



Vitamin D deficiency in Indian child-mother pair and evaluation of calcium with or without oral vitamin D supplementation: exploring a “suitable” intervention strategy

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Abstract

Background: Vitamin D deficiency (VDD) prevails in epidemic proportions in the Indian sub continent and other developing countries. The absence of vitamin D fortified food and a strong culture obstacle for exposure to sunlight are major causes of VDD in India. Periodically administering large doses of vitamin D supplementation is the only option to prevent VDD.

Aim: Our aim was to evaluate calcium and/ or vitamin D supplementation of Indian child-mother pairs.

Methods: This study was a double blind, randomized controlled trial. Intervention: Group A received a total of 3500 mg of Shelcal tablets periodically, while Group B received a total of 600,000 IU of Calcirol sachets along with 3500 mg of Shelcal tablets for children, and 900,000 IU of Calcirol sachets with 3500 mg of Shelcal tablets for mothers at 15-day intervals from day 0 to day 90. We examined the vitamin D status at baseline, 90 days post-intervention, and after a 90-day follow-up, i.e., on day 0 and day 180.

Results: At baseline, 65% children and 85% mothers were Vitamin D deficient [25(OH)D<50nmol/L]. Significant increases were observed in serum 25(OH)D levels by 23.55 and 17.2, respectively, in children and mothers after 90 days of supplementing with calcium and vitamin D. Both groups (Group A and Group B) of child-mother pairs showed a decrease in calcium and alkaline phosphatase levels. After 90 days post-intervention and 90 days phone follow-up, only 21.4% children and 16.7% mothers were vitamin D sufficient.

Conclusion: The supplementation of calcium with vitamin D for 90 days resulted in a significant increase in serum 25(OH)D levels in both child-mother pairs.

Keywords: Vitamin D, 25-hydroxy vitamin D, alkaline phosphatase, hypovitaminosis D, Calcirol sachet

Introduction

Vitamin D deficiency (VDD) is a global public health problem affecting about 50% of the worldwide population (Holick & Chen 2008) [8]. Cultural and social taboos often dictate lifestyle patterns, such as clothing choices, that may limit sun exposure. The socioeconomically backward (or disadvantaged) people constitute a large percentage of the population in India. (Agarwal *et al.* 2002) [1]. The underprivileged generally suffer from overall poor nutrition, and access to most vitamin D supplements is limited. The typical dressing style followed in India, which allows for sun exposure, seems like a distant reality for urban Indians working indoors (Gupta & Gupta 2014) [6]. Therefore, they require more vitamin D. Vitamin D can be obtained from three sources: sun exposure, vitamin D supplements, and vitamin D fortified foods. However, traditional practices, desire for fair skin, the lack of vitamin D in food, and the unorganized nature of milk fortification make it impractical to rely solely on these sources.

Vitamin D is a fat-soluble vitamin playing a vital role in human physiology. In West Bengal, VDD as determined by (based on) low blood level of 25-Hydroxy vitamin D [25(OH)D] is common in under 7 years old children and their mothers. This situation is similar to the situation in many regions of India (Gupta and Gupta 2014) [6]. Tests

were conducted on families from low socio-economic group (urban poor) from the metropolitan city of Kolkata. Exposure to sunlight is minimal and is avoided by most people in India. Obtaining sufficient vitamin D from natural food sources alone is difficult. Therefore, consuming vitamin D fortified food and some exposure to sunlight are essential for maintaining a good vitamin D status. Vitamin D and Calcium recommendations often coincide as these nutrients are metabolically interrelated and serve endocrine functions (Institute of Medicine 1997).

The objective of this study was to assess vitamin D deficiency and evaluate calcium supplementation with or without vitamin D of Indian children and their mothers.

Materials and methods

Subjects

This study was a double blind Randomized Controlled Trial (RCT) conducted at the Infectious Disease (ID) hospital, Kolkata, India. Healthy non pregnant women aged 18 to 40 yrs old and their children 1 to less than 7 yrs were enrolled in this study. We recruited these children and their mothers from the family welfare clinic of a large charitable Government Hospital called “ID Hospital”, which has an outdoor clinic in the city of Kolkata, India, after obtaining written informed consent from the women. They came from

the municipal urban communities in the metropolitan city of Kolkata, India. The socio-economic and demographic features of these families are shown in Table-1. The eligibility criteria for inclusion in the study were children aged 1-7 years, mothers aged 18-40 years, families living

within a 10 km radius of the study center, and the exclusion criteria were unwillingness to give written informed consent and taking vitamin supplements. The study protocol was approved by the Ethics Review Committee of Society for Applied Studies, Kolkata, India.

Table 1: Socio economic status of the family.

Variables	Number (%)
House with cement floor wall & roof	7 (14%)
Lives and cooks in one room	33 (66%)
Family income, \$/month median (quarter)	96 (60,120)
Mothers Education	
Illiterate	4 (8%)
Can sign only	1 (2%)
1-5 years of school	6 (12%)
6-10 years of school	33 (66%)
>10 years of school	6 (12%)
Fathers Education	
Illiterate	3 (6%)
Can sign only	5 (10%)
1-5 years of school	7 (14%)
6-10 years of school	27 (54%)
>10 years of school	8 (16%)
Mothers Occupation	
House wife	42 (84%)
Maid servant	2 (4%)
Cooking	1 (2%)
Tuition	2 (4%)
Others	3 (6%)
Fathers Occupation	
Driver	11 (22%)
Business	10 (20%)
Govt. Job	2 (4%)
Private Company	6 (12%)
Hand Gloves making	5 (10%)
Others	16 (32%)
Family income per month	
Rs. Up to 3000/-	3 (6%)
Rs. 3100/- to 5000/-	10 (20%)
Rs. above 5000/- to 10000/-	28 (56%)
Rs. 10000/- and above	9 (18%)
Smoking	
Father	24(48%)
Mother	1(2%)
Alcohol addicted	
Father	23(46%)
other	0

Intervention

Among the study group, only subjects with vitamin D deficiency (25(OH)D < 50 nmol/L) were provided doses. They were divided into two groups: Group A and Group B. Doses were provided at 15-day intervals, from day 0 to day 90, a total of 7 times. Group A comprised 50% of the subjects, while the remaining 50% belonged to Group B. In Group A, each child and mother were provided with Shelcal tablets 3500 mg (50 mg per follow-up). Similarly, in Group B, the child was provided with Calcirol sachet 600,000 IU along with Shelcal tablets 3500 mg, and the mother was

provided with Calcirol sachet 900,000 IU along with Shelcal tablets 3500 mg with water.

After completing the doses for 3 months, their information was collected by phone follow-up every 15 days for up to 160 days. At 180 days, blood samples were drawn to estimate serum calcium (Ca), 25-hydroxy vitamin D [25(OH)D], and alkaline phosphatase.

Statistical Analysis

Analysis was performed by using Stata software (version 11.2). The baseline data were presented as mean and

standard deviation for continuous variables and in proportion for categorical variables. Vitamin D status was assessed using the following plasma 25(OH)D cut-off points: hypovitaminosis D. The results were presented as geometric mean and their 95% confidence intervals. Pair t-test was used to compare the baseline and 6 months levels of 25(OH)D, Serum Calcium, and serum alkaline phosphatase of two groups of Group A and Group B. The prevalence of vitamin D was defined as severe deficiency (25(OH)D < 25 nmol/L), deficiency (25(OH)D ≥ 25 & < 50 nmol/L), insufficiency (25(OH)D ≥ 50 & < 75 nmol/L), and sufficiency (25(OH)D ≥ 75 nmol/L).

Sample Size

We recruited children aged 1 to 7 years and women aged 18 to 40 years old. For the 6-month follow-up, we used a 90% confidence level ($\alpha = 0.1$) and 80% power ($\beta = 0.2$) to detect a difference in the occurrence of our study group. The study group was divided into two groups: Group A and Group B. Group B received 600,000 IU and 900,000 IU of vitamin D supplement with 3500 mg of calcium for the child and mother, respectively, under direct observation (DOT). Both the child and mother of Group A received only 3500 mg calcium. The estimated sample size in each group would be 80% confidence and 50% power ($\alpha=0.05$, $1-\beta=0.8$), requiring a sample of forty-eight subjects in each group. We added 2% to this number, resulting in a sample size of 50 children and 50 mothers.

Results

This study involved mother and child pairs from the urban and peri-urban communities of Kolkata, India. A total of 48 children and their mothers completed the initial study. The

mean age of the children was 38.93 (SD 20.25) months, and the mean age of the mothers was 25.78 (SD 4.08) years. Similarly, the mean BMI of children and mothers were 17.85 (SD 13.15) kg/m² and 23.66 (SD 4.57) kg/m², respectively. The BMI of children and mothers both were statistically highly significant ($p=0.006$). The baseline median serum level of 25(OH)D for child mother pair (Group A, Group B) were (40.83, 37.74) nmol/L and (42.01, 37.43) nmol/L of children and mothers, respectively. After supplementation, the 25(OH)D level of child mother pair was compared with the baseline (Fig 1(a) and Fig1(b)). The prevalence of severe deficiency, deficiency, and insufficiency of vitamin D in children and mothers were 10.3%, 56.3%, 29.2%, and 16.7%, 68.8%, 14.6%, respectively. Doses were provided to only vitamin D deficient children and their mothers. A total of 31 children and 41 mothers were vitamin D deficient and were provided their 1st dose. Six children and seven mothers dropped out of the follow-up. The total completed dose of 90 days was received by 28 children and 34 mothers. After completing the dose, 3 children and 5 mothers dropped out of the follow-up. Furthermore, 3 mothers and 6 children did not agree to have their blood drawn at 180 days. Lastly, a total of 26 mothers and 19 children completed the study and had their blood drawn.

The effect of Calcium with or without vitamin D supplementation on biochemical parameters is presented in Table 2. Individuals receiving supplemental calcium with or without vitamin D had an increase in 25(OH)D levels of (6.61, 23.55) nmol/L and (16.15, 17.20) nmol/L of (Group A, Group B) of child and mother respectively. Comparing Group A of mothers and children it was observed that after supplementation, 25(OH)D had increased in both mothers and children. In Group A children, 25(OH) increased non significantly ($p=0.4$), but it was significantly ($p=0.047$) increased for mothers.

Regarding everyday sunlight exposure of mothers, 11.5% of mothers were exposed to less than 15 minutes of sunlight, 80.8% were exposed to 15 to 30 minutes and 7.7% had more than 30 minutes sun exposure per day. There was a statistically significant association between sunlight exposure and vitamin D levels ($p=0.03$).

Table 2: Effect of calcium with or without vitamin D supplementation on baseline serum calcium, serum alkaline phosphatase and serum 25 hydroxy vitamin D of non pregnant women of child bearing age and their children.

Variable	Group A (Only Calcium supplement)					Group B (Calcium + Vitamin D supplement)					
	Child	Baseline (mean±SD)	After supplement (mean±SD)	Mean Difference	95% CI	p-value	Baseline (mean±SD)	After supplement (mean±SD)	Mean Difference	95% CI	p-value
25(OH)D (nmol/L)		39.64±6.73	46.25±18.83	-6.61	-23.86 to 10.64	0.4	36.29±10.15	59.84±19.10	-23.55	-41.53 to -5.57	0.02
Alkaline Phosphate (U/L)		260.88 ± 56.09	239.25± 48.87	21.63	-8.84 to 52.09	0.14	266.44±50	241.78±76.12	24.67	-55.85 to 105.18	0.50
Calcium (mg/dL)		10.05±0.41	9.74±0.41	0.31	-0.13 to 0.76	0.14	9.83±0.32	9.71±0.29	0.12	-0.20 to 0.45	0.42
Mother											
25(OH)D (nmol/L)		36.20±12.7	52.27±24.9	-16.15	-32.10 to -0.2	0.047	34.86± 9.76	52.07±18.92	17.20	-30.51 to -3.89	0.02
Alkaline Phosphate		87.86±21.28	83.36±17.74	4.5	-20.80 to 14.23	0.2	97.92±28.23	80±24.05	17.92	11.60 to 24.24	0.0001
(U/L) Calcium (mg/dL)		9.45±0.27	9.33±0.28	-0.12	-0.03 to 0.27	0.10	9.52±0.49	9.26±0.46	0.26	-0.002 to 0.52	0.051

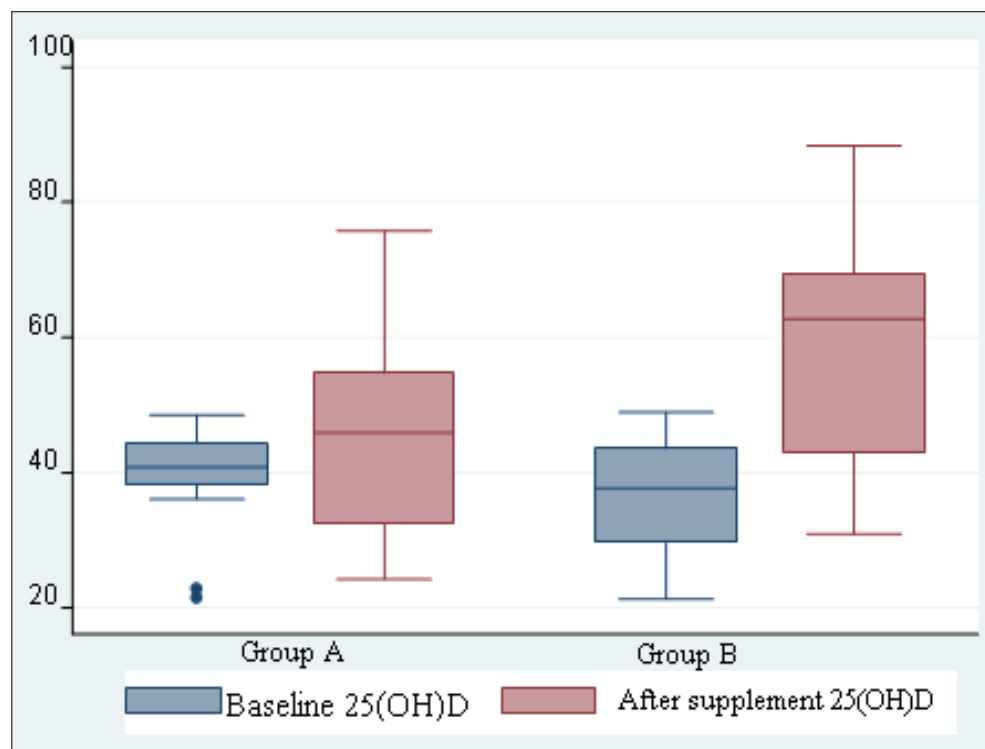


Fig 1(a): Children 25(OH)D status

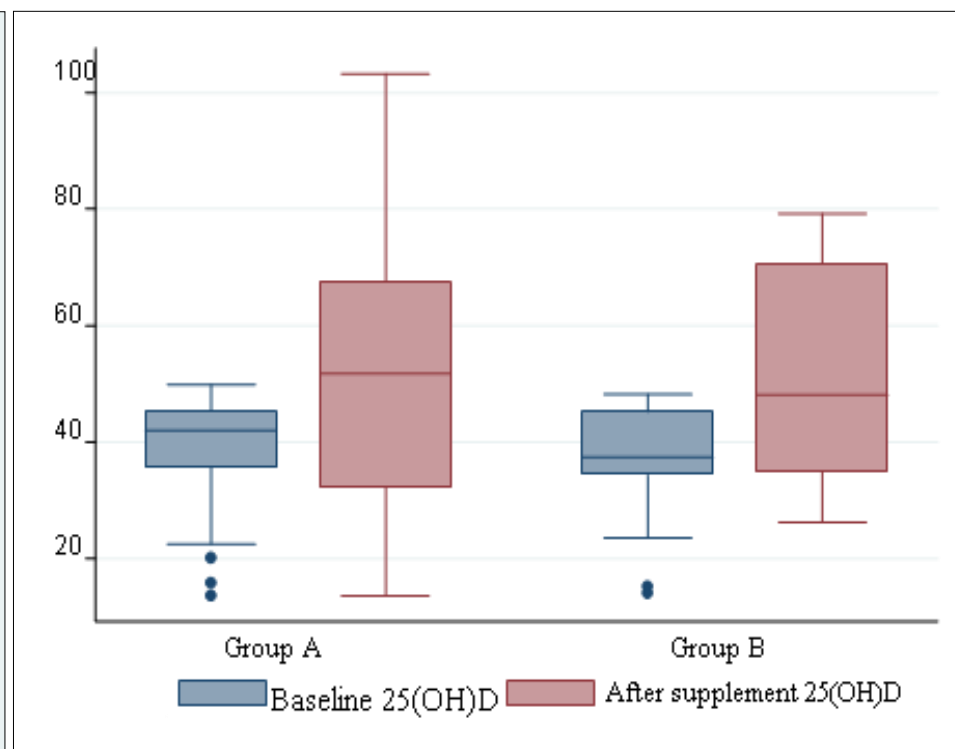


Fig 1(b): Mother 25(OH)D status

Discussion

Many studies all over the world including India have shown high to very high prevalence rate of vitamin D deficiency in infants and children (and also their mothers) (Holick 2008^[8], Aparna *et al.* 2018^[2], Marwaha *et al.* 2010)^[11]. In our previous pilot study involving 28 children under five and their mothers in India, we found a high proportion of them to have very low to low blood 25(OH)D level (Sen *et al.* 2018)^[15]. 50-90% of children suffer from vitamin D deficiency or insufficiency primarily due to lower dietary calcium intake, dark skin color, inadequate sunlight exposure, and changing lifestyles (Balasubramanian *et al.* 2013). However, in our study, 66-96% of children and 85-100% of mothers were vitamin D deficient or insufficient, confirming the poor vitamin D status of Indian mothers and their children.

In West Bengal, Vitamin D deficiency, as determined by (based on) low blood levels of 25(OH)D, is common both in under seven children and their mothers. This situation is similar to many regions in India (Holick 2012, Chacem *et al.* 2020)^[8, 9]. Tests were conducted on low socio-economic group (urban poor) from the metropolitan city of Kolkata. The diet of this population provides little, if any, vitamin D or its precursors. Fortification of a staple food with Vitamin D is the most viable population-based strategy to achieve vitamin D sufficiency. However, there is no food fortification with vitamin D in India. Exposure to sunlight is minimal and is avoided by most people in India. Supplementation is the only option to address this health problem. (Khadilkar *et al.* 2022).

A negative correlation between 25(OH)D and ALP was observed in both children and mothers, confirming the findings of other studies. Children and mothers in both Group A and Group B, after vitamin D supplementation, showed effectiveness in increasing 25(OH)D levels and decreasing ALP levels. However, calcium alone was less effective in both mothers and children in both groups, as serum calcium levels decreased non-significantly from baseline to 180 days.

In a study from the United Arab Emirates (UAE), vitamin D supplementation of 2000 IU/Day or 60000 IU/month in lactating women for 3 months was effective in achieving a serum 25(OH)D concentration > 50 nmol/L (Saddi *et al.* 2007)^[14]. In our previous trial study, we showed that large-dose vitamin D supplementation (60000 IU and 720000 IU for child and mother respectively) was safe, as no toxicity reports were observed after such supplementation (Sen *et al.* 2018)^[15].

However, a study from 2008 showed that short-term 25(OH)D oral supplementation over 8 weeks increased 25(OH)D levels to > 50 nmol/L, but at the end of 12 months, the levels were < 50 nmol/L (Goswami *et al.* 2008)^[5]. In our study, after completing the doses for 90 days, 81% of children and 67% of mothers achieved 25(OH)D levels > 50 nmol/L. But, in our previous study, we observed that all children and their mothers, except one child and one mother, had 25(OH)D levels > 50 nmol/L after 7 days of completing the doses. Therefore, serum 25(OH)D levels depend on the completion of vitamin D supplementation and the number of days elapsed before blood samples are drawn. If completion and blood sample drawing are delayed, 25(OH)D levels will decrease.

In India, 50-90% of children suffer from vitamin D deficiency or insufficiency, primarily due to lower dietary

calcium intake, dark skin color, and inadequate sunlight exposure (Chacham *et al.* 2020)^[4]. Several studies worldwide, including in India, have shown a high prevalence of vitamin D deficiency in children and mothers (Holick 2007, Sachan *et al.* 2005)^[13]. In our study, 67% of children and 85% of mothers had vitamin D deficiency. In a country like India where there is no fortification of food with vitamin D, supplementation remains an important alternative for improving the vitamin D status of individuals.

In our study, sunlight exposure of children revealed that 13.3% were exposed to less than 15 minutes of sunlight, 60% were exposed to 15-30 minutes, and 26.7% had more than 30 minutes of sun exposure. However, there was no statistically significant association between vitamin D levels and sun exposure ($p=0.07$).

In the urban low socio-economic status of children in Kolkata, there is minimal sunlight exposure, malnutrition, low dietary calcium intake, religious customs, and atmospheric pollution, all of which contribute to increased skin pigmentation and a high prevalence of vitamin D deficiency. Therefore, 65-85% of children and mothers in this area were found to be vitamin D deficient. While vitamin D is not a panacea, it may be an important, cost-effective, and safe adjuvant therapy for many diseases and stages of life, including pregnancy, childhood, and old age. A yearly dose may be the most suitable mode to comply with, serving as an "Interim Intervention Strategy" until a food fortification program with vitamin D is in place.

In conclusion, the preliminary findings suggest that calcium with vitamin D supplementation is more effective than calcium supplementation alone in children and mothers. Additionally, vitamin D supplementation appears to be more effective for children than for mothers. This study will provide large-scale intervention with calcium and vitamin D supplementation to prevent hypovitaminosis D in Indian mothers and children.

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