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ARAŞTIRMA / RESEARCH

Zoledronic acid improves bone mineral density in β-thalassemia patients with osteoporosis: a case control study

Zoledronik asit osteoporozlu β-talasemi hastalarında kemik mineral yoğunluğunu arttırır: Bir vaka kontrol çalışması

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Abstract

Purpose: This study addresses the efficacy of zoledronic acid treatment in osteoporotic patients with β-thalassemia.

Materials and Methods: Thirty-nine thalassemic patients were enrolled in this study. Bone mineral density was measured using Dual energy x-ray absorbtiometry (DEXA), and biochemical markers of osteoporosis in serum and urine were measured at 0, 6, and 12 months of treatment. Patients with thalassemia were randomly received either zoledronic acid 4 mg IV once every six months or calcitriol 0.25 mcg / day for 12 months. A subgroup analysis was also performed according to gonadal status.

Results: There were 21 males and 18 females, mean age of 23.1 ± 1.3 and bone age of 16.3±0.35 years. At the end of 12 months, bone mineral densities of the lumbar vertebra and femoral neck significantly increased in the zoledronic acid group. In patients with hypogonadism who received hormone replacement therapy, t-scores significantly increased. Serum NTX levels indicated significant increase in both treatment groups compared with the control group at the end of the treatment. A significant increase was obtained in urinary NTX level in hypogonadal patients who received zoledronic acid, and in serum NTX and IGF-1 levels in normogonadal patients who received calcitriol.

Conclusion: In our study, zoledronic acid resulted in significant improvement in bone mineral density and t-scores. This improvement was in correlation with IGF-1 levels.

Keywords: β -thalassemia, osteoporosis, zoledronic acid, DEXA, serum and urinary NTX, IGF-I

Öz

Amaç: Bu çalışmada osteoporozu olan β-talasemi hastalarında zoledronik asit tedavisinin etkinliği incelenmiştir.

Gereç ve Yöntem: Çalışmaya 33 β-talasemili hasta ile kontrol grubu olarak yaş ve cinsiyetleri uyumlu 25 sağlıklı gönüllü alındı. Hastalarda 0. 6. ve 12.aylarda DEXA ve serum ve idrarda osteoporozun bazı biyokimyasal belirteçleri ölçüldü. Talasemili hastalar 12 ay süreyle her altı ayda bir 4 mg İV zoledronik asit ve 0.25 mcg/gün kalsitriol alanlar olmak üzere rastgele iki ayrı gruba ayrıldı. Gonadal fonksiyonlarına göre de iki alt gruba ayrılarak takip edildiler.

Bulgular: Hastalar yaş ortalaması 23.1±1.3 ve kemik yaşı ortalaması 16.3±0.35 yıl olan 21 erkek, 18 kadından oluşmaktaydı. On iki ay sonunda zoledronik asit grubunda lumbar vertebra ve femur boynu kemik mineral yoğunluğu anlamlı artış gösterdi. Hipogonadik olup hormon replasmanı yapılan hastalarda lumbar vertebra ve femur boynu t-skoru anlamlı artış gösterdi. Serum NTX düzeyleri tedavinin sonunda her iki tedavi grubunda da kontrol grubuna göre belirgin olarak yükselme gösterdi. Hipogonadik hastalar içinde zolendronik asit alanlarda idrar NTXdüzeylerinde, normogonadik hastalar içinde kalsitriol alanlarda idrar NTX ve serum IGF-1 düzeylerinde anlamlı artış saptandı.

Sonuç: Çalışmamızda zoledronik asit kemik mineral yoğunluğu ve t-skorlarında belirgin düzelmeler sağlamıştır. Bu düzelme IGF-1 düzeyi ile korelasyon göstermiştir.

Anahtar kelimeler: β-talasemi, zoledronik asit, osteoporoz, DEXA, serum ve idrar, NTX, IGF-I

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INTRODUCTION

Thalassemia-associated osteoporosis is among the most commonly observed complications of the disease seen as a result of various factors such as hypogonadism, delayed puberty, bone marrow expansion, iron overload, desferrioxamine (DFO) toxicity, genetic factors, calcium, Vitamin D and GH /IGF-1 deficiencies, hypoparathyroidism, hypothyroidism and decreased physical activity[1,2,3,4,5]. Severity of the bone disease in thalassemia is associated with the underlying type of thalassemia and its treatment. It was reported that hemoglobin could prevent most bone related complications by increasing to 9.5 g / dl [6]. Bone mineral increase begins in childhood and dramatically increases in adolescence until sexual maturation develops[6].

According to World Health Organization 2004 criteria, osteopenia and osteoporosis values were classified as for adult women, but these criteria was used on studies in patients with thalassemia. It was shown that in β- thalassemic patients who have treated with appropriate transfusion and chelation therapy, the incidence of osteoporosis and vertebral fracture were found to be higher in both sexes compared to age-matched control groups. Osteoporosis is detected in 42% of patients with appropriate therapy of β-thalassemia. Lumbar vertebrae and femur are affected in male patients, while vertebral involvement is observed in females. In β-thalassemic patients who have receiving appropriate treatment, in studies which are using biochemical parameters were found bone formation decreased, but resorption increased. Since bone loss continues in adult thalassemia, early diagnosis and treatment is important.

Delayed puberty is present in approximately 70% of patients with thalassemia and is the most common endocrine anomaly[7]. Deterioration of the hypothalamic-pituitary-gonadal axis is one of the most obvious causes of abnormal sexual development in patients with thalassemia. More severe osteoporotic findings are observed in the femur and vertebral levels in the gonadal insufficiency of the thalassemia than in the eugenadal. These findings support the importance of sex hormones on bone tissue and the importance of hormone replacement with early sex steroids[8]. In recent years there have been persuasive reports indicating the efficacy and tolerability of bisphosphonates in beta-thalassemic patients. Several therapies including calcium, vitamin D, calcitonin, sex steroids, and strontium ranelate provide contribution to bone growth and remodeling[9-11]. Furthermore, in recently one phase 2b study was showed that denosumab is a fully human monoclonal antibody against the receptor activator of nuclear factor kappa-B ligand (RANKL) that has been licensed for the treatment of different types of osteoporosis[12,13]. Although this drug has been used for hypercalcemia in hematological malignancies, there is insufficient evidence for its use in osteoporotic patients with β- thalassemia.

Unfortunately, rapid bone resorption and osteoporosis are develops in β- thalassemic patients despite the therapeutic approach such as adequate blood transfusion, appropriate iron chelation and hormone replacement therapy[14]. The increased bone resorption observed in patients with thalassemia related osteoporosis has led to the use of bisphosphonates in this subset of patients, as bisphosphonates are potent inhibitors of osteoclastic bone resorption[15,16].

Zoledronic acid, a strong bisphosphonate molecule, inhibits bone resorption in primary and secondary osteoporosis even when it is used in low dose. In this study, we tried to investigate the effect of zoledronic acid treatment on markers of bone turnover and mineral density in osteoporotic patients with β-thalassemia. In our study, we emphasized also the importance of hormone replacement therapy (HRT) as well as zoledronic acid treatment by classifying osteoporotic patients with β- thalassemia according to their gonadal status.

MATERIALS AND METHODS

A total of 39 patients with transfusion-dependent β-thalassemia of which six had thalassemia-intermedia (TI), followed up by Çukurova University, Faculty of Medicine, Hematology Unit were enrolled in this study. Local Ethics Committee of our institution approved the study and written informed consents were given by all patients.

Forty osteoporotic patients were enrolled in the study. A patient with the diagnosis of TI was excluded from the study because her age impaired the mean. Thirty-nine patients were included in the study and 18 of them were female and 21 were male. Mean age of them were 23.1± 2.1 years. All patients were on chelation therapy with desferrioxamine (DFO) 40-60 mg/kg/day for 5-6 days/week. Twenty-five

healthy controls (13 female) with a mean age of 26.4 ± 0.9 were also included. In this randomised controlled trial patients are assigned to two treatment groups. These groups were similar in terms of age, bone age, hemoglobin level, osteoporosis level and gonadal status.

Blood samples and laboratory analysis

In our study, biochemical tests in urine were collected from 24-hours urine samples collected from patients. In order to measure N-terminal cross-linking telopeptide of type I collagen (NTX) levels, serum samples taken from patients at 0, 6 and 12 months were stored at -70 ° and urine at -20 °C. Urine NTX results were corrected for urine creatinine in patients with β-thalassemia. In each visit other than laboratory investigations such as hematological and biochemical indices, we obtained samples for serum and urinary levels of NTX, osteocalcin, insulin-like growth factor (IGF-I) levels, serum parathormone (PTH) and TSH levels, sex hormones including follicle stimulating hormone (FSH), luteinizing hormone (LH), estrogen, testosterone, dehydroepiandrosterone-sulfate (DHEAS) levels. N-terminal cross-linking telopeptide of type I collagen (NTX) levels in serum and urine were measured by enzyme-linked immunosorbent assay (ELISA) method (Osteomark, Ostex International Inc., Seattle, WA, USA).

Serum osteocalcin levels were determined by electrochemiluminescence method (Roche Diagnostic Elecsys E-170, Germany). Twenty-four hour urine OH-proline levels were measured by colorimetric-manual method (FAR S.r.l., Italy). Serum IGF-I levels were measured by ELISA method (Quantikine, R & D Systems Inc., USA) at 0 and 12 months of the study.

Assessment of gonadal status

Pubertal assessments were made according to Tanner Scale. The Tanner scale (also known as the Tanner stages) is a scale of physical development in children, adolescents and adults. Tanner stages are evaluated as 5 stages in both sex. These parameters are height, testicle size, pubic hair, penis size and other secondary sex characters in male patients, and annual growth, pubic hair, breast development, menarch age in female patients [17].

Drug administration

Group 1 patients received zoledronic acid intravenously (IV) at a dose of 4 mg, every 6 months for one year. In the other group, calcitriol 0.25 mcg was administered by the oral route on a daily basis. All the patients in both groups received 1000 mg elemental calcium supplement once a day during the study period. Patients in both groups were further stratified according to their gonadal status. Six and eight patients received sex HRT, respectively, in the zoledronic and calcitriol treatment groups. A patient in the calcitriol group left the treatment on his own initiative without receiving any therapy. As HRT, men received testosterone (every 3 weeks intra-muscular testosterone 250 mg), and women received estrogen-progesterone (3 mg drospirenone + 0.03 mg ethnylestradiol p.o./daily; 21 days daily and 7 days break) treatment.

During the study, patients were allowed to take blood transfusions and the medications which of needed, such as DFO, elemental calcium, zinc and folic acid. Zoledronic acid was administered IV for at least 15 minutes. Patients were followed up for hypocalcemia, hypomagnesemia and hypophosphatemia.

No serious side effects were seen in patients. All patients were evaluated at six-monthly intervals during one year. Height, weight and bone age measurements were performed in all patients. Transfusion and iron chelation therapy datas were recorded. A patient aged seventeen died due to congestive heart failure and pneumonia 10 months after the completion of the study.

Bone mineral density measurements

Osteoporosis was diagnosed as a t-score <-2.5 and z-score <-2 in bone mineral density (BMD) measured using Dual-energy X-ray absorptiometry (DEXA) according to the standard criteria of the WHO 18. Dual-energy X-ray absorptiometry is considered the gold standard for the diagnosis of osteoporosis. Osteoporosis is diagnosed when the BMD is less than or equal to 2.5 standard deviations below that of a young (30–40-year-old) healthy adult women reference population. This is translated as a t-score (t-score <-2.5). Osteopenia is the determination of t-score between (-1) and (-2.5) 18,19.

Bone mineral density measurements of the our patients were performed at six-monthly intervals on antero-posterior and lateral lumbar vertebrae (L1-L4) and left femoral neck (FN) using DEXA (Norland, XR 46, USA). Bone mineral density results were expressed as g / cm2 and t-score. The coefficient of

variation (CV) was 1% for lumbar vertebrae and 1.2% for femoral neck.

Statistical analysis

All data were analysed using SPSS (version 10.0) package program. Values were expressed as mean ±SEM. Mean values were compared using Mann-Whitney U and Wilcoxon Signed Rank test. For comparison of repeated measurements according to treatment groups, "repeated measures analysis of variance" was used. Correlation between biochemical parameters and BMD was studied using Spearman's rank correlation test, and p values ≤ 0.05 were considered significant.

RESULTS

Clinical characteristics of the study population are presented in Table 1. There was a slight female preponderance in the calcitriol arm compared to zoledranate group (Table 1) (p=0.05). In patients with TI included in the study, mean age was 34.1±5.6 years, bone age was 18.1±1.0 years, total number of transfusions was 66±26 units, DFO starting age was 28±7 years, splenectomy age 29±6 years, baseline Hb level 8.8±0.6 g/dl, and baseline IGF-I level was 0.98±0.26 ng/ml. Pre-treatment laboratory findings for all patients in both treatment arm was given in Table 1. Baseline levels of NTX, osteocalcin, IGF-I, PTH and BMD results are shown in Table 2. Baseline IGF-1 level was significantly lower in both group of patients compared to healthy controls (3.1± 0.25 ng/ml) (p<0.001).

Table 1. Baseline clinical characteristics

	Zoledronate group	Calcitriol group	P
Age (years)	22.3 ± 1.2	23.9 ± 2.4	0.7
Gender (M/F)	7/12	15/5	0.05
Hypogonadic (n)	6	8	0.1
Height (cm)	157 ± 3.3	158 ± 2.2	0.6
Bone age (years)	19.1	18.8	0.8
Weight (kg)	45.6 ± 2.1	50.5 ± 1.7	0.07
Hemoglobin (g/dl)	7.2 ± 0.2	7.2 ± 0.4	0.9
Ca (mg/dl)	9.5 ± 0.2	9.1 ± 0.2	0.7
ALP (U/l)	340 ± 32	317 ± 27	0.8
P (mg/dl)	5.1 ± 0.2	5.2 ± 0.4	0.6

When zoledronic acid and calcitriol treatment groups were compared in terms of baseline hemoglobin, ferritin and other biochemical parameters including serum Ca, P, ALP there were no significant difference between the groups (p>0.05). Baseline serum NTX levels were significantly elevated in both treatment groups whereas baseline urinary NTX levels were also significantly lower compared to healthy controls (serum NTX 4.6 ± 0.8 U/ml and 518±118 nMBCE [nmol/l BCE (Bone Collagen Equivalents)], in healthy controls, respectively, p=0.01 and p=0.014) (Table 2). When BMD changes were compared by gender, BMD was found to be significantly lower in male patients than in female patients (p=0.013 for lumbar vertebrae (LV) and p<0.001 for FN).

Table 2. Baseline BMD measurements and laboratory characteristics *

	Zoledronate group	Calcitriol group	P
Serum NTX (U/ml)	24.6 ± 2.7	15.7 ± 2.8	0.03
Urinary NTX (NcBME/mMol)	95.6 ± 63.7	226 ± 94	0.3
Osteocalcin (ng/ml)	19.5 ± 2.3	27.5 ± 3.5	0.07
Urinary OH-proline (mg/day/m²)	27.6 ± 6.2	26.4 ± 3.8	0.3
Ferritin (ng/ml)	1551 ± 408	2545 ± 648	0.2
PTH (pg/ml)	35.1 ± 3.7	34.6 ± 6	0.5
1,25-(OH)2-vitamin D3 (pg/ml)	32.7 ± 1.7	42.7 ± 3.7	0.06
IGF-I (ng/ml)	1.02 ± 0.08	1.0 ± 0.1	0.9
Lumbar vertebra BMD (t-score) (g/cm²)	 -4.03 ± 0.3 0.62 ± 0.03	 -3.77 ± 0.22 0.65 ± 0.02	0.2
Femoral neck BMD (t-score) (g/cm²)	 -1.3 ± 0.3 0.74 ± 0.03	 -0.94 ± 0.7 0.80 ± 0.03	0.1

IGF-1 Insulin-like growth factor-1, *NTX* N-terminal cross-linking telopeptide of type I collagen, BMD bone mineral density. *Data are given as mean±SEM

The effects of zoledronic acid and calcitriol on study parameters are presented in Table 3. In the zoledronic acid and calcitriol groups, a meaningful recovery was achieved in t-scores in DEXA measurements of the LV and FN. There was a progressive increase in BMD after treatment in both groups (Table 3). The increase in LV BMD was statistically significant at 12 months of zoledronic acid treatment (p=0.032). Within the calcitriol treated group BMD of the FN was significantly elevated (p=0.038). Among bone turnover markers, in zoledronate treated group osteocalcin levels were significantly elevated at the end of 12 months (p=0.04). Serum NTX was not changed in the same group while urinary NTX levels were significantly elevated (p =0.04). Within the calcitriol group urinary NTX levels were also found to be elevated though non-significantly. IGF-1 levels were significantly increased in both groups (p=0.04 and 0.006, respectively) at the end of 12 months of treatment.

Table 3. Effects of both therapy groups on bone turnover parameters and BMD[a]

	Zoledronate group			Calcitriol group		
	Baseline	**6th month**	**12th month**	**Baseline**	**6th month**	**12th month**
Osteocalcin	19.5 ± 2.3	20.4 ± 2.8	25.0 ± 6.0*	27.5 ± 3.5	32.3 ± 6.1	32.7 ± 4.7
Serum NTX	24.6 ± 2.8	28.2 ± 3.6	22.4 ± 4.0	15.7 ± 2.8	21.7 ± 2.9	25.4 ± 3.8
Urinary NTX	95.6 ± 63.7	342.5 ± 160	361 ± 104 *	226 ± 94	144 ± 50.7	461 ± 91*
1,25(OH)2 D	32.7 ± 1.7	33.9 ± 2.8	24.9 ± 2.3*	42.7 ± 3.7	30.0±2.0*	23.6 ± 1.7*
PTH	35.1 ± 3.7	49.2 ± 7.1*	50.4 ± 8.0*	34.6 ± 6	33 ± 5.8	41.1 ± 6.2
IGF-I	1.1 ± 0.1	-	1.5 ± 0.1*	1.0 ± 0.1	-	1.7 ± 0.2*
Ca	9.5 ± 0.2	9.4 ± 0.2	9.5 ± 0.1	9.1 ± 0.2	9.3 ± 0.3	9.5 ± 0.2*
P	5.1 ± 0.2	5.2 ± 0.2	4.9 ± 0.3*	5.2 ± 0.4	5.2 ± 0.4	5.3 ± 0.4
ALP	340 ± 31	308 ± 40	296 ± 36	317 ± 27	357 ± 36	342 ± 44
UOHP	27.6 ± 6.2	25.5 ± 4,5	22.7 ± 4.2	26.4 ± 3.8	27.1 ± 3.3	29.2 ± 5.6
UCa	87.6±41	107±31	142±53	130±40	134±44	119±29
BMD (g/cm²)						
LV	0.62 ± 0.03	0.67 ± 0.03*	0.67 ± 0.04 *	0.65±0.02	0.67 ± 0.02	0.66 ±0.025
FN	0.74 ± 0.03	0.77 ± 0.05	0.80 ± 0.07	0.80 ±0.03	0.80 ± 0.03	0.91 ±0.048*
BMD(t-score)						
LV	-4.03 ± 0.3	-3.9 ± 0.3	-3.6 ± 0.4	-3.7± 0.2	-3.6 ± 0.2	-3.6± 0.2*
FN	-1.3 ± 0.3	-1.35± 0.3	-0.4 ± 0.6	-0.4± 0.3	-0.5 ± 0.2	0.1± 0.3

[a]Normal ranges: Osteocalcin (24-70 ng/ml); serum NTX (7.7-19.3 U/ml); urinary NTX (NcBME/mMol); 1.25 (OH)2vitD3 (18.7-47 pg/ml); PTH (10-69 pg/ml); UOHP (Urinary OH-proline) (0.4-1.3 g/day); UCa (Urinary Calcium): 100-320 mg/day; IGF-I(ng/ml); Ca (8.8-10.2 mg/dl); P (2.7-4.5 mg/dl); ALP (5-240 U/l), LV: Lumbar vertebrae, FN: Femoral neck

There were 14 hypogonadic patients within the study population (6 patients in zoledronate and 8 in calcitriol groups). Pubertal development was found to be below Tanner 3 in 25 patients (65%). There were no significant differences in terms of age, gender, height and weight between hypogonadic and normogonadic patients. Alkaline phosphatase levels and ferritin levels were significantly higher in hypogonadic patients compared to normogonadics (p=0.04 and 0.02, respectively). The effects of zoledronic acid and calcitriol on study parameters according to gonadal status is presented in Table 4 and 5. Addition of hormone replacement therapy in hypogonadic thalassemic patients had resulted in significant elevation of BMD parameters (Table 5). The analysis involving efficacy of therapy in addition to HRT favored zoledronic acid treatment. Lumbar vertebrae BMD either as t-score or g/cm², both elevated significantly (p=0.04 and 0.05, respectively) in hypogonadic patients treated with zoledronic acid compared to calcitriol treated patients (p ≥0.05). In normogonadic patients although there was an improvement at the end of 12 months of treatment the difference was statistically significant for the FN t-score measurements (p=0.04). In normogonadic patients BMD parameters did not differ according to the treatment arm. There was a positive correlation between serum N-telopeptides and PTH level in patients with thalassemia (r=0.353, p=0.03). Patients who received more erythrocyte transfusions had higher serum NTX levels (r=0.439, p=0.007).

Table 4. Effects of both therapies on bone turnover parameters according to gonadal status

			OC	SNTx	UNTx	UOHP	IGF-I
Zoledronic acid	Hypogonadic	Baseline	25.7±4.9	29±4.3	45±39	33.5±9.7	0.9±0.3
		12th month	32.4±14	26±6.1	635±212*	16.4±2.5	1.6±0.3
	Normogonadic	Baseline	17.5±2.5	22.6±3.4	121±94	25±8	1.1±0.1
		12th month	20.9±5.7	20±5.5	178±46	26.5±6.4	1.4±0.1
Calcitriol	Hypogonadic	Baseline	29±5.1	10.1±2.6	438±205	28.5±5.7	1.1±0.2
		12th month	32.5±7.4	32.6±4.4*	380±122	33.7±9.7	1.5±0.3
	Normogonadic	Baseline	25±5.0	19.7±4.1	72±25	25±5.4	0.9±0.1
		12th month	33±6.5	19.6±5.3	510±127 *	26±7.0	1.9±0.2*

*p<0.05

Table 5. BMD changes of both therapy groups according to gonadal status (mean ± SEM)

			Lumbar vertebra		Femoral neck	
			t-score	g/cm²	t-score	g/cm²
Zoledronic acid	Hypogonadic	Baseline	-5.0±0.3	0.52±0.02	-2.2±1.0	0.66±0.06
		12th month	-4.5±0.2*	0.58±0.02	-0.7±1.3	0.81±0.17
	Normogonadic	Baseline	-3.6±0.4	0.66±0.04	-1.0±0.4	0.77±0.04
		12th month	-2.9±0.5	0.72±0.05	-0.2±0.5	0.79±0.07
Calcitriol	Hypogonadic	Baseline	-3.6±0.3	0.64±0.03	-0.4±0.3	0.80±0.04
		12th month	-3.6±0.3	0.65±0.03	0.2±0.4	0.90±0.07
	Normogonadic	Baseline	-3.8±0.3	0.65±0.03	-0.5±0.4	0.81±0.06
		12th month	-3.7±0.2	0.68±0.03	0.04±0.5	0.93±0.06

*p<0.05

DISCUSSION

Increased and ineffective erythropoiesis causes expansion of bone marrow cavity, reduction in cortical and trabecular bone tissue, and hence, osteoporosis in beta-thalassemia patients[9]. Additionally, other factors including delayed puberty, endocrine dysfunction and iron overload contribute to the increased bone resorption. As a result of better supportive care of these patients in recent years life expectancy had significantly increased which resulted in increased prevalence of disease-related complications. Osteoporosis is observed in 50% of thalassemic patients. In our patients, osteoporosis was diagnosed according to WHO's standard diagnostic criteria[18,19]. Several studies showed that in patients who receive good transfusion and HRT, osteoporosis develops generally in the LV, and the FN is also affected in those who receive a poor therapy[5,20,21]. Both LV and FN BMD measurements indicated osteoporosis in our patients. Hypogonadism and insufficient pubertal development in most of our patients likely have contributed to the pathogenesis. Bisphosphonates, potent inhibitors of bone resorption, have shown to be effective in primary and glucocorticoid-induced osteoporosis[22]. Increased resorption phase in bone metabolism in thalassemia suggests that antiresorptive drugs such as BPs might be used in treatment in order to limit bone turnover and prevent fracture risk[23]. In our study we tried to investigate the efficacy of zoledronic acid in the treatment of thalassemia-associated osteoporosis. After 12 months, patients treated with zoledronic acid demonstrated significantly higher increases in LV BMD (9.4 %) compared to calcitriol treated patients (3.4%). There are two randomized controlled trials testing zoledronic acid in thalassemia-associated osteoporosis[15,24]. In the study performed by Voskaridou et al. zoledronic acid was tested at two different doses 4 mg i.v. every 3 months and 4 mg i.v. every 6 months[15]. Zoledronic acid demonstrated significant improvement in BMD of LV and FN at both dose levels. In the other study zoledronic acid 4 mg every 3 months for 2 years resulted again in where 1 mg IV zoledronic acid was administered at 3-month intervals for a year, and significant recoveries were found in biochemical parameters and BMD (t-score) measurements[25]. In a meta-analysis, it was reported that transfusion sessions of zoledronic acid doses could be performed concomitantly, and hence, zoledronic acid was a promising bisphosphonate [26]. Yiğitoğlu et al. saw meaningful recoveries in lumbar BMD values of two sisters with thalassemia major whom they treated with 4 mg IV zoledronic acid at

intervals for 6 years (one of them had several major fractures), and reported durable efficacy during the follow-up period[27]. However, despite positive results favoring zoledronic acid treatment, it should be noted that the frequency of osteonecrosis of the jaw might increase as the dose and frequency of administration increase [10, 28]. The recent detection of certain markers in urine and serum of patients with bone diseases has led to better understanding of the pathophysiology. It is well-known that ALP of bone origin and osteocalcin are the most sensitive markers of bone formation, and urinary NTX levels are postulated as the best marker of bone resorption[29]. In a study performed by Lasco et al, it was found that urinary pyridinoline and deoxypyridinoline levels, bone resorption markers, were higher in patients with β-thalassemia than in healthy controls, whereas osteocalcin, IGF-I, IGFBG-III levels were lower than in controls. In the same study, LV and FN BMD values were also low, and these values showed correlation with the bone formation markers cited above[30]. Low serum IGF-I levels in patients with beta-thalassemia were found to be correlated with osteopenia and osteoporosis, unbalanced bone turnover, increased resorptive phase and reduced bone formation. In our study, baseline serum IGF-I levels were significantly lower in both treatment groups than in the control group, and showed a significant increase in both treatment groups after the treatment. According to the findings of our study, we may suggest that bone formation and resorption are increased in patients with β-thalassemia, and bone formation is increased and resorption is slow in healthy controls.

In our study we have used biochemical markers are used to evaluate effectiveness of osteoporosis treatment. There was a significant improvement in osteocalcin levels in zoledronate group and IGF-1 levels were increase in both treatment arms at the end of 12 months. Effects on bone resorptive markers were not sufficiently observed in our cohort probably due to the small sample size and short duration of the study. In a major study where zoledronic acid was used in a dose of 4 mg/year, it was observed that the dose did not sufficiently affect bone resorption, and the subsequent studies were generally performed using a dose of 5 mg/year. In this study, zoledronic acid was used at a moderate dose level compared to other osteoporosis studies where zoledronic acid is used. After a year of follow-up, despite the significant recovery in both groups in BMD and t-scores, it may be a better approach to correlate the absence of recovery in biochemical parameters with the shorter term of treatment. Yet, Vaskoridau et al. reported that recovery in bone resorption markers continued even 2 years beyond discontinuation of therapy in a study where they treated patients with zoledronic acid[15,31].

We have tried to monitor the efficacy of our treatment by serial measurements of various biomarkers. NTX measured either in serum or urine are often used as a biomarker of bone resorption while evaluating efficacy of treatment. While there was a decrease in serum NTX levels in zoledronate treated patients, in other group of patients who were treated with calcitriol, serum NTX levels were elevated. These changes did not reach statistically significant levels in both groups. It was surprising to find urinary NTX levels elevated at the end of treatment period in both of our study groups. Rosen et al. had previously demonstrated that the interpretation of changes in biochemical markers of bone turnover after treatment with antiresorptive therapy could be problematic[32]. They explained the fluctuations in NTX levels by spontaneous long-term variability in these markers even in the absence of any interventions. Additionally they have stated that these markers differ in degree to which they decline with antiresorptive therapy. Therefore, it is evident that even if NTX levels are sensitive markers to monitor efficacy of antiresorptive therapies they are not specific. Thus NTX levels may provide limited evidence of treatment effect as also partially reflected in our study[29,32]. We think that it might be more appropriate to use serum C-terminal telopeptide level which is known to be a more specific marker to measure the rate of bone turnover, as well as bone specific ALP levels[33].

Hormone replacement therapy (HRT) has an important role in the treatment and prevention of osteoporosis in patients with β-thalassemia[5,21]. As expected, despite that age, gender and other auxological parameters did not differ between hypogonadic and normogonadic patients with thalassemia, baseline BMD values were lower in hypogonadic patients. In our study, significant improvements were seen in LV BMD values of hypogonadal patients treated with zoledronic acid at the end of the treatment, whereas no change was observed in BMD values of hypogonadal patients treated with calcitriol. At the end of our study, hypogonadal patients who received zoledronic acid and HRT were the patients who most benefited from

the treatment. Zoledronic acid was well-tolerated and there were no serious adverse effects observed with our regimen. Our results suggest that zoledronic acid is an effective choice in treatment of osteoporosis in thalassemia-associated osteoporosis. An important restriction was that the duration of our study was insufficient and the lack of BMD data of the healthy control group.

Zoledronic acid appears to be an effective treatment option that can be used safely in the treatment of thalassemia-associated osteoporosis. Further research is needed to establish long-term safety and efficacy of zoledronic acid.

Yazar Katkıları: Çalışma konsepti/Tasarımı: MEO, EG; Veri toplama: MEO; Veri analizi ve yorumlama: MEO, EG; Yazı taslağı: MEO, EG; İçeriğin eleştirel incelenmesi: EG, AT; Son onay ve sorumluluk: MEO, EG, AT; Teknik ve malzeme desteği: AT; Süpervizyon: EG; Fon sağlama (mevcut ise): yok.
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REFERENCES

1. Perisano C, Marzetti E, Spinelli MS, Calla CAM, Graci C, Maccauro G. Physiopathology of bone modifications in β-thalassemia. anemia. 2012;2012:320737.
2. Terpos E, Voskaridou E. Treatment options for thalassemia patients with osteoporosis. Ann N Y Acad Sci 2010;1202:237-43.
3. Piga A. Impact of bone disease and pain in thalassemia. Hematology Am Soc Hematol Educ Program. 2017;1:272-7.
4. Jensen CE, Tuck SM, Agnew JE, Koneru S, Morris RW, Yardumian A et al. High prevalence of low bone mass in thalassaemia major. Br J Haematol. 1998;103:911-5.
5. Giusti A, Pinto V, Forni GL, Pilotto A. Management of beta-thalassemia-associated osteoporosis. Ann N Y Acad Sci. 2016;1368:73-81.
6. Wonke B. Bone disease in β-thalassemia major. Br J Haematol. 1998;103:897-901.
7. Italian Working Group on Endocrine Complications in Non-Endocrine Diseases. Multicentre study on prevalence of endocrine complications in thalassaemia major. Clin Endocrinol (Oxf). 1995;42:581-6.
8. Lasco A, Morabito N, Gaudio A, Crisafulli A, Meo A, Denuzzo G. Osteoporosis and β-thalassemia major: Role of the IGF-I/IGFBP-III axis. J Endocrinol İnvest. 2002;25:338-44.
9. Mahachoklertwattana P, Chuansumrit A, Sirisrirot R, Chouptum L. Bone mineral density, biochemical and hormonal profiles in suboptimally treated children and adolescents with β-thalassemia disease. Clin Endocrinol. 2003;58:273-9.
10. Doggrell SA. Zoledronic acid once-yearly: what role in the prevention of non-vertebral osteoporotic fractures? Therapeutics. 2010;2:539–47.
11. Morabito N, Catalano A, Gaudio A, Morini E, Bruno LM, Basile G et al. Effects of strontium ranelate on bone mass and bone turnover in women with thalassemia major-related osteoporosis. J Bone Miner Metab. 2016;34:540-6.
12. Voskaridou E, Ntanasis-Stathopoulos I, Papaefstathiou A, Christoulas D, Dimopoulou M, Repa K et al. Denosumab in transfusion-dependent thalassemia osteoporosis: a randomized, placebo-controlled, double-blind phase 2b clinical trial. Blood Adv. 2018;2:2837–47.
13. Papapoulos S, Lippuner K, Roux C, Lin CJF, Kendler DL, Lewiecki EM et al. The effect of 8 or 5 years of denosumab treatment in postmenopausal women with osteoporosis: results from the FREEDOM Extension study. Osteoporos Int. 2015;26:2773-83.
14. Baldini M, Forti S, Marcon A, Ulivieri FM, Orsatti A, Tampieri B et al. Endocrine and bone disease in appropriately treated adult patients with beta-thalassemia major. Ann Hematol. 2010;89:1207-13.
15. Voskaridou E, Anagnostopoulos A, Konstantopoulos K, Stoupa E, Spyropoulou E, Kiamouris C et al. Zoledronic acid for the treatment of osteoporosis in patients with beta-thalassemia: results from a single-center, randomized,placebo-controlled trial. Haematologica. 2006 91:1193-202.
16. Pennisi P, Pizzarelli G, Spina M, Riccobene S, Fiore CE. Quantitative ultrasound of bone and clodronate effects in thalassemia-induced osteoporosis. J Bone Miner Metab. 2003;21:402-8.
17. Marshall WA, Tanner JM. Variations in the pattern of pubertal changes inboys. Arch.Dis Child. 1970;4:23.
18. Who scientific group on the assessment of osteoporosis at primary health care level Summary Meeting Report Brussels, Belgium. 5-7 May 2004.
19. Mirza F, Canalis E. Management of endocrine disease: Secondary osteoporosis: pathophysiology and management. Eur J Endocrinol. 2015;173:R131–51.
20. Saki N, Abroun S, Salari F, Rahim F, Shahjahani M, Mohammadi-Asl J. Molecular aspects of bone resorption in β-thalassemia major. Cell J. 2015;17:193-200.
21. Lasco A, Morabito N, Gaudio A, Buemi M, Wasniewska M, Frisina N. Effects of hormonal replacement therapy on bone metabolism in young

adults with beta-thalassemia major. Osteoporosis Int. 2001;12:570-5.
22. Guisti A. Bisphosphonates in the management of thalassemia-associated osteoporosis: a systematic review of randomised controlled trials. J Bone Miner Metab. 2014;32:606–15.
23. Pietra P, Pizzarelli G, Spina M, Riccobene S, Fiore CE. Quantitative ultrasound of bone and clodronate effects in thalassemia-induced osteoporosis. J Bone Miner Metab. 2003;21:402-8.
24. Gilfillan CP, Strauss BJ, Rodda CP, Bowden DK, Kean AM, Obaid M. A randomized, double-blind, placebo-controlled trial of intravenous zoledronic acid in the treatment of thalassemia-associated osteopenia. Calcif Tissue Int. 2006;79:138-44.
25. Perifanis V, Vyzantiadis T, Vakalopoulou S, Tziomalos K, Garypidou V, Athanassiou-Metaxa M. Treatment of beta-thalassaemia-associated osteoporosis with zoledronic acid. Br J Haematol. 2004;125:91-2.
26. Mamtani M, Kulkarni H. Bone recovery after zoledronate therapy in thalassemia-induced osteoporosis: a meta-analysis and systematic review. Osteoporos Int. 2010;21:183-7.
27. Yigitoglu PH, Guzel R, Evran M, Gurkan E. Use of zoledronic acid in thalassemia-induced osteoporosis:two case reports and review of the literature. Turk J Phys Med Rehab. 2012;58:154-8.
28. Khan AA, Sándor GK, Dore E, Morrison AD, Alsahli M, Amin F et al. Canadian Task Force on osteonecrosis of the Jaw. Bisphosphonate associated osteonecrosis of the jaw. J Rheumatol. 2009;36:478-90.
29. Withold W. Monitoring of bone turnover; biological, preanalytical and technical criteria in the assesment of biochemical markers. Eur J Clin Chem Clin Biochem. 1996;34:785-99.
30. Lasco A, Morabito N, Gaudio A, Crisafulli A, Meo A, Denuzzo G. Osteoporosis and β-thalassemia major: Role of the IGF-I/IGFBP-III axis. J Endocrinol İnvest. 2002;25:338-44.
31. Voskaridou, E, Christoulas D, Konstantinidou M, Tsiftsakis E, Alexakos P, Terpos E. Continuous improvement of bone mineral density two years post zoledronic acid discontinuation in patients with thalassemia-induced osteoporosis: long term follow up a randomized, placebo-controlled trial. Haematologica. 2008;93:1588–90.
32. Rosen HN, Moses AC, Garber J, Ross DS, Lee SL, Greenspan SL. Utility of biochemical markers of bone turnover in the follow-up of patients treated with bisphosphonates. Calcif Tissue Int. 1998;63:363-8.
33. Rosen HN, Moses AC, Garber J, Iloputaife ID, Ross DS, Lee SL et al. Serum CTX: a new marker of bone resorption that shows treatment effect more often than other markers because of low coefficient of variability and large changes with bisphosphonate therapy. Calcif Tissue Int. 2000;66:100-3.