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Research Article

EVALUATION OF OSTEOPATHY IN PATIENTS WITH BETA-THALASSEMIA MAJOR USING DIFFERENT IRON CHELATION THERAPIES

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ABSTRACT

Objective: We aim to assess the bone mineral density (BMD) and bone biochemical parameters in Iraqi patients with β-thalassemia major (β-TM).

Methods: Dual-energy X-ray absorptiometry scan was used to evaluate bone density and interpreted about Z-score which compares to the BMD of age-, sex-, and ethnicity-matched reference population. Biochemical parameters such as calcium, 25-OH Vitamin D, parathyroid hormone, and serum ferritin (SF) evaluated.

Results: No statistical difference in SF between pediatrics and adults was determined; however, 66 patients were having their SF between 1000 and 2500 ng/ml and 122 patients with SF >2500 ng/ml. Calcium and Vitamin D levels are low in both adults and pediatrics. The bone status shows high percentages of osteoporosis 62% and 54.5% for pediatrics and adults, respectively, as well as osteopenia 27% and 34.3% for both pediatric and adults and to a lesser extent normal bone status 11% for each**.**

Conclusion: Osteopathy has a high prevalence in Iraqi patients with β-TM and should receive an optimal transfusion and chelation therapy to prevent bone expansion. Calcium and Vitamin D should be routinely determined to prevent deficiency.

Keywords: Beta-thalassemia major, Osteoporosis, Osteopenia, Deferoxamine, Parathyroid.

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INTRODUCTION

Beta-thalassemia syndrome is a group of Hereditary Blood Disorders that are mainly characterized by the reduction or absence of beta-globin chain synthesis, resulting in a reduction of hemoglobin in red blood cells (RBCs), decreased the production of RBCs and consequently anemia [1,2]**.** Beta-thalassemia usually inherited as an autosomal recessive disease, more than 200 β-thalassemia mutations have been identified, in some of which severe anemia is produced [3]. In Iraq, there is a high prevalence of β-thalassemia major (β-TM) (the prevalence rate in 2015 was 27.4/100,000 populations) [4]. β-TM refers to a severe phenotype which occurs when patients are homozygous or compounds heterozygous for β chain mutation (β+/β0, β0/β0), patients commonly present with symptoms within the first 2 years of life [5,6]**.** The cornerstone management for patients with β-TM is based on lifelong transfusion and iron chelation [7]. Transfusion aims to correct anemia, suppress ineffective erythropoiesis and to inhibit gastrointestinal iron absorption [8]. Normally humans have no mechanism for excreting excess iron in iron overload conditions [9].

Physiologically two distinct cell types are in charge of the maintenance and renewal of the bone: The osteoblasts which are responsible for bone formation and the osteoclasts which are responsible for bone resorption and remodeling [10]. The essential pathway that links osteoclast-mediated bone resorption with osteoblast-mediated bone formation consists of a paracrine system that comprises receptor activator of nuclear factor-κB ligand (RANKL), its RANK, and soluble protein osteoprotegerin (OPG). RANKL produced by osteoblasts and their precursors bind to the RANK receptor, promoting osteoclast differentiation and proliferation. OPG acts as a decoy receptor to block the action of RANKL. This system provides a balance between bone formation and resorption and through which a wide variety of biological mediators such as hormones, cytokines, and growth factors affect bone homeostasis. RANKL enhances osteoclastic function that elevated in β-TM patients, while OPG and OPG/RANKL ratio reduced, associated with low bone mineral density (BMD), this gives evidence that OPG/RANKL system plays a key role in the pathogenesis of osteoporosis in β-TM [10,11].

Ineffective erythropoiesis results in erythroid hyperplasia and marrow expansion secondary to extramedullary hematopoiesis [6], resulting in expansion of the medulla, thinning of cortical bone and resorption of cancellous bone causing a generalized loss of BMD [11]. Deposition of iron in the bones impairs osteoid maturation and inhibits mineralization locally; this occurs by a mechanism that includes the incorporation of iron into calcium hydroxyapatite crystals which consequently affects their growth [12]. Deferoxamine (DFO) is an iron chelator used to reduce iron overload in patients with thalassemia, but on the other hand, it was found to have harmful effects on bone status. It exerts a direct effect by interfering with bone growth and by altering bone metabolism due to chelation of trace metals. DFO exerts deleterious effects on osteoblasts through inhibition of deoxyribonucleic acid (DNA) synthesis, osteoblasts proliferation, and differentiation of osteoblastic precursors and in patients receiving high doses it enhances osteoblasts apoptosis [13,14].

Parathyroid hormone (PTH) secreted by the parathyroid gland and functions in calcium homeostasis together with Vitamin D and calcitonin, PTH maintains calcium levels within normal range by facilitating its absorption from the gastrointestinal tract, by phosphorous excretion and calcium reabsorption from the kidney, and by bone resorption. PTH also plays a role in the conversion of Vitamin D to its active form (1, 25-dihydroxycholecalciferol) in the kidneys [15].

Iron deposition within the parathyroid gland occurs in patients with iron overload due to recurrent transfusions and results in hypoparathyroidism (HPT), particularly after 10 years of age, as

a consequence of that low PTH and Vitamin D ensued. HPT is also a leading cause of hypocalcemia, and several neurological complications may evolve such as tetany, seizures, and paresthesia [16,17].

METHODS

Patient selection and study design

The present study designed as a single-center, cross-sectional, observational study. The study performed at AL-Karama Teaching Hospital/Department of Hereditary Blood Disorders (Thalassemia Center). The participant patients in this study were all with β-TM trait who were attending Thalassemia Centre at Al-Karama Hospital for recurrent blood transfusion and for receiving their iron chelation therapy. A total of 201 patients approved to enroll in the study, written informed consent taken from all patients (and for underaged patients the written informed consent also taken from their legal guardian). The patients divided into two main groups: Group A: Patients on DFO are subdivided into two groups: Children and adolescents (8–18 years) and adults (19 and more) and Group B: Patients on deferasirox (DFX) are subdivided into two groups: Children and adolescents (8–18 years) and adults (19 and more).

The sample size calculated using Raosoft sample calculator (online tool), a total of 600 patients with β-TM were registered at AL-Karama Thalassemia Centre; hence, a sample size of approximately 200 patients should be included assuming a margin of error of 5% and a confidence level of 95%.

Method of bone status evaluation

About 5 ml of fasting pre-transfusional venous blood collected and serum stored at −20°C after separation. Serum ferritin (SF) level was measured using Cobas e 411 (Roche diagnostic equipment). The ELISA kits were used to determine serum levels of calcium and 25-OH Vitamin D. Immunoenzymatic assay used for the evaluation of PTH. Dual-energy X-ray absorptiometry (DEXA) scan of the lumbar spine region done as an index of bone density. BMD is expressed in grams of minerals per scanned square centimeters (g/cm^2) and interpreted in relationship to Z-score which compares to the BMD of age, sex, and ethnicity of the matched reference population [18,19]. In the assessment of the lumbar spine, the region of interests placed on the L1-L4 vertebral bodies [20]. Patients with Z-score of -1SD or greater are considered normal, Z-scores between −1 and −2.5 are osteopenic (low bone mass), Z-scores of −2.5 or less are considered osteoporotic, and severely osteoporotic patients are those having Z-score of <−2.5 with fragility fracture [21].

Statistical analysis

Discrete variables presented using their numbers and percentages; Chi-square test was used to analyze the discrete variables (or Fisher exact test when Chi-square test is not valid; due to low sample size <20 and if two or more with an expected frequency is <5). Two samples t-test was used to analyze the differences in means between two groups (if both follow a normal distribution with no significant outlier). Binary logistic regression analysis was used to calculate the odds ratio and their 95% confidence intervals when the outcome can categorize into two binary levels, and if appropriate probability plot used to present the relationship. Linear regression analysis was performed to assess the relationship between different variables. If one or both of the variables were following a normal distribution, Pearson correlation is used. If both did not follow a normal distribution, the Spearman correlation used. Scatter plot was used to present the regression analysis. Correlation coefficient or standardized beta (r) is a representative of magnitude and direction of the relationship; 0.00–0.29=little or no correlation; 0.3–0.49=weak; 0.5–0.69=moderate; 0.7–0.89=strong; and 0.9–1.00=very strong. The negative sign indicates an inverse relationship, while a positive sign indicates a direct relationship. Statistics is a Software Package 22 (Chicago, IL), Minitab 17.1.0 software package was used to make the statistical analysis, p-value considered to be statistically significant if <0.05.

RESULTS

The demographic data and disease characteristics illustrated in Table 1; a total of 201 patients were approved to include in the study. In this study, the patients included fall into three groups: Children (8–12), n=40; (19.9%), adolescents (13–18), n=78; (38.8%), and adults (>19), n=83; (41.3%), but children and adolescents were considered as a one category (pediatric) during the study. The frequency of their attendance was every 2 weeks, and their age of onset on blood transfusion was approximately 13.7±9.0 months.

Although there is no statistical difference in SF between pediatrics and adults, 66 patients were having their SF between 1000 and 2500 ng/ml and 122 patients with SF >2500. Calcium and Vitamin D levels are low in both adults and pediatrics. The bone status shows high percentages of osteoporosis 62% and 54.5% for both pediatrics and adults, respectively, as well as osteopenia 27% and 34.3% for both pediatrics and adults and to a lesser extent normal bone status 11% for each as illustrated in Tables 2 and 3.

Advanced age predicts a decrease in BMD and osteoporosis insignificantly. However, males having higher BMD in comparison to females; there was no significant effect on BMD. DFX inversely associated with osteoporosis as illustrated in Table 4. Increase in iron overload predicts osteoporosis, but the relationship is insignificant.

In univariate analysis advance age, DFO and elevated ferritin predict osteoporosis (Table 5).

There was no significant correlation between the different variable in Table 6 with osteoporosis in the adult.

DISCUSSION

Osteopathy affects 40–50% of the patients with thalassemia major. The mechanism of osteopathy in β-TM is multifactorial and comprises the effects of anemia on bone health, secondary to iron deposition

Table 1: Demographic data and disease characteristics

Variables	Value
Number	201
Age (years), mean±SD	19.1±7.3
8–12 years	40 (19.9%)
13–18 years	78 (38.8%)
≥19 years	83 (41.3%)
Gender	
Female	107 (53.2%)
Male	94 (46.8%)
BMI (kg/m^2), mean±SD	21.4±4.0
The frequency of attendance (days), mean±SD	14.7±4.3
Age at which disease diagnosed (months), mean±SD	13.7±9.0

SD: Standard deviation

Table 2: Assessment of bone status and various biochemical parameters

Variables	Paediatrics*	Adults	p-value
Number	100	101	-
Ferritin (ng/mL)	3,957.9±2,914.0	3,761.7±2,452.9	0.606
<1000	7 (7.0%)	6 (5.9%)	0.737
1000–2500	35 (35.0%)	31 (30.7%)	
>2500	58 (58.0%)	64 (63.4%)	
Calcium (mmol/L)	2.06±0.23	2.1±0.2	0.227
Vitamin D (ng/mL)	10.5±8.5	11.4±9.1	0.498
PTH (pg/mL)	35.7±27.1	34.0±23.4	0.644
Z score	−2.7±1.4	−2.5±1.2	0.186
Bone status			
Normal (>1 SD)	11 (11.0%)	11 (11.1%)	0.509
Osteopenia (−1)–(−2.5 SD)	27 (27.0%)	34 (34.3%)	
Osteoporosis (<−2.5 SD)	62 (62.0%)	54 (54.5%)	

*Pediatrics (children and adolescents). PTH: Parathyroid hormone

(hemosiderosis) of various endocrine glands, liver dysfunction, chronic illness affecting physical health, and chelation therapy inducing toxicity. Recently, the receptor activator of nuclear ƙB (RANK)/RANK ligand and OPG system has been found to be the dominant pathway affecting osteoclastic activation and proliferation and plays a major role in thalassemia-induced osteoporosis [22,23].

DEXA is a non-invasive method for the assessment of BMD; in the present study, the osteopenia was in 27% of pediatrics and 34.3% of adults (totally 30.35%), while osteoporosis was 62% in pediatrics and 54.5% in adults (totally 57.7%), and the total normal BMD was in only 10.9%. The results of the Z score in our study were comparable to Valizadeh *et al*. [19] and Merchant *et al*. [24]. Our findings state that there is no significant difference in Z score between adults and pediatrics which appears to be in sharp contrast to Mohseni *et al*. [25], in which they concluded that low BMD in pediatrics is less common than adults. However, in our study, there was a non-significant direct relationship between advancing in age and decrease in BMD and osteoporosis this was in agreement with AL-Amir *et al*. [26]; however, in pediatrics advance in age significantly (in univariate analysis) and dependently (in multivariate analysis) predict osteoporosis which is in agreement with Merchant *et al.* [24] and Izadyar *et al*. [27] who reported a statistically significant relationship between age and BMD; nevertheless, Izadyar *et al*. have the same findings regarding gender in which both of us found no significant association between gender and BMD, and our finding regarding gender was in disagreement with Mohseni *et al.*, who reported a significant effect of gender on BMD in which males had higher BMD, but their findings undermined because of its ineffectiveness on Z score [25].

Table 3: SF levels and bone status among patients using DFO and DFX

Variables	DFO	DFX	p-value
Number	99	102	-
Ferritin	5028.8±3006.5	2724.1±1699.2	<0.001 [S.]
<1000	3 (3.0%)	10 (9.8%)	<0.001 [S.]
1000–2500	19 (19.2%)	47 (46.1%)	
>2500	77 (77.8%)	45 (44.1%)	
Z score	−2.8±1.2	−2.4±1.4	0.014 [S.]
Bone status			
Normal	5 (5.1%)	17 (16.8%)	0.009 [S.]
Osteopenia	27 (27.6%)	34 (33.7%)	
Osteoporosis	66 (67.3%)	50 (49.5%)	

DFO: Deferoxamine, DFX: Deferasirox

Table 4: Predictors of osteoporosis for all patients

Variables	Osteoporosis	
	OR (95% CI)	p-value
Age	1.009 (0.971–1049)	0.647
Gender	1.420 (0.805–2.504)	0.226
BMI	0.960 (0.894–1.030)	0.253
Treatment (DFX)	0.475 (0.268–0.845)	0.011 [S.]
Splenectomy	2.083 (0.970–4.470)	0.060
Ferritin	1.000 (1.000–1.000)	0.149
Calcium	0.102 (0.964–1.260)	0.156
Vitamin D	0.974 (0.943–1.006)	0.113
PTH	1.010 (0.998–1.023)	0.109

OR: Odds ratio, CI: Confidence interval, BMI: Body mass index, PTH: Parathyroid hormone, DFX: Deferasirox

The effect of splenectomy on BMD was not significant (p=0.06) which was in agreement with Merchant *et al*. [24] up to our knowledge, the effect of splenectomy on BMD was not studied more often. In adults, the increase in SF predicts insignificantly (p=0.544) osteoporosis while in pediatrics SF is a dependent and significant predictor of osteoporosis, this was consistent with a study conducted on 70 children and adolescents by Nesheli and Farahanian [28] which reported a significant relationship between SF and bone density. The lack of significant correlation between SF and BMD in adults could attribute to SF tolerance, SF measurements may vary at different times, and other possibilities are small sample size, measurements error, and type of chelator used. The mechanism of iron toxicity on the bone is elucidated by its local effect which impairs osteoid maturation and bone mineralization which occurs by iron incorporation into calcium hydroxyapatite crystals which are important information of normal bones in which these crystals deposited on a proteinaceous collagen matrix [12].

DFO is considered a significant predictor for osteopathy, osteopenia and osteoporosis were higher in patients using DFO, and this could attribute to low compliance with DFO and high SF levels in these patients. DFO exerts a deleterious effect on bone through its direct effect by altering bone metabolism by chelating trace metals. DFO affects osteoblasts through inhibition of DNA synthesis, osteoblasts proliferation, and differentiation of osteoblastic precursors and in patients receiving high doses it enhances osteoblasts apoptosis [13].

The reason behinds low BMD and high prevalence of osteoporosis in both populations in the present study attributed to high SF levels, low calcium and Vitamin D levels, poor nutritional supports, limited physical activity, and inappropriate chelation. The impaired calcium hemostasis is thought to be a consequence of iron overload, Vitamin D deficiency, and HPT [29]. According to Shah study [30], hypocalcemia in states of iron overload is usually chronic and asymptomatic. Hypocalcemia (resulting from HPT) occurs through increasing calcium loss through urine and decrease intestinal absorption of calcium. The reported calcium levels in Salva's study were comparable to our levels.

The PTH levels in this study were various range from 8.6 to 62.8 pg/ml in pediatrics and from 10.6 to 57.4 in adults, the PTH levels in our study are comparable to many studies [26,31]. The development of HPT is mainly considered a consequence of iron overload, Pirinççioğlu and Söker [31] found a positive correlation between SF levels and PTH, while Chern and Lin [32] found that the development of HPT is not correlated with SF as many patients developed HPT while their SF

Table 5: Predictors of osteoporosis for pediatric patients

Variables	Univariate		Multivariate	
	OR (95% CI)	p-value	OR (95% CI)	p-value
Age	1.169 (1.034–1.320)	0.012 [S.]	1.090 (0.919–1.293)	0.31
Gender	1.111 (0.495–2.493)	0.798	-	-
BMI	0.959 (0.870–1.057)	0.398	-	-
Treatment (DFX)	0.333 (0.142–0.780)	0.011 [S.]	0.576 (0.133–2.505)	0.462
Ferritin	1.000 (1.000–1.000)	0.022 [S.]	1.000 (1.000–1.000)	0.459
Calcium	1.091 (0.943–1.262)	0.240	-	-
Vitamin D	0.987 (0.941–1.035)	0.581	-	-
PTH	1.011 (0.993–1.030)	0.217	-	-

R^2 (Cox and Snell)=0.282, PTH: Parathyroid hormone, BMI: body mass index, DFX: Deferasirox, OR: Odds ratio, CI: Confidence interval

Table 6: Predictors of osteoporosis for adult patients

Variables	Osteoporosis	
	OR (95% CI)	p-value
Age	1.029 (0.957–1.107)	0.440
Gender	1.774 (0.793–3.968)	0.163
BMI	0.967 (0.871–1.073)	0.523
Treatment (DFX)	0.640 (0.289–1.419)	0.272
Splenectomy	1.818 (0.757–4.366)	0.181
Ferritin	1.000 (1.000–1.000)	0.544
Calcium	0.493 (0.079–3.090)	0.450
Vitamin D	0.964 (0.922–1.009)	0.118
PTH	1.009 (0.991–1.026)	0.326

PTH: Parathyroid hormone, BMI: body mass index, DFX: Deferasirox, OR: Odds ratio, CI: Confidence interval

levels were lower than other patients. HPT development in their study was found to be dependent on patients' related factors and tendencies. Several mechanisms were postulated to describe the glandular damage by iron overload which mainly occurs in the second or third decade of life: 1 - the formation of free radicals and lipid peroxidation causing mitochondrial, lysosomal, and sarcolemmal membrane damage, 2 - number of surface transferrin receptors in the cell, and 3 - the ability of the cell to protect itself against iron species [31,33].

Vitamin D is crucial for calcium and phosphorous absorption. Vitamin D has a direct relation to BMD, and maximum BMD achieved when the 25-OH-D level is 40 ng/ml or more [34]. In our study, serum Vitamin D levels are apparently deficient in both pediatrics and adults (10.5±8.5 in pediatrics and 11.4±9.1 in adults) which are comparable to Akhouri and Neha [35] and Merchant *et al.* [24] Vitamin D deficiency in patients with β-TM attributed to nutritional deficiency and hepatic impairment due to hemosiderosis which results in defective hydroxylation of Vitamin D to 25-OH-Vitamin D, levels of Vitamin D >30 ng/ml is considered normal and levels between 10-20 ng/ml are considered deficient and levels of 21–29 ng/ml is considered insufficient [24,34].

CONCLUSION

Osteopathy (osteopenia and osteoporosis) has a high prevalence in Iraqi patients with β-TM. Poor chelation, undernourishment, calcium and Vitamin D deficiency, and secondary HPT are the leading cause of osteopathy. Therapeutic intervention should initiate in the case of Vitamin D and calcium deficiency. Annual BMD evaluation is crucial to avoid osteopathy in thalassemia patients in Iraq.

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AUTHORS' CONTRIBUTION

All authors contribute equally in the making of this manuscript.

CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest.

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