BONE MINERAL DENSITY IN BETA THALASSEMIA MAJOR PATIENTS*
Beta talassemi majörlü hastalarda kemik mineral dansitesi

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Abstract

Purpose: *Investigation of bone mineral density (BMD) in thalassemia major patients, and its relationship with anthropometric, hematologic, biochemical and hormonal parameters.*

Patients and Methods: *Twenty-two transfusion dependent thalassemic children aged between 2.5 and 18 years (13 female, 9 male) were studied. Their weight, height, triceps skinfold thickness and midarm circumference were measured and the body mass index was calculated. Hemoglobin, serum levels of calcium, phosphorus, alkaline phosphatase, triglyceride, cholesterol, vitamin A, vitamin E, β-carotene, and ferritin, were studied in all children. Bone mineral density was measured with dual energy X-ray absorbtiometry method from L1-L4 spine and left femoral neck in all patients.*

Results: *Mean BMD for lumbar spine and left femoral neck was 0.53±0.07 g/cm^2 (0.401- 0.617 g/cm^2) and 0.58± 0.08 g/cm^2 (0.396 - 0.740 g/cm2) respectively. These values were lower than BMD values of age and sex matched healthy children. Age, height, weight, midarm circumference (not for femoral BMD) and triglyceride values correlated positively with BMD of both sites, but ferritin negatively correlated.*

Conclusion: *BMD values of thalassemic patients were significantly lower than the values of healthy children. Hypertransfusion, effective chelation and aggressive dietary management could positively affect BMD in these patients.*

Key Words: *Anthropometry, Bone mineral density, Thalassemia*

Özet

Amaç: *Beta talasemi majorlü hastalarda kemik mineral dansitesini ölçmek ve bunun antropometrik, biyokimyasal ve hormonal parametrelerle ilişkisini araştırmaktır.*

Hastalar ve Yöntem: *Yaşları 2.5 ile 18 arasında olan 13'ü kız, 9'u erkek 22 talasemi majorlü hasta çalışmaya alındı. Hastaların ağırlık, boy, triseps cilt kıvrım kalınlığı, ortakol çevresi ölçüldü ve vücut kitle indeksi hesaplandı. Tüm hastalardan hemoglobin, serum kalsiyum, fosfor, alkalen fosfataz, trigliserid, kolesterol, vitamin A, vitamin E, β-karoten ve ferritin, düzeyleri çalışıldı. Kemik mineral dansitesi, dual enerji x-ray absorpsiyometri (DEXA) yöntemi ile, L1-L4 vertebralar ve sol femur boynundan ölçüldü.*

Sonuçlar: *Lomber vertebralar ve sol femur boynundan ölçülen ortalama kemik mineral dansiteleri sırasıyla: 0.53±0.07 g/cm^2 (0.401- 0.617 g/cm^2) ve 0.58±0.08 g/cm2 (0.396 - 0.740 g/cm^2) bulundu. Bu değerler hastalara yaş ve cinsiyet olarak benzer sağlıklı çocukların değerlerinden düşük idi. Yaş, ağırlık, boy, ortakol çevresi ve trigliserid düzeyleri her iki bölgeden elde edilen kemik mineral dansitesi değerleri ile pozitif korelasyon gösterirken, ferritin düzeyleri negatif korelasyon gösterdi.*

Sonuç: *Talasemili hastaların kemik mineral dansitesi değerleri sağlıklı, çocuklardan düşük bulundu. Hipertransfüzyon, etkili şelasyon tedavisi ve yoğun nütrisyonel desteğin bu hastalarda kemik mineral dansitesi değerlerini olumlu etkileyeceğini düşünüyoruz.*

Anahtar Kelimeler: *Antropometri, Kemik mineral dansitesi, Talasemi*

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Optimal acquisition of bone mineral during growth contributes to the adequecy of the bone mineral content (BMC) throughout life (1). Bone mineral acquisition is affected by many factors including age, genetic determinants, sexual maturation, amount of weight-bearing physical activity and dietary calcium (2-5). Measurement of BMC can be performed using several methods, including

standard radiography, radiogrammetry, computed tomography, and single and dual photon absorbtiometry (1,6). Dual energy X-ray absorbtiometry (DEXA) has the advantage of requiring low radiation, and ability to take measurements from different sites and also allows discrimination of bone mineral from soft tissue and air interfaces (6).

Patients with beta thalassemia major (BTM) frequently have bone disorders of multifactorial etiology. The main reason of the bone changes in BTM is chronic expansion of red marrow leading to widening of the medullary space, cortical thinning and trabecular atrophia (7). Other possible factors which affect bones in BTM patients are hypoparathyroidism, changes in vitamin D (vit D) metabolism, deficiencies of calcitonin, osteocalcine, sex steroids, vitamin C and defective activity of growth hormone (GH) / insulin-like growth factor-1 (IGF-1) / insulin-like growth factor binding protein-3 (IGFBP-3) system and desferrioxamine (DFO) toxicity (8-19).

In this study, we investigated bone mineral densities (BMD) of BTM patients and the relationship with some anthropometric, hematologic, biochemical and hormonal factors.

PATIENTS AND METHODS

This study was prospectively conducted in pediatric hematology department of Erciyes University Medical faculty between January and October 1999. The study group consisted of 22 transfusion-dependent BTM patients aged between 2.5 and 18 years (13 female, 9 male). Their first transfusion ages were 3 months-3 years (1.17±0.76 year). They were on normal diet without additional calcium or vitamins. They were on irregular chelation therapy with DFO. They had required blood transfusion with packed red cell at 3 or 4 weeks. Written consent was obtained from parents of patients before study. Their weight, height, triceps skinfold thickness (TST) and midarm circumference (MC) were measured, and body mass index (BMI) then calculated according to weight (kg)/ [height (m)]^2 formula. Blood samples for all analysis were obtained immediately before transfusion. Hemoglobin (Hb) levels and serum levels of calcium (Ca), phosphorus (P), alkaline phosphatase (ALP), triglyceride (TG) and cholesterol (CLS) were analysed by standard hematologic and biochemical methods. Ferritin was assayed by IRMA method (RADIM, Italy). Serum levels of vitamin A (vit A), vitamin E (vit E) and β-carotene were analyzed by spectrophotometric method (20. 21). Bone mineral

Table I. Demographic, anthropometric, hematologic and biochemical features of patients

Variable		Mean±SD*	Variable	Mean±SD
Age	Male(9)	8.89±4.47	**Ferritin** (ng/ml)	1664± 857
	Female(13)	8.15±2.91	**Ca** (mg/dl)	9.85± .64
	Total(22)	8.45± 3.55	**P** (mg/dl)	4.99± .83
Weight (kg)		24.32±7.88	**ALP** (U/L)	326± 137
Height (cm)		118.77±17.37	**TG** (mg/dl)	198± 115
BMI (kg/m^2)		16.84±1.36	**CLS** (mg/dl)	121± 46
TST (mm)		7.95±2.68	**Vit A** (g/dl)	30.0± 2.4
MC (cm)		17.55± 1.79	**Vit E** (mg/dl)	0.74± 0.04
Hb (gr/dl)		8.26± 1.08	**β-carotene** (g/dl)	71± 17

CLS: 112-200 mg/dl, TG: 40-120 mg/dl, vit A: 25-60 g/dl, β-carotene: 35-115 g/dl, vit E: 0.70-0.80 mg/dl. Weight, height, BMI, TST and MC vary with age
**SD: Standard Deviation*

density (BMD) was measured by DEXA method (Hologic QDR-4500 A) from L1 to L4 spine, and left femoral neck in all patients. The average of the L1 to L4 spinal measurement was expressed as spinal BMD. Vertebral BMD values of the patients were compared with the densitometer's standards and BMD values in normal children, measured using the same method (DEXA) (22,23). Patients' left femoral neck BMDs were compared with young adult standards and BMD values in healthy children measured by the same method (DEXA) (24). We could not obtain BMD measurements from left femoral neck for children less than 9 years old.

Statistical analysis were performed using SPSS for Windows Release 5.0 and relation of the parameters with BMD values were evaluated with correlation, p values below 0.05 were accepted as significant.

Table II. Bone mineral density and standard deviation scores of patients

Patient No	Age(year)/Sex	BMD1 (g/cm2)	SS1	BMD2 (g/cm2)	SS2
1	8/F	0.447	-2.36	0.569	-
2	7/M	0.575	-.28	0.658	-
3	11/F	0.587	-1.48	0.609	-2.85
4	9.5/M	0.438	-2.60	0.529	-4.09
5	13.5/M	0.605	-2.19	0.627	-2.46
6	11/F	0.614	-2.15	0.727	-1.68
7	9.5/M	0.584	-1.14	0.740	-2.17
8	5.5/F	0.460	-1.46	0.520	-
9	11/F	0.601	-1.81	0.540	-3.54
10	8/M	0.617	-.12	0.540	-
11	6/F	0.578	.01	0.573	-
12	11/F	0.570	-1.81	0.634	-2.52
13	5.5/M	0.471	-1.33	0.494	-
14	5/M	0.468	-1.13	0.546	-
15	5/F	0.468	-1.13	0.468	-
16	7.5/F	0.545	-1.01	0.631	-
17	7.5/F	0.507	-1.49	0.595	-
18	8/F	0.457	-2.23	0.561	-
19	18/M	0.587	-2.85	0.657	-3.42
20	4/M	0.508	-.22	0.572	-
21	12/F	0.527	-2.73	0.555	-3.40
22	2.5/F	0.401	-1.27	0.396	-

BMD1:spinal bone mineral density of the patients, BMD2:femoral bone mineral density of the patients
SS1: standart deviation score of spinal bone mineral density of the patients according to their ages
SS2: standart deviation score of femoral bone mineral density of the patients according to their ages

Table III. Correlation of bone mineral density with some parameters

	Spinal		Femoral	
Variable	**r***	**p value**	**r***	**p value**
Age	0.6069	0.003	0.5755	0.005
Weight	0.6898	0.000	0.6190	0.002
Height	0.6718	0.001	0.7080	0.000
MC	0.5206	0.013		
Ferritin	-0.4689	0.028	-0.4323	0.045
Triglycerid	0.4652	0.029	0.4530	0.034

**r: correlation coefficient*

RESULTS

Age and sex properties, and hematological, biochemical and hormonal results of patients were presented in Table I. Median percentile for weight was 18 (3-90 percentile; five of the patients were less than 3rd percentile) and for height was 10 (3-50 percentile; 10 of the patients were less than 3rd percentile). BMI percentile for age and sex ranged from 3 to 97 percentile (mean 50 percentile). Because the height percentiles of the patients were markedly lower than weight percentiles, BMI values generally remained in normal ranges. The average level of pretransfusion hemoglobin was 8.26±1.8 g/dl. Serum levels of ferritin ranged from 222.7 to 4000 ng/ml. Fourteen of 22 patients had 1500 ng/ml or higher values of serum ferritin because of irregular chelating therapy. TST and MC measurements were generally parallel to weight. Serum levels of Ca, P, ALP, TG and CLS were within normal limits. Only two patients had slightly low values of serum vit E. Serum vit A and beta carotene concentrations were in normal ranges in all patients. Mean BMD for lumbal spine and left femoral neck were 0.53±0.07 g/cm^2 (0.401- 0.617 g/cm^2) and 0.58±0.08 g/cm^2 (0.396 - 0.740 g/cm^2) respectively (Table II). Spinal and femoral BMD were correlated with anthropometric and biochemical parameters. Age, height, weight, MC (not for femoral BMD) and triglyceride values correlated positively with BMD of both sites, but ferritin correlated negatively (Table III).

DISCUSSION

Patients with BTM frequently have osteopenia measured using DEXA and other methods (9,10,14,25,26). Bone changes in BTM are of multifactorial origin. Red marrow hyperplasia, because of chronic anemia and hypoxia, leads to widening of medullary space, cortical thinning and trabecular atrophy. These changes were more striking before hypertransfusion (27). Treatment of BTM patients with hypertransfusion and desferrioxamine decreased the natural history of bone changes but did not eradicate them (27). Hypoparathyroidism, changes in vit D metabolism, alterations of serum osteocalcine, calcitonin concentrations, sex steroid deficiency, defective GH/IGF-1/IGFBP-3 axis and DFO toxicity also play a very important role in the bone structure (8-19)

Moulas et al (13) reported that serum 25-OH vit D and 24,25 $(OH)_2$ vit D decreased, and 1,25 $(OH)_2$ vit D, osteocalcine, PTH, Ca, P and ALP were unchanged in BTM patients. Similar changes were

observed by Dandona et al (12) i.e. 25-OH vit D concentrations were lower than controls and 1.25 $(OH)_2$ vit D, PTH and osteocalcine concentrations were similar. Soliman et al (14) reported that BTM patients have lower BMD than constitutional short stature (CSS) children. They noticed that five of their 30 thalassemic patients had hypocalcemia, two of the five had hypoparathyroidism and three had rickets. Aloia et al (8) reported both vit D and PTH deficiencies in thalassemic patients. Zamboni et al (16) demonstrated that serum Ca, P, PTH, 25-OH vit D, $1,25(OH)_2$ vit D concentrations and urinary cAMP excretion were lower than the control group, whereas serum calcitonin and urinary P and OH-prolin levels were higher. Canatan et al (11) treated BTM patients with salmon calcitonin for one year and observed improvement in bone pain and osteoporosis.

In our patients, BMD values were low for same age and sex. Serum levels of Ca, P and ALP were within normal ranges. No parameter regarding vit D metabolism was studied in these patients.

Bone mineralization normally increases with growth and accelerates with puberty. Maximum bone mineral acquisition is achieved with the onset of puberty and most of the adult bone content is acquired during this period (5,9,28,29). Delayed growth and puberty is a major feature of BTM. Growth hormone and related proteins (IGF-1 and IGFBP-3), and sex steroids have been evaluated in several studies (14,17,30). Soliman et al (17) showed that serum IGF-1 and IGFBP-3 concentrations were lower in BTM patients than in controls. BMD was highly correlated with circulating IGF-1 and IGFBP-3 concentrations. Serum GH response to provocation was defective in 40 % patients. Serum ferritin concentration correlated with GH peak response to provocation and circulating IGF-1, and IGFBP-3 concentrations. In the IGF generation test after GH injection, thalassemic children had significantly lower IGF-1 and IGFBP-3 levels than CSS and GHD patients. After one year therapy with hGH, there was a marked acceleration of growth velocity in GH deficient short BTM patients, but this acceleration was slower than CSS and GHD patients. They concluded that some children with BTM have defective GH/IGF-1/IGFBP-3 axis and suggest the presence of partial resistance to GH.

Beta thalassemia major patients with hypogonadism had low BMD values and treatment with transdermal estrogen (for females) or hCG (for males) resulted in impairment in BMD (9). Filosa et al (25) reported that patients with thalassemia had lower BMD than controls, and severe form of the disease corresponded to severe reduction in BMD and to hypogonadism. Güler et al (31) found that GH deficiency was 43.4 % and partial GH deficiency was 21.7 % in a previous study in our patients. All patients were at the prepubertal age except for two, and both had pubertal problems. Eighty-two % of our patients have low levels of IGF-1 in the serum for same age and sex in a the previous study (31).

Desferrioxamine therapy (toxicity) may induce some bone changes and growth failure in thalassemic children. Flattening of the vertebral bodies, widened growth plates, circumferential metaphyseal osseous defect, sharp zones of provisional calcification and rachitic-like changes in long bones were reported in DFO treated patients. These findings were observed especially in early started treatment and in patients treated with high dose DFO (18,19).

In this study we found that BTM patients had low BMD, measured from lumbar spine and left femoral neck. Seven of the 22 patients had L1-L4 lumbar spine BMD values of less than 2 standard deviation for same age and sex. Eleven had BMD values between (-1) and (-2) standard deviation. Our patients' BMDs were also compared to BMD values in Glastre (22), Ponder (23) and Maynard's (24) studies and similar results were obtained. There is very limited data for left femoral neck BMD for healthy children, so we compared the left femoral neck BMD values of the patients whose ages were 8 or above similarly to Maynard's report (24). Seven of the nine patients whose ages were 8 or above had BMD values of less than 2 standard deviation for

same age and sex. The other two patients had standard deviation between (-1) and (-2). Our patients' BMD values for lumbal spine and left femoral neck correlated to each other similarly to Henderson's report (32). BMD values correlated with age weight, height, MC and serum triglyceride, and but not surprisingly, inversely correlated with serum ferritine. Spinal BMDs negatively (not significantly) correlated with pretransfusion hemoglobin values (p= .062). We conclude that BMD values from lumbal spine and upper femur of BTM patients were significantly lower than those of healthy children. Age, weight, height, serum ferritin and pretransfusion hemoglobin (weak effect on spinal BMD) are effective parameters on BMD. Hypertransfusion (higher pretransfusion Hb), effective chelation and aggressive dietary management could positively affect BMD in BTM children.

REFERENCES

1. *Fassler ALJ, Bonjour JP. Osteoporosis as a pediatric problem. Pediatr Clin North Am 1995;42:811-824.*
2. *Jouanny P, Guillemin F, Kuntz C, Jeandel C, Pourel J. Enviromental and genetic factors affecting bone mass: Similarity of bone density among members of healthy families. Arthritis Rhuem 1995;38:61-67.*
3. *Lonzer MD, Imrie R, Rogers D, Worley D, Licata A, Secic M. Effects of heredity, age weight, puberty, activity and calcium intake on bone mineral density in children. Clin Pediatr 1996;35:185-189.*
4. *Johnson ML, Gong G, Kimberling W, Recker SM, Kimmel DB, Recker RB. Linkage of a gene causing high bone mass to human chromosome 11 (11q12-13). Am J Hum Genet 1997;60:1326-1332.*
5. *Rubin K, Schirduan V, Gendreau P, Sarfarazi M, Mendola R, Dalsky G. Predictors of axial and peripheral bone mineral density in healthy children and adolescents, with special attention to the role of puberty. J Pediatr 1993;123:863-870.*
6. *Kimmel P. Radiologic methods to evaluate bone mineral content. Ann Intern Med 1984;100:908-911.*
7. *Rioja L, Girot R, Garabedian M, Cournot-Witmer G. Bone disease in children with homozygous beta-thalassemia. Bone Miner 1990;8:69-86.*
8. *Aloia JF, Ostuni JA, Yeh JK, Zaino EC. Combined vitamin D parathyroid defect in thalassemia major. Arch Int Med 1982;142:831-832.*
9. *Anapliotou ML, Kastanias IT, Psara P, Evangelou EA, Liparaki M, Dimitriou P. The contribution of hypogonadism to the development of osteoporosis in thalassemia major: new therapeutic approaches. Clin Endocrinol (Oxford) 1995;42:279-287.*
10. *Bisbocci D, Livorno P, Modina P et al. Osteodystrophy in thalassemia major. Ann Ital Med Int 1993;8: 224-226. (Abstract)*
11. *Canatan D, Akar N, Arcasoy A. Effects of calcitonin therapy on osteoporosis in patients with thalassemia.Acta Haematol 1995;93:20-24.*
12. *Dandona P, Menon RK, Houlder S, Thomas M, Hoffbrand AV, Flynn DM. Serum 1,25 dihydroxyvitamin D and osteocalcin concentrations in thalassemia major. Arch Dis Child 1987; 62: 474-477.*
13. *Moulas A, Challa A, Chaliasos N, Lapatsanis PD. Vitamin D metabolites (25-hydroxyvitamin D, 24,25-dihydroxyvitamin D and 1,25-dihydroxyvitamin D) and osteocalcin in beta-thalassemia. Acta Paediatr 1997;86:594-599.*
14. *Soliman AT, El Banna N, Abdel Fattah M, ElZalabani MM, Ansari BM. Bone mineral density in prepubertal children with beta-thalassemia: Correlation with growth and hormonal data. Metabolism 1998;47:541-548.*
15. *Zaino EC, Yeh JK, Aloia J. Defective vitamin D metabolism in thalassemia major. Ann N Y Acad Sci 1985;445:127-134.*
16. *Zamboni G, Marradi P, Tagliaro F, Dorizzi R, Tato L. Parathyroid hormone, calcitonin and vitamin D metabolites in beta-thalassemia major. Eur J Pediatr 1986; 145: 133-136.*
17. *Soliman AT, El Banna N, Ansari BM. GH*

response to provocation and circulating IGF-1 and IGFBP-3 concentrations, the IGF-1 generation test and clinical response to GH therapy in children with beta-thalassemia. Eur J Endocrinol 1998;138:394-400.

18. Brill PW, Winchester P, Giardina PJ, Cunningham-Rundles S. Deferoxamine-induced bone dysplasia in patients with thalassemia major. AJR Am J Roentgenol 1991;156:561-565.
19. Olivieri NF, Koren G, Harris J et al. Growth failure and bony changes induced by deferoxamine. Am J Pediatr Hematol Oncol 1992;14: 48-56.
20. Needl JB, Pearson WN. Macro and micro methods for the determination serum vitamin A using trifluoroacetic acid. J Nutr 1962; 79: 454-462.
21. Rindi G. Rapid colorimetric method for the determination of tocopherol and tocopherylacetat in plasma. Special reprint of International Review of Vitamin Research. 1958; 28: 225-234.
22. Glastre C, Braillon P, David L, Cochat P, Meunier PJ, Delmas PD. Measurement of bone mineral content of the lumbar spine by dual energy X-ray absorbtiometry in normal children: Correlations with growth parameters. J Clin Endocrinol Metab 1990;70:1330-1333.
23. Ponder SW, McCormick DP, Fawcett HD, Palmer JL, McKernan MG, Brouhard BH. Spinal bone mineral density in children aged 5.00 through 11.99 years. AJDC 1990;144:1346-1348.
24. Maynard LM, Guo SS, Chumlea WC et al. Total-body and regional bone mineral content and areal bone mineral density in children aged 8-18 y: The fels longitudinal study. Am J Clin Nutr 1998;68:1111-1117.
25. Filosa A, Di Maio S, Vocca S, Saviano A, Esposito G, Pagano L. Longitudinal monitoring of bone mineral density in thalassemic patients. Genetic structure and osteoporosis. Acta Peadiatr 1997;86:342-346.
26. Chao T, Hwang B. The correlation of serum ferritin level to Ca-P metabolism and bone density in thalassemic patients. J Pediatr Endocrinol Metab 1996; 9:609-611.
27. Orzincolo C, Castaldi G, Bariani L, Scutellari PN. The evolutionary effects of therapy on skeletal lesions in beta-thalassemia. Radiol Med (Torino) 1994;87:381-388. (Abstract)
28. Bonjour JP, Theintz G, Buchs B, Slosman D, Rizzoli R. Critical years and stages of puberty for spinal and femoral bone mass accumulation during adolescence. J Clin Endocrinol Metab 1991;73:555-563.
29. McKay HA, Bailey DA, Mirwald RL, Davison KS, Faulkner RA. Peak bone mineral accrual and age at menarche in adolescent girls: A 6-year longitudinal study. J Pediatr 1998;133:682-687.
30. Cavallo L, Gurrado R, Gallo F, Zacchino C, De Mattia D, Tato L. Growth deficiency in polytransfused beta-thalassemia patients is not growth hormone dependent. Clin Endocrinol (Oxf) 1997;46:701-706.
31. Güler E. Endocrine complications in beta thalassemia major patients. Doctoral thesis, Kayseri, 1998.
32. Henderson RC. The correlation between dual-energy X-ray absorbtiometry measures of bone density in the proximal femur and lumbar spine of children. Skeletal Radiol 1997; 26:544-547.