

Effect of Vitamin D Supplementation on Bone Turnover Markers in Children and Adolescents from North India

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Abstract

Objectives: Vitamin D is known to play an important role in bone mineral metabolism. Its deficiency may affect growth and status of bone markers in children. Hence, we undertook to study the status of bone markers in children with vitamin D deficiency (VDD) and impact of vitamin D3 supplementation on them. **Materials and Methods:** Total 468 out of 615 children and adolescents with VDD, who were given either of the three doses (600, 1000, and 2000) of vitamin D supplementation, were included in the study. These 468 children with pre- and postsupplementation preserved samples with available anthropometry, serum biochemistry, 25-hydroxy-vitamin D, and parathormone were evaluated for bone formation (procollagen type 1 amino-terminal propeptide [P1NP]) and resorption (β -cross laps [CTx]) markers. **Results:** The mean age and body mass index of these children were 11.3 ± 2.3 years (boys: 11.5 ± 2.4 ; girls: 12.2 ± 1.2 years; $P = 0.03$) and 18.1 ± 3.8 kg/m² (boys: 18.2 ± 3.9 ; girls: 17.6 ± 3.2 kg/m²; $P = 0.208$), respectively. There were 8.8% subjects with severe, 42.7% with moderate, and 48.5% with mild VDD. There was a significant decline in serum P1NP (from 691 ± 233 ng/ml to 640 ± 259 ng/ml, $P < 0.001$) and CTx (from 1.67 ± 0.53 ng/ml to 1.39 ± 0.51 ng/ml, $P < 0.001$) following supplementation. Though decline in serum P1NP and CTx levels was observed in both boys and girls, among all three supplementation groups and VDD categories, the effect was more marked in serum CTx than P1NP levels. **Conclusions:** Vitamin D supplementation in VDD children resulted in decrease in both bone formation (P1NP) and resorption (CTx). The impact, however, was more marked on bone resorption than bone formation.

Keywords: β -Cross laps, bone markers, parathormone, procollagen type-1 amino-terminal propeptide, serum 25OHD, vitamin D deficiency

INTRODUCTION

Vitamin D is required by all age groups to not only maintain calcium homeostasis, bone mineralization, and skeletal health but also derive many extraskelatal benefits.^[1] Presently, vitamin D deficiency (VDD) is recognized as a global epidemic.^[2,3] Despite adequate sunshine, VDD has been reported among all age groups from India.^[4-9] It is attributed to poor sun exposure, dark skin complexion, atmospheric pollution, vegetarian foods habits, absence of food fortification with vitamin D, and poor intake of vitamin D supplements.^[4,10] There is defective bone formation and mineralization in subjects with VDD. Increasing severity of VDD results in secondary hyperparathyroidism with resultant increased formation of active vitamin D for maintaining normal serum calcium levels. This would lead to increase in the bone resorption and increase in bone resorption markers.^[11,12] Hence, it is logical to

hypothesize that vitamin D supplementation in VDD children and adolescents will result in increase in bone formation and decrease in bone resorption markers. However, the available reports in literature are conflicting. Vitamin D supplementation has been shown to decrease bone resorption markers in some studies,^[13-15] whereas one study reported decrease in both formation and resorption markers.^[16] Most studies, however, reported no change in bone resorption or formation markers with vitamin D supplementation.^[17-24] A negative association between serum 25-hydroxy-vitamin D (25OHD) and resorption markers reported among children and adolescents with VDD

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or insufficiency in cross-sectional studies,^[25-29] no longer persisted following vitamin D supplementation.^[29] Likewise, studies conducted among vitamin D sufficient adolescents did not reveal any association between serum 25OHD and bone markers.^[20-24,30]

In view of widely prevalent VDD in children and adolescents in India,^[6,7] and conflicting observations in literature with regard to relationship between vitamin D and bone markers, we undertook this study to evaluate the correlation between serum 25OHD and bone markers and impact of vitamin D supplementation on serum bone formation [procollagen type 1 amino-terminal propeptide (P1NP)] and bone resorption [β -cross laps (CTx)] markers among VDD children and adolescents.

MATERIALS AND METHODS

The present study was a subpart of another study performed in four fee paying schools in Delhi (Latitude N 28.38°, E 77.12°), India, with consent from school authorities, parents/guardians, and verbal assent from children. Parents were asked to sign the consent form after they were provided with the details of the study in the patient information sheet and their interaction with the principal investigator to clear their doubts. The details of screening, selection of subjects, and supplementation with vitamin D in the original study are shown in consort diagram [Figure 1]. Subjects suffering from any systemic illness, consuming drugs affecting bone mineral metabolism such as calcium, vitamin D, glucocorticoids, antitubercular or antiepileptics, or inability to consume vitamin D capsules orally, and having serum 25OHD >20 ng/ml were excluded from the study. Subjects were cluster randomized and divided in three groups for supplementation with soft gelatin vitamin D3 capsules (D-rise®) every day for a period of 6 months (Group “A” –600 IU, Group “B” –1000 IU, and Group “C” –2000 IU). Supplementation of vitamin D was done for six working days per week under supervision of teachers and investigating staff. For Sundays and planned school holidays, parents were provided with the vitamin D capsules to be supplemented at home and asked to maintain monthly record. The study protocol was approved by Institute Ethical committee of the institute. This trial was registered as clinical trial registration number: CTRI: 2017/01/007681.

Anthropometry measurements such as height, weight, and body mass index (BMI) were noted at baseline. Height was measured to the nearest 0.1 cm using portable wall mounted stadiometer (Holten’s Stadiometer, 200 cm/78 in, Model WS045, Narang Medical Limited, Delhi, India) with subjects standing straight with head held in the Frankfurt plane. Weight, without shoes and light clothes on, was measured to the nearest 0.1 kg, using an electronic scale (EQUINOX Digital weighing machine, Model EB6171, Equinox Overseas Private Limited, New Delhi, India). BMI was defined as the ratio of body weight-to-height square and was expressed in kg/m². Weight categories were defined by revised criteria by Indian

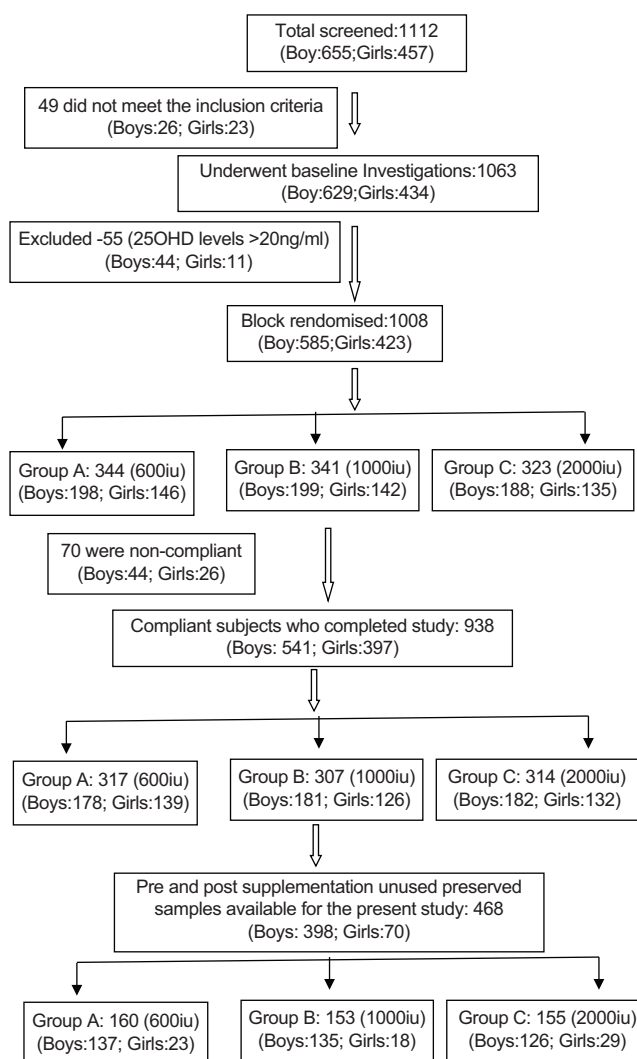


Figure 1: Consort flow diagram

Association of Pediatrics. Participants above adult equivalent of BMI of 23 were defined as overweight and those above adult equivalent of BMI of 27 were defined as obese.^[31]

Blood samples were collected in the fasting state between 08:00 h and 09:00 h, centrifuged and serum separated into four aliquots at the study site, and transported in dry ice (–60°C) to the laboratory where they were stored in –20°C. Serum calcium, phosphorus, and alkaline phosphatase (ALP) were estimated on the same day, whereas serum 25OHD and parathormone (PTH) were estimated within 1 week of sample collection. Total 468 subjects, whose untouched baseline and postsupplementation samples were available for measurement of serum P1NP and CTx later, were included in the study. Serum calcium, serum phosphate, and ALP were measured by commercially available kit using automated biochemistry analyzer Cobas C-501 (Roche Diagnostics, Mannheim, Germany). The normal range for serum total calcium for 2–12 years was 8.8–10.8 and 8.4–10.5 mg/dl for 12–18-year-old children with analytical sensitivity 0.2 mg/dl, inorganic phosphorus was 3.1–5.3 mg/dl in 7–12-year-old and 2.8–4.8 in 13–16-year-old children with

analytical sensitivity 0.3 mg/dl, and ALP for 10–<13 years: 129–417 U/l, 13–<15 years: 57–254 U/l, 15–<18 years: 50–117 U/l for girls; and 10–<13 years: 129–417 U/l, 13–<15 years: 116–468 U/l, 15–<18 years: 82–331 U/l for boys with analytical sensitivity 5 U/l. Serum P1NP (reference range: 5–1200 ng/ml), CTx (reference range: 0.01–6.0 ng/ml), and PTH (reference range: 10–65 pg/ml, interquartile range 34.31 pg/ml) were measured by commercially available kits (Roche Diagnostics GmbH, Sandhofer Strasse 116, D-68305, Mannheim, Germany). Intra- and interassay coefficient of variations were <5% for all assay. The serum 25OHD was assayed using chemiluminescence method (Diasorin, Stillwater, MN, USA). Intra- and interassay coefficient of variation was 3.5% and 5% for serum 25OHD. Serum 25OHD level of <20 ng/ml was defined as VDD.^[32] VDD was further classified as severe (25OHD <5 ng/ml), moderate (25OHD <10 ng/ml), and mild (25OHD <20 ng/ml).^[33] Blood samples were repeated 6 months after intervention.

Statistical analysis

Analysis was performed using SPSS 20.0 (Chicago, IL, USA). Descriptive statistics were calculated as mean and standard deviations (95% confidence interval). Differences among various parameters between genders were analyzed by Student's *t* test. Analysis of variance was used to study the difference in the mean of various parameters, among other groups. Paired *t* test was applied to calculate significance level of various parameters pre- and postsupplementation. Serum PTH was not normally distributed, hence expressed as median and range, and was analyzed with Mann–Whitney, Kruskal–Wallis, and Wilcoxon signed-rank test. Bonferroni correction was used to assess significance of bone markers between quartiles of PTH. Pearson's correlation was used to evaluate relation between various parameters and serum P1NP and CTx levels. A *P* < 0.05 was considered statistically significant.

RESULTS

The baseline demographic and biochemical characteristics of 468 study subjects as per gender and VDD categories are shown in Tables 1 and 2, respectively. The mean age and BMI of the children were 11.3 ± 2.3 years (boys: 11.5 ± 2.4; girls: 12.2 ± 1.2 years; *P* = 0.03) and 18.1 ± 3.8 kg/m² (boys: 18.2 ± 3.9; girls: 17.6 ± 3.2 kg/m²; *P* = 0.208), respectively. Boys had significantly higher BMI, serum calcium, phosphates, and CTx levels whereas girls had higher serum ALP and PTH levels [Table 1]. Fifty-three participants (11.3%) were obese [boys: 67 (11.5%); girls: 20 (4.7%)] and 88 (18.8%) were overweight [boys: 113 (19.3%); girls: 74 (17.5%)]. There were 8.8% (41) participants with severe, 42.7% (200) with moderate, and 48.5% (227) with mild VDD. There was no significant difference in various parameters except BMI among the three VDD groups [Table 2].

Serum P1NP status

A significant overall decrease in mean baseline serum P1NP level from 691 ± 233 to 640 ± 259 ng/ml (paired *t* test < 0.0001)

Table 1: Basic characteristics of study population according to gender

Parameters	Boys	Girls	<i>P</i>
Number	398 (85%)	70 (15%)	
Age (years)	11.5±2.4	12.2±1.2	0.030
Height (m)	1.44±0.14	1.47±0.09	0.047
Weight (kg)	38.6±14.2	38.1±9.0	0.753
BMI (kg/m ²)	18.2±3.9	17.6±3.2	0.208
S. calcium (mg/dl)	9.9±0.4	9.7±0.6	<0.0001
S. phosphates (mg/dl)	4.8±0.5	4.5±0.7	<0.0001
S. ALP (U/l)	261±83	292±128	0.010
25OHD (ng/ml)	10.6±3.8	11.1±3.6	0.289
S. PTH (pg/ml) [#]	52±26 (46, 13-246)	68±33 (72, 13-261)	0.003
Serum P1NP (ng/ml)	689±225	701±278	0.685
Serum CTx (ng/ml)	1.70±0.51	1.47±0.60	0.001

[#]Serum PTH values (median, range) analyzed by Mann-Whitney test

was noted following supplementation [Table 3]. This decrease was observed in both boys (689 ± 225 vs. 646 ± 250 ng/ml; *P* = 0.003) and girls (701 ± 278 vs. 607 ± 300 ng/ml *P* < 0.001), different supplementation groups, and all three VDD categories except in mild VDD category where it did not achieve statistical significance [Table 4]. Similar decline in serum P1NP was also observed in all weight categories postsupplementation, but statistical significance was achieved in only normal weight children.

Baseline and postsupplementation values of serum P1NP within the group were no different in boys and girls, different weight categories, supplementation groups, and vitamin D deficient categories [Table 4]. Significant incremental response in serum P1NP levels with increasing serum PTH values from 1st to 4th PTH quartiles was noted both at baseline and postsupplementation. However, significant decline in serum P1NP following supplementation was limited to 1st to 4th PTH quartiles only [Table 5 and Figure 2].

Serum CTx status

The overall mean baseline serum CTx (1.67 ± 0.53 ng/ml) decreased significantly following supplementation (1.39 ± 0.50 ng/ml, *P* < 0.001). This significant reduction in serum CTx was observed only in boys (1.70 ± 0.51 vs. 1.38 ± 0.51 ng/ml, *P* < 0.0001) but not in girls (1.47 ± 0.60 vs. 1.40 ± 0.55 ng/ml, *P* = 0.273), all weight categories, supplementation groups, and VDD categories in paired analysis [Table 4].

No intragroup differences were observed in the baseline and postsupplementation values of serum CTx among different supplementation groups and VDD categories. Similarly, no significant difference was noted in different weight categories in the presupplementation values, but a significant difference did show up between normal weight and obese/overweight subjects following supplementation [Table 4, *P* < 0.007].

Unlike P1NP, serum CTx values did not differ among different quartiles of PTH at baseline, but the values showed significant incremental increase from 1st to 4th quartile following supplementation. The serum CTx values were significantly

Table 2: Basic characteristics of study population according to vitamin D status

Parameters	Severe VDD (25OHD ≤5 ng/ml)	Moderate VDD (25OHD >5-≤10 ng/ml)	Mild VDD (25OHD >10-≤20 ng/ml)	P for trend
Number	41 (8.8%)	200 (42.7%)	227 (48.5%)	
Age (years)	11.5±2.8 (10.6-12.3)	11.5±2.4 (11.2-11.9)	11.7±2.1 (11.4-12.0)	0.622
Height (m)	1.46±0.16 (1.40-1.51)	1.44±0.15 (1.42-1.46)	1.44±0.13 (1.42-1.46)	0.729
Weight (kg)	41.6±19.1 (35.6-47.6)	39.6±14.4 (37.6-41.6)	37.1±11.3 (35.6-38.6)	0.056
BMI (kg/m ²)	18.8±5.4 (17.1-20.5)	18.6±4.0 (18.0-19.1)	17.6±3.1 (17.2-18.0)	0.014
S. calcium (mg/dl)	9.8±0.4 (9.7-9.9)	9.8±0.5 (9.8-9.9)	9.8±0.5 (9.8-9.9)	0.819
S. phosphates (mg/dl)	4.8±0.5 (4.6-4.9)	4.8±0.6 (4.7-4.8)	4.7±0.6 (4.6-4.8)	0.802
S. ALP (U/l)	259±67 (238-280)	268±100 (254-282)	267±88 (253-276)	0.822
25OHD (ng/ml)	4.4±0.5 (4.2-4.6)	8.3±1.3 (8.1-8.4)	13.9±2.8 (13.6-14.2)	<0.0001
S. PTH (pg/ml) [#]	70±34 (69, 19-214)	59±32 (51, 17-261)	50±21 (45, 13-123)	<0.0001
Serum PINP (ng/ml)	738±221 (668-808)	693±227 (662-725)	680±239 (648-711)	0.329
Serum CTx (ng/ml)	1.68±0.48 (1.53-1.83)	1.66±0.51 (1.58-1.73)	1.67±0.56 (1.60-1.75)	0.927

[#]Serum PTH values (median, range) analyzed by Kruskal-Wallis test

Table 3: Changes in parameters pre- and post-vitamin D supplementation

Parameters	Presupplementation	Postsupplementation	Paired t-test
Height (m)	1.44±0.14	1.47±0.14	<0.0001
Weight (kg)	38.6±13.5	40.8±13.8	<0.0001
BMI (kg/m ²)	18.1±3.8	18.5±3.9	<0.0001
S. calcium (mg/dl)	9.8±0.5	9.8±0.5	0.064
S. phosphates (mg/dl)	4.7±0.6	4.9±0.6	<0.0001
S. ALP (U/l)	265±92	260±82	0.087
25OHD (ng/ml)	10.7±3.8	29.5±10.0	<0.0001
S. PTH (pg/ml) [#]	49 (13-261)	35 (10-147)	<0.0001
PINP (ng/ml)	691±233	640±259	<0.0001
CTx (ng/ml)	1.67±0.53	1.39±0.51	<0.0001

[#]Serum PTH values expressed as median (range) and analyzed by Wilcoxon signed-rank test

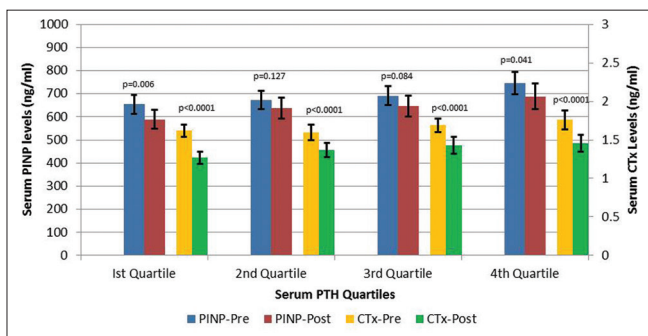


Figure 2: Bone formation (PINP) and bone resorption marker (CTx) (Pre- and post vitamin D supplementation) according to serum parathormone quartiles

higher in the 4th quartile of PTH when compared to the 1st quartile [Table 5 and Figure 2].

Correlation between serum 25OHD and bone markers

Baseline serum PINP levels were positively correlated with serum phosphates ($r = 0.271$, $P < 0.0001$), ALP ($r = 0.364$, $P < 0.0001$), PTH ($r = 0.271$, $P < 0.0001$), and CTx ($r = 0.465$, $P < 0.0001$) but not with age ($r = -0.036$, $P = 0.434$), BMI ($r = -0.090$, $P = 0.052$), serum calcium ($r = -0.058$, $P = 0.207$), and baseline 25OHD ($r = -0.089$, $P = 0.055$).

Baseline serum CTx levels were also positively correlated with age ($r = 0.096$, $P = 0.038$), serum phosphates ($r = 0.270$, $P < 0.0001$), ALP ($r = 0.233$, $P < 0.0001$), PTH ($r = 0.163$, $P < 0.0001$), and PINP ($r = 0.465$, $P < 0.0001$). It was not correlated with BMI ($r = -0.026$, $P = 0.570$), calcium ($r = -0.093$, $P = 0.055$), and baseline 25OHD ($r = -0.025$, $P = 0.585$).

Postsupplementation serum PINP and CTx levels maintained similar correlations in above mentioned parameters except for BMI which now had acquired a significant negative correlation with serum PINP ($r = -0.109$, $P = 0.018$) and CTx ($r = -0.1146$, $P = 0.002$) levels.

DISCUSSION

Vitamin D is an essential micronutrient not only for bone health but also for normal growth and development in children and adolescents. Its deficiency causes rickets in children, which is characterized by defective bone formation and mineralization and increased bone resorption due to secondary hyperparathyroidism required to maintain calcium homeostasis. With this background, it is reasonable to assume that vitamin D supplementation in VDD should affect both bone formation and resorption. Impact of supplementation on

Table 4: Effect of vitamin D supplementation on serum levels of P1NP and CTx in various groups

Bone markers				P for trend
Vitamin D supplementation	600 IU (A)	1000 IU (B)	2000 IU (C)	
Number	n=160	n=153	n=155	
Serum P1NP (ng/ml)				
Baseline	688±231 (652-724)	697±215 (663-732)	687±254 (647-727)	0.913
Postsupplementation	643±250 (603-681)	637±248 (597-676)	642±278 (597-686)	0.978
Paired P	0.028	0.005	0.048	
Serum CTx (ng/ml)				
Baseline	1.66±0.49 (1.58-1.73)	1.64±0.51 (1.55-1.72)	1.70±0.58 (1.61-1.80)	0.512
Postsupplementation	1.37±0.52 (1.29-1.45)	1.36±0.50 (1.28-1.44)	1.41±0.53 (1.33-1.50)	0.622
Paired P	<0.0001	<0.0001	<0.0001	
VDD categories				
	Severe VDD (25OHD ≤5 ng/ml)	Moderate VDD (25OHD >5-≤10 ng/ml)	Mild VDD (25OHD >10-≤20 ng/ml)	
Number	n=41	n=200	n=227	
Serum P1NP (ng/ml)				
Baseline	688±231 (652-724)	697±215 (663-732)	687±254 (647-727)	0.913
Postsupplementation	623±251 (544-702)	635±250 (600-670)	648±269 (613-683)	0.798
Paired P	0.009	0.002	0.074	
Serum CTx (ng/ml)				
Baseline	1.68±0.48 (1.53-1.83)	1.66±0.51 (1.58-1.73)	1.67±0.56 (1.60-1.75)	0.927
Postsupplementation	1.32±0.48 (1.17-1.47)	1.39±0.55 (1.31-1.47)	1.39±0.50 (1.32-1.45)	0.686
Paired P	0.001	<0.0001	<0.0001	
Weight categories				
	Normal	Overweight	Obesity	
Number	n=327	n=88	n=53	
Serum P1NP (ng/ml)				
Baseline	702±234 (676-727)	666±235 (648-711)	664±223 (602-725)	0.291
Postsupplementation	650±261 (621-678)	618±257 (563-672)	619±245 (552-687)	0.483
Paired P	0.001	0.092	0.238	
Serum CTx (ng/ml)				
Baseline	1.68±0.55 (1.58-1.73)	1.63±0.50 (1.55-1.72)	1.66±0.44 (1.61-1.80)	0.715
Postsupplementation	1.43±0.50 (1.38-1.49)	1.25±0.53 (1.14-1.36)	1.30±0.53 (1.16-1.49)	0.007
Paired P	<0.0001	<0.0001	<0.0001	

Table 5: Bone markers according to serum PTH quartiles

Parameters	1st Quartile ≤36.5 pg/ml)	2nd Quartile >36.5-48.7 pg/ml)	3rd Quartile >48.7-67.8 pg/ml)	4th Quartile >67.8 pg/ml)	P for trend
Number	117	118	116	117	
Serum P1NP (ng/ml)					
Baseline	654±222 (613-695)	672±221 (632-713)	691±222 (650-732)	746±259* (698-793)	0.018
Postsupplementation	589±216 (550-629)	637±256 (591-684)	647±248 (601-692)	688±301* (632-743)	0.035
Paired P	0.006	0.127	0.084	0.041	
Serum CTx (ng/ml)					
Baseline	1.62±0.44 (1.54-1.70)	1.60±0.53 (1.50-1.69)	1.69±0.49 (1.60-1.78)	1.76±0.63 (1.64-1.88)	0.081
Postsupplementation	1.27±0.41 (1.20-1.35)	1.37±0.49 (1.28-1.46)	1.43±0.56 (1.33-1.54)	1.46±0.57* (1.35-1.56)	0.028
Paired P	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

*The differences between 4th quartile and 1st quartile were significant after Bonferroni correction - Baseline P1NP ($P=0.003$), postsupplementation P1NP ($P=0.004$), and CTx ($P=0.034$)

bone turn over can be seen early by evaluating bone formation and resorption markers, but its effect on bone density and fracture rate would require a large, long-term population-based study.^[18] The effect of vitamin D3 supplementation on bone markers in children, however, has not been well studied.

Significant decrease in serum P1NP, observed in boys and girls following vitamin D supplementation, was also reported in another study involving VDD youths with HIV, who were supplemented 120,000 IU/month for 12 months.^[16] In contrast, vitamin D supplementation among 14 children with rickets

resulted in a transient rise initially (2–4 weeks) followed by reduction in serum P1NP with continued supplementation.^[15] Most studies, however, have reported no effect on bone formation markers (bone-specific ALP, serum osteocalcin, serum P1NP) following vitamin D supplementation.^[13-14,17-25] Absence of any impact on serum P1NP may be either due to smaller supplemental doses (100–600 IU) of vitamin D^[17,20-24], shorter duration of supplementation (4–12 weeks),^[18,20,23,24] or higher baseline serum 25OHD levels^[14,18,21-24] before starting supplementation, when compared with the present study.

The decrease in serum P1NP was significant in all three supplementation groups and VDD categories except in mild category. This can be explained based on relationship of serum P1NP with serum PTH levels. There were increasing levels of serum P1NP from 1st to 4th quartile of PTH along with a strong positive correlation with PTH ($r = 0.271$, $P < 0.0001$). Though there was a parallel decrease in serum P1NP and PTH following supplementation, decrease in serum P1NP levels was significant only in the 1st and 4th quartiles of PTH. This suggests that bone remodeling requires optimal levels of serum PTH. A low level of PTH may be associated with low bone formation and high levels of PTH with high bone turnover. Hence, suppression of PTH with vitamin D supplementation is associated with decrease in bone formation in 1st PTH quartile and decrease in bone turnover with decrease in serum PTH in 4th quartile. This would also explain absence of significant change in serum P1NP in mild VDD group (serum PTH range 12.6–122.8 with mean on 49.8 ± 21.5 pg/ml), which is associated with PTH levels in optimal range. Absence of correlation between serum P1NP and baseline serum 25OHD, observed in the present study, was also reported in a German and Swiss adolescents.^[25,30]

The significant decrease in serum CTx was observed in all three supplementation groups, VDD categories, and weight categories. Viljakainen *et al.*^[14] similarly reported decrease in bone resorption marker (urinary deoxypyridinoline) following vitamin D supplementation for 1 year in 212 girls. A decrease in bone resorption markers was also observed in young jockeys^[13] and adolescents with HIV.^[16] Barocelli *et al.*,^[15] in contrast, described a transient rise in bone resorption markers [cross-linked carboxyterminal telopeptide of type I collagen and cross-linked *N*-telopeptides of type I collagen (NTX)] in 14 children with rickets after 2–4 weeks of vitamin D supplementation, followed by a gradual decline with continued supplementation. Most studies, however, reported no change in bone resorption markers after vitamin D supplementation.^[17-24] The explanation for no change in serum CTx is similar to what has been described for serum P1NP.

Absence of correlation between serum CTx and baseline serum 25OHD ($r = -0.025$, $P = 0.585$) also observed in one other study.^[30] This was in contrast to a large population-based study reporting an inverse correlation between serum CTx and serum 25OHD.^[25] Lack of correlation between serum CTx values and VDD categories similarly differed from the reported lower

urinary deoxypyridinoline values in Chinese girls with VDD when compared to those with normal serum 25OHD levels.^[26]

Serum PTH was a strong predictor of serum CTx ($r = 0.163$, $P < 0.0001$) and showed an incremental trend with increasing quartile of PTH in pre- and postsupplementation assessment. Rajakumar *et al.*^[27] also found PTH to be a significant predictor of serum CTx levels after adjusting for race, season, Tanner stage, dietary calcium, skin color, and BMI.

Baseline serum P1NP and CTx levels were not significantly different among weight categories. Why significant decrease in P1NP in normal weight children as against significant decline in serum CTx in overweight and obese kids, following supplementation, is not understood? On the contrary, Rajakumar *et al.*^[34] showed significant decrease in serum osteocalcin, BAP, and urine NTX in normal and obese children.

In present study, two interesting findings were (a) reduction in bone formation and resorption markers post-vitamin D supplementation, and (b) decline in bone resorption marker was significantly more than the bone formation marker in all the groups analyzed. This raises question about conventional understanding that VDD causes rickets and osteomalacia due to defective bone formation and mineralization. If it was due to defective bone formation, these markers (a) should have increased after vitamin D supplementation, and (b) baseline bone formation markers should have been lower in severe VDD compared to mild VDD subjects. The results, however, are contrary to the above assumptions. In the absence of differences in bone resorption marker among VDD categories, the decrease in serum CTx was significantly greater as compared to serum P1NP, suggesting that participants with VDD had more abnormalities of bone resorption than bone formation. Since decline in both bone formation and resorption following vitamin D supplementation suggests decrease in bone turnover, it may not be out of context to say that rickets and osteomalacia are diseases primarily of high bone turnover rather than defective bone formation. This high bone turnover is predicted by serum PTH levels rather than serum 25OHD levels. This is further corroborated by significantly high serum P1NP and serum CTx in 4th quartile of PTH when compared to other quartiles pre- and postsupplementation, and a strong positive correlation between serum P1NP and CTx ($r = 0.465$, $P < 0.0001$). Thus, it can be hypothesized that VDD is characterized by high bone turnover (bone resorption > bone formation), which manifests in the presence of high PTH levels. A similar observation had been reported by us in our previous study, where bone mineral density among adolescents became significantly lower at higher quartiles of PTH but was not affected by various levels of serum 25OHD.^[35] This study further emphasizes the fact that while defining VDD, serum PTH and/or bone markers should be taken into consideration.^[36,37]

The main strength of our study was a large cohort of Indian school children with VDD evaluated for bone turnover markers and impact of vitamin D supplementation in them. The major limitations of the study were (a) noninclusion of

control/placebo arm and (b) inability to assess their lifestyle and pubertal status due to social and administrative reasons.

CONCLUSIONS

Vitamin D supplementation leads to decrease in both bone formation (PINP) and resorption (CTX) markers in children and adolescents with VDD. This effect is seen with all doses of vitamin D supplementation (600, 1000, and 2000 IU), in all VDD and weight categories. The effect is more marked on bone resorption when compared to bone formation. This suggests that VDD is characterized by bone resorption more than bone formation, which is akin to postmenopausal osteoporosis. Hence, chronic VDD in children and adolescents may predispose them to risk of osteoporosis at an early age.^[38,39]

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Conflicts of interest

There are no conflicts of interest.

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