Bone metabolism and mineral density in patients with beta-thalassemia major

Umit Dundar, MD, Alphan Kupesiz, MD, Sebahat Ozdem, MD, Erdal Gilgil, MD, Tiraje Tuncer, MD, Akif Yesilipek, MD, Meral Gultekin, MD.

ABSTRACT

Objectives: To evaluate bone metabolism in patients with beta-thalassemia major and to determine the factors associated with the development of osteoporosis.

Methods: We studied 25 patients with thalassemia major with a mean age of 18.4 years (range 5-31) and aged and gender matched 24 healthy controls who were attending the outpatient physical medicine and rehabilitation clinic of Akdeniz University Hospital between January 2004 and March 2004 in Turkey. Bone mineral density (BMD) of lumbar spine (L1-L4) and proximal femur were determined using dual x-ray absorptiometry (DXA). Venous blood samples were obtained for determination of blood cell count and markers of bone formation and resorption.

Results: The BMD values, both at lumbar and femoral neck levels were significantly lower in patients compared to controls. Serum N-telopeptide level was slightly higher, whereas osteocalcin was slightly lower in patients; however, these values were not statistically significant. Plasma levels of insulin like growth factor-1 (IGF-I) and insulin like growth factor binding protein-3 (IGFBP-3) were significantly lower in patients. Also, serum levels of estradiol and progesterone in females, luteinizing hormone and follicle-stimulating hormone in both gender were significantly lower in patients. Serum levels of free testosterone and total testosterone were lower in patients, but not statistically significant. Patients also had significantly higher serum phosphorus levels, and lower serum calcitonin levels compared to controls.

Conclusion: The BMD is decreased in thalassemic patients. Growth retardation, growth hormone / IGF-I / IGFBP-3 axis dysfunction, gonadal dysfunction and hypothalomo-pituitary-gonadal axis dysfunction may be responsible for the development of osteoporosis in the patients with beta-thalassemia major.

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From the Department of Physical Medicine and Rehabilitation (Dundar), Faculty of Medicine, Kocatepe University, Afyonkarahisar, Departments of Pediatric Hematology and Oncology (Kupesiz, Yesilipek), Microbiology (Ozdem, Gultekin), and Physical Medicine and Rehabilitation (Gilgil, Tuncer), Faculty of Medicine, Akdeniz University, Antalya, Turkey.

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Address correspondence and reprint request to: Dr. Umit Dundar, Assistant Professor, Department of Physical Medicine and Rehabilitation, Faculty of Medicine, Kocatepe University, 03200 Afyonkarahisar, Turkey. Tel. +90 (272) 2171753. Fax. +90 (272) 2172029. E-mail: umitfir@yahoo.com

The β-thalassemia major is an inherited blood disorder that leads to life threatening anemia and requires regular blood transfusion and iron-chelating therapy throughout life from early childhood. The combination of transfusion and chelation therapy has dramatically extended the life expectancy of thalassemia patients who can now survive into their fourth and fifth decades of life.[1] With advances in transfusion management beginning in the 1960s, there has been marked improvement in terms of skeletal development and normal cosmetic facial and long bone appearance. But osteopenia severe osteoporosis remains a serious complication even in well-transfused and iron chelated patients. Prior to institution of aggressive transfusion regimens, fractures occurred primarily in the long bones and sometimes it was associated with trauma. After the introduction of aggressive transfusion, the pattern changed, with less involvement of long bone and an increase in vertebral compression fractures especially in older patients.[2,3] Bone disease in patients with thalassemia major is a multifactorial and the process was poorly understood. Major pathogenetic factors for the development of low bone mineral density (BMD) in thalassemic subjects appear to be; chronic hypoxemia and medullary expansion, defective growth affecting both height and weight, abnormal calcium-phosphate homeostasis, delayed or lack of pubertal development and decreased sex steroid secretion, low plasma vitamin D concentration, genetic predisposition, abnormal growth hormone (GH)/insulin-like growth factor-1 (IGF-I)/insulin-like growth factor binding protein-3

(IGFBP-3) axis and, other factors such as secondary hypoparathyroidism hypothyroidism and diabetes may represent additional mechanisms.[3-6] The aim of this study was to evaluate the bone metabolism and to determine the factors associated with the development of osteoporosis in patients with β-thalassemia major.

Methods. We studied 25 patients with beta-thalassemia major (15 women and 10 men) with a mean age of 18.4±6.7 years (range 5-31), who were attending the outpatient physical medicine and rehabilitation clinic of Akdeniz University Hospital, between January 2004 and March 2004 in Antalya, Turkey. The age and gender matched 24 healthy subjects participated in our study as controls. All patients have had regular blood transfusions to keep the hemoglobin concentration above 10 g/dl and received subcutaneous desferrioxamine 40 mg/kg, 5-6 times per week. We excluded patients with thyroid disease, parathyroid disease, evident skeletal abnormalities and a history of corticosteroid and anticonvulsant drug use. Bone mineral density was measured in all patients and controls by dual energy x-ray absorptiometry (DXA, HOLOGIC Q DR 4500 W) both at the lumbar spine (antero-posterior projection of L1-L4) and the proximal femur (neck and total score). The instrument was calibrated daily according to the manufacturer's instructions. The BMD data were expressed as grams per centimeter square and standard deviation (SD) scores and this was compared with BMD values of controls. Osteopenia is a T-score between -1 and -2,5 and osteoporosis is equals or below -2,5 (World Health Organization [WHO] study group, 1994). The WHO definition of osteoporosis relates only to adult women and not to children. Thus, we expressed values as Z-score; a Z-score represents the number of SD above or below the age and gender matched mean reference value (National Health and Nutrition Examination Survey III reference population database for United States of America to calculate Z-scores).[29] Approval for the study was granted by the Local Ethical Committee of the University.

Biochemical analyses. Following an overnight fasting (at least 8 hours), venous blood samples were obtained for determination of blood cell count and markers of bone metabolism which included: bone resorption markers serum N-telopeptide of collagen type I (Ntx), bone formation markers (osteocalcin, alkaline phospatase), hormones (free triiodothyronine [free T3], free thyroxine [free T4], thyroid stimulating hormone [TSH], parathyroid hormone [PTH], luteinizing hormone [LH], follicle-stimulating hormone [FSH], estradiol, progesterone, total and free testosterone, IGF-I, IGFBP-3, adrenocorticotropic hormone [ACTH], cortisol, calcitonin, routine biochemical markers (calcium, phosphorus, magnesium) and 25-hydroxyvitamin D. A urine sample was obtained to calculate urinary calcium/creatinine ratio. Blood cell count was determined on the same day, then the serum was separated from the red blood cells by centrifugation and kept frozen at -20°C until analysis. Free and total testosterone was studied only in males. Estradiol and progesterone were studied only in females. The level of TSH, free T3, free T4, LH, FSH, total testosterone, progesterone, PTH, cortisol, osteocalcin, and estradiol were measured by electrochemiluminescence immunoassay. The level of alkaline phospatase was measured by standardized colorimetric method. The level of phosphorus was measured by ammonium phosphomolybdate colorimetric method. The level of magnesium was measured by xylidyl blue colorimetric method. The level of serum Ntx was measured by enzyme-linked immunosorbent assay. The level of serum and urinary calcium was measured by o-cresolphthalein endpoint colorimetric method. The level of urinary creatinine was measured by jaffe method. The level of 25-hydroxyvitamin D, IGF-I, ACTH and free testosterone were measured by radioimmunoassay. The level of calcitonin and IGFBP-3 was measured by immunoradiometric method. All patients signed an informed consent before participating in the study.

Statistical analysis. All parametric results were expressed as mean±SD for each group. Statistical analyses were performed using the unpaired t test to compare the mean concentration among the patients and controls when data were normally distributed and Wilcoxon test were used when data were not normally distributed. For correlation analysis, Pearson coefficient of correlation was used. A probability value of less than 0.05 was assumed as significant.

Results. The demographic features of our patients and controls are summarized in **Table 1**. The gender, age, weight and body mass index did not differ significantly among the patients and controls. However, the mean height of the patients with β-thalassemia major were significantly lower than the height of the controls (p=0.016). Dual energy x-ray absorptiometry showed that patients with β-thalassemia major had a significant reduction of BMD compared to the controls (**Table 2**). Hormonal and biochemical characteristics of the patients and controls are shown in **Table 3**. A marker of bone resorption, serum Ntx level was slightly higher in the patients, whereas a marker of bone formation, osteocalcin was slightly lower in patients. However, these values were not statistically significant. Plasma levels of IGF-I and IGFBP-3 were significantly lower in patients (p<0.001). When we made correlation analysis of IGF-I, IGFBP-3 with BMD was made, we found that IGF-I, IGFBP-3 showed a good correlation with BMD (**Table 4**). Serum levels of estradiol and progesterone of

females, LH and FSH of both males and females were significantly lower in patients (p<0.001). Serum levels of free and total testosterone were lower in patients compared to controls, but this difference was not statistically significant. Patients had significantly higher serum phosphorus levels and lower serum calcitonin levels (p<0.01). Other biochemical parameters did not differ among thalassemics and controls.

Discussion. The increased survival of the patients with β-thalassemia major brought on by more efficient therapy, made bone metabolism study absolutely necessary, because the related morbidity varies widely across the patient population and often imposes a customized treatment.[7] In our study, BMD values both at lumbar spine and proximal femur (femoral neck and total score) were significantly lower in patients compared to controls. Also, several studies have shown decreased bone density in thalassemic patients.[1-8] Etiology of decreased BMD in thalassemic patients is thought to be multifactorial and one of them seems to be growth retardation. In our study, height of the patients was

Table 1 - Demographic features of the patients with β-thalassemia major and controls.

Parameter	Patients	Controls	*P* value
Gender			0.77
Female	15	13	
Male	10	11	
Age (years)	18.4 ± 6.7	18.1 ± 6.1	0.88
Weight (kg)	42.8 ± 12.4	48.9 ± 11.5	0.09
Height (m)	1.48 ± 0.15	1.59 ± 0.15	0.016
BMI (kg/m²)	18.9 ± 2.5	19.0 ± 2.2	0.95

BMD - body mass index

Table 2 - The BMD values of the patient with β-thalassemia major and controls.

BMD values	Patients	Controls	*P* value
BMD of L1-L4			
-gm/ cm²	0.663 ± 0.168	0,851 ± 0,146	<0.001
-Z-sore	-2.66 ± 1.16	-0,92 ± 0,89	<0.001
BMD of femoral neck			
-gm/ cm²	0.695 ± 0.119	0,785 ± 0,130	0.02
-Z-sore	-2.32 ± 1.17	-1,02 ± 1,27	0.04
BMD of proximal femur as total score			
-gm/ cm²	0.726 ± 0.133	0,886 ± 0,134	<0.001
-Z-sore	-2.50 ± 1.07	-0,47 ± 1,13	0.001

BMD - bone mineral density, L1 - Lumbar spine 1, L4 - lumbar spine 4, Z-core - represents the number of standard deviations above or below the age and sex matched mean reference value

Table 3 - Hormonal and biochemical characteristics of the patients and controls.

Characteristics	Patients	Controls	*P* value
Calcium (mg/dl)	10.2 ± 0.9	10.3 ± 0.9	0.521
Phosphorus (mg/dl)	5.4 ± 1.2	4.6 ± 0.8	0.008
Magnesium (mg/dl)	2.1 ± 0.3	2.1 ± 0.2	0.379
Alkaline phosphatase (U/l)	341.3 ± 148.2	311.9 ± 201.7	0.144
Free T3 (pg/ml)	3.9 ± 0.7	4.2 ± 0.6	0.250
Free T4 (ng/ml)	1.4 ± 0.3	1.3 ± 0.2	0.352
TSH (uIU/ml)	2.5 ± 1.1	2.4 ± 1.2	0.582
PTH (uIU/ml)	35.8 ± 19.9	32.3 ± 12.6	0.841
Osteocalcin (ng/ml)	37.6 ± 27.1	60.4 ± 44.4	0.055
Serum N-telopeptide (nm BCE)	42.2 ± 32.8	28.7 ± 16.7	0.164
Free testosterone (pg/ml)*	2.9 ± 3.8	10.1 ± 10.2	0.053
Total testosterone (ng/ml)*	2.1 ± 3.9	3.6 ± 3.3	0.139
Estradiol (pg/ml)†	19.4 ± 15.6	72.1 ± 51.1	<0.001
Progesterone (ng/ml)†	0.3 ± 0.2	2.7 ± 3.3	<0.001
LH (mIU/ml)	1.6 ± 2.4	4.3 ± 3.7	<0.001
FSH (mIU/ml)	2.0 ± 2.1	4.1 ± 2.2	<0.001
IGF-I (ng/ml)	203.5 ± 142.8	625.9 ± 299.9	<0.001
IGFBP-3 (ng/ml)	2292.48 ± 946.65	3763.08 ± 514.66	<0.001
25-hydroxyvitamin D (ng/ml)	37.9 ± 16.1	42.4 ± 21.6	0.569
Calcitonin (pg/ml)	5.9 ± 2.1	9.1 ± 4.9	0.007
Cortisol (µg/dl)	13.4 ± 6.6	13.1 ± 5.6	0.873
Urinary calcium/ creatinine ratio	0.063 ± 0.07	0.074 ± 0.05	0.182
ACTH (pg/ml)	68.5 ± 22.4	83.3 ± 34.2	0.085

*calculated only for male, †calculated only for female, Free T3 - free triiodothyronine, Free T4 - free thyroxine, TSH - thyroid stimulating hormone, PTH - parathyroid hormone, LH - luteinizing hormone, FSH - follicle-stimulating hormone, IGF-I - Insulin-like growth factor-1, IGFBP-3 - insulin-like growth factor binding protein-3, ACTH - adrenocorticotropic hormone

Table 4 - Correlation of IGF-I, IGFBP-3 with BMD.

BMD values	IGF-I	IGFBP-3
BMD of L1-L4 (gm/ cm²) (r)	0.565	0.620
p value	<0.001	<0.001
BMD of femoral neck (gm/ cm²) (r)	0.508	0.507
p value	<0.001	<0.001
BMD of proximal femur (gm/ cm²) (r)	0.593	0.642
p value	<0.001	<0.001

BMD - bone mineral density, IGF-I - insulin-like growth factor-1, IGFBP-3 - insulin-like growth factor binding protein-3, L1 - Lumbar spine 1, L4 - Lumbar spine 4

significantly lower than controls. The skeletal bone mass is the result of a balance between the amount of bone gained during growth and subsequent bone loss. In thalassemic patients, both these processes would be altered. Jensen et al[10] found that young (<12 years) thalassemic patients have low BMD, which suggests that the peak bone mass achieved was also adversely affected.[9,10] So, growth retardation by altering peak mass may contribute to the development of low BMD in thalassemic patients. Several studies showed that thalassemics had an unbalanced bone turn-over with an increased resorption phase and decreased or normal formation phase.[4,7,9] But in another study, Lala et al[11] found that bone turnover in thalassemic patients was not different in controls. In our study, serum Ntx level was slightly higher in the patients, whereas osteocalcin was slightly lower in the patients, but these values were not statistically significant. These results may show that, a slight increase in bone resorption and a slight decrease in bone formation may cause the unbalanced bone turnover and eventually may result in the development of low BMD in the patients with thalassemia major. Hypogonadism, which is often observed in these patients, can also play an important role in the development of osteoporosis.[3,4,7,12-14] In our study, serum levels of estradiol and progesterone in females and, LH and FSH in both males and females were significantly lower in patients. Serum levels of free testosterone and total testosterone were lower in patients than in controls, but not statistically significant. This result may be due to the small number of subjects included in our study. Bone metabolism and skeletal consolidation result from a complex sequence of hormonal changes in interaction with nutritional factors, where the concerted action of GH, IGF-I and sex hormones and their receptors, besides other factors are responsible for timing and attainment of skeletal consolidation.[3] Estrogen replacement therapy for women is the most effective preventive measure against postmenopausal osteoporosis.[15] It is noteworthy that therapeutic correction of hypogonadism with appropriate hormone replacement therapy in these thalassemic patients has failed to protect them from low bone mass, although it results in some suppression of bone turnover.[12] This could suggest in these patients a possible and not yet well-clarified target organ resistance, or the presence of extragonadal factors such as low level of IGF-I.[3,4] During a normal pubertal growth spurt, sex steroids increase GH secretion with a subsequent increase of IGF-I levels. Sex steroids and GH contribute approximately 50% of the height gain.[16-19] The IGF-I is a potent stimulator of linear growth and a major determinant of bone mineralization. The exogenous administration of IGF-I has been shown to increase growth and bone formation in humans and animals.[20-23] This effect is potentiated when IGF-I is combined with IGFBP-3.[24] In our study, plasma levels of IGF-I and IGFBP-3 were significantly lower in patients. Also, correlation analysis showed that IGF-I and IGFB-3 have a good association with BMD in thalasemic patients. In our patients, low level of IGF-I and IGFBP-3 and a significant association of these growth factors with BMD and linear growth (height of our patients) suggested a major role of GH/ IGF-I/ IGFBP-3 axis in the pathogenesis of osteoporosis. Our thalassemic patients also had significantly higher serum phosphorus levels compared to the control group. There are several disorders causing hyperphosphatemia; chronic renal failure, neoplasm, excess phosphate intake, laxative usage, acidosis, hyperthyroidism, and hypoparathyroidism. In patients with β-thalassemia major, secondary to accumulation of iron in the parathyroid gland, rarely hypoparathyroidism develops.[25] In our study, mean PTH level did not differ among thalassemics and controls. However, hyperphosphatemia in our patients may estimate the development of iron deposition in parathyroid gland and during that time, it causes the development of hypoparathyroidism. In our study, thalassemic patients also had significantly lower serum calcitonin levels compared to controls. This result was very interesting. In a previous study, Alagna et al[26] found that serum calcitonin level did not differ among thalassemics and controls.[26] Calcitonin is an effective inhibitor of osteoclastic bone resorption. It has direct effect on osteoclast, causing contraction of cytoplasmic membrane, a decrease in area of osteoclast ruffled border and inhibition of osteoclast's capacity to resorb bone.[27] In this respect, Canatan et al[28] used calcitonin in the treatment of osteoporosis in the patiens with β-thalassemia major. After one year of treatment, osteoporosis improved, number of fractures decreased in the treatment group.[28] In our study, decreased calcitonin concentration in the patients may contribute to the development of relatively increased bone resorption.

In conclusion, the BMD scores of proximal femur and lumbar vertebra in patient group were found significantly lower than control group. Etiology of low BMD in thalassemic patients is thought to be multifactorial. Growth retardation, GH/IGF-I/ IGFBP-3 axis dysfunction, gonadal dysfunction, and hypothalomo-pituitary-gonadal axis dysfunction may be responsible for the development of osteoporosis in the patients with β-thalassemia major.

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