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Research Article

Bone turnover, areal BMD, and bone microarchitecture by second-generation high-resolution peripheral quantitative computed tomography in transfusion-dependent thalassemia

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Abstract

Thalassemic osteopathy includes low bone mass and impaired bone microarchitecture. We aimed to evaluate the prevalence and determinants of bone quantity (osteoporosis) and quality (microarchitecture) in a cohort of adult patients with transfusion-dependent thalassemia (TDT). Patients with TDT ($n = 63$) and age- and BMI-matched controls ($n = 63$) were recruited in the study. Areal bone mineral density (BMD) was measured using DXA Hologic scanner. P1NP and β-CTX were estimated by electrochemiluminescence assay. Bone geometry and volumetric BMD (vBMD) were estimated by second-generation high-resolution peripheral quantitative computed tomography. Bone turnover marker β-CTX was significantly lower in the TDT group, but there was no difference in P1NP levels. Low bone mass ($Z \leq -2$) was present in greater proportion of patients both at lumbar spine (LS) (54 vs 0%; $p = .001$) and femoral neck (FN) (33 vs 8%; $p = .001$). Hypogonadism was associated with low BMD at FN (OR 10.0; 95% CI, 1.2–86; $p = .01$) and low hemoglobin with low BMD at LS (OR 1.58; 95% CI, 0.96–2.60; $p = .07$). The mean trabecular bone score was also significantly lower in patients compared with controls (1.261 ± 0.072 vs 1.389 ± 0.058). Total, cortical and trabecular vBMD were significantly lower in cases than controls. The trabecular number and cortical thickness were significantly lower and trabecular separation higher in cases than controls. Adults with TDT have significantly lower areal, cortical and trabecular vBMD. The bone microarchitecture is also significantly impaired in terms of lower number and wider spacing of trabeculae as well as lower cortical thickness and area at both radius and tibia.

Keywords: thalassemia, HR-pQCT, osteoporosis, ferritin, iron overload, bone microarchitecture

Lay Summary

Bone quantity and quality are likely to be affected in patients with thalassemia who are dependent on regular blood transfusions. This study evaluated bone health in adults with transfusion-dependent thalassemia (TDT) by comparing bone density and microarchitecture with age- and BMI-matched controls. Adults with TDT had significantly lower bone mineral density (both areal and volumetric), suggesting low bone quantity compared to healthy controls. Trabecular and cortical microarchitecture were also adversely affected, suggesting impaired bone quality. Overall, the study highlights that adults with TDT have significantly compromised bone quantity and quality, increasing their risk for osteoporosis and related fractures.

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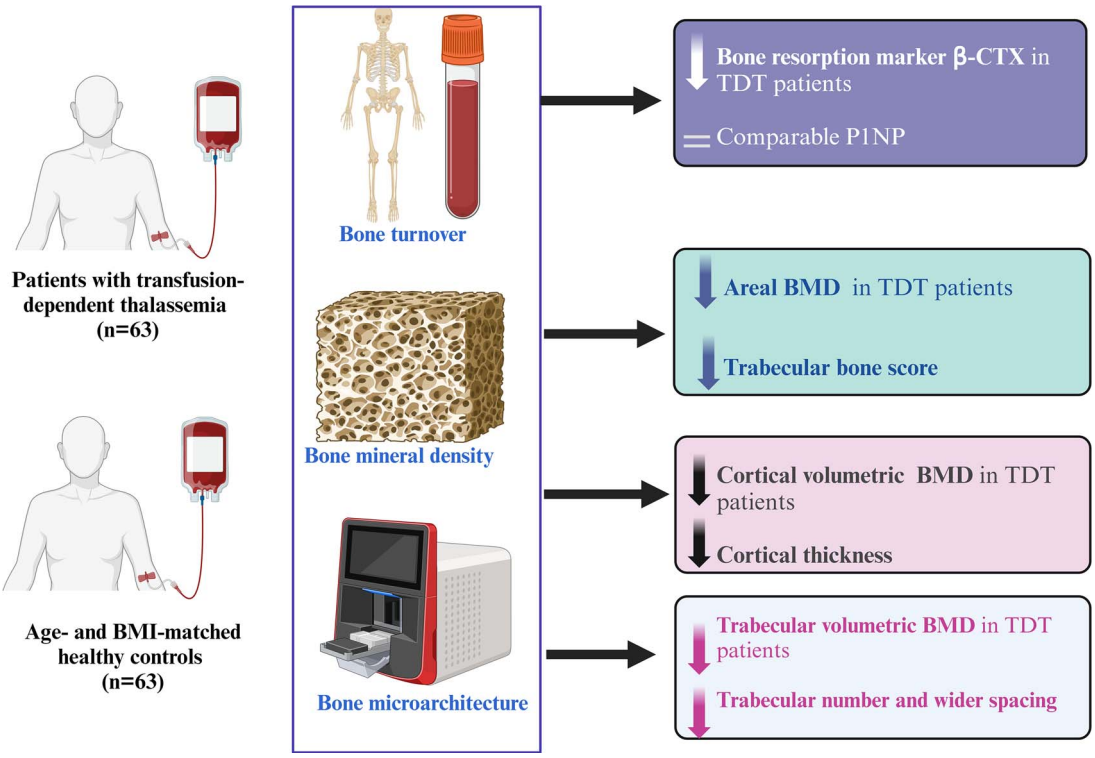
Graphical Abstract



Introduction

Thalassemia refers to a group of inherited hemoglobinopathies arising due to anomalous synthesis of the hemoglobin molecule. ß-thalassaemia is characterized by reduction in one or more ß-globin chains, which leads to defective haemoglobin production and damage to red cells from the effects of alpha (α)-globin subunits that are produced in excess. Nearly 1% to 1.5% of the world's population is affected by ß-thalassaemia (affected or carrier).[1] India contributes to a nearly 25% of the global thalassemia burden,[2] translating to an overall estimated prevalence of 100 000 patients diagnosed with β-thalassemia.[2] Depending on the severity of ß-globin chain deficiency, the ß-thalassemias are classified as thalassemia major (most severe and requiring regular blood transfusions), intermedia (milder form, requiring fewer transfusions), or minor (heterozygous carrier which is almost always clinically silent).

Thalassemia-related bone disease, otherwise called thalassemic osteopathy is an entity that includes low bone mass, fractures and impaired bone microarchitecture. It is an important contributor to morbidity in patients with transfusion-dependent thalassemia (TDT), despite the advances in transfusion and chelation strategies. The prevalence of low bone mass and osteoporosis is variably reported in different studies, ranging from 50% to 74% at lumbar spine and 10% to 38% at femoral neck.[3–6]

Various factors are attributed as causative for the high prevalence of low bone mass and osteoporosis in these individuals. Both disease and therapy related factors are reportedly associated with poor bone mass in TDT. Firstly, peak bone mass acquisition is poor in these patients.[7,8] Secondly, there is the adverse impact of chronic anemia, bone marrow expansion due to ineffective erythropoiesis and iron overload on bone turnover, mass and microarchitecture.[7,9] Further, there are issues pertaining to calcium absorption as well as multiple endocrinopathies, especially hypogonadism, growth hormone deficiency and diabetes mellitus, all of which compound the problem.[10,11] Alterations in the receptor activator of nuclear factor kappa-ß (RANK)/RANK ligand/osteoprotegerin and the Wnt/β-catenin systems as well as genetic factors (collagen type 1 alpha 1, and vitamin D receptor gene polymorphisms) have also been reported.[12]

Osteoporosis, by definition, entails not only reduced bone mineral density but also impaired bone microarchitecture. However, there is scant evidence on the bone microarchitecture in patients with TDT. This is nevertheless important to not only provide mechanistic insights into thalassemic osteopathy but also optimize therapeutic options. The current study was designed to evaluate the prevalence and determinants of low bone mass, osteoporosis and microarchitectural variables using high-resolution peripheral quantitative computed tomography (HR-pQCT).

Materials and methods

The study was conducted at a single tertiary care center in India. Consecutive patients with thalassemia ($n = 63$) were recruited from the Adult Thalassemia Clinic, Department of Clinical Hematology & Medical Oncology and age- and BMI-matched controls ($n = 63$) were recruited from the outpatient clinic, department of Endocrinology. All patients were transfusion-dependent and had ß-thalassaemia major.

Clinical and demographic variables included age at diagnosis (initiation of blood transfusion), age at initiation of chelation therapy, and average annual blood transfusions. Biochemical measurements related to bone and mineral

metabolism (including serum intact PTH), liver and renal function tests and ferritin were performed in a fasting plasma sample (0800 h-0900 h) just prior to the scheduled blood transfusion. Bone turnover markers including P1NP (Procollagen 1 Intact N-Terminal Propeptide) and β-CTX (β C-terminal telopeptide), were estimated using electrochemiluminescence (ECLIA) (eCOBAS 8000, Roche diagnostics). Serum ferritin was measured by ECLIA (ecobas e601, Roche diagnostics.

Bone mineral density (BMD), T- and Z- scores were measured at lumbar spine (LS), femoral neck (FN) and distal end of radius (DER) using the DXA Hologic scanner (HOLOGIC Discovery A, QDR 4500; Hologic, Inc., Bedford, MA). Considering the young age of the cohort, patients were classified as having either low bone mass (Z score < -2) or normal bone mass. All patients had epiphyseal fusion, so there was no scope for further increase in height. Also, none of the subjects had significant vertebral kyphosis clinically or radiologically to suggest loss of height due to osteoporosis vertebral fractures. For TDT patients with severe short stature (height < 3rd centile), bone mineral apparent density was calculated using the UW calculator (https://courses.washington.edu/bonephys/opBMAD.html). Bone geometry and volumetric bone mineral density were estimated by HRpQCT (XtremeCT II, Scanco Medical AG, Switzerland) to image bone microarchitecture in vivo at peripheral skeletal sites (distal radius and distal tibia) in patients and controls. After proper positioning, the appropriate scan region was immobilized in a cast. An initial two-dimensional scout view obtained at a fixed distance (9 mm from the reference line at the endplate of the radius and 22 mm from the reference line at the tibial plafond). Individual images were graded and only those scans grade 3 or less, were included in the final analysis. In case of a grade 4 or 5 image (moderate to complete disruption of the cortex, smearing of trabeculae or large, horizontal streaks), repeat acquisition was performed and analysis was done only if the repeat scan was grade 3 or lower in quality.[13]

All patients received folic acid, zinc supplementation, calcium (1000 mg daily), and cholecalciferol supplementation (60 000 IU monthly). Iron chelator (deferiprone or deferasirox) was prescribed according to the level of serum ferritin, as advised by the hematologist. Appropriate hormone replacements were done in all hypogonadal and hypothyroid subjects. None of them received growth hormone (GH) replacement. Those with diabetes were managed with multiple subcutaneous insulin injections. Patients with osteoporosis received anti-resorptive therapy in the form of zoledronic acid, as fixed protocol (4 mg every three monthly in first year followed by one injection yearly for 2 years).[14]

Statistical analysis

Statistical analysis was performed using the Statistical Package for Social Sciences 22.0 software program (IBM Statistics 22.0). Quantitative variables were assessed for normality using the Kolmogorov–Smirnov method and then classified as either parametric or non-parametric. The Student's t-test was used to compare the means of two groups for parametric data and Mann–Whitney U test was used to compare the median values for non-parametric data. Categorical variables were compared between the groups using Pearson chi-square or Fisher's exact test. Spearman correlation co-efficient (r) was used to measure the association between various variables and BMD at LS as well as FN. Patients with low BMD at either LS or FN were compared with those with normal BMD at these sites. Logistic regression analysis was performed to calculate the odds ratios for variables associated with low BMD at LS and FN, and p-values $<.05$ were considered significant. Data are presented as n (%), median (quartile q25–q75), or mean $\pm$ standard deviation (SD).

Results

The thalassemia cohort comprised 63 patients, with a male preponderance (70%). There were 63 age- and BMI-matched controls, again with a greater proportion of males (57%) (Table 1). The median average ferritin was 3310 ng/mL (IQR 697-5342) and the last measured ferritin was 1696 ng/mL (554-5348). Patients received a mean of 21 ± 10 blood transfusions annually. The average age of initiation of chelation therapy was 4 (3-8) years and greater proportion of patients received deferasirox (80.3%) as compared to deferiprone (56.8%). Both agents were required in 41.1% of the patients. Gonadal hormone replacement was ongoing in 14/20 (70%) of women and 22/45 (49%) of men.

The mean serum calcium in cases (8.7 ± 0.8 mg/dL) was significantly lower than the controls (9.3 ± 0.4 mg/dL) ($P<.0001$). The mean 25(OH)D was significantly higher in cases (31.9 ± 15.0 ng/mL) than controls (24.4 ± 18.5 ng/mL) ($p = .01$). Among cases, 48% had vitamin D deficiency [25(OH)D < 30 ng/mL], while among controls, the prevalence of vitamin D deficiency was 69.6%. Bone formation marker (P1NP) was comparable between cases and controls. However, the bone resorption marker (β-CTX) was significantly lower in TDT cases as opposed to controls (median being 258 (130 – 608) in TDT cases versus 426 (294 – 610) in controls, $p = .04$). Alkaline phosphatase was higher in the cases (108.9 ± 61.3 IU/l) as opposed to controls (vs 77.4 ± 31.4 IU/l, $p = .003$). Overall, hepatic siderosis was present in 76.9% of patients, with 28.8% patients having severe siderosis. Of those with high ALP, 80% had hepatic siderosis on T2*MRI of the liver.

Hypoparathyroidism was present in 20% (13/63) of the TDT cohort. Those with hypoparathyroidism had significantly lower calcium and PTH, but higher phosphate and 25(OH)D levels as compared with controls (Table SS1). Those without hypoparathyroidism also had significantly lower calcium and PTH but higher phosphate and 25(OH)D levels as compared with controls (Table SS2).

Low bone mass ($Z \leq -2$) was present in a greater proportion of patients at the lumbar spine (54%) as compared with the femoral neck (33%) (Table 2). The prevalence of low bone mass was higher in patients' vis-à-vis controls at both lumbar spine (54% vs 0%) and femoral neck (33% vs 8%). The mean T-scores at lumbar spine and at femoral neck were significantly lower in cases than controls. Similarly, the mean Z-scores at lumbar spine and at femoral neck were significantly lower in thalassemia patients versus age- and BMI-matched controls. The mean T- and Z-scores were also significantly lower at the distal-third of radius, but the proportion of patients with low bone mass was lower in TDT patients as opposed to controls. The trabecular bone score was significantly lower and the proportion of patients with degraded microarchitecture higher, in patients with TDT as compared to age- and BMI-matched controls.

Table 1. Demographic, calcemic, and bone turnover markers in transfusion-dependent thalassemia.

Parameter	Cases ($n=63$)	Controls ($n=63$)	p-value
Age (yr)	26.4 ± 6.4	27.0 ± 5.4	.54
BMI (kg/m^2)	20.4 ± 3.6	21.4 ± 3.3	.12
Male: Female	2.3:1	1.3:1	.008
Calcium (mg/dL)	8.7 ± 0.8	9.3 ± 0.4	.001
Inorganic phosphate (mg/dL)	4.0 ± 1.1	3.4 ± 0.6	.007
Alkaline phosphatase (IU/L)	108.9 ± 61.3	77.4 ± 31.4	.003
Albumin (g/dL)	4.8 ± 0.9	4.8 ± 0.2	.79
25(OH)D (ng/mL)	31.9 ± 15	24.4 ± 18.5	.01
PTH (pg/mL)	26.7 ± 17.8	51.8 ± 19.6	.001
P1NP (ng/mL)	54 (20–85)	52 (40–74)	.57
β-CTX (pg/mL)	258 (130–608)	426 (294–610)	.04

Values are expressed in mean ± SD or median (q25-q75), as appropriate. Abbreviations: BMI, Body mass index; 25(OH)D, 25-hydroxy vitamin D; PTH, Parathyroid hormone; P1NP, Procollagen type 1 N-terminal propeptide; β-CTX, β-CrossLaps of type I collagen-containing cross-linked C-telopeptide.

Table 2. Areal BMD and prevalence of low BMD in patients with transfusion-dependent thalassemia.

Parameter	Cases ($n=63$)	Controls ($n=63$)	p-value
Femoral neck (FN) variables			
FN BMD (g/cm^2)	**0.667 ± 0.236**	**0.921 ± 0.150**	**.001**
FN T score	**−1.6 ± 1.0**	**−0.5 ± 0.7**	**.001**
FN Z score	**−1.6 ± 1.1**	**−0.5 ± 0.7**	**.001**
BMD status at FN based on Z score			
Normal	**38/57 (66.7%)**	**55/60 (91.7%)**	**.00001**
Low bone mass	**19/57 (33.3%)**	**5/60 (8.3%)**	
Lumbar spine (LS) variables			
LS BMD (g/cm^2)	**0.708 ± 0.249**	**0.955 ± 0.179**	**.001**
LS T score	**−2.1 ± 1.2**	**−0.9 ± 1.0**	**.001**
LS Z score	**−2.2 ± 1.4**	**−0.8 ± 0.9**	**.002**
BMD status at LS based on Z score			
Normal	**26/57 (45.6%)**	**62/62 (100%)**	**.00001**
Low bone mass	**31/57 (54.4%)**	**0**	
Distal end of radius (DER) variables			
DER BMD (g/cm^2)	**0.494 ± 0.101**	**0.558 ± 0.116**	**.001**
DER T score	**−3.4 ± 1.4**	**−1.2 ± 1.5**	**.002**
DER Z score	**−3.2 ± 1.3**	**−0.9 ± 1.5**	**.001**
BMD status at DER based on Z score			
Normal	**11/41 (26.8%)**	**8/48 (16.7%)**	**.007**
Low bone mass	**30/41 (73.2%)**	**40/48 (83.3%)**	
Trabecular bone score			
TBS	**1.261 ± 0.072**	**1.389 ± 0.058**	**.0001**

Values are expressed in mean ± SD or frequency (%), as appropriate.

Bone microarchitectural analyses revealed significantly lower total as well as cortical and trabecular volumetric bone mineral density in cases as opposed to controls (Table 3). On bone geometry analysis, the cortical bone area was also significantly lower in cases than controls, both at radius and tibia. The trabecular bone area, on the other hand, was higher at the radius ($p=.17$) and tibia ($p=.006$). Cortical thickness at radius and tibia were also significantly lower in cases than controls (Table 3). Representative images of a patient with TDT and an age- and BMI-matched control depicting significant lower cortical thickness are shown in Figure 1. The trabeculae were lower in number and more widely separated at both radius and tibia ($p<.05$) in patients as compared to controls. Trabecular bone fraction, denoting the relative proportion of trabecular component in relation to the whole bone, was also significantly lower in cases than controls. Representative images of the same pair of patient and control depicting increased trabecular separation are shown in Figure 2. Figure 3 depicts the differences in the trabecular microarchitecture between a patient with β-thalassemia major who succumbed to septic shock and an age- and gender-matched control.

We did not find any significant correlation between the average ferritin value and BMD at LS, FN, or DER. There was a weak positive correlation between last measured ferritin and BMD at LS (Spearman $r=0.28$, $p=.07$), at FN ($r=0.23$, $p=.15$) and at DER ($r=0.28$, $p=.10$). There was moderate positive correlation between hemoglobin and spine BMD ($r=0.36$, $p=.006$) but none between Hb and femoral neck BMD ($r=0.17$, $p=.2$). There was no significant correlation between BMD at any site and age or duration of disease. Those with low BMD at LS had significantly lower hemoglobin as compared to those with normal BMD (8.8 ± 1.3 vs 9.4 ± 1.0; $p=.04$), without any significant differences in age, BMI, age at diagnosis, ferritin, hypogonadism, growth hormone deficiency, and number of hormone deficiencies (Table SS3). Those with low BMD at FN had higher prevalence of hypogonadism as compared with those with normal BMD (93%

Table 3. Volumetric BMD and trabecular and cortical microarchitecture in patients with transfusion-dependent thalassemia as compared to controls.

Parameter	Cases ($n = 63$)	Controls ($n = 63$)	p-value
Bone volumetric density			
Radius Total volumetric BMD (mg HA/ccm)	251.9 ± 83.2	357.5 ± 80.8	<.0001
Radius Trabecular volumetric BMD (mg HA/ccm)	135.3 ± 75.6	171.7 ± 45.9	.001
Radius Meta volumetric BMD (mg HA/ccm)	187.5 ± 75.1	229.8 ± 45.0	.003
Radius Inner volumetric BMD (mg HA/ccm)	100.1 ± 77.2	131.8 ± 47.6	.007
Radius Cortical volumetric BMD (mg HA/ccm)	805.3 ± 127.5	890.3 ± 87.5	.001
Tibia Total volumetric BMD (mg HA/ccm)	211.8 ± 72.1	324.3 ± 50.0	.001
Tibia Trabecular volumetric BMD (mg HA/ccm)	121.3 ± 60.7	169.4 ± 45.0	.0001
Tibia Meta volumetric BMD (mg HA/ccm)	159.4 ± 93.9	242.0 ± 40.6	.0001
Tibia Inner volumetric BMD (mg HA/ccm)	88.0 ± 60.2	125.0 ± 40.1	.001
Tibia Cortical volumetric BMD (mg HA/ccm)	876.7 ± 98.7	939.3 ± 60.2	.002
Bone geometry			
Radius total area (mm^2)	273.6 ± 102.5	276.7 ± 83.4	.85
Radius trabecular area (mm^2)	233.5 ± 69.0	211.7 ± 76.1	.17
Radius cortical area (mm^2)	43.7 ± 12.7	68.7 ± 15.8	.001
Tibia total area (mm^2)	736.3 ± 183.2	651.4 ± 145.0	.005
Tibia trabecular area (mm^2)	657.5 ± 186.1	533.5 ± 134.5	0.006
Tibia cortical area (mm^2)	84.2 ± 20.0	128.3 ± 23.4	0.001
Bone structure			
Radius Trabecular number (1/mm)	1.075 ± 0.447	1.515 ± 0.288	0.001
Radius Trabecular thickness (mm)	0.235 ± 0.036	0.241 ± 0.024	0.31
Radius Trabecular separation (mm)	1.290 ± 1.380	0.629 ± 0.136	0.003
Radius TV/BV trabecular bone fraction (mm)	0.187 ± 0.091	0.248 ± 0.067	0.001
Radius Cortical thickness (mm)	0.776 ± 0.267	1.192 ± 0.249	0.001
Radius Cortical porosity	0.005 ± 0.005	0.004 ± 0.003	0.63
Radius Cortical pore diameter (mm)	0.142 ± 0.039	0.147 ± 0.027	0.36
Tibia Trabecular number (1/mm)	0.962 ± 0.350	1.237 ± 0.211	0.0001
Tibia Trabecular thickness (mm)	0.248 ± 0.038	0.264 ± 0.020	0.0004
Tibia Trabecular separation (mm)	1.281 ± 0.974	0.783 ± 0.140	0.001
Tibia TV/BV trabecular bone fraction (mm)	0.174 ± 0.063	0.359 ± 0.053	0.0001
Tibia Cortical thickness (mm)	0.927 ± 0.255	1.494 ± 0.231	0.0001
Tibia Cortical porosity	0.009 ± 0.009	0.013 ± 0.008	0.002
Tibia Cortical pore diameter (mm)	0.191 ± 0.036	0.213 ± 0.050	0.006

Values are expressed in mean ± SD.

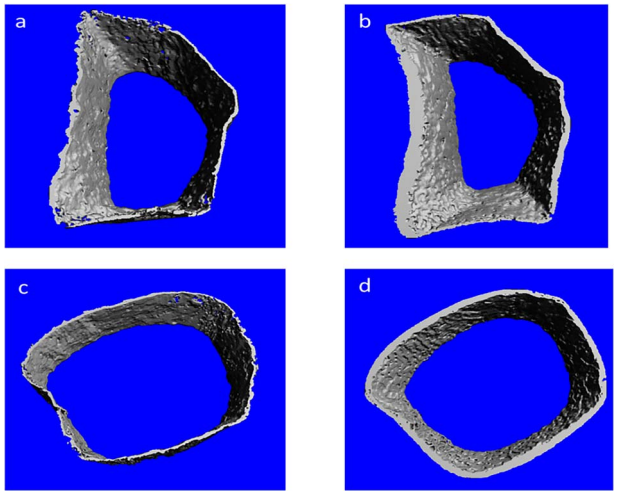


Figure 1. Panel of photographs depicting the cortical microarchitecture in a patient with thalassemia and an age-and BMI-matched control. The top panel depicts the significantly lower cortical thickness and volumetric BMD at the radius (a) and tibia (c) in the patient versus the control at both radius (b) and tibia (d).

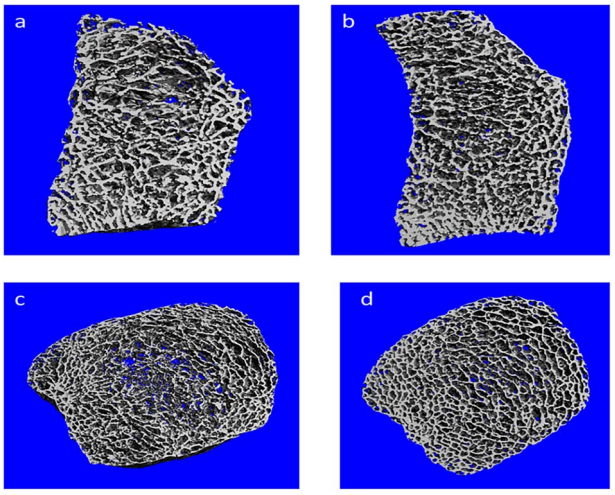


Figure 2. Panel of photographs depicting the trabecular microarchitecture in a patient with thalassemia and an age-and BMI-matched control. The top panel shows reduced trabecular number and increased separation at the radius in the patient (a) versus the control (b) at the radius. Similarly, more significant changes are noted at the tibia in the patient (c) as compared with the control (d).

vs 60%, $p = .01$), without any differences in other variables (Table SS4). Binary logistic regression revealed hypogonadism as a significant predictor of low BMD at FN [odds ratio 10.0 (95% CI, 1.2–86.0; $p = .01$)] and low hemoglobin as a predictor of low BMD at LS [(odds ratio 1.58 (95% CI, 0.96–2.6; $p = .07$)]. Overall, the only predictor of low BMD at either site (LS or FN) was low hemoglobin [odds ratio 2.58 (95% CI, 1.18–5.64; $p = .01$)].

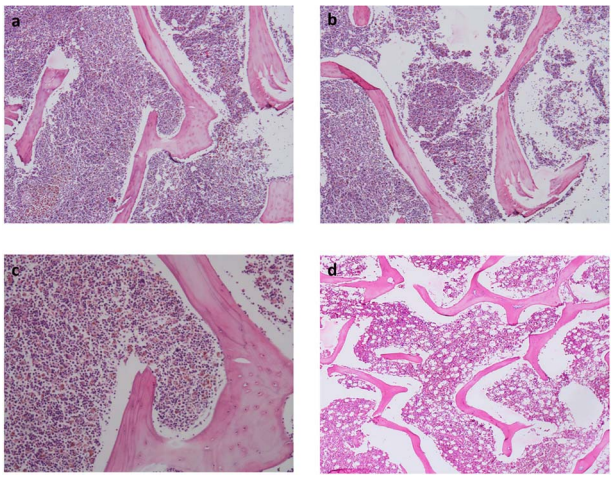


Figure 3. Photomicrographs of iliac crest biopsy in a patient with β-thalassemia major who succumbed to septic shock showing (a) reduced number and thinning of bony trabeculae and bone mass, (b) fragmentation of bony trabeculae with widened marrow spaces, (c) reduced lamellations, and (d) control showing well-spaced and normal thickness of bony trabeculae (hematoxylin & eosin stain).

Both patients with and without hypoparathyroidism had significantly lower T- and Z-scores and TBS as compared to controls. Further, volumetric BMD (total, cortical, and trabecular) were also significantly lower in both subgroups as compared with controls. Significantly higher trabecular number and separation, lower trabecular thickness and cortical thickness were present in both subgroups as compared to controls.

Fractures were evaluated in the patients and classified as either fragility fractures (clinically silent or resulting from low trauma) or related to trauma. Post-traumatic fractures were sustained in 10 patients; none had a minimal trauma fracture.

Discussion

The current study provides evidence regarding the comprehensive evaluation of osteoporosis and impaired bone microarchitecture in patients with transfusion-dependent thalassemia. The study is, to the best of literature search, the first to evaluate bone microarchitecture and geometry in cortical and trabecular compartments in a large adult cohort of transfusion-dependent thalassemia. We noted significantly lower areal BMD and greater prevalence of low bone mass at the lumbar spine and femoral neck in patients with thalassemia. Low bone mass/osteoporosis was more prevalent at the lumbar spine as compared to the femoral neck in these patients. We observed impairment in multiple microarchitectural variables, such as lower volumetric bone mineral density (total, cortical, and trabecular), cortical bone area and cortical thickness in thalassemia patients. The trabecular compartment was also affected in terms of lower trabecular number and greater separation, at both radius and tibia in thalassemia versus age- and BMI-matched controls. The current study therefore provides clinical evidence of deterioration in multiple variables of both bone quantity and quality, in patients with TDT, despite being managed with regular chelation therapy, calcium and vitamin D supplementation.

Thalassemia is an inherited condition characterized by chronic anemia necessitating repeated blood transfusions which results in iron-overload and consequent complications. Bone fragility and osteoporosis are an important, yet under-recognized complication of this condition.[10] Further, increasing life expectancy is perhaps another contributor to this complication, which affects nearly half of TDT patients.[5,6] Since the skeleton forms the structural basis for normal hematopoiesis, it is intricately linked to the disease process in thalassemia as well as its endocrine co-morbidities and therapy (iron chelation). Increased demand on erythropoiesis leads to marrow expansion into bone, thereby thinning the areal and volumetric bone density, as also suggested by the fact that regular transfusions favors osteoblast function and bone formation.[15,16] However, it is interesting to note that the iron-overload and even toxicity of chelation therapy has also been attributed to as a factor for skeletal fragility in thalassemia. The mechanisms underlying iron-overload associated osteoporosis involves tartrate-resistant acid phosphatase expression in osteoclasts which has been reported to directly correlate with ferritin levels, liver iron concentration as well as reduced intestinal calcium absorption via reduced α-klotho and increased reactive oxygen species which impair bone remodelling.[17–19] Iron chelators also adversely impact bone by inhibiting osteoblast proliferation and enhancing its apoptosis as well as promoting hypercalciuria.[20,21]

In the current study, bone mineral parameter analysis revealed significantly lower serum calcium in TDT patients despite optimal prescription of calcium and sufficient levels of 25(OH)D and was true for both patients with and without hypoparathyroidism. The low calcium levels in TDT could be attributed to the hypercalciuria, the competitive interaction between iron and calcium for hepcidin or the competitive interaction between calcium and zinc for divalent metal transporter 1, as all patients were on zinc supplementation.[18,20,22] Interestingly, serum 25(OH)D levels were significantly higher as compared with the controls, but this was possible due to the ongoing regular supplementation in these patients. The PTH was significantly lower in the TDT patients. This was again true for patients with as well as without hypoparathyroidism. This is possibly due to the fact that even TDT patients without hypoparathyroidism had a possible subclinical hypoparathyroid state, with relative preservation of serum calcium as has been previously reported.[23] Though hypoparathyroidism is usually associated with preserved or even high BMD, TDT patients in this study had lower volumetric BMD, possibly because of the fact that hypoparathyroidism is a late complication in the disease-course of TDT and the lower BMD is attributable to long standing disease with consequent poor acquisition of peak bone mass. Bone turnover markers (BTM) revealed significantly lower β-CTX in cases as compared with controls. There is variable evidence on BTMs in TDT, with studies showing reduced osteocalcin,[24] no significant difference in β-CTX[25] or lower P1NP and higher β-CTX.[26] Other studies have reported no significant difference in BTMs at baseline (prior to intervention with anti-resorptive agents).[27] However, in the current study, TDT patients had lower β-CTX, probably because of regular zoledronic acid use in them, leading to appropriate suppression of bone turnover as a consequence. Further, the alkaline phosphatase levels were higher in TDT patients without any significant difference in P1NP, signifying contribution of liver related ALP, rather than bone specific ALP to the circulating ALP level.

Areal BMD analysis revealed a significantly higher prevalence of low bone mass in TDT patients as opposed to

controls. Low bone mass in the current study was defined on the basis of Z-scores, considering the young age of the cohort. The findings from the study are in concordance with previous studies reporting a high prevalence of low bone mass and/or osteoporosis in these individuals.[4,5,9,27] Low bone mass has been reported to have an early onset in TDT patients, even in well-transfused and optimally chelated patients,[28] starting from suboptimal acquisition of peak bone mass, and aggravated by iron-overload, endocrinopathies (hypogonadism, growth hormone deficiency, and diabetes mellitus), and chelating agents. We observed a greater skeletal deficit in terms of low bone mass at the lumbar spine as opposed to femoral neck. This can be attributed to the fact that lumbar spine is more metabolically active than femoral neck and is more sensitive to an adverse metabolic milieu. Predictors of low BMD were analysed individually at LS and FN, revealing a significantly higher odds of low BMD at either site with low hemoglobin and low BMD at FN with hypogonadism. The association of hypogonadism with low BMD is previously reported.[29] However, we did not observe any significant association with either current age or age at diagnosis with low BMD, likely due to comparable results between those with and without low BMD at LS and FN. The mean BMI was lower in those with low BMD at both LS and FN, but it did not attain statistical significance, as reported in prior studies.[29]

Bone microarchitecture analysis has been performed in TDT patients in very few studies.[24,30,31] However, these studies used either quantitative CT or peripheral quantitative CT, with QCT studies being inconclusive and pQCT studies showing some alterations in volumetric BMD and cortical thickness.[31,32] Interestingly, in terms of compartments, patients were found to have lower total radial vBMD, cortical vBMD but slightly higher radial trabecular vBMD,[32] and this was attributed to erythroid hyperplasia and marrow expansion. Similarly, lower volumetric BMD and lower cortical thickness at the tibia were reported in young thalassemia patients.[24] To the best of our knowledge, HRpQCT evaluation of bone microarchitecture, structure and geometry have not been performed hitherto. We found significantly lower volumetric BMD at both radius and tibia overall as well as in the cortical and trabecular compartments. But the trabecular area was higher at tibia in TDT patients, possibly reminiscent of marrow expansion into bone. The cortical area at both sites was significantly lower in cases. However, the trabeculae were lower in number and more widely separated at both sites in TDT patients, pointing towards a microarchitectural deficit and poor bone quality in these patients. Cortical thickness was lower at both sites in patients and controls, again possibly due to the relative marrow expansion into the bone. We believe that the knowledge gained from the current study will likely enhance understanding of the exact etiopathogenesis underlying low BMD and impaired microarchitecture in patients with TDT, and possibly aid in guiding therapeutic decisions for choice of bone-active agents in them.

The strengths of the study include evaluation of bone microarchitecture by HRpQCT, comparison with age- and BMI-matched controls due to the known variation of bone quantity and quality with age and adiposity. However, there are certain limitations, including small cohort, cross-sectional analysis, and non-availability of variables such as FGF23, bone-specific alkaline phosphatase, RANKL/OPG or sclerostin, which may be attempted in the future. Another possible limitation is that the groups were not matched for gender. However, the fact that there was significantly lower BMD, T- and Z-scores at all three sites as well as lower volumetric BMD and impaired cortical and trabecular microarchitecture in TDT patients despite male predominance, possibly implies that the observations would be similar even with gender-matched cases and controls. Furthermore, the fact that patients were treated with zoledronic acid as part of institutional protocol could be the reason for a very low prevalence of fragility fractures. The results of the current study suggest the use of zoledronic acid as an effective measure in patients with TDT, to reduce fragility fractures. However, the evidence would be more robust by designing a two-arm randomized controlled trial with and without the use of zoledronic acid in a prospective fashion.

Conclusion

Patients with transfusion-dependent thalassemia have significantly lower areal and volumetric BMD (both cortical and trabecular), despite chelation therapy, calcium, and vitamin D supplementation. The bone microarchitecture is also significantly impaired in terms of lower number and wider spacing of trabeculae as well as lower cortical thickness and area at both radius and tibia.

Author contributions

Liza Das (Formal analysis, Investigation, Methodology, Validation, Visualization, Writing—original draft, Writing—review & editing [co-first]), Alka Khadwal (Data curation, Investigation, Methodology, Project administration, Resources, Writing—review & editing [co-first]), Pankaj Malhotra (Methodology, Project administration, Resources, Software, Supervision, Validation, Writing—review & editing), Jayaditya Ghosh (Data curation, Formal analysis), Vandana Dhiman (Data curation, Investigation), Vivek Sharma (Methodology, Resources), Shallu Singhmar (Data curation, Resources), Chirag Ahuja (Investigation, Resources), Uma Saikia (Resources, Writing—review & editing), Sanjay Bhadada (Methodology, Project administration, Resources, Supervision, Validation, Writing—review & editing), and Pinaki Dutta (Conceptualization, Methodology, Project administration, Resources, Validation, Writing—review & editing).

Supplementary material

Supplementary material is available at *JBMR Plus* online.

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Conflicts of interest

The authors have no conflicts of interest to declare.

Data availability

The data underlying this article are available in the article and in its online supplementary material.

Ethical approval

The study was approved by the Institutional Ethics Committee (PGI/IEC-INT/2022/62).

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