RESEARCH PAPER

# Effect of Vitamin D and Calcium Supplementation on Bone Mineral Content in Children with Thalassemia

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**Objective**: To evaluate the effect of vitamin D and calcium supplementation for osteoprotection in thalassemia. **Methods**: 29 children (age 2-12 y) were supplemented with oral vitamin D (1000 IU/d) and calcium (500 mg/d) for 1 year. The dual energy *X*-ray absorptiometry (DXA) was done to assess bone mineral content at baseline and 12 months. Serum 25-hydroxy vitamin D, intact parathyroid hormone, osteocalcin, calcium, phosphate, alkaline phosphatase, and spot urine deoxypyridinoline (DPD)/creatinine were done at baseline, 6 months and 12 months. **Results**: The mean (SD) bone mineral content increased from baseline value of 8.4 (2.8) g to 10.8 (3.5) g ($P$<0.001). The mean (SD) vitamin D level increased from baseline value of 16.0 (5.8) ng/mL to 23.4 (6.6) ng/mL ($P$<0.001). The change in serum osteocalcin and spot urine DPD/creatinine ratio were not significant ($P$=0.062). **Conclusions**: Oral vitamin D and calcium supplementation increases bone mineral content in children with thalassemia.

**Keywords**: *Absorptiometry, Bone density, Cholecalciferol, Prevention.*

Children with transfusion-dependent beta thalassemia have poor bone health inspite of optimal management. The frequency of osteoporosis even in well-treated thalassemia patients is 40-80% [1-3]. The pathogenesis of low bone mass is multi-factorial, including progressive marrow expansion, chronic hypoxia, direct iron toxicity, the effect of iron chelators, endocrinopathies, and nutritional deficiency [4-6]. Vitamin D deficiency is also an important cause of osteoporosis in these children [6]. Though vitamin D deficiency and reduced bone mineral content (BMC) have been reported in various studies [3,4,7], the exact role of vitamin D and calcium supplementation for osteoprotection in thalassemia has not been adequately evaluated.

## METHODS

This study was conducted in a tertiary institute in Puducherry, India, between September 2015 and February 2017. The study was approved by Institute Ethics Committee (Human studies). Children aged 2-12 years with thalassemia major or intermedia were included in the study. Children with hypocalcemic seizures, tetany, rickets, renal stones, chronic kidney disease, chronic liver disease and those receiving phenytoin, immuno-suppressants or furosemide were excluded. Assuming an intervention-attributable change in BMC of 20% with 95% confidence, the minimal sample size required was 24. Accounting for 20% attrition, the sample size was decided as 30**.**

The BMC and bone mineral density (BMD) were estimated at lumbar spine (L1-L4) at enrolment and after one year of vitamin D and calcium supplementation using Dual-Energy *X*-Ray Absorptiometry (DXA) scanner (bone densitometer, Discovery Wi, Hologic Company, USA). The instrument was calibrated with phantoms supplied by the manufacturer and the precision of measurement was within 1%.

Serum 25-hydroxy vitamin D 25(OH)D, intact parathyroid hormone (iPTH), calcium, phosphate, alkaline phosphatase (ALP), albumin, osteocalcin, and spot urine deoxypyridinoline (DPD)/creatinine ratio were measured at enrolment, 6 months and 12 months. Serum 25(OH)D and iPTH were estimated by chemiluminescence method (ADVIA Centaur). Serum osteocalcin and urine DPD were also estimated by chemiluminescence (IMMULITE 1000). The reportable range, assay sensitivity, intra-assay and inter-assay co-efficient of variation of 25(OH)D, iPTH, serum osteocalcin and urine DPD are presented in ***Table*** **I**.

Children with baseline serum 25(OH)D level below 20 ng/mL were treated with oral vitamin D 2000 IU/day for initial 6 weeks followed by 1000 IU/day for remaining period of study. Children with baseline 25(OH)D level

**TABLE I** PERFORMANCE CHARACTERISTICS OF SERUM 25 OH VITAMIN D, iPTH, OSTEOCALCIN AND URINE DPD

| *Parameter* | *Reportable range* | *Assay sensitivity* | *Intra-assay CV* | *Inter-assay CV* |
|---|---|---|---|---|
| Serum 25(OH) vitamin D (mg/mL) | 4.5-150 | 4.2 | < 7% | < 11% |
| Serum iPTH; pg/mL | 4.6-2000 | 4.6 | < 5% | < 7% |
| Serum Osteocalcin; ng/mL | 2-200 | 0.55 | < 4% | < 10% |
| Urine DPD; nmoL | 7-300 | 6 | < 15% | <20 % |

*25(OH)D: 25-hydroxy vitamin D; CV: coefficient of variation; iPTH: intact parathyroid hormone; DPD: deoxypyridinoline.*

above 20 ng/mL received vitamin D 1000 IU/day for a period of 12 months [8]. All the children in the study group received 500 mg calcium carbonate for a period of 12 months. All enrolled children were monitored monthly and received standard thalassemia care, including blood transfusion, chelation therapy and growth monitoring. We calculated height-for-age Z score (HAZ) for BMC for children in the 5 to 12 years age group using online calculator (*https://zscore.research.chop.edu/bmd Calcula tor.php*). Urine calcium creatinine ratio was monitored monthly to avoid iatrogenic hypercalcemia. For children aged above 5 years, thyroid function test and fasting blood sugar were also done.

*Statistical analysis*: Data were expressed as mean and standard deviation. Paired Student *t* test was used for outcome variables before and after intervention. Proportions were compared using Chi-square test. Data were analyzed using SPSS version 20.0 (SPSS, Inc., Chicago). *P* value <0.05 was considered as significant.

## RESULTS

Thirty children with thalassemia were assessed for eligibility and enrolled by consecutive sampling. One child was lost to follow-up, and was excluded from analysis. Remaining 29 patients (19 boys) were followed up monthly for a period of one year. Mean (SD) age at enrollment was 5 (1.4) years. Seen (24%) patients were not receiving iron chelation, 17 (59%) were receiving deferasirox monotherapy and 5 (17%) were receiving deferasirox and deferiprone combination therapy. Six (21%) patients had serum ferritin <500 ng/mL, 10 (34%) patients had ferritin level between 501-1650 ng/mL, and 13 (45%) patients had values >1650 ng/mL. No patient had increased calcium creatinine ratio. None of the included children had hypothyroidism or diabeties mellitus.

The changes in bone health parameters and anthropometry are presented in ***Table* II**. At baseline, low HAZ for BMC (Z score ≥2) was seen in 11 (68%) children, and after one year only one child had low HAZ. Hypoparathyroidism (iPTH <10 pg/mL) was seen in one child at baseline that improved by the end of study. Hyperparathyroidism (>65 pg/mL) was seen in four children at baseline and end of study. None of the patients developed hypercalciuria or hypercalcemia during the course of the study.

The factors like age, gender, anthropometry, duration of disease, number of previous transfusions, serum ferritin, calcium profile, iPTH, 25-OH Vitamin D and bone turn-over markers had no significant association with the bone health status.

## DISCUSSION

Children with thalassemia are at risk for osteoporosis, and any measure to improve bone health will prevent fracture and other related complications. This study showed that vitamin D and calcium supplementation significantly improve BMC and BMD as well as serum 25(OH)D and calcium levels, without any adverse effects.

This study displayed 69% prevalence of low bone mass, as defined as BMC Z-score ≥ –2 [9,10], as compared to 55-62% in previous studies [2,3]. The difference may be due to decreased prevalence of malnutrition and better thalassemia care in high-income countries. The treatment of low bone mass in thalassemia patients was studied in many interventional studies predominantly using bisphosphonates, along with calcium and vitamin D, which showed positive effect on bone health [11,12]. In our study, we supplemented only with oral vitamin D and calcium, which may be a safer and cheaper strategy.

A high frequency of hypovitaminasis D in children with thalassemia can be explained by liver iron deposition, decreased sunlight exposure and less physical activity due to disease burden [13]. Though bone turnover markers are extremely useful in the management of osteoporosis [14], we did not find any significant reduction in these markers. This may be related to inadequate sample size of our study for these parameters.

A potential limitation of this study is absence of unsupplemented controls. However, as proportion of children with low BMC and BMD- Z score (adjusted to

**WHAT THIS STUDY ADDS?**

- Calcium and vitamin D supplementation over a period of one year improves the vitamin D level and bone mineral content in children with thalassemia.

**TABLE II** CHANGE IN BONE HEALTH PARAMETERS

| *Parameter* | *Baseline (n=29) Mean (SD)* | *After one year (n=29) Mean (SD)* | *P value* |
|---|---|---|---|
| Low weight for age(Z <-2), *n* (%) | 16 (55%) | 13 (45%) | 0.599 |
| Low height for age (Z <-2), *n* (%) | 14 (48%) | 15 (52%) | 0.792 |
| BMC (g) | 8.4 (2.8) | 10.8 (3.5) | <0.001 |
| BMD (g/cm$^2$) | 0.4 (0.0) | 0.43 (0.0) | <0.001 |
| *Low BMC (Z ≤ -2), *n* (%) | 20 (69%) | 11 (38%) | 0.034 |
| *Low BMD (Z ≤-2), n (%) | 20 (69%) | 11 (38%) | 0.034 |
| Serum 25-OH vitamin D (ng/mL) | 16.0 (5.8) | 23.4 (6.6) | <0.001 |
| Vitamin D deficiency (12-20 ng/mL), *n* (%) | 12 (41%) | 07(24%) | <0.001 |
| Vitamin D insufficiency (<12.0ng/mL), *n* (%) | 09 (31%) | 0 (0%) | <0.001 |
| Serum Osteocalcin (ng/mL) | 13.0 (6.7) | 10.3 (7.5) | 0.062 |
| Spot Urine DPD $^{\#}$/creatinine ratio (nmol DPD $^{\#}$/ mmol Cr) | 5.0 (0.9) | 4.9 (1.2) | 0.614 |
| Serum Albumin Corrected Calcium (mg/dL) | 8.9 (0.8) | 9.6 (0.7) | <0.001 |
| Serum Phosphate (mg/dL) | 4.8 (1.2) | 5.4 (0.7) | 0.027 |
| Serum iPTH (pg/mL) | 37.3 (19.5) | 42.0 (18.3) | 0.216 |
| Serum ALP (IU/L)$^{\#}$ | 436 (228) | 400 (141) | 0.376 |

*BMC: bone mineral content; BMD: bone mineral density; DPD: deoxypyridinoline; iPTH: intact parathyroid hormone; ALP: alkaline phosphatase; *Adjusted for height.*

age and height) decreased significantly with supplementation, it is less likely that the rise in BMC is entirely due to growth related mineralization. Even at the end of study, many children included in this study still had low bone mass and vitamin D insufficiency, and hence further controlled trials are required to optimize the dose of vitamin D and calcium supplementation for osteoprotection effect in children with thalassemia.

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