood 25OHD levels in both groups, but median values (10.0 and 7.8 ng/mL) were nearer to respective mean values and were comparable. The cord blood 25OHD levels were 61.1% and 62.9% of their mothers' 25OHD levels, respectively, in the study and control groups. Overall, 96.4% ($n = 106$) infants had vitamin D deficiency (25OHD <20 ng/mL) at birth comprising 94.33% ($n = 50$) infants in the study group and 98.24% ($n = 56$) infants in the control group ($p = 0.350$); among them, severe hypovitaminosis D was present in 62.26% ($n = 33$) and 71.92% ($n = 41$) infants in the study and control group ($p = 0.314$), respectively.

The mean serum 25OHD levels of mothers in the study and control groups at recruitment were 16.2 ± 9.3 ng/mL (interquartile range 8.8–20.1 ng/mL) and 14.1 ± 7.1 ng/mL (interquartile range 9.4–18.1 ng/mL), respectively, ($p = 0.359$). Thirty-eight (71.69%) and 47 mothers (82.45%) had serum 25OH vitamin D3 < 20 ng/mL on the day following delivery in the study group and control group, respectively, ($p = 0.255$) and severe hypovitaminosis D was found in similar number of mothers ($p = 1.000$) in two groups (Table 2).

Serum vitamin D levels of mothers and infants at 6 months

The mean serum 25OHD levels of infants (29.1 ± 14.6 ng/mL) born to mothers supplemented with vitamin D3 were significantly higher ($p = 0.00$) than infants (15.7 ± 17.7 ng/mL) of unsupplemented mothers at 6 months of age. Half of the infants ($n = 56/110$; 50.9%) had serum 25OHD levels <20 ng/mL and 76.8% of them were born to mothers who were not supplemented with vitamin D3 during early postpartum period. Severe hypovitaminosis D was found in 4 infants (7.54%) in the study group and 25 infants (43.85%) in the control group at 6 months, and the difference was highly significant ($p = 0.000$). The serum 25OHD levels of vitamin D-supplemented mothers were almost double than those of vitamin D-unsupplemented mothers (Table 3). On the pattern of their infants, the mothers who were given vitamin D3 in the early postpartum period manifested vitamin D deficiency and severe vitamin D deficiency in significantly fewer cases ($p = 0.000$) compared to vitamin D-unsupplemented mothers (Tables 4 and 5).

Vitamin D safety profile in mothers and infants

Urinary calcium and creatinine ratio in the mothers at 14 weeks was 0.17 ± 0.12 and 0.16 ± 0.11 in the study and control group, respectively. At 6 months, it was found to be 0.14 ± 0.11 and 0.13 ± 0.11 in the study and control group,
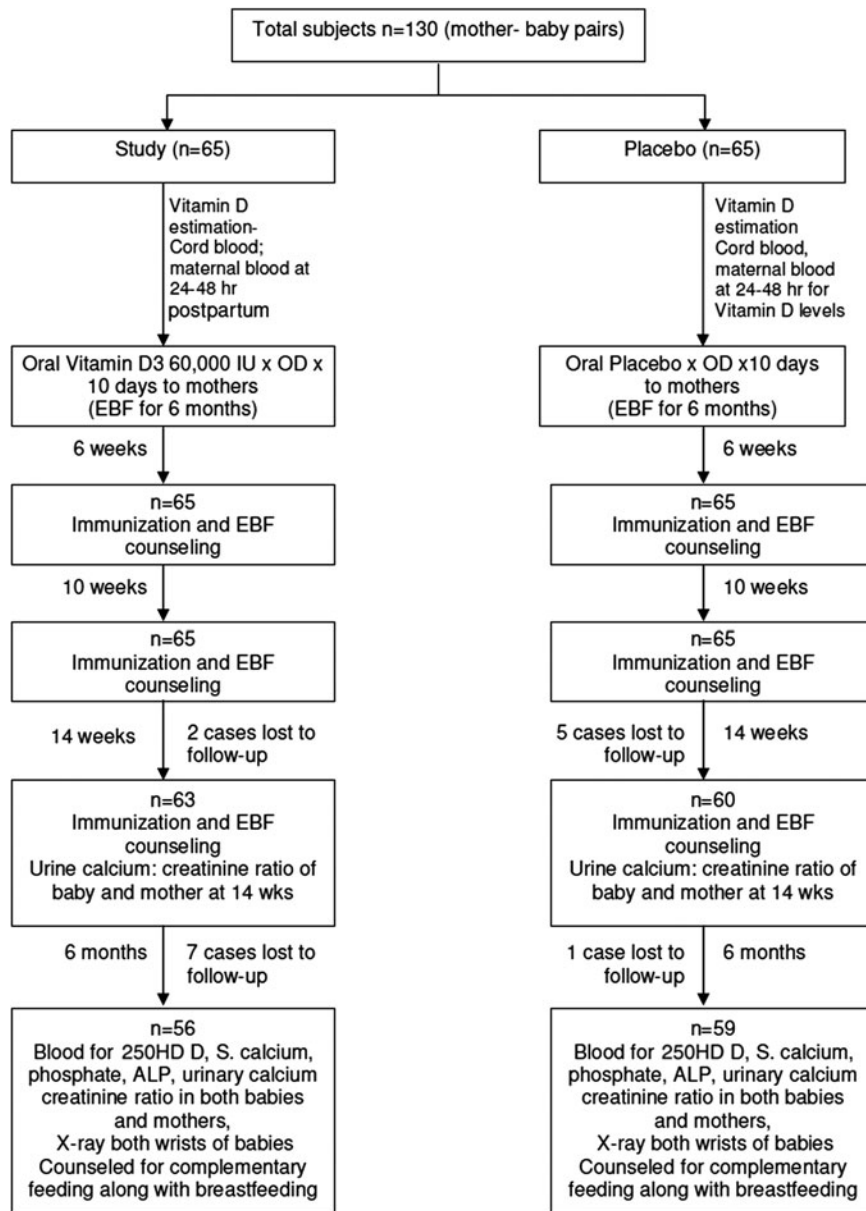
FIG. 1. Study flowchart.

TABLE 1. MATERNAL CHARACTERISTICS ($N = 115$)

Parameters	Study group (n = 56)	Control group (n = 59)	p
Age (years)	24.32 ± 2.650	24.51 ± 3.569	0.769
Parity (numbers)	1.57 ± 0.684	1.56 ± 0.702	0.926
Gestation (weeks)	38.96 ± 0.571	38.81 ± 0.706	0.210
Weight (kg)	54.16 ± 7.478	55.69 ± 6.826	0.253
Height (cm)	155.68 ± 3.347	156.56 ± 4.304	0.225
BMI	22.46 ± 2.656	22.76 ± 2.595	0.543
Diet			
Vegetarian diet, $n = 57$ (49.57%)	31 (55.4%)	26 (44.1%)	0.265
Mixed diet, $n = 58$ (50.43%)	25 (44.6%)	33 (55.9%)	
Dress			
Partially covered, $n = 104$ (90.43%)	52 (92.86%)	52 (88.14%)	0.295
Fully covered, $n = 11$ (9.56%)	4 (7.14%)	7 (11.86%)	
Normal delivery, $n = 103$ (89.56%)	49 (87.5%)	54 (91.53%)	0.551
LSCS, $n = 12$ (10.44%)	7 (12.5%)	5 (8.47%)	
Primary education, $n = 20$ (17.39%)	9 (16.07%)	11 (18.64%)	0.268
Secondary education, $n = 64$ (55.65%)	28 (50%)	36 (61.01%)	
Graduate, $n = 31$ (26.95%)	19 (33.92%)	12 (20.33%)	

BMI, body mass index; LSCS, lower section cesarean section.

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TABLE 2. SERUM 25OHD LEVELS OF MOTHERS AND INFANTS AT BASELINE

| | Serum 25OHD levels at recruitment (ng/mL)[a] | | | |
| | Mother | | Infant | |
Parameters	Study (n = 53)	Control (n = 57)	Study (n = 53)	Control (n = 57)
Mean ± SD	16.26 ± 9.36	14.15 ± 7.11	9.94 ± 5.77	8.91 ± 5.12
Median	14.44	12.56	10.02	7.86
Inter quartile range	8.84–20.13	9.42–18.10	4.94–13.36	5.32–12.12
Percentile				
25	8.84	9.42	4.94	5.32
50	14.44	12.56	10.02	7.86
75	20.13	18.10	13.36	12.12
Geometric mean (95% CI)	13.81 (16.20–11.72)	12.53 (14.30–10.98)	8.28 (6.96–9.86)	7.53 (6.45–8.82)
p	0.359		0.433	

[a]Serum 25OHD levels could be measured in 53 and 57 mother-infant pairs, respectively, in study and control group.

respectively ($p = 0.812$). In infants also, the urinary calcium and creatinine ratio was always <0.5 well below the safety value of <2 at 14 weeks and 6 months in both groups ($p = 0.968$) (Table 6).

Infants with radiological and biochemical rickets at 6 months

Mean serum calcium ($p = 0.824$) and phosphorus ($p = 0.456$) levels in the infants of vitamin D-supplemented and vitamin D-unsupplemented mothers were comparable at 6 month of age. However, mean serum ALP was significantly high ($p = 0.028$) in the control group showing that infants were deficient in bone mineralization and 10 infants (16.94%) exhibited biochemical rickets. Four infants developed radiological rickets at 6 months of age, two infants each in the study group (3.6%) and control group (3.4%). No child in the study group had biochemical rickets.

Discussion

Our study was conducted on sufficient numbers ($n = 115$) and adequate follow-up of mother-infant pairs of full-term gestation, who were randomized into two groups. In the study group, mothers received oral 600,000 IU of vitamin D3 one day after delivery in equally divided doses over 10 days.

Mothers in the control group received placebo. Age, weight, height, BMI, socioeconomic status, dressing preference, dietary habit, sunlight exposure, educational qualifications, and parity and mode of delivery in mothers were comparable in both groups. Similarly, infants born to vitamin D-supplemented and vitamin D-unsupplemented mothers had similar characteristics at birth and follow-up. This study confirms once again that large number of pregnant and lactating women do suffer from hypovitaminosis D. On the day following delivery, almost 3/4th parturient mothers were deficient in vitamin D stores and about 1/3rd of them in the study and control group were suffering from severe hypovitaminosis D. Similarly, almost all infants had low levels of 25OHD in the cord blood with 75% of them suffering from severe hypovitaminosis D [25OHD <11 ng/mL] and showed direct correlation with the maternal 25OHD levels. Our observations are in conformity with an earlier study from our institution that has reported vitamin D deficiency in 70% lactating mothers at 10 weeks postpartum and maternal vitamin D levels showed direct correlation with those of infants at 6 weeks and 6 months of age.[8] A large number of reports from different geographical areas, including countries with high sunshine, are available in the literature, highlighting the high prevalence of vitamin D deficiency in women during pregnancy and lactation and their children.[10,18–26]

TABLE 3. SERUM 25OHD LEVELS OF MOTHERS AND INFANTS AT 6 MONTHS

| | Serum 25OHD levels (ng/mL)[a] | | | |
| | Mother | | Infant | |
Parameters	Study (n = 53)	Control (n = 57)	Study (n = 53)	Control (n = 57)
Mean ± SD	40.33 ± 21.66	22.95 ± 20.18	29.19 ± 14.67	15.73 ± 17.73
Median	34.50	17.0	28.16	12.22
Inter quartile range	28.07–45.42	13.16–29.70	20.28–34.91	8.95–19.85
Percentile				
25	28.07	13.16	20.28	9.42
50	34.50	17.0	28.16	12.22
75	45.42	29.70	34.91	19.85
Geometric mean (95% CI)	34.85 (30.05–40.41)	18.77 (16.20–21.65)	24.46 (20.37–29.40)	11.80 (9.88–14.08)
p	<0.00		<0.00	

[a]Serum 25OHD levels could be measured in 53 and 57 mother-infant pairs, respectively, in study and control group.

TABLE 4. SERUM 25OHD <20 NG/ML IN
MOTHER-INFANT PAIRS AFTER 6 MONTHS

Mothers

Serum 25OHD levels	Study group (n=53)	Control group (n=57)	p[a]
<20 ng/mL	5 (9.43%)[a]	33 (57.89%)[a]	0.000
Mean ± SD	14.05 ± 2.40	12.96 ± 4.07	
Median	14.32	13.39	

Infants

Serum 25OHD levels	Study (n=53) (ng/mL)	Control (n=57) (ng/mL)	p[a]
<20 ng/mL	13 (24.52%)[a]	43 (75.43%)[a]	0.000
Mean ± SD	13.19 ± 6.11	10.34 ± 5.11	
Median	15.92	10.26	

[a]Intergroup significance.

In a review from the United States of 22 case reports during the period 1986–2003, which included 166 patients with nutritional rickets, it was found that 96% of the children with rickets were breastfed, and only 5% of them were reported to have received vitamin D supplementation.[27] Jain et al. reached the conclusion following an Indian study that prevalence of vitamin D deficiency was high in breastfed infants and their mothers, and 30% infants exhibited radiological rickets.[22] Exclusively breastfed infants required vitamin D supplementation in all seasons even in a country like Greece with abundant sunshine, where foods are not supplemented, to maintain adequate levels of vitamin D, reported by Challa et al.[28] The maternal and infant serum vitamin D levels exhibit significant correlation implying that such a high incidence of hypovitaminosis D in infants at birth and infancy is due to vitamin D deficiency in the mothers during pregnancy and lactation.[7,8,18,22,23] This raises a pertinent question whether we must supplement only nursing infant or mother, or both? An infant obtains vitamin D from three sources: by placental transfer during intrauterine period and after birth through

TABLE 5. SERUM 25OHD <11 NG/ML IN
MOTHER-INFANT PAIRS AFTER 6 MONTHS

Mothers

Serum 25OHD levels	Study (n=53) (ng/mL)	Control (n=57) (ng/mL)	p
<11 ng/mL	1 (1.88%)[a]	9 (15.78%)[a]	0.017[a]
Mean ± SD	10.78	7.58 ± 3.18	
Median	10.78	9.68	

Infants

Serum 25OHD levels	Study (n=53) (ng/mL)	Control (n=57) (ng/mL)	p
<11 ng/mL	4 (7.54%)[a]	25 (43.85%)[a]	0.000[a]
Mean ± SD	5.29 ± 3.56	6.89 ± 3.21	
Median	4.53	7.32	

[a]Intergroup significance.

TABLE 6. URINARY CALCIUM AND CREATININE
RATIO IN MOTHERS AND INFANTS

	U cal/creat at 14 weeks		U cal/creat at 6 months		
	Study (n=56)	Control (n=59)	Study group (n=56)	Control group (n=59)	p
Mother	0.17 ± 0.12	0.16 ± 0.11	0.14 ± 0.10	0.13 ± 0.11	0.812
Infant	0.30 ± 0.20	0.29 ± 0.20	0.33 ± 0.19	0.32 ± 0.19	0.968

U cal/creat, urinary calcium and creatinine.

breast milk and sunlight exposure.[29–31] Maternal vitamin D deficiency during lactation, related to poor vitamin D stores during pregnancy, lack of sun exposure, and low intake of vitamin D from diet, contributes to low breast milk vitamin D contents and, therefore, poor supply of vitamin D to the exclusively breastfed infant leading to its deficiency. The newborn infant born to a vitamin D-replenished mother is protected from vitamin D deficiency for the first few months of life as 25OHD crosses the placenta readily and neonatal levels of vitamin D approximate two-thirds of maternal serum concentrations with a half-life of ~3 weeks.[32,33]

Ala-houhala found that daily supplementation of 2000 IU to lactating mothers raised serum vitamin D levels of infants to a sufficient level and was comparable to daily 400 IU vitamin D supplementation to infants.[20] Wagner and colleagues observed that a dose of vitamin D as high as 6400 IU/day for 6 months was safe and raised infants' serum vitamin D levels significantly, and the effect was comparable to daily supplementation of 300 IU to infants.[11] Similar observations have been made by others[7,34]; it has also been shown that intermittent supplementation of high-dose vitamin D to mothers during lactation is equally effective as daily low-dose vitamin D supplementation in sufficiently raising serum vitamin D levels in infants and is better in terms of compliance (Oberhelman et al.).[35]

We found that giving 600,000 IU vitamin D_3 to mothers during early postpartum period significantly raised the mean serum 25OHD levels of exclusively breastfed infants at 6 months of age and their mothers compared to infants of unsupplemented mothers, and the number of infants with serum 25OHD levels <20 and <11 ng/mL were significantly low among them. This would have been possible only due to significant rise in vitamin D levels in the maternal blood, which actually happened in our study, and subsequent transfer of higher amount of vitamin D in the breast milk of supplemented group. We could not measure vitamin D in the breast milk. However, it is known that vitamin D supplementation to mothers in the lactation period raises breast milk vitamin D.[7,11,36] In fact human milk reflects the vitamin D status of the mother.[37] It is known how vitamin D gets access to human milk; a vitamin D binding protein has been postulated to mediate transfer of 25OHD in the human milk.[33] There was no clinical evidence of intoxication of hypervitaminosis D either in mothers or infants due to hypercalcemia in the form of lethargy, vomiting, constipation, seizures, and hypotonia and poor feeding either in the mothers or infants of vitamin D supplementation group. The urinary calcium and creatinine ratios were also within normal limits, although it was measured after 14 weeks and 6 months of vitamin D

administration when peak levels of vitamin D might have receded.

There is increasing realization that a continuum is required in maintaining vitamin D levels in the mother from pregnancy to lactation to prevent hypovitaminosis D in the nursing infants and young children.[38] In the case of maternal vitamin D deficiency, human milk contents decrease, resulting in inadequate supply of vitamin D to the nursing infant. In contrast, when maternal vitamin D sufficiency is achieved, human milk attains an antirachitic activity equivalent to infant oral supplementation.[39] Supplementation of lactating mother is a better approach as it improves vitamin D status of both infants and mothers and is safe for both. Supplementation of nursing infant, on the other hand, would have improved vitamin D levels of the infant only and acceptability and compliance of supplementation,[40] appropriate dosage,[41] and safety issues would remain as no long-term studies on dosage and safety of vitamin D supplementation to infants from early neonatal period are presently known.

In our study, four infants developed radiological rickets at 6 months of age, two each in the study and control group, respectively. At the same time, 10 infants born to vitamin D-unsupplemented mothers developed biochemical rickets. The cord blood 25OHD levels were low in all four infants. However, there was no significant difference in the serum 25OHD levels of mothers of infants with radiological or biochemical rickets in both study and control groups at recruitment. Some authors have suggested that mothers of infants developing nutritional rickets are deficient in vitamin D levels compared to nonrachitic children.[42,43] The limitation of the study is that we could not measure 25OHD levels in the breast milk. Another shortcoming is that optimum dose and duration of maternal vitamin D supplementation cannot be ascertained.

Conclusion

Supplementation of mothers with 60,000 IU vitamin D3 (cholecalciferol) daily, given orally for 10 days starting after 24 hours of delivery, significantly raised the serum 25OHD levels of their exclusively breastfed infants at 6 months of age in comparison to unsupplemented mothers. The number of infants with serum 25OHD levels <20 and <11 ng/mL at 6 months of age was also significantly less in vitamin D-supplemented group and prevented development of biochemical rickets in them.

Acknowledgments

Authors' Contributions

P.N. searched literature, prepared first draft, and collected data; M.M.A.F. conceptualized and designed study, supervised data collection, and prepared final draft; P.B. reviewed literature and helped to write discussion; S.V.M. measured vitamin D and critically analyzed results. All authors approved final article.

Disclosure Statement

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