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Increment in vitamin D level and bone mineral accrual in children with vitamin D deficiency

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Purpose: To compare different regimens of vitamin D with respect to its serum increment levels and bone mineral accrual in vitamin D-deficient children.

Methods: Children identified as being vitamin D deficient (serum levels<20 ng/mL) were divided into 3 treatment groups by stratified block randomization (group 1, 4,000 IU/day of vitamin D3 plus 50 mg/kg/day calcium for 12 weeks; group 2, 30,000 IU/wk of vitamin D3 plus 50 mg/kg/day calcium for 12 weeks; and group 3, 300,000 IU of vitamin D3 once intramuscularly plus 50 mg/kg/day calcium). After regimen completion, each child received a maintenance dose of 400 IU/day vitamin D3 plus 50 mg/kg/day calcium. Their serum vitamin D level was measured after 3 and 12 months. Total body less head bone mineral concentration (BMC) and total body less head bone mineral density (BMD) were measured after 12 months.

Results: The mean increment in serum vitamin D levels from baseline to 3 months was significantly higher in group 3 than in groups 1 and 2, but the levels from 3 to 12 months were almost similar among all 3 groups. There were no significant differences among the 3 groups with respect to percentage increase of BMD and BMC.

Conclusion: The injectable form of vitamin D was more efficacious than the oral forms in increasing the serum level to the normal range. All 3 regimens were equally effective in increasing the BMC and BMD. The 400 IU/day maintenance dose was sufficient to keep the serum level within the normal range.

Key words: Vitamin D, Calcium, Deficiency, Bone mineral accrual, Bone mineral density

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Introduction

Till 1990s, vitamin D deficiency was not heard of in India. The Indian population is neither over light nor excessively dark. But until 2000, no systematic study had been done for directly assessing body Vitamin D status in India. In 2000, for the first time serum 25-hydroxy vitamin was measured using a sensitive and specific assay in apparently healthy subjects in Delhi and it was shown that significant hypovitaminosis D was present in 90% of the subjects[1]. Thereafter, many studies from various parts of our country have revealed widespread vitamin D deficiency in India in all age groups. Recent data indicate that vitamin D deficiency is pandemic and even the healthy and the young are affected.

The American Academy of Pediatric in their revised guidelines (2008), has stated that on the basis of the available evidence, serum 25-hydroxy vitamin D concentration in children should be >50 nmol/L (20 ng/mL).

In a hospital based study from Delhi, toddlers with a mean age 1.6 years who were brought to a tertiary care center with a history of delayed walking were studied. Sixty

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percent of these children were diagnosed to have nutritional rickets[1]. In a study looking at the serum 25-hydroxy vitamin D levels in slum children of Delhi, authors found that the percentage prevalence of vitamin D deficiency was over 80%[2]. A study from Pune described the role of sunlight exposure in determining the vitamin D status of under privileged toddlers. Toddlers from underprivileged areas, who were deprived of sunlight had a much greater incidence of hypovitaminosis D (77%) and frank rickets than the control group (16 4%)[3]. These studies highlight the fact that vitamin D deficiency is very common in Indian toddlers.

Stoss therapy recommends parenteral or large oral dose of vitamin D3, which has been shown to cause sustained higher levels of serum 25-hydroxy vitamin D, especially the regimen with 6,00,000 IU. The safety of Stoss therapy has been confirmed in many studies and it can lead to hypercalcemia only at very high doses. Doses of 1,50,000 to 3,00,000 IU are effective with minimal side effects (Table 1).

Given the high prevalence of vitamin D deficiency in North India, we planned this study to assess the effect of daily, weekly and monthly regimens on serum vitamin D level and bone mineral accrual in vitamin D deficient children.

Materials and methods

1. Design and setting

A prospective randomized clinical trial was conducted in Department of Pediatrics, Ganesh Shankar Vidyarthi Memorial Medical College, L.L.R and associated hospital, Kanpur. The duration of the study was 15 months.

2. Sample size

A pilot study was done in the Department of Pediatrics, Ganesh Shankar Vidyarthi Memorial Medical College, Kanpur. A small sample of 10 children was studied. Five children received 4,000 IU/day of vitamin D3 for 12 weeks along with calcium (50 mg/kg/day), and 5 children received 3,00,000 IU of vitamin D3 once intramuscular along with calcium (50 mg/kg/day). In the first group, one recovered from vitamin D deficiency whereas in the second group, three recovered from vitamin D deficiency after 1 month. A difference of 40% was observed in the 2 groups. Therefore, the trial was designed at 80% power, at α level of 0.05 to detect a 40% difference between the treatment groups, taking a baseline vitamin D deficiency prevalence of 80%. The sample size was calculated to be using the formula for calculation of sample size comparing 2 proportions[4].

3. Ethical consideration

Ethical clearance was taken from the Institutional Ethical Committee of Ganesh Shankar Vidyarthi Memorial Medical College, Kanpur

4. Selection of cases

Children attending pediatrics outpatient department in Ganesh Shankar Vidyarthi Memorial Medical College during the study period were included in the study based on the following criteria:

5. Inclusion criteria

1) All children between 2 to 5 years age group

2) Having vitamin D deficiency based on the criteria Serum vitamin D level (<20 ng/mL)

3) Parents had given informed written consent.

6. Exclusion criteria

1) Children with chronic illness.

2) Children on steroid and other factor influencing vitamin D in children.

3) Acute illness <2 weeks.

7. Randomization

Subjects eligible for the study were divided in 2 strata based on gender (male or female). Stratified randomization with a block size of 3 was used to assign patients to groups 1, 2, and 3. Group 1 received 4,000 IU/day of vitamin D3 for 12 weeks along with calcium carbonate (50-mg elemental calcium/kg/day), group 2 received 30,000 IU/wk of vitamin D3 for 12 weeks along with calcium carbonate (50-mg elemental calcium/kg/day), and group 3 received 3,00,000 IU of vitamin D3 once intramuscular along with calcium carbonate (50-mg elemental calcium/kg/day). Calcium carbonate was the calcium preparation used in the dosage of 50-mg elemental calcium/kg/day). After completion of 3 regimens, children of each group received maintenance dose of 400 IU/day vitamin D3 along with calcium (50 mg/kg/day). After 3 months and 12 months, their serum vitamin D was measured.

Table 1. Recommended treatment regimens of vitamin D supplementation

Group	Daily regimen (8–12 weeks)	Weekly regimen (8–12 weeks)	Stoss therapy[15] (oral or intramuscular)	Maintenance
<1 month	1,000 IU	50,000 IU	-	400–1,000 IU
1–12 months	1,000–5,000 IU	50,000 IU	1,00,000–6,00,000 IU over 1–5 days (preferably 3,00,000 IU)	400–1,000 IU
1–18 years	5,000 IU	50,000 IU	3,00,000–6,00,000 IU over 1–5 days	600–1,000 IU
>18 years	6,000 IU	50,000 IU	3,00,000–6,00,000 IU over 1–5 days	1,500–2,000 IU

The randomization lists were computer-generated prior to the start of the study and kept confidential. It was a single blind study and patients involved in the study were unaware of assignment to treatment groups.

After 3 months, serum vitamin D level was measured. After 12 months, total body less head bone mineral concentration (BMC), total body less head bone mineral density (BMD) and serum vitamin D level was measured. The increase in bone mineral content after 1 year was noted.

8. Procedure of estimation of vitamin D

Vitamin D estimation was done by chemiluminescence immunoassay (CLIA), which is a quantitative immunoassay method used for the determination of total 25-hydroxy vitamin D in serum on a fully automated analyser, CLIA was done using autoanalyser (Architect i1000SR, Abott Laboratories, Abbott Park, IL, USA), available in the Department of Biochemistry situated in the hospital premises. So the sample was collected and transported to the laboratory immediately after collection without any delay.

9. Procedure for measuring BMD and BMC

Bone densitometry was performed using dual X-ray absorptiometry with a single Hologic Delphi A model bone densitometer (Hologic Inc., Marlborough, MA, USA) with the manufacturer's software for pediatrics (Hologic ver. 12.3; Hologic Inc.). Scans were performed using the manufacturer's protocol. Weekly monitoring was done for Scanner calibration and long-term stability using anthropomorphic whole-body phantoms (Hologic Inc.). Precision for BMD and BMC was <2.5% for the whole-body phantom by DEXA scan.

10. Statistical analysis

Data was compiled using Microsoft Excel and analyzed using SPSS ver. 17.0 (SPSS Inc., Chicago, IL, USA). Quantitative variables were analyzed using mean and standard deviation. comparison between the 3 groups was done using one way analysis of variance (ANOVA). Comparison between the baseline, 3-month, and 12-month values was done using repeated measure ANOVA. Two tailed *P* value less than 0.05 was considered significant. Paired *t* test was used to analyze the difference in quantitative variables before and after vitamin D supplementation.

Results

A total of 19 subjects each was included in groups 1, 2, and 3, respectively. In group 1, 3 subjects migrated and were lost to follow up and one subject opted out of the study. In group 2, 4 subjects were lost to follow up. In group 3, 4 subjects opted out of the study. Mean age of subjects in group 1 was 3+0.9 years, group 2 was 3.2+0.7 years, and in group 3 was 3.4+1.4 years and this difference was not statistically significant. Similarly, the difference in mean serum calcium, mean serum phosphorus, and baseline vitamin D levels of subjects in groups 1, 2, and 3 was not found to be statistically significant. Mean baseline BMD of subjects in group 1 was 0.522+0.028, group 2 was 0.518+ 0.04, and in group 3 was 0.546+0.097 and this difference was statistically significant. Similarly, the difference in mean baseline BMC of subjects in groups 1, 2, and 3 was not found to be statistically significant (Table 2).

It was observed that the mean increment in serum vitamin D level between baseline and after 3 months was highest in group 3 (20.13) in comparison to group 2 (14.82) and group 1 (14.42) and this difference was found to be statistically significant (Tables 3, 4). The mean increment in serum vitamin D level from 3 months to 12 months was almost similar in all the groups (group 1, 19.56; group 2, 22.04; and group 3, 23.46) and this was not found to be statistically significant. The mean increment in serum vitamin D level from baseline to 12 months was highest in group 3 was 43.58 whereas in group 2 and group 1 was 36.86 and 33.98, respectively. Mean increment in serum vitamin D level from baseline to 12 months between the different groups was found to

Table 2. Baseline characteristics of study subjects in the 3 groups

Characteristic	Group 1 (n=15)	Group 2 (n=15)	Group 3 (n=15)	*P* value
Age (yr)	3.0±0.9	3.2±0.7	3.4±1.4	0.595
Serum calcium (mEq/L)	4.32±0.33	4.28±0.17	4.33±0.31	0.882
Serim phosphorus (mEq/L)	4.24±0.48	4.23±0.08	4.12±0.06	0.592
Vitamin D (ng/mL)	13.60±5.67	14.26±4.65	12.93±6.08	0.805
BMD (g/cm^2)	0.52±0.03	0.52±0.04	0.55±1.00	0.430
BMC (g)	140.88±56.27	158.16±93.88	192.92±109.06	0.277

Values are presented as mean±standard deviation.
Group 1, 4,000 IU/day of vitamin D3 plus 50 mg/kg/day calcium for 12 weeks; group 2, 30,000 IU/wk of vitamin D3 plus 50 mg/kg/day calcium for 12 weeks; group 3, 300,000 IU of vitamin D3 once intramuscularly plus 50 mg/kg/day calcium; BMD, total body less head bone mineral density; BMC, total body less head bone mineral concentration.
One-way analysis of variance. *P*<0.05, statistically significant differences between the groups.

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Table 3. Comparison of vitamin D levels at baseline and 3 months and 1 year after vitamin D supplementation

Group	Serum vitamin D level (ng/mL)			*P* value
	Baseline	3 Months	1 Year	
1 (n=15)	13.60±5.67	28.02±7.26	47.58±6.16	<0.0001*
2 (n=15)	14.26±4.65	29.08±3.66	51.12±1.87	<0.0001*
3 (n=15)	12.93±6.08	33.06±10.21	56.52±4.49	<0.0001*

Values are presented as mean±standard deviation.
Group 1, 4,000 IU/day of vitamin D3 plus 50 mg/kg/day calcium for 12 weeks; group 2, 30,000 IU/wk of vitamin D3 plus 50 mg/kg/day calcium for 12 weeks; group 3, 300,000 IU of vitamin D3 once intramuscularly plus 50 mg/kg/day calcium.
Repeated measures analysis of variance.
*P<0.05, statistically significant differences between the groups.

Table 4. Comparison of mean differences in serum vitamin D values after vitamin D supplementation in the 3 groups

Mean difference	Group 1 (n=15)	Group 2 (n=15)	Group 3 (n=15)	*P* value
Baseline to 3 months	14.42±4.31	14.82±2.01	20.13±6.78	0.003*
3 Months to 1 year	19.56±3.20	22.04±2.39	23.46±7.88	0.119
Baseline to 1 year	33.98±1.54	36.86±2.97	43.58±4.64	<0.001*

Values are presented as mean±standard deviation.
Group 1, 4,000 IU/day of vitamin D3 plus 50 mg/kg/day calcium for 12 weeks; group 2, 30,000 IU/wk of vitamin D3 plus 50 mg/kg/day calcium for 12 weeks; group 3, 300,000 IU of vitamin D3 once intramuscularly plus 50 mg/kg/day calcium.
One-way analysis of variance.
*P<0.05, statistically significant differences between the groups.

be statistically significant.

In group 1, at baseline mean BMD was 0.522+0.028 and after 1 year mean BMD was 0.541+0.021. This difference was found to be statistically significant. There was a 3.63% increase in the mean BMD after vitamin D supplementation in group 1 (Tables 5-7). Similarly in groups 2 and 3 the difference between mean BMD at baseline and at 1 year was found to be statistically significant. There was a 7.9% and 1.28% increase in the mean BMD after vitamin D supplementation in groups 2 and 3, respectively. But the comparison between the different groups for percentage increment in BMD was not found to be statistically significant. In group 1, at baseline mean BMC was 140.88+56.27 and after 1 year mean BMC was 206.4+59.83. This difference was found to be statistically significant. In group 2 at baseline mean BMC was 158+93.88 and after 1 year mean BMC was 231+103.79. This difference was statistically significant. In group 3 at baseline mean BMC was 109.06+261.4 and after 1 year, mean BMC was 153.43+104.04. This difference was also found to be statistically significant. Increment in BMC level from baseline to 12 months was 46.5%, 46.45%, and 40% in groups 1, 2, and 3, respectively. But the comparison between the different groups for percentage increment in BMC was not found to be statistically significant.

Table 5. Comparison of BMD at baseline and after 1 year in all 3 groups

Group	BMD (g/cm^2)		*P* value
	Baseline	After 1 year	
1 (n=15)	0.522±0.028	0.541±0.021	<0.025*
2 (n=15)	0.517±0.0440	0.558±0.072	<0.0001*
3 (n=15)	0.546±0.0972	0.553±0.0612	<0.122

Values are presented as mean±standard deviation.
Group 1, 4,000 IU/day of vitamin D3 plus 50 mg/kg/day calcium for 12 weeks; group 2, 30,000 IU/wk of vitamin D3 plus 50 mg/kg/day calcium for 12 weeks; group 3, 300,000 IU of vitamin D3 once intramuscularly plus 50 mg/kg/day calcium; BMD, total body less head bone mineral density.
Paired *t* test.
*P<0.05, statistically significant differences between the groups.

Table 6. Comparison of BMC at baseline and after 1 year in all 3 groups

Group	BMC (g)		*P* value
	Baseline	After 1 year	
1 (n=15)	140.88±56.27	206.4±59.83	<0.0001*
2 (n=15)	158±93.88	231±103.79	<0.0001*
3 (n=15)	109.06±261.4	153.43±104.04	<0.0001*

Values are presented as mean±standard deviation.
Group 1, 4,000 IU/day of vitamin D3 plus 50 mg/kg/day calcium for 12 weeks; group 2, 30,000 IU/wk of vitamin D3 plus 50 mg/kg/day calcium for 12 weeks; group 3, 300,000 IU of vitamin D3 once intramuscularly plus 50 mg/kg/day calcium; BMC, total body less head bone mineral concentration.
Paired *t* test.
*P<0.05, statistically significant differences between the groups.

Table 7. Comparison of the mean differences in BMD and BMC values after Vitamin D supplementation in the 3 groups

Mean difference	Group 1 (n=15)	Group 2 (n=15)	Group 3 (n=15)	*P* value
BMD (baseline to 1 year)	0.019±0.023	0.041±0.036	0.007±0.090	>0.05
BMC (baseline to 1 year)	65.58±36.37	72.84±32.05	43.83±41.77	>0.05

Values are presented as mean±standard deviation.
Group 1, 4,000 IU/day of vitamin D3 plus 50 mg/kg/day calcium for 12 weeks; group 2, 30,000 IU/wk of vitamin D3 plus 50 mg/kg/day calcium for 12 weeks; group 3, 300,000 IU of vitamin D3 once intramuscularly plus 50 mg/kg/day calcium; BMD, total body less head bone mineral density; BMC, total body less head bone mineral concentration.
One-way analysis of variance.
*P<0.05, statistically significant differences between the groups.

Discussion

In this study, we observed that the mean increment in serum vitamin D level from baseline to 3 months was highest in the group receiving injectable form of vitamin D in comparison to the other groups receiving oral supplementation. There were no undesirable side effects observed in either group and both oral and injectable forms of treatment were well-tolerated. This effect may be due to better compliance for injectable form of vitamin D. Similarly Billoo et al.[5] showed that difference between the oral and injectable forms of vitamin-D (cholecalciferol) was significant and injectable form was shown to be more effective. Hackman et

al.[6] showed that injectable high-dose regimen may be an effective and cheap alternative for patients with vitamin D deficiency. Mittal et al.[7] showed that the intramuscular dose of 3,00,000 IU vitamin D was safer and more efficacious than an intramuscular dose of 6,00,000 IU. Our finding was in contradiction to the finding of Mondal et al.[8] who concluded that staggered oral and one-time intramuscular administration of 600,000 IU vitamin D are equally effective and safe in treatment of nutritional rickets.

In this study, we found that the increment in serum vitamin D from 3 months to 12 months was almost similar in all the groups and the comparison between different groups was not found to be statistically significant. This finding suggests that the 400 IU was sufficient in maintaining vitamin D level in the normal range in all the three groups after initial loading dose of vitamin D.

Although in our study, we found that the BMD increased from baseline to 12 months by 7.9% in group 2, 3.36% in group 3 and 1.28% in group 1, the comparison between the groups for increment in BMD was insignificant. Previously no study was done on this topic so we lack data for comparison. There are very few studies which revealed that there is a significant effect of vitamin D supplementation BMD. Kalra et al.[9] concluded that 658 IU vitamin D was required to achieve an improvement in the densitometric parameters, in particular the increase in the BMD in the lumbar spine. Winzenberg et al.[10] showed that total body bone mineral content and lumbar spine bone mineral density were roughly equivalent to 2.6% and 1.7% greater from baseline in the vitamin D supplemented group.

Viljakainen et al.[11], showed that daily supplementation of 400 IU of vitamin D resulted in 2% increment in hip BMD from baseline and 3% increment in lumbar spine BMD. Thacher et al.[12] showed that 1,000 mg of calcium along with the 200 IU of vitamin D brought about a 1% increase in BMC and a 4% increase in forearm BMD. El-Hajj Fuleihan et al.[13] showed that the 14,000 IU/wk brought about a 4% increase in forearm BMD in adolescent girls.

In the present study, there was an increase in BMC from baseline to 12 months of 46.5% in group 1, 46.45% in group 2 and 40% in group 3 and there was no statistically significant difference between the 3 groups. Viljakainen et al.[11], showed that the bone mineral augmentation in femur occur by 17.2% in the group receiving 400 IU daily vitamin D supplementation. Du et al.[14], showed that daily supplementation of 133 IU of vitamin D resulted in a 1% increase in total body less head BMC. This difference in the findings may be due to difference in dosing schedule of vitamin D supplementation.

One limitation of our study was that the sample size was relatively small and many subjects were lost to follow up. Another limitation was that the duration of study was only 1 year. Therefore, we suggest that more studies should be conducted with a larger sample size and for a longer duration to validate the effect of vitamin D supplementation on vitamin D levels and bone mineral accrual in the pediatric age group.

In conclusion, the findings of this randomized clinical trial revealed that in vitamin D deficient children, the a single intramuscular dose of 3,00,000 IU of vitamin D was more efficacious over oral therapeutic regimens (4,000 IU/day or 30,000 IU/wk) in increasing the vitamin D level to normal range and thereafter 400 IU daily oral dose of vitamin D was sufficient in maintaining the vitamin D level within the normal range. All 3 regimens brought about an increase in BMD and BMC. We observed that the mean increment in BMD and BMC from baseline to 12 months in the 3 different regimens was equivalent. Therefore, all the 3 regimens were equally effective in increasing BMD and BMC.

Conflict of interest

No potential conflict of interest relevant to this article was reported.

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