Original Article

Assessment of the efficacy of 400 IU of oral Vitamin D3 supplementation for term healthy breastfed Indian infants

Kaifi Siddiqui[1], Anwar Hasan Siddiqui[2], Sheeba Farooqui[3], Pradeep Kumar Ranbijuli[4]

From [1]Assistant Professor, Department of Pediatrics, Hamdard Institute of Medical Sciences, New Delhi, [2]Assistant Professor, Department of Physiology, Jawaharlal Nehru Medical College, Aligarh Muslim University, Aligarh, Uttar Pradesh, [3]Resident, Department of Medical Genetics, Kasturba Medical College, Manipal, Karnataka, [4]Additional Chief Health Director, Department of Pediatrics, Jagjivan Ram Railway Hospital, Mumbai, Maharashtra, India

ABSTRACT

Introduction: Vitamin D is essential for growing children and its deficiency may have a catastrophic effect on growing children. Various reports state that Vitamin D deficiency is widely prevalent in the Indian population including infants and children. **Objective:** The objective of the study was to assess the efficacy of 400 IU of oral Vitamin D3 supplementation for term healthy breastfed Indian infants. **Materials and Methods:** The prospective, double-blinded cross-sectional study included 100 newborns divided into two randomized groups, A and B through a computer-generated program. Group A received 1 ml (400IU) Vitamin D drops daily for 3 months along with the breast milk whereas the Group B was only breastfed. Serum Vitamin D, Calcium, and alkaline phosphatase levels were measured in both the groups at birth and 3 months follow-up. **Results:** At birth no significant difference was observed in the serum level of Vitamin D, calcium and alkaline phosphatase in both the groups; however, at 3 month follow-up visit the Group A babies who were supplemented with Vitamin D 400 IU/day had a significantly more Vitamin D level (27.61 ± 11.95 ng/ml) compared with the babies of the non-supplemented group (10.56 ± 7.34 ng/ml). In the supplemented group, 46.7% had normal Vitamin D levels (>30 ng/ml), 20% had insufficiency (20–30 ng/ml) and 33.3% were deficient (<20 ng/ml). In babies who were non-supplemented, none had normal Vitamin D levels (>30 ng/ml), 13.3% had insufficiency (20–30 ng/ml), and 86.7% were deficient (<20 ng/ml). Group A babies also had significantly more serum calcium (9.81 ± 0.82 mg/dl) as compared to the Group B babies (8.52 ± 0.88). No significant difference was observed in alkaline phosphatase levels in both the group. **Conclusion:** In view of the high prevalence of maternal Vitamin D deficiency and the consequent low levels in the breast-fed infants do require supplementation of Vitamin D of at least 400 IU/day starting soon after birth.

Keyword: Breastfed infants, New born, Sunlight exposure, Supplements, Vitamin D

Vitamin D has been traditionally known as an anti-rachitic factor or sunshine vitamin. In 1922, Edward Mellanby discovered Vitamin D while researching a disease called rickets. Besides its pivotal role in calcium homeostasis and bone mineral metabolism, Vitamin D in the endocrine system is now recognized to subserve a wide range of fundamental biological functions in cell differentiation, inhibition of cell growth, and immunomodulation [1-3]. Vitamin D modulates the transcription of cell cycle proteins, which decrease cell proliferation and increase cell differentiation of several specialized cells of the body, namely, osteoclastic precursors, enterocytes, and keratinocytes [4,5].

Vitamin D status during pregnancy appears to play a role in fetal skeletal development, tooth enamel formation, and general fetal growth and development [6,7]. The Vitamin D stores of the newborn depend entirely on the Vitamin D stores of the mother. If the mother is Vitamin D deficient, the infant will be deficient because of decreased maternal-fetal transfer of Vitamin D. Resurgence of prolonged exclusive breastfeeding has led to a coincident increase in the incidence of Vitamin D deficiency in infancy. Worldwide public health authorities recommend exclusive breastfeeding for the first 6 months of life for all infants. However, breast milk does not generally supply the adequate amount of Vitamin D [8] due to which, breastfed infants are at risk of Vitamin D deficiency [9].

Infants who are breastfed but do not receive supplemental Vitamin D or adequate sunlight exposure are at an increased risk of developing Vitamin D deficiency or rickets [10,11]. Human milk typically contains Vitamin D concentration of 25 IU/L or less [12]. Thus, the recommended adequate intake of Vitamin D cannot be met with human milk as the sole source of Vitamin D for the breastfeeding infant; although, there is evidence that

Access this article online

Received - 05 February 2021
Initial Review - 24 February 2021
Accepted - 04 March 2021

DOI: 10.32677/IJCH.2021.v08.i03.004

Quick Response code

Correspondence to: Sheeba Farooqui, Room No. 220A, Sonia Block, KMC Campus, Manipal - 576 104, Karnataka, India. E-mail: sheebafarooqui@gmail.com

limited sunlight exposure prevents rickets in many breastfed infants [13,14].

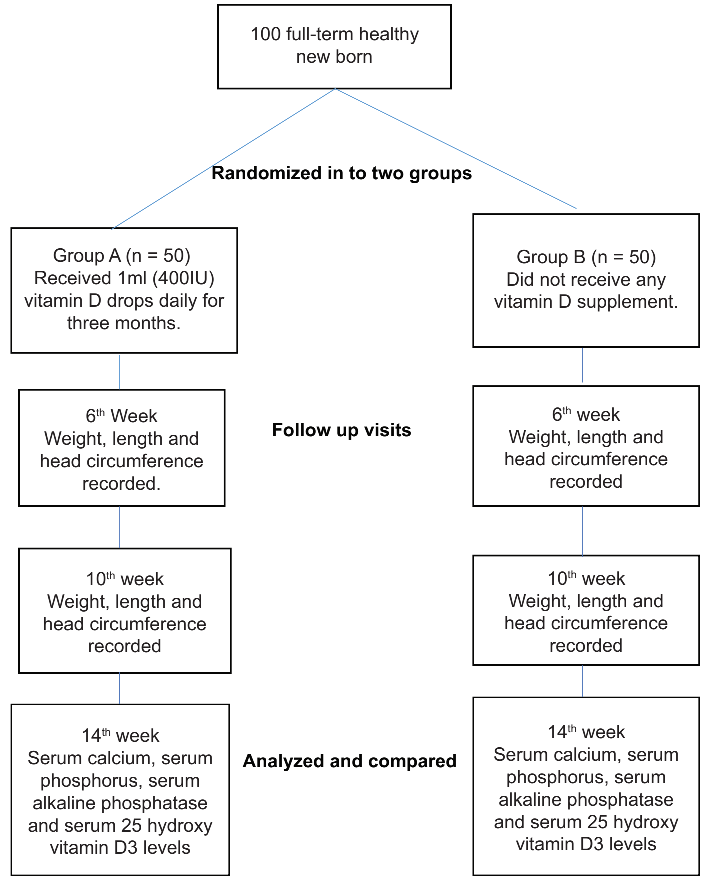
In a study by Rothberg *et al*. [15], lactating mothers were administered daily supplements of 500 and 1000 IU of Vitamin D from delivery and despite this, 25(OH) D levels of their infants measured at 6 weeks were not altered. In contrast, daily supplements of 400 IU of Vitamin D to infants significantly increased the 25(OH) D levels at 6 weeks. A study from Finland had concluded that when the mothers were supplemented with 2000 IU of Vitamin D, their infants had 25(OH) D levels similar to those receiving daily supplements of 400 IU of Vitamin D indicating that it is more efficient to supplement infants rather than their mothers [16].

A recent Indian study reported Vitamin D deficiency in 66.7% of infants and 81.1% of mothers [17]. Given the high prevalence of Vitamin D deficiency in pregnant Indian females and their babies, the problem of Vitamin D deficiency is likely to worsen among non-supplemented breastfed Indian babies. Poor exposure to sunlight, dark skin and low Vitamin D status of Indian babies and mother would mean that the doses required for Indian babies may be higher than what has been recommended for their counterparts in developed countries. Moreover, there is a lack of Indian data validating the efficacy of Vitamin D supplementation in the AAP recommended dose (400 IU) for term healthy newborns in India. This study aimed to assess the efficacy of 400 IU of oral Vitamin D3 supplementation for term healthy breastfed Indian infants at 3 months of age.

MATERIALS AND METHODS

The prospective, double-blinded cross-sectional study was conducted in the Department of Pediatrics in a tertiary care hospital from April 2016 to March 2017 after obtaining ethical clearance from the Institutional Ethics Committee (D.No.1287/FM). The study population comprised 130 full-term healthy babies divided into two groups of 65 each, one serving as the test group and the other as the control group. A minimum sample size with 42 participants was estimated for each group to detect a difference of units in indices to be clinically significant with a power of 80% at 95% confidence interval. The subject selection was strictly based on the inclusion and the exclusion criteria as mentioned below. Twenty-five subjects were lost to follow-up and the final analysis was done on 100 subjects with 50 in each group.

Healthy, full-term, appropriate for gestational age (>25th centile for gestational age as per Indian reference charts) babies, born to healthy mothers between 18 and 35 years of age, and who were exclusively breast-fed, were included in this study. Exclusion criteria were presence of chronic disease in mother; unwillingness or inability to continue breastfeeding, any gross congenital anomaly in the infant or illness requiring hospitalization in neonatal period, **r**esiding more than 100 km or unlikely to follow-up at the hospital, and mothers taking vitamin supplements.



Babies were randomized to two groups of 50 each: Group A received one ml (400IU) Vitamin D drops daily for 3 months. For the rationalization of the study same brand of Vitamin D was prescribed to all the babies and the mothers were advised to strictly adhere to the brand. Group B did not receive any dose of Vitamin D. Randomization was done in blocks. The randomization sequence was generated by a computer program and maintained by a colleague not directly involved in this trial. Subjects were allocated to either of the two groups. Exclusive breastfeeding was advised to both the groups.

Maternal history including age, parity, community, dressing style, dietary history, profession, socioeconomic status, details of calcium, and Vitamin D supplements intake during pregnancy and any significant illness or event during the antenatal period was recorded. Baby's clinical and anthropometric data, that is, weight, height, and head circumference were recorded at birth and each follow-up visits at 6th, 10th, and 14th weeks. At each of the follow-up visits, counseling for vaccination and exclusive breastfeeding was reinforced and compliance with Vitamin D in the supplemented group was ascertained. The non-supplemented group was checked for any use of Vitamin D supplements from other sources. Details regarding the mode of feeding, supplementation of any vitamin or calcium, duration, and type of clothing worn during sunlight exposure in the infants were recorded.

About 3 ml of baby's venous blood sample was collected at birth and at follow-up visit on 14th week (~3 months of age) for the estimation of serum calcium, phosphorus, alkaline phosphatase, and 25-OH Vitamin D3 (25OHD) levels. The serum level of 25OHD was measured by radioimmunoassay (kits from M/s DiaSorin Inc., USA). Serum calcium, phosphorus, and alkaline phosphatase were analyzed using calorimetry enzyme kits.

Statistical analysis was performed using software Statistical Package for the Social Science version 25.0 for Windows (IBM Corp. USA). One-sample Kolmogorov–Smirnov tests were performed to check whether distribution of variables was normal. Continuous variable was expressed as mean ± standard deviation. Independent sample t-test was adopted for comparison of differences of data with normal distribution and Mann–Whitney test was used for comparison of differences for data that displayed a skewed distribution. All tests were two tailed, confidence intervals were calculated at 95% level and a p-value of <0.05 was considered significant.

RESULTS

Both the study groups (Group A and Group B) were comparable in age, sex, weight at birth, gestation, and mode of delivery. No statistically significant difference was observed in the demographic data (Table 1). Serum levels of biochemical parameters, namely, Vitamin D, calcium, phosphorus, and alkaline phosphate measured at birth showed no statistically significant difference in both the groups (Table 2).

At 3 months follow-up, serum levels of Vitamin D and calcium were significantly more in Group A than Group B as demonstrated in Table 3. An observation was made between sun exposure duration and serum Vitamin D levels in both the group. It was seen that in both the groups' sun exposure of >15 min a day led to increase in serum Vitamin D levels. However, the intra group difference of serum Vitamin D in sun exposed versus non-exposed was not statistically significant (Fig. 1).

DISCUSSION

Vitamin D deficiency is a major public health problem. Globally, nearly 80–90% population is Vitamin D deficient. Vitamin D deficiency is prevalent even in settings of widespread prenatal vitamin use, as prenatal vitamins contain doses of Vitamin D that is too low to meaningfully raise serum 25-OH D [18]. Poor Vitamin D status during pregnancy has been associated with preeclampsia, gestational diabetes, and bacterial vaginosis, preterm labor and Intrauterine growth restriction as well as offspring rickets reduced bone density, asthma and schizophrenia.

We found that all the babies who had not been given Vitamin D supplements were deficient in Vitamin D. All the mothers, despite receiving supplements of 400–500IU of Vitamin D in pregnancy and while breast feeding had insufficient levels of Vitamin D. 53% of the mothers had elevated alkaline phosphatase which is a biochemical indicator of osteomalacia.

The Vitamin D stores of the newborn depend entirely on the Vitamin D stores of the mother. If the mother is Vitamin D deficient, the infant will be deficient because of decreased maternal fetal transfer of Vitamin D [6]. Many studies show a direct positive correlation between maternal and cord blood Vitamin D levels. Salle BL in her study reported immediate postnatal Vitamin D levels of 28±2 ng/ml in mothers and 23±2 ng /ml in their term babies cord samples; (r = +0.97) [19]. A study showed infants born to Vitamin D replete mothers become Vitamin D deficient after 8 weeks of life if not supplemented with Vitamin D. [20]

Table 1: Demographic variables of infants at birth

Variable	Group A (Vit. D supplement)	Group B (Non-supplement)
Number	50	50
Gender		
Male	31	28
Female	19	22
Weight (g)	2934±426	2942±368
Birth weight categories (in g)		
<2000	0	0
2000–2499	13 (26%)	11 (22%)
≥2500	37 (74%)	39 (78%)
Gestation (weeks)	38.6±1.1	37.8±1.4
Mode of delivery		
Normal	33 (66%)	36 (72%)
LSCS	17 (34%)	14 (28%)

Table 2: Serum levels of biochemical parameters in both groups at birth (0 weeks)

Variable	Group A (n=50)	Group B (n=50)	"p-value"
Vit. D (ng/ml)	5.29±3.01	6.05±3.01	0.684
Calcium (mg/dl)	9.00±0.94	8.89±1.03	0.72
Phosphorus (IU/l)	5.52±1.06	5.95±0.90	0.584
Alkaline phosphatase (IU/L)	151.53±43.24	156.87±46.24	0.431

Table 3: Serum levels of biochemical parameters in both groups at 3 months (14 weeks)

Variable	Group A (n=50)	Group B (n=50)	"p-value"
Vit. D (ng/ml)	27.61±11.95	10.56±7.34	0.001*
Babies with Vitamin D levels			
>30 ng/ml	23 (46%)	0 (0%)	
20–30 ng/ml	13 (26%)	8 (16%)	
10–20 ng/ml	11 (22%)	15 (30%)	
<10 ng/ml	3 (6%)	27 (54%)	
Calcium (mg/dl)	9.81±0.82	8.52±0.88	0.002*
Babies WITH calcium levels			
>8 mg/dl	43 (86%)	37 (74%)	
<8 mg/dl	7 (14%)	13 (26%)	
Phosphorus (IU/l)	5.52±1.06	5.95±0.90	
Alkaline phosphatase (IU/L)	151.53±43.24	156.87±46.24	0.144

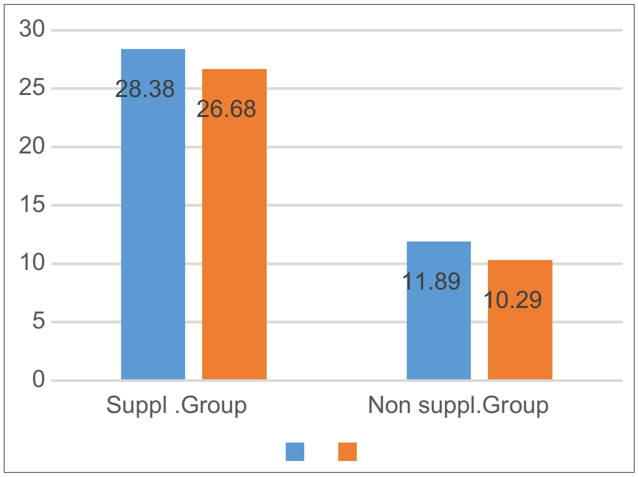


Figure 1: Serum levels of Vitamin D (ng/ml), recorded at 3 months in both groups in relation to exposure to sunlight

Cancela *et al*. reported that circulating Vitamin D concentrations in breastfed infants are directly related to the Vitamin D content of the mothers' milk and also concluded that breast milk is an inadequate source of Vitamin D, [21] The Vitamin D content of human milk has been defined with sensitive assay technology to range between 20 and 70 IU/L. These studies also showed that dietary maternal Vitamin D supplementation and ultraviolet (UV) light exposure increase the Vitamin D content of breast milk [22,23]. If a lactating mother has a limited exposure to UV light, a limited Vitamin D intake, the Vitamin D content of her milk will be low.

Rothberg *et al*. showed that supplementing mothers with 500 IU–1000 IU of Vitamin D from delivery, did not affect 25-OHD levels of infants at 6 weeks [15]. Heaney *et al*. found that supplementation of pregnant women with 400 IU Vitamin D would only increase circulating 25OHD concentrations by 2.8 ng/mL after 5 months [24]. According to the data available on supplementation of lactating women, it seems that the milk of supplemented mothers alone may provide the infant with sufficient Vitamin D only if the dose given to the mother is large enough-2000 IU/Day which would be equivalent to the infant receiving 400 IU of Vitamin D directly. In our study, babies supplemented with Vitamin D had a higher mean Vitamin D levels than in non-supplemented group (27.61±11.95 vs. 10.56±7.34 ng/ml). In accordance with the current study, Ismail *et al*., reported Vitamin D mean level of infants supplemented with 400 IU Vitamin D 32.25±14.75 ng/ml [12].

Limitation of the study was the relatively small size of the study and control group which limited the determination of measured clinical parameters. The results, therefore, can be considered to show only the trend and not any firm conclusion. The fact that we used an urban convenience sample may also limit the generalizability of these findings. Finally, information on nutrition and health habits for mother and children was obtained by self-report, which has inherent limitations. Nevertheless, the result will be justified by further research on large sample size.

CONCLUSION

The current study data add to accumulating evidence that Vitamin D deficiency, a preventable health problem, is common among infants and toddlers in India. There was a substantial increase in Vitamin D levels of the babies when supplemented with 400 IU/day of Vitamin D in addition to supplementing their mothers with Vitamin D. The Indian Academy of Paediatrics and other organizations involved in maternal and child healthcare need to emphasize the need for Vitamin D supplementation to pregnant and lactating women and also to exclusively breast fed babies.

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Funding: None; Conflicts of Interest: None Stated.

How to cite this article: Siddiqui K, Siddiqui AH, Farooqui S, Ranbijuli PK. Assessment of the efficacy of 400 IU of oral Vitamin D3 supplementation for term healthy breastfed Indian infants. Indian J Child Health. 2021; 8(3):122-126.