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Assessment of Bone Mineral Density and Hormonal Profiles in Beta Thalassemia Major Patients: A Study in Erbil Thalassemia Center

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Abstract: **Objectives:** This study assessed the prevalence of low bone mineral density and its association with hormonal profiles in adult patients with β-thalassemia major. **Methods:** A cross-sectional study of 141 adults (≥18 years) with β-thalassemia major was conducted at the Erbil Thalassemia Center from September 2024 to March 2025. Bone mineral density was measured using DXA (lumbar spine, femoral neck) and classified using Z-scores: osteopenia (-1 to -2.5) and osteoporosis (≤ -2.5). Serum levels of ferritin, sex hormones, thyroid function, PTH, and vitamin D were analyzed. Statistical analyses were performed using SPSS version 21.0. Non-normally distributed continuous variables were presented as medians (IQRs) and analyzed using the Kruskal–Wallis and Spearman's correlation tests. Statistical significance was set at $p<0.05$. **Results:** The mean age ±SD was 24.3±5.5 years; 68 (48.2%) were male. Severe iron overload was found (median ferritin: 4255 ng/dL). Osteoporosis prevalence was high: 79 (56.0%) at the spine and 58 (41.1%) at the femoral neck. Sex hormone disturbances were significantly associated with bone mineral density (BMD) at both sites. For vertebral BMD, patients with osteoporosis had significantly lower median testosterone (1.8 ng/mL (95% CI: 0.5–3.2); $p = 0.045$), estradiol (13.0 pg/mL (95% CI: 5.0–22.0); $p = 0.028$), and LH (2.5 mIU/mL (95% CI: 1.5–3.5); $p = 0.008$) compared to those with normal BMD. For hip BMD, similar patterns were observed, with lower testosterone (1.6 ng/mL (95% CI: 0.5–3.0); $p = 0.005$), LH (2.4 mIU/mL (95% CI: 1.5–3.5); $p = 0.006$), and FSH (3.0 mIU/mL (95% CI: 2.0–5.0); $p = 0.043$) in patients with osteoporosis. **Conclusion:** Osteoporosis is highly prevalent in adult β-thalassemia major patients and is significantly related to hypogonadism.

Key Words: β-Thalassemia Major, Bone Mineral Density, Osteoporosis, Hypogonadism, Endocrine Complications

INTRODUCTION

β-Thalassemia major (β-TM) is a severe, transfusion-dependent hereditary anemia resulting from mutations in the β-globin gene [1]. While it is globally distributed, its prevalence is notably high in regions such as the Mediterranean, the Middle East, and Central and South Asia, including Iraq [2]. Lifelong transfusion therapy, essential for patient survival, inevitably leads to iron overload. This, in turn, drives a complex pathophysiology involving endocrinopathies and progressive organ damage [3-6].

One of the most debilitating long-term complications of β-TM is osteoporosis, a condition characterized by reduced bone mineral density and increased fracture risk [7,8]. The pathogenesis of bone disease in this population is multifactorial. Chronic ineffective erythropoiesis leads to massive expansion of bone marrow, causing mechanical disruption of trabecular architecture and cortical thinning [7]. Direct iron toxicity further impairs skeletal health by depositing in bone cells, altering osteoblast and osteoclast activity, and disrupting normal bone remodeling [5,6]. Additional contributing factors include chronic anemia-induced hypoxia, nutritional deficiencies, and physical inactivity. Endocrine dysfunction, particularly hypogonadism resulting from iron deposition in the pituitary and gonads, represents another established mechanism [5,6]. Despite advances in transfusion regimens and iron chelation therapy that have markedly improved survival, osteoporosis

remains highly prevalent and constitutes a major source of morbidity, chronic pain, and impaired quality of life in adult patients [7,8].

In Iraq and the broader Middle Eastern region, β-TM constitutes a significant public health burden due to high carrier rates and consanguineous marriage patterns [2,4]. While the general risk of osteoporosis in thalassemia is well recognized, comprehensive local data specifically linking bone mineral density with detailed endocrine profiles—particularly gonadal function in the region—remain scarce. Existing studies from the region have largely reported BMD measurements alone or examined limited biochemical parameters without systematic hormonal assessment [9-12]. This gap in knowledge limits the development of evidence-based screening and management protocols tailored to regional patient populations. Furthermore, there is a lack of understanding regarding the specific contribution of hypogonadism to bone loss among adult β-TM patients in this geographic context.

Therefore, this study aims to assess the prevalence of osteopenia and osteoporosis and to assess their association with hormonal profiles, with a specific focus on sex hormones, in a cohort of adult β-TM patients managed at a major tertiary care center in Erbil, Iraq. We hypothesize that: (1) the prevalence of osteoporosis and osteopenia will be high in this adult β-TM cohort, (2) hypogonadism, as reflected by low sex hormone levels, will be significantly associated with reduced BMD at both lumbar spine and femoral neck sites.

METHODS

This cross-sectional study was conducted at the Erbil Thalassemia Care Center (ETCC) in Erbil province from September 2024 to March 2025. This ETCC is the major tertiary center for the medical care of thalassemia patients in Erbil and the region. Patients were eligible if they were (1) aged 18 years or older, (2) diagnosed with β-thalassemia major (β-TM) via clinical presentation and confirmed by hemoglobin electrophoresis, and (3) undergoing a regular program of blood transfusions and iron chelation therapy at the center. Exclusion criteria included diagnoses of α-thalassemia, sickle cell disease, β-thalassemia intermedia, pregnancy, or a history of bone marrow transplantation.

The study protocol was approved by the Research Ethics Committee of the College of Medicine, Hawler Medical University (Approval Code: 30 on August 6, 2024). All participants provided written informed consent.

Of the 225 patients initially screened, 84 were excluded because they were under 18 years of age. The final study cohort comprised 141 adult β-TM patients.

Data were collected via a review of electronic medical records and structured face-to-face interviews using separate data sheets for male and female participants to capture gender-specific variables.

The collected variables included:

- Demographic and Clinical: Age, sex, marital status, family history of thalassemia, smoking status, transfusion history, iron chelation regimen, history of puberty (age at onset), surgical history (e.g., splenectomy), medication/supplement use, and self-reported compliance
- Anthropometric: Weight and height were measured during a clinical visit to calculate body mass index (BMI)
- Hematological and biochemical parameters: Blood samples were drawn after a 12-hour fast and at least 15 days post-transfusion. Samples were centrifuged at the ETCC, and serum was stored at -70°C until analysis. Analyses performed at the center included: complete blood count (CBC), liver function tests (LFTs), including alanine transaminase (ALT), aspartate aminotransferase (AST), and bilirubin, renal function tests (urea, creatinine), fasting blood glucose, hepatitis C antibodies, and ferritin (measured using a DIOLIS30i analyzer)
- Endocrine and bone metabolic parameters: Frozen serum aliquots were transported in a cool box to the Galiyawa Endocrine Center, a public center for analysis using an automated analyzer. Measured parameters included: calcium, phosphate, alkaline phosphatase, thyroid-stimulating hormone (TSH), free thyroxine (FT4), luteinizing hormone (LH), follicle-stimulating hormone (FSH), parathyroid hormone (PTH), 25-hydroxyvitamin D, total testosterone (males), estradiol (females), growth hormone (GH), and insulin-like growth factor-I (IGF-I)

The BMD (g/cm²) was measured at the lumbar spine (L1–L4) and the left femoral neck using dual-energy X-ray absorptiometry (DXA) with an Osteosys machine (Osteosys Co., Ltd., Seoul, Korea) at ETCC. Given that our study population consists of adults under 50 years of age (mean age 24.3±5.5 years), the WHO T-score-based diagnostic criteria for osteoporosis, which are intended for postmenopausal women and men aged 50 years and older [13], are not directly applicable. Accordingly, we used Z-scores (age- and sex-matched BMD values) for classification, as recommended by the International Society for Clinical Densitometry (ISCD) for premenopausal women and men under 50 years of age [14,15]. Z-scores were classified as: Normal BMD: Z-score ≥ –1.0, Osteopenia: Z-score between –1.0 and –2.5, and Osteoporosis: Z-score ≤ –2.5. This approach is consistent with established clinical guidelines for BMD interpretation in young adult populations [14,15].

The outcome measures were as follows:

- Primary Outcome: The prevalence of low BMD (osteopenia or osteoporosis) at the lumbar spine and femoral neck
- Secondary Outcomes: The associations between BMD (Z-scores) and hormonal profiles (sex hormones, TSH, FT4, PTH, vitamin D) and mineral levels (calcium, phosphate)

- Endocrine disorders were classified based on biochemical abnormalities and clinical presentation as follows:
- **Hypogonadism:** Biochemical hypogonadism was defined as testosterone <3.0 ng/mL in males or estradiol <20 pg/mL in premenopausal females. Clinical hypogonadism was defined as a biochemical abnormality with documented clinical features (delayed puberty, primary or secondary amenorrhea, reduced libido, infertility, or signs of androgen/estrogen deficiency on clinical examination)
- **Hypothyroidism:** Biochemical hypothyroidism was defined as TSH >4.2 µIU/mL. Clinical (overt) hypothyroidism was defined as elevated TSH with low FT4 (<12 pmol/L) and clinical features (fatigue, weight gain, cold intolerance). Subclinical hypothyroidism was defined as elevated TSH with normal FT4 and no or minimal clinical symptoms [16]
- **Growth hormone deficiency:** Biochemical GH deficiency was defined as GH <1.0 ng/mL with low IGF-1 (<50 ng/mL for age). Clinical GH deficiency was defined as short stature (height Z-score<-2) with biochemical confirmation
- **Hypoparathyroidism:** Biochemical hypoparathyroidism was defined as PTH <15 pg/mL with low serum calcium (<8.5 mg/dL). Clinical hypoparathyroidism requires biochemical abnormality with symptoms or signs of hypocalcemia (paresthesia, muscle cramps, Chvostek or Trousseau signs)
- Short stature was defined as a height Z-score<–2 relative to age-matched references
- Diabetes status was categorized based on fasting blood glucose: normal (<100 mg/dL), prediabetes (100–125 mg/dL), and diabetes (≥126 mg/dL)

Reference ranges for hormonal assays (provided by the Galiyawa Endocrine Center) were as follows: total testosterone (males, adult): 2.5–9.0 ng/mL, estradiol (females, follicular phase): 20–150 pg/mL, LH: 1.0–12.0 mIU/mL, FSH: 1.0–8.0 mIU/mL (male), 1.5–10.0 mIU/mL (female), TSH: 0.4–4.2 µIU/mL, FT4: 12–22 pmol/L, PTH: 10–65 pg/mL, vitamin D (25-hydroxy): 30–100 ng/mL (sufficient), 20–29 ng/mL (insufficient), <20 ng/mL (deficient), IGF-1: age- and sex-adjusted ranges were used according to the manufacturer's reference. Transfusion adequacy was defined as maintaining a pre-transfusion hemoglobin level >9 g/dL.

The sample size was estimated using G*Power software (version 3.1) for a Kruskal–Wallis test (three groups: normal, osteopenia, osteoporosis) with an alpha of 0.05, power of 0.80, and a medium effect size (f = 0.25). The calculated sample size was 159. Our final sample of 141 was below the target by 18 participants (11.3%) due to logistical constraints during the recruitment period. Post-hoc power analysis based on observed effect sizes indicated achieved power of 0.75 for three-group Kruskal-Wallis comparisons, 0.96 for Spearman's correlation analyses, and 0.51–0.56 for sex-stratified two-group comparisons.

Data were analyzed using the Statistical Package for Social Sciences (SPSS) version 21.0 (IBM Corp., Armonk, NY). The normality of continuous variables was assessed using the Kolmogorov–Smirnov and Shapiro–Wilk tests. Non-normally distributed data were presented as median (interquartile range, IQR). Categorical variables are presented as frequencies and percentages. Group comparisons for continuous variables were performed using the Kruskal–Wallis test. Spearman's rank correlation was used to assess relationships between continuous variables. A two-tailed p-value<0.05 was considered statistically significant.

RESULTS

Out of the 141 patients included in the analysis, 68 (48.2%) were male, and 73 (51.8%) were female, with a mean age±standard deviation (SD) of 24±5 years. The majority of the patients were single, 124 (88.0%), and non-smokers, 128 (90.8%). The distribution of vertebral BMD categories did not differ significantly by sex (p = 0.082), marital status (p = 0.533), or smoking status (p = 0.557).

Analysis of hip bone mineral density revealed a comparable pattern to that of the vertebral findings. The prevalence of normal, osteopenic, and osteoporotic BMD at the hip site was similarly distributed between males, 10 (14.7%), 29 (42.6%), 29 (42.6%), and females, 9(12.3%), 35 (47.9%), 29 (39.7%) (p = 0.803), respectively. No statistically significant associations were observed across demographic variables (Table 1).

The majority of patients were on deferasirox monotherapy (54.6%), followed by deferiprone (36.2%), deferoxamine (5.7%), and combination therapy (3.5%). Most patients (95.0%) received transfusions at intervals of more than 4 weeks. Splenectomy was performed in 52.5% of patients, and 46.8% were HCV-positive.

Endocrine disorders were highly prevalent in this cohort. Hypogonadism was present in 57.4% of males (testosterone <3.0 ng/mL) and 78.6% of females (estradiol <20 pg/mL), with an overall prevalence of 68.1%. Overt hypothyroidism was documented in 1.4% of patients, while subclinical hypothyroidism was observed in 9.9%. Diabetes mellitus was present in 7.8% of patients, and impaired glucose tolerance was found in 9.2%. Short stature was documented in 57.9% of patients, and hypoparathyroidism was present in 12.1% (Table 2).

Given the small number of patients with normal vertebral BMD (n = 3), we repeated the analysis after combining normal and osteopenic groups into a single "non-osteoporotic" group (n = 62) and compared it with the osteoporosis group (n = 79) using the Mann–Whitney U test. The findings remained consistent: osteoporotic patients had significantly lower median testosterone (1.27 vs. 3.90 ng/mL, p = 0.032), estradiol (10.00 vs. 11.20 pg/mL, p = 0.028), and LH (1.23 vs. 4.15 mIU/mL, p = 0.003). FSH showed a non-significant trend (2.09 vs. 3.18 mIU/mL, p = 0.065) (Table 3).

Table 1: Associations between Demographic Characteristics and Bone Mineral Density Status at Vertebral and Hip Sites (N = 141)

Site	Characteristic	Category	Total N (%)	Normal n (%)	Osteopenia n (%)	Osteoporosis n (%)	p-value
Vertebral	Age (Years)	Mean±SD	24±5	21±4	25±5	24±6	0.316*
	Sex	Male	68 (48.2)	3 (4.4)	24 (35.3)	41 (60.3)	0.082
		Female	73 (51.8)	0 (0.0)	35 (47.9)	38 (52.1)	
	Marital Status	Single	124 (88.0)	3 (2.4)	50 (40.3)	71 (57.3)	0.533
		Married	17 (12.0)	0 (0.0)	9 (52.9)	8 (47.1)	
	Smoking Status	Smoker	13 (9.2)	0 (0.0)	4 (30.8)	9 (69.2)	0.557
		Non-smoker	128 (90.8)	3 (2.3)	55 (43.0)	70 (54.7)	
Hip	Age (Years)	Mean±SD	24±5	23±4	24±5	25±6	0.725*
	Sex	Male	68 (48.2)	10 (14.7)	29 (42.6)	29 (42.6)	0.803
		Female	73 (51.8)	9 (12.3)	35 (47.9)	29 (39.7)	
	Marital Status	Single	124 (88.0)	16 (12.9)	57 (46.0)	51 (41.1)	0.850
		Married	17 (12.0)	3 (17.6)	7 (41.2)	7 (41.2)	
	Smoking Status	Smoker	13 (9.2)	1 (7.7)	6 (46.2)	6 (46.2)	0.801
		Non-smoker	128 (90.8)	18 (14.1)	58 (45.3)	52 (40.6)	

BMD: bone mineral density, SD: standard deviation. *p-value derived from Kruskal-Wallis test. All other p-values are from Pearson's Chi-Square test (Fisher's Exact Test confirmed non-significance for all). The normal BMD vertebral group had a very small sample size (n = 3, 2.1%), which reflects the exceptionally high prevalence of osteoporosis in this population (56.0% at the spine)

Table 2: Clinical Characteristics of the Study Population (N = 141)

Characteristic	Category	N (%)
Chelation Regimen	Deferasirox	77 (54.6)
	Deferiprone	51 (36.2)
	Deferoxamine	8 (5.7)
	Combination therapy	5 (3.5)
Transfusion Frequency	Every 2 weeks	7 (5.0)
	More than 4 weeks	134 (95.0)
Splenectomy	Yes	74 (52.5)
	No	67 (47.5)
HCV Status	Positive	66 (46.8)
	Negative	75 (53.2)
Hypogonadism (male)†	Present	39/68 (57.4)
Hypogonadism (female)‡	Present	55/70 (78.6)
Hypogonadism (total)	Present	94/138 (68.1)
Overt Hypothyroidism	Present	2 (1.4)
Subclinical Hypothyroidism	Present	14 (9.9)
Diabetes Mellitus	Present	11 (7.8)
Impaired Glucose Tolerance	Present	13 (9.2)
Short Stature	Present	81/140 (57.9)
Hypoparathyroidism	Present	17 (12.1)

† Testosterone <3.0 ng/mL, ‡ Estradiol <20 pg/mL. Values represent biochemical abnormalities based on laboratory reference ranges. Clinical endocrine disorders were diagnosed by the attending physician based on combined biochemical and clinical findings

Table 3: Sensitivity Analysis - Comparison of Hormonal Levels Between Non-osteoporotic and Osteoporotic Groups (Mann-Whitney U Test)

Hormone	Non-osteoporotic Median (IQR)	Osteoporotic Median (IQR)	p-value
Testosterone (ng/mL)†	3.90 (0.90–5.00)	1.27 (0.11–3.73)	0.032
Estradiol (pg/mL)‡	11.20 (10.00–30.75)	10.00 (8.00–20.00)	0.028
LH (mIU/mL)	4.15 (1.29–6.33)	1.23 (0.60–4.30)	0.003
FSH (mIU/mL)	3.18 (1.36–7.52)	2.09 (0.76–5.90)	0.065

†Testosterone: Males only (n = 68), ‡ Estradiol: Females only (n = 70)

Abnormal BMD was highly prevalent in this study, though the distribution varied by site. At the vertebra, the majority of patients, 79 (56.0%), were osteoporotic, with a minimal proportion in the normal range, 3 (2.1%). At the hip, the prevalence of osteoporosis was lower, 58 (41.1%), and normal BMD was more frequently observed, 19 (13.5%). (Figure 1).

Regarding the influence of key clinical management factors on vertebral bone, 74 (52.5%) of patients had a history of splenectomy. Neither splenectomy status nor HCV serostatus, 66 (46.8% positive), was associated with a different distribution of vertebral BMD categories (p = 0.946), (p = 0.945), respectively. Furthermore, the majority of patients had normal BMI, 96 (68.1%), with no significant association observed across underweight, normal, and overweight/obese categories (p = 0.542). Furthermore, splenectomy status did not significantly affect hip BMD category distribution (p = 0.891). While a lower proportion of HCV-positive patients had normal hip BMD compared to HCV-negative patients, 6 (9.1%) vs. 13 (17.3%), this difference did not reach statistical significance (p = 0.308). BMI category also showed no statistically significant association with hip BMD outcomes (p = 0.562), with the proportion of normal BMD ranging from 2 (6.1%) in the overweight/obese group to 2 (16.7%) in the underweight group (Table 4a, Table 4b).

Table 4a: Associations between Clinical Characteristics (Splenectomy, HCV, and BMI) and Bone Mineral Density Status at Vertebral Sites (N = 141)

Site	Characteristic	Category	N (%)	Normal n (%)	Osteopenic n (%)	Osteoporotic n (%)	p-value
Vertebral	Splenectomy	No	67 (47.5)	1 (1.5)	29 (43.3)	37 (55.2)	0.946
		Yes	74 (52.5)	2 (2.7)	30 (40.5)	42 (56.8)	
	HCV Status	Negative	75 (53.2)	2 (2.7)	32 (42.7)	41 (54.7)	0.945
		Positive	66 (46.8)	1 (1.5)	27 (40.9)	38 (57.6)	
	BMI Category	Underweight	12 (8.5)	0 (0.0)	6 (50.0)	6 (50.0)	0.542*
		Normal	96 (68.1)	3 (3.1)	35 (36.5)	58 (60.4)	
		Overweight/Obese	33 (23.4)	0 (0.0)	18 (54.5)	15 (45.5)	

Table 4b: Associations between Clinical Characteristics (Splenectomy, HCV, BMI) and Bone Mineral Density Status at Hip Sites (N = 141)

Site	Characteristic	Category	N (%)	Normal n (%)	Osteopenic n (%)	Osteoporotic n (%)	p-value
Hip	Splenectomy	No	67 (47.5)	10 (14.9)	30 (44.8)	27 (40.3)	0.891
		Yes	74 (52.5)	9 (12.2)	34 (45.9)	31 (41.9)	
	HCV Status	Negative	75 (53.2)	13 (17.3)	31 (41.3)	31 (41.3)	0.308
		Positive	66 (46.8)	6 (9.1)	33 (50.0)	27 (40.9)	
	BMI Category	Underweight	12 (8.5)	2 (16.7)	6 (50.0)	4 (33.3)	0.562*
		Normal	96 (68.1)	15 (15.6)	38 (39.6)	43 (44.8)	
		Overweight/Obese	33 (23.4)	2 (6.1)	20 (60.6)	11 (33.3)	

Table 5: Biochemical Markers across Bone Mineral Density Categories (N = 141)

Variable	BMD Site	Normal Median (IQR)	Osteopenia Median (IQR)	Osteoporosis Median (IQR)	p-value
Hemoglobin (g/dL)	Vertebra	9.00 [N/A]	8.90 [1.20]	9.00 [1.10]	0.756
	Hip	8.90 [0.80]	9.05 [1.05]	8.85 [1.03]	0.644
Serum Ferritin (ng/mL)	Vertebra	1200.0 [N/A]	2952.0 [6458.0]	2482.0 [4871.0]	0.322
	Hip	1876.0 [7105.0]	3127.5 [5508.0]	2491.0 [3873.3]	0.546
Vitamin D (ng/mL)	Vertebra	17.80 [N/A]	16.70 [13.00]	18.00 [13.00]	0.935
	Hip	15.00 [21.00]	19.00 [14.00]	16.45 [11.00]	0.178

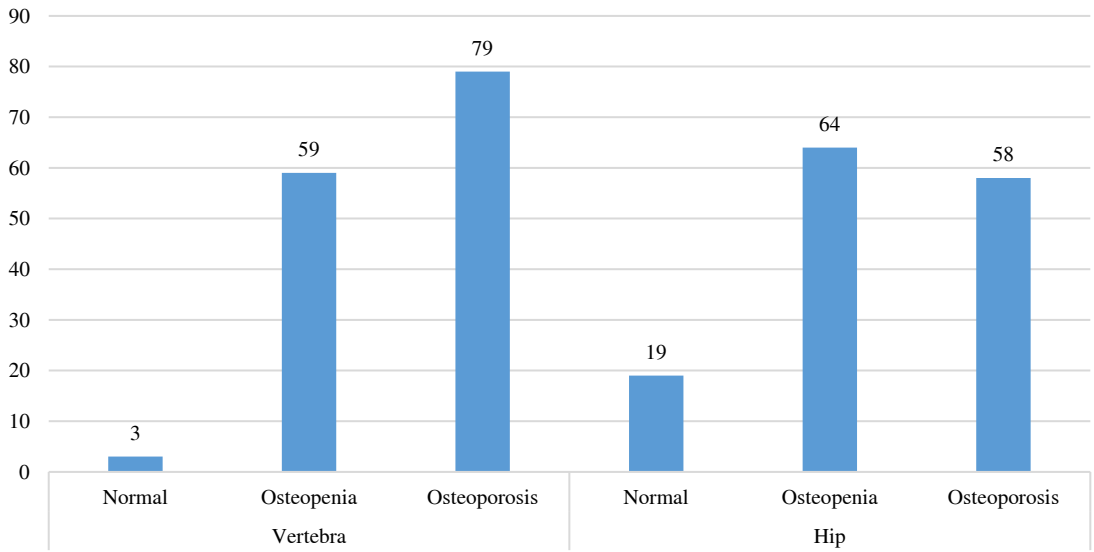

Figure 1: Prevalence of Bone Density Status for the Vertebra and Hip Regions (N =141)

The distribution of key biochemical markers across BMD categories is presented in Table 5. Serum ferritin levels were markedly elevated across all patient groups, with median values ranging from 1200 to 3128 ng/mL across BMD categories, confirming severe iron overload in this thalassemia cohort. Vitamin D levels showed widespread insufficiency across all groups (median range: 15–19 ng/mL), with a non-significant trend toward lower values in the osteoporotic group at the hip (16.45 ng/mL) compared to the osteopenic group (19.00 ng/mL, p = 0.178). However, no statistically significant differences were found in the median levels of hemoglobin, serum ferritin, or vitamin D across the normal, osteopenic, and osteoporotic groups at either the vertebral or hip sites (all p-values > 0.05).

Hormonal and biochemical markers were compared across vertebral BMD categories. Sex hormone disturbances were prominent, with significantly lower median testosterone levels in males with osteoporosis (1.8 (0.5–3.2) ng/mL) compared to other categories (p = 0.045) and lower estradiol in females with osteoporosis

Table 6: Hormonal and Biochemical Markers Across Vertebral BMD Categories (N = 141)

Variable	N	Median (IQR)*	Mean rank (Normal)	Mean rank (Osteopenia)	Mean rank Osteoporosis)	p-value
GH (ng/mL)	141	0.8 (0.4–1.5)	60.0	73.2	69.8	0.791
IGF-1 (ng/mL)	141	95 (64–156)	81.5	75.1	67.5	0.504
Testosterone (ng/mL)**	71	1.8 (0.5–3.2)	52.0	41.7	31.1	0.045
Estradiol (pg/mL)**	70	13.0 (5.0–22.0)	–	41.3	30.6	0.028
FSH (mIU/mL)	141	3.0 (2.0–5.0)	87.0	77.7	65.4	0.169
LH (mIU/mL)	141	2.5 (1.5–3.5)	106.5	81.2	62.0	0.008
TSH (mIU/L)	141	3.2 (2.0–5.0)	55.8	66.2	75.2	0.355
FT4 (pmol/L)	141	8.5 (7.0–11.0)	63.2	69.2	72.7	0.836
PTH (pg/mL)	141	14.0 (9.0–19.0)	91.3	69.0	71.8	0.632
Creatinine (mg/dL)	141	0.6 (0.5–0.8)	101.7	75.3	66.7	0.188
Calcium (mg/dL)	141	9.3 (8.8–9.7)	80.8	63.7	76.1	0.188
Phosphate (mg/dL)	141	5.5 (4.8–6.2)	75.0	72.8	69.5	0.880
ALP (U/L)	141	330 (250–430)	83.5	62.1	77.2	0.087

*Medians and IQRs are added for interpretability based on descriptives (not shown in ranks). ** Testosterone: males only (n = 71). Estradiol: females only (n = 70). Reference Ranges: Testosterone (male): 2.5–9.0 ng/mL, Estradiol (female, follicular): 20–150 pg/mL, LH: 1.0–12.0 mIU/mL, FSH (male): 1.0–8.0 mIU/mL, FSH (female): 1.5–10.0 mIU/mL

Table 7: Hormonal and biochemical markers across hip BMD categories (n = 141)

Variable	N	Median (IQR)*	Mean rank (Normal)	Mean rank (Osteopenia)	Mean rank (Osteoporosis)	p-value
GH (ng/mL)	141	0.8 (0.4–1.5)	74.5	65.5	75.9	0.340
IGF-1 (ng/mL)	141	95 (64–156)	66.7	78.8	63.8	0.112
Testosterone (ng/mL)**	71	1.6 (0.5–3.0)	53.4	34.1	30.8	0.005
Estradiol (pg/mL)**	70	12.5 (5.0–21.0)	39.9	38.3	31.2	0.314
FSH (mIU/mL)	141	3.0 (2.0–5.0)	90.7	71.8	63.7	0.043
LH (mIU/mL)	141	2.4 (1.5–3.5)	94.8	73.0	61.0	0.006
TSH (mIU/L)	141	3.1 (2.0–5.0)	53.3	72.8	74.8	0.123
FT4 (pmol/L)	141	8.5 (7.0–11.0)	73.5	71.5	69.7	0.930
PTH (pg/mL)	141	14.0 (9.0–19.0)	76.5	69.5	70.8	0.807
Creatinine (mg/dL)	141	0.6 (0.5–0.8)	89.0	70.0	66.2	0.097
Calcium (mg/dL)	141	9.3 (8.8–9.7)	70.2	66.7	76.0	0.448
Phosphate (mg/dL)	141	5.5 (4.8–6.2)	76.7	76.2	63.3	0.177
ALP (U/L)	141	330 (250–430)	70.7	63.3	79.6	0.091

* Medians and IQRs are added for interpretability based on descriptives (not shown in ranks). ** Testosterone: males only (n = 71). Estradiol: females only (n = 70). Reference ranges: Testosterone (male): 2.5–9.0 ng/mL, Estradiol (female, follicular): 20–150 pg/mL, LH: 1.0–12.0 mIU/mL, FSH (male): 1.0–8.0 mIU/mL, FSH (female): 1.5–10.0 mIU/mL

(13.0 (5.0–22.0) pg/mL, p = 0.028). LH also varied significantly across groups (p = 0.008) (Table 6).

For hip BMD categories, a similar pattern was observed for sex hormones. Testosterone levels were significantly lower in the osteoporosis group (median: 1.6 [0.5–3.0] ng/mL, p = 0.005), and both LH (p = 0.006) and FSH (p = 0.043) varied significantly across categories. (Table 7).

DISCUSSION

This study aimed to assess the prevalence of osteopenia and osteoporosis and to assess their association with hormonal profiles, with a specific focus on sex hormones, in a cohort of adult β-TM patients managed at a major tertiary care center in Erbil, Iraq. The prevalence of low BMD was strikingly high at both skeletal sites. This finding aligns with the established literature, which identifies skeletal morbidity as a primary determinant of long-term morbidity in this population [12]. The pathophysiology is multifactorial, involving chronic anemia, marrow expansion, endocrine dysfunction—particularly hypogonadism—and the direct toxic effects of iron overload on bone remodeling [17]. The lack of significant association between BMD and demographic factors such as age, sex, marital status, or smoking history suggests that the underlying disease process and its complications, rather than common demographic variables, are the principal drivers of bone loss in β-TM (8,20). This finding is clinically important as it implies that all adult β-TM patients, regardless of demographic profile, are at substantial risk for osteoporosis and should undergo routine BMD screening.

A notable finding in our study is that estradiol was significantly associated with vertebral BMD (p = 0.028) but not with hip BMD (p = 0.314) in female patients. This site-specific difference warrants discussion.

The spine has a higher proportion of trabecular bone (approximately 50-70%) compared to the hip, which is predominantly cortical bone (approximately 80%) (Riggs et al., 2004). Trabecular bone is more metabolically active and more sensitive to hormonal changes, particularly estrogen deficiency (Khosla et al., 2012). Estrogen suppresses osteoclast-mediated bone resorption through effects on RANKL and OPG, and its withdrawal leads to accelerated trabecular bone loss. This is consistent with our finding that vertebral BMD was more severely affected overall (56% osteoporosis vs. 41% at the hip) and showed stronger associations with estradiol levels.

Furthermore, the differential effect of estradiol at the spine versus hip may reflect differences in bone turnover rates at these sites. Trabecular bone has a higher surface-to-volume ratio and undergoes more rapid remodeling than

cortical bone, making it more susceptible to the effects of hormonal changes (Seeman, 2008). This is particularly relevant in β-TM, where ineffective erythropoiesis and marrow hyperplasia disproportionately affect the spine.

These findings suggest that estrogen deficiency may be a more important determinant of trabecular bone loss (as measured at the spine) than cortical bone loss (as measured at the hip) in female β-TM patients. This has clinical implications: DXA screening at the spine may be more sensitive for detecting estrogen-deficiency-related bone loss in this population, and estrogen replacement therapy may be particularly beneficial for preserving vertebral bone mass.

Consistent with previous reports, osteoporosis was more prevalent at the lumbar spine (56.0%) than at the femoral neck (41.1%) [16–19]. This site-specific severity is likely attributable to the spine's high trabecular bone content and large marrow space, making it more susceptible to ineffective erythropoiesis, marrow hyperplasia, and iron-induced endocrine dysfunction [7,8]. Studies from other Middle Eastern and Mediterranean regions have reported vertebral osteoporosis rates ranging from 30% to 70% [12,15]. Our analysis found no significant association between BMD status and demographic factors such as age, sex, marital status, or smoking history, suggesting that the underlying disease process and its complications, rather than common demographic variables, are the principal drivers of bone loss in β-TM [20,21].

The high prevalence of hypogonadism observed in our cohort (68.1%) is consistent with previous reports in β-thalassemia major populations, where rates range from 40% to 80% depending on the population and diagnostic criteria used [22,23]. The higher prevalence in females (78.6%) compared to males (57.4%) may reflect differences in gonadal susceptibility to iron deposition or differences in hormonal reference ranges used to define hypogonadism.

The most significant finding of this study is the strong association between hypogonadism and low BMD. We observed significantly lower levels of testosterone ($p = 0.045$), estradiol ($p = 0.028$), and LH ($p = 0.008$) in patients with vertebral osteoporosis. This pattern was mirrored at the hip, with significantly lower levels of testosterone ($p = 0.005$), LH ($p = 0.006$), and FSH ($p = 0.043$) in the osteoporotic group. These results confirm that gonadal dysfunction is a central mechanism in the development of osteoporosis in β-TM, as sex hormones are critical regulators of osteoblast and osteoclast activity [24]. Our findings support earlier research indicating that hypogonadism, present in a high percentage of transfusion-dependent thalassemia patients, is a key predictor of reduced BMD and increased fracture risk [23,26]. The observed differences in sex hormone levels between osteoporotic and non-osteoporotic patients are clinically meaningful. Median testosterone was approximately 44% lower in patients with vertebral osteoporosis (1.8 vs. 3.2 ng/mL), and LH was approximately 40% lower (2.5 vs. 4.15 mIU/mL). These reductions are consistent with the established role of hypogonadism in bone loss.

Although GH and IGF-1 levels were measured, we found no significant association with BMD categories at either the vertebral (GH: $p = 0.791$, IGF-1: $p = 0.504$) or hip (GH: $p = 0.340$, IGF-1: $p = 0.112$) sites. This finding is somewhat unexpected given the well-established anabolic effects of the GH/IGF-1 axis on bone metabolism, where GH stimulates osteoblast proliferation, and IGF-1 promotes bone matrix synthesis [27,28].

Several factors may explain this lack of association. First, the median IGF-1 level in our cohort (95 ng/mL) was within the lower normal range for adults, possibly reflecting the combined effects of chronic disease, iron overload, malnutrition, and hepatic dysfunction—all of which are common in β-TM and can impair GH/IGF-1 secretion [29]. Second, the cross-sectional design may not capture the cumulative effects of GH/IGF-1 deficiency over time. Third, the relatively small number of patients with severe GH deficiency in our cohort (as suggested by the median GH level of 0.8 ng/mL) may have limited our ability to detect an association. Finally, it is possible that hypogonadism, rather than GH deficiency, is the predominant endocrine driver of bone loss in this adult population, as supported by our significant findings for sex hormones [24].

These findings suggest that while GH/IGF-1 axis evaluation remains clinically important, the assessment and management of hypogonadism should be prioritized in adult β-TM patients presenting with low BMD. Future prospective studies incorporating provocative GH testing and measuring IGF-1 binding proteins may provide more definitive insights into the role of the GH/IGF-1 axis in bone disease in β-TM.

Despite the high prevalence of iron overload and vitamin D insufficiency in our cohort, we found no statistically significant association between serum ferritin or vitamin D levels and BMD at either skeletal site. This finding is consistent with some previous studies in β-thalassemia [8,30] but contrasts with others that have reported inverse correlations between ferritin and BMD [10].

Several explanations may account for this lack of association. First, serum ferritin, while widely available, is an acute-phase reactant that can be elevated by inflammation, infection, and hepatic dysfunction, all of which are common in β-TM [31]. It does not accurately reflect tissue-specific iron deposition, particularly in the pituitary gland, gonads, and bone marrow, where iron exerts its most deleterious endocrine and skeletal effects [32]. The uniformly high ferritin levels across all BMD groups in our cohort (median range: 1200–3128 ng/mL) suggest that once iron overload exceeds a threshold, additional ferritin elevation may not further worsen BMD, or that other factors—particularly hypogonadism—become the dominant determinants of bone loss.

Second, chronic iron overload may exert indirect effects on bone that are not captured in cross-sectional analyses. Iron deposition in the pituitary gland and gonads leads to hypogonadism, which we found to be strongly associated with low BMD [29]. Furthermore, iron overload promotes oxidative stress, which can impair osteoblast differentiation

and function while enhancing osteoclast-mediated bone resorption through RANKL/RANK/OPG pathway dysregulation [16]. The cumulative effect of iron toxicity over decades may be more important than current ferritin levels in determining bone health. Our cross-sectional design may not capture these cumulative effects.

Third, vitamin D insufficiency was nearly universal in our cohort (91.5%), with uniformly low levels across all BMD groups. This narrow range of vitamin D values may have limited our ability to detect an association with BMD. Additionally, the effects of vitamin D on bone may be overshadowed by the more profound impact of hypogonadism and direct iron toxicity on bone metabolism in this population. Nevertheless, the high prevalence of vitamin D insufficiency is clinically concerning and warrants routine supplementation regardless of its association with BMD [25].

Importantly, these findings do not diminish the clinical significance of iron overload or vitamin D deficiency in β-TM. Rather, they suggest that in patients with established severe iron overload, the endocrine consequences of iron deposition (particularly hypogonadism) may be more proximate determinants of bone loss than the iron burden itself. This highlights the importance of comprehensive endocrine evaluation and targeted intervention in addition to iron chelation therapy.

These findings have important implications for healthcare planning in the Kurdistan Region of Iraq and similar resource-limited settings. The high prevalence of hypogonadism-related osteoporosis (56% at spine) underscores the need for: (1) establishment of routine endocrine screening protocols for all adult β-TM patients, (2) training of hematology staff in basic endocrine assessment, (3) development of referral pathways to endocrinology services, and (4) consideration of hormone replacement therapy where appropriate. Furthermore, our data suggest that DXA screening, though not universally available, should be prioritized for patients with evidence of hypogonadism. These recommendations are particularly relevant given the limited endocrine resources in the region and the growing adult β-TM population.

Limitation and Recommendation

Several limitations of this study should be acknowledged. First, the cross-sectional design precludes establishing causality between hormonal abnormalities and bone loss. Second, the final sample size (n = 141) was below the calculated target of 159, and post-hoc power analysis revealed that the study was underpowered to detect medium effect sizes across the three BMD groups (power = 0.752). The very small number of patients with normal vertebral BMD (n = 3, 2.1%) further limited the statistical reliability of comparisons involving this category. To address this, we performed sensitivity analyses combining the normal and osteopenic groups (non-osteoporotic, n = 62), which confirmed the significant associations between testosterone ($p = 0.032$), estradiol ($p = 0.028$), and LH ($p = 0.003$) with vertebral BMD.

Third, serum ferritin, while the most widely available marker of iron overload, has limitations as a surrogate for tissue iron burden. Ferritin levels can be influenced by inflammation, acute phase response, and hepatic dysfunction (31). MRI-based quantification of liver iron concentration (LIC) and cardiac iron (T2*) would have provided a more accurate assessment of total body iron burden and organ-specific deposition (32). This is a significant limitation, particularly given the strong association between iron overload and endocrine dysfunction. Future studies in this population should incorporate MRI-based iron quantification to better define the relationship between tissue iron deposition, endocrine function, and bone health.

Fourth, bone density was assessed using DXA, which provides areal BMD (g/cm^2) that can be influenced by bone size, potentially underestimating BMD in patients with short stature or bone deformities. Fifth, multiple statistical comparisons were performed when examining the various endocrine and biochemical parameters. Because these analyses were hypothesis-driven and exploratory in nature, formal adjustment for multiple testing was not applied (33). Therefore, findings with borderline statistical significance should be interpreted with caution and require confirmation in larger prospective studies. However, the consistent pattern of lower sex hormone levels across multiple hormones (testosterone, estradiol, LH, FSH) and both skeletal sites (vertebral and hip) supports the biological plausibility of the observed associations.

Finally, this study was conducted at a single center in Erbil, Iraq, which may limit the generalizability of findings to other populations and settings. Despite these limitations, the study provides valuable local data on the prevalence of osteoporosis and its endocrine associations in adult β-TM patients in a region where such comprehensive data are scarce.

CONCLUSION

Osteoporosis and osteopenia are prevalent findings in adults with β-TM, with the lumbar spine being most severely affected. Hypogonadism emerged as the hormonal abnormality most strongly associated with low BMD at both skeletal sites. These results highlight an urgent need for proactive, integrated management. For adult β-TM patients, we recommend: (1) regular DXA screening to detect bone loss, (2) comprehensive endocrine evaluation, and (3) multidisciplinary care involving hematologists, endocrinologists, and allied health professionals to address anemia, iron overload, endocrine dysfunction, and bone health.

Ethical Consideration

The study protocol was approved by the Research Ethics Committee of the College of Medicine, Hawler Medical University (Approval Code: 30 on August 6, 2024). All participants provided written informed consent.

Author Contributions

Conceptualization, B.Z. and M.Q.M., methodology, B.Z. and M.Q.M., software, not applicable, validation, B.Z. and

M.Q.M., formal analysis, B.Z., investigation, B.Z., resources, B.Z., data curation, B.Z., writing—original draft preparation, B.Z., writing—review and editing, B.Z. and M.Q.M., visualization, not applicable, supervision, M.Q.M., project administration, B.Z., funding acquisition, not applicable. All authors have read and agreed to the published version of the manuscript.

Informed Consent Statement

Informed consent was obtained from all subjects involved in the study.

Data Availability Statement

The datasets generated and analyzed during the current study are available from the corresponding author upon reasonable request.

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Conflict of Interest

The authors declare that they have no conflicts of interest.

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Declaration of Generative AI and AI-Assisted Technologies in the Manuscript Preparation Process

During the preparation of this work, the authors used (ChatGPT-4), solely for language polishing, SPSS guidance, and formatting assistance. All scientific content, data analysis, clinical interpretations, and conclusions are the original work of the authors. After using this tool/service, the authors reviewed and edited the content as needed and take full responsibility for the content of the published article.

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