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Original Article

Effects of Hormonal Replacement Therapy on Bone Metabolism in Young Adults with Beta-thalassemia Major

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Abstract. The aim of our cross-sectional study was to evaluate the effects of hormonal replacement therapy (HRT) on a population of young thalassemics in order to understand better the role of hypogonadism in the balance of bone metabolism. Markers of bone turnover and bone mineral density (BMD) were measured in 40 young patients (mean age 19.8 $\pm$ 4.5 years) with beta-thalassemia major: 20 subjects were biochemically eugonadal, since they were undergoing HRT (group A, treated patients), and 20 were hypogonadic, having suspended HRT (group B, untreated patients). We also examined 20 healthy control subjects (group C) matched for age, anthropometric features and sex to the study groups. Our study shows that young thalassemic patients exhibit a significant loss of cortical and trabecular bone [aBMD L2–L4: 0.886 $\pm$ 0.052 g/cm^2 (group A), 0.726 $\pm$ 0.040 g/cm^2 (group B), 1.083 $\pm$ 0.090 g/cm^2 (group C); aBMD femoral neck: 0.890 $\pm$ 0.071 g/cm^2 (group A), 0.700 $\pm$ 0.065 g/cm^2 (group B), 0.934 $\pm$ 0.076 g/cm^2 (group C)]. Osteoporosis is only observed at the lumbar level in treated thalassemic patients, while in untreated patients it involves the femoral neck also. Bone turnover in thalassemic patients is higher in the resorptive phase, than in the neoformation phase and this is more marked in hypogonadicuntreated patients. In conclusion, our data demonstrate the important role played by hypogonadism in the development and deterioration of osteopenia/osteoporosis in thalassemia major. Consequently, sex hormone replacement therapy represents an appropriate tool in the prevention and treatment of osteoporosis in thalassemics, probably together with bisphosphonates in cases with severely increased bone resorption.

Keywords: HRT; Hypogonadism; Osteoporosis; Thalassemia

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Introduction

Optimized transfusional treatment and iron chelation programs have greatly increased the life expectancy of patients with beta-thalassemia major, who can now survive until their third or fourth decade. However, aging leads to the development of additional clinical problems in these patients, such as osteopenia and osteoporosis, which are frequently the cause of pathologic fractures and limb deformities [1,2]. So far, little information is available concerning the prevalence of bone mass alterations and the factors responsible for the development of osteopenia and osteoporosis in beta-thalassemia [3,4]. In a previous study, we found that 48% of female thalassemic patients have osteopenia and 38% have osteoporosis, while the corresponding male data are 76% and 18% (Lasco et al., unpublished data). Red marrow overstimulation and hyperplasia, which are common beta-thalassemia features, hemochromatosis determined by massive transfusional therapy [5], vitamin D deficiency due to iron overload [6], chronic liver disease, alterations in the growth hormone/insulin-like growth factor I (GH/IGF-I) axis [7] and genetic predisposition contribute to the pathogenesis of this form of osteoporosis. It is well known that several hormones, included sex steroids, mediate bone growth and

remodeling [8–12] and that beta-thalassemic patients often have gonadal impairment [13–15]. Moreover, sexual hormone replacement therapy has beneficial effects on bone metabolism [16–18]: male subjects with delayed puberty [19], hypogonadism of any cause and female patients with long-standing secondary amenorrhea show a substantial reduction in bone mass. This can be improved by adequate HRT. On the basis of these considerations, we aimed to evaluate the bone mass and turnover parameters and their association with concomitant gonadal function in beta-thalassemic patients.

Materials and Methods

Patients

Sixty-eight patients with beta-thalassemia major attending the Centre for the Prevention and Cure of Osteoporosis of the Department of Internal Medicine of Messina University were initially recruited for this study. From the overall series, 28 patients (12 males) were not admitted to the study on the basis of the following exclusion criteria: thyroid disease (5 cases), diabetes mellitus (4), other endocrine disorders (3), malabsorption (1), liver disease (6), spinal radiological abnormalities (4), steroid treatment (2), anticonvulsant treatment (1), smoking (1), HIV-positivity (1). The remaining patients (40 subjects) were included in our cross-sectional study and separated into two groups on the basis of their 17β-estradiol and testosterone levels. Group A (sex-hormone-treated thalassemics) consisted of males with plasma levels of testosterone >8 nmol/l and females with levels of 17β-estradiol >75 pmol/l; the remaining patients constituted group B (untreated thalassemics). Group A consisted of 20 patients (10 males, 10 females); all were without evidence of hypogonadism, since they had been undergoing hormonal sex hormone replacement therapy for 3.0 $\pm$ 2.1 years, with a good compliance. Of these, 7 patients had spontaneous puberty before hormonal therapy was begun. Group B consisted of 20 patients (10 males, 10 females); all displayed evident hypogonadism, since they had undergone HRT only for 0.3 $\pm$ 0.1 years with a low compliance, and had suspended it 2.9 $\pm$ 1.7 years previously. None of them had spontaneous puberty. One patient in group A (sex-hormone-treated thalassemics) and 4 in group B (untreated thalassemics) at the time of the present study were still growing and had not attained their final height. In addition, we recruited 20 young volunteers from the hotel school of Messina. These were healthy subjects matched for age, height, weight and sex to the patients, and were placed in group C (10 males, 10 females). Diagnosis of homozygous beta-thalassaemia was made using hemoglobin electrophoresis to identify variant hemoglobins [20].

The therapeutic scheme of HRT in group A was as follows: metil-testosterone, 100 mg i.m. every 28 days (Testovis Depot) for male patients; 17β-estradiol 50 μg/day (Epiestrol 50) for 21 days every month and medroxyprogesterone acetate 10 mg (Farlutal 10) for the last 11 days of the menstrual cycle for female patients. This therapeutic scheme was executed continuously.

Patients had been maintained on a regular transfusion program since early childhood, according to a monthly regimen with the aim of maintaining pretransfusional hemoglobin levels above 1.55 mmol/l. All thalassemic patients were subjected to an iron chelation program with desferioxamine (Desferal), administered subcutaneously by minipump, via an 8 h infusion each day. The dosage varied from 40 to 60 mg/kg per day and was recommended to be used 5 nights each week. Compliance with chelation therapy according to serum ferritin levels was monitored for 1 year and considered acceptable in all patients.

The study was performed according to the principles of the Declaration of Helsinki, and informed consent was obtained in every case.

Methods

Bone Mass Measurements. Bone mineral density (BMD) was measured by dual-energy X-ray absorptiometry (DXA; SOPHOS L-XR-A, Sopha Medical Italia) at the lumbar spine (L2–L4) and the proximal femur (neck and Ward's triangle) in the anteroposterior projection. The values were expressed as the BMD area (aBMD L2–L4 and aBMD femoral neck, g/cm^2). In order to reduce the influence of bone size on BMD measurements in the growing skeleton, the apparent volumetric density of the lumbar spine (vBMD L2–L4, g/cm^3) was calculated using the following formula: vBMD = aBMD $\times$ [4/($\pi \times$ width)] [21]. The instrument was calibrated on a daily basis according to the manufacturer's instructions. Reproducibility was calculated as a coefficient of variation (CV) obtained by weekly measurements of a standard phantom on the instrument and by repeated measurements obtained in three patients of different ages. The CV of our instrument is 0.5% with the standard phantom; in vivo we calculated a CV of 1.1% for the lumbar spine, 1.5% for the femoral neck and 3.2% for Ward's triangle.

Biochemical Analyses. Fasting blood samples were taken for the measurement of serum calcium (Ca), phosphorus (P), albumin, creatinine (Creat), alkaline phosphatase (ALP), alanine aminotransferase (ALT), bilirubin, intact parathyroid hormone (PTH), osteocalcin (BGP), 25-hydroxyvitamin D (25-OHD), follicle stimulating hormone (FSH), luteinizing hormone (LH), 17β-estradiol (E_2), testosterone (T), ferritin and hemoglobin (Hb). A 2 h fasting morning urine sample was collected at the same time for measurements of calcium, creatinine, hydro-

xyproline (HOP) and pyridinium crosslinks [pyridinoline (PYR) and deoxypyridinoline (D-PYR)].

Ca (normal range 2.2–2.6 mmol/l), P (normal range 1.2-1.6 mmol/l), Creat (normal range 63–133 μmol/l in serum and 0.13–0.22 mmol/kg body weight per 24 h in urine), bilirubin (normal range 5.1–17 μmol), albumin (normal range 35–55 g/l), ALT (normal range 3–26 IU/l), ALP (normal range 10–32 IU/l) and Hb (normal range 2.1–2.6 mmol/l) were determined by automated routine procedures. BGP (normal range 3.36–6.86 pmol/ml), PTH (normal range 1.17–6.77 pmol/l) and 25-OHD (normal range 25–125 nmol/l) were measured by an IRMA (BOUTY, Italiana Laboratori Bouty Milan, Italy). FSH (normal range 4–30 IU/l), LH (normal range for men 6-18 IU/l, for women 5–20 IU/l in follicular phase of menstrual cycle), E_2 (normal range 73–367 pmol/l for the follicular phase of menstrual cycle), T (normal range 9.5–30 nmol/l) and ferritin (normal range 3–12 mmol/l) were evalulated by a solid-phase immunoassay (Roche Diagnostics, Milan, Italy). HOP (normal range 12–32 mmol/mol urinary creatinine), PYR (normal range 26–91 pmol/μmol urinary creatinine) and D–PYR (normal range 3–21 pmol/μmol urinary creatinine) were measured by HPLC (BIORAD Diagnostics, Richmond, CA). Diagnosis of hypogonadism was made according to levels of 17β-estradiol for female patients (<75 pmol/l) and testosterone for male patients (<8 nmol/l). Bone age was evaluated using the radiographic atlas of Greulich and Pyle [22]. Height was measured with a Harpenden stadiometer and expressed as standard deviation scores (SDS) according to Tanner and Whitehouse [23]. Pubertal status was assessed by means of Tanner's method [24,25]. Testicular volume was determined by using a caliper to measure the horizontal and vertical axis and applying the following formula:

$$V = 4/3\ \mu\ (a/2)^2\ b/2$$

where a is the horizontal axis and b vertical axis (in cm). Volumes were expressed in milliliters as the mean of both testicles [26].

Statistical Analysis

Statistical analyses were performed using the Statview 512 statistical package. All values were expressed as mean $\pm$ SD. Comparisons between groups were performed by Student's t-test for unpaired data when the data were normally distributed, or by nonparametric statistical analysis (Wilcoxon rank sum test) when they were not. A p value of <0.05 was considered significant. Multiple regression analysis was performed using BMD values (aBMD L2–L4, vBMD L2–L4 and aBMD femoral neck) as the dependent variables, and all the other clinical and biochemical data as independent variables.

Results

The clinical characteristics of the subjects are presented in Table 1. The chronological age and body mass index (BMI) did not differ significantly among the three study groups. However, the bone age in group B (untreated thalassemics) was lower than in the other two groups, in both male and female patients. Testicular volume was significantly lower in male thalassemics than in controls ($p<0.001$). Hormonal data are shown in Table 2. Thalassemic patients had secondary hypogonadotropic hypogonadism, but levels of testosterone and 17β-estradiol were significantly lower than controls only in group B ($p<0.001$). Biochemical data are presented in Table 3. Serum levels of albumin, creatinine, calcium and phosphorus and urinary levels of creatinine did not differ significantly among the study groups. Levels of alanine aminotransferase, bilirubin and ferritin were significantly higher in thalassemic patients than in controls ($p<0.001$), with no significant differences between group A and B patients.

In comparison with the control group, all thalassemic patients showed increased bone turnover. Levels of pyridinoline, deoxypyridinoline, hydroxyproline and

Table 1. Clinical characteristics of patients and controls

Subjects	n	Chronological age (years)	Bone age (years)	Height (SDS)	BMI (kg/m^2)	Pubertal stage	Testicular volume (ml)
Male patients							
Group A	10	19.5 $\pm$ 2.1	17.1 $\pm$ 0.9*	-1.26 ± 0.32	22 $\pm$ 2	G4/P4–G5/P5	12 $\pm$ 2*
Group B	10	20.2 $\pm$ 3.4	15.3 $\pm$ 2.2**	-1.34 ± 0.30	21 $\pm$ 3	G3/P3	11 $\pm$ 1*
Group C	10	18.3 $\pm$ 4.2	17.2 $\pm$ 1.7 *p=0.02 A vs B **p=0.04 B vs C	-0.80 ± 0.40	23 $\pm$ 4	G5/P5	21 $\pm$ 3 *p<0.001 A and B vs C
Female patients							
Group A	10	20.4 $\pm$ 4.1	16.2 $\pm$ 0.9*	-1.20 ± 0.23	20 $\pm$ 3	B4/P4-B5/P5	
Group B	10	19.3 $\pm$ 5.3	14.1 $\pm$ 1.2*	-1.35 ± 0.30	22 $\pm$ 4	B3/P3	
Group C	10	19.1 $\pm$ 5.5	17.4 $\pm$ 1.7 *p<0.001 B vs A and C	-0.71 ± 0.31	23 $\pm$ 4	B5/P5	

All values are expressed as mean $\pm$ SD.

Table 2. Hormonal parameters of patients and controls

	FSH (IU/l)	LH (IU/l)	$17\beta E_2$ (pmol/l)	Testosterone (nmol/l)
Male patients[a]				
Group A	2.2 ± 1.4*	2.4 ± 1.5*	–	18.2 ± 4.0**
Group B	1.6 ± 0.5**	1.5 ± 0.6**	–	5.1 ± 1.3*
Group C	6.1 ± 2.6 *p<0.001 A vs C **p=0.006 B vs C	6.3 ± 2.8 *p<0.001 A vs C **p=0.006 B vs C	–	20.4 ± 5.7 *p<0.001 B vs C **p<0.001 A vs B
Female patients[b]				
Group A	2.0 ± 1.2*	1.9 ± 1.4*	111.9 ± 18.6**	–
Group B	1.5 ± 0.8**	1.8 ± 0.9**	43.8 ± 25.5*	–
Group C	4.2 ± 1.5 *p=0.002 A vs C **p<0.001 B vs C	4.7 ± 1.5 *p<0.001 A vs C **p<0.001 B vs C	127.7 ± 29.2 *p<0.001 B vs C **p<0.001 A vs B	–

All values are expressed as mean ± SD.
FSH, follicle stimulating hormone; LH, luteinizing hormone; $17\beta E_2$, 17β-estradiol; T, testosterone.
[a] Dosage on the 12th day after the last injection of metil-testosterone for group A.
[b] Dosage on the 7th day of induced or spontaneous menstrual cycle for groups A and C.

Table 3. Biochemical parameters of patients and controls

	ALT (IU/l)	Bilirubin (μmol/l)	Albumin (g/l)	Ca (mmol/l)	P (mmol/l)	Creat (μmol/l)	Urinary Creat (mmol*kg^{-1}/24h)	Hb (mmol/l)	Ferritin (mmol/l)
Group A	15 ± 1.8*	48 ± 3.1*	40 ± 2.9	2.50 ± 0.07	1.53 ± 0.10	84.5 ± 4.7	0.18 ± 0.02	1.60 ± 0.7*	58.44 ± 49.70*
Group B	16 ± 1.4*	47 ± 2.8*	38 ± 1.8	2.45 ± 0.05	1.52 ± 0.09	85.1 ± 3.4	0.17 ± 0.03	1.55 ± 0.8**	72.34 ± 57.88*
Group C	10 ± 2.1 *p<0.001 A and B vs C	10 ± 1.9 *p<0.001 A and B vs C	39 ± 2.1	2.48 ± 0.08	1.54 ± 0.08	83.7 ± 5.4	0.19 ± 0.04	2.32 ± 0.8 *p=0.04 A vs C **p=0.01 B vs C	5.20 ± 2.98 *p<0.001 A and B vs C

All values are expressed as mean ± SD.
ALT, alanine aminotransferase; Ca, calcium; P, phosphorus; Creat, creatinine; Hb, hemoglobin.

Table 4. Parameters of bone turnover

	Calciuria (mmol/mmol urinary creatinine)	PYR (pmol/μmol urinary creatinine)	D-PYR (pmol/μmol urinary creatinine)	HOP (mmol/mol urinary creatinine)	ALP (IU/l)	BGP (pmol/ml)	PTH (pmol/l)	25-OHD (nmol/l)
Group A	0.58 ± 0.14*	77.9 ± 15.8*(**)	20.9 ± 3.8*(**)	37.1 ± 23.9*	41.5 ± 6.9*(***)	5.1 ± 1.1*	4.4 ± 0.7	70.8 ± 10.1
Group B	0.63 ± 0.23*	90.3 ± 14.7*	25.8 ± 3.2*	45.9 ± 23.7**	34.4 ± 4.6**	4.4 ± 1.0	4.6 ± 0.8	71.2 ± 11.3
Group C	0.26 ± 0.08 *p<0.001 A and B vs C	48.5 ± 12.3 *p<0.001 A and B vs C **p=0.04 A vs B	14.8 ± 5.7 *p<0.001 A and B vs C **p=0.002 A vs B	22.6 ± 11.9 *p=0.02 A vs C **p<0.001 B vs C	29.1 ± 6.0 *p<0.001 A vs C **p=0.02 B vs C ***p=0.008 A vs B	4.2 ± 1.4 *p=0.03 A vs C	4.9 ± 1.2	78.8 ± 21.5

All values are expressed as mean ± SD.
PYR, pyridinoline; D-PYR, deoxypyridinoline; HOP, hydroxyproline; ALP, alkaline phosphatase; BGP, osteocalcin; PTH, parathyroid hormone; 25-OHD, 25-hydroxyvitamin D.

calciuria were significantly higher in both thalassemic groups than in controls, with a major alteration in group B (untreated thalassemics). Levels of BGP and ALP were significantly enhanced in group A (treated thalassemics), while this increase was not statistically significant in group B (untreated thalassemics) (Table 4). Levels of PTH and 25-OHD were not significantly different between patients and controls. aBMD and vBMD of the lumbar spine (L2–L4) were reduced in both groups of patients compared with controls. However, this reduction was higher in group B than in group A. aBMD was significantly reduced at the femoral

Table 5. Bone mineral density

	aBMD L2–L4 (g/cm^2)	vBMD L2–L4 (g/cm^3)	aBMD femoral neck (g/cm^2)
Group A	0.886 ± 0.052*(**)	0.283 ± 0.016*(**)	0.890 ± 0.071**
Group B	0.726 ± 0.040*	0.231 ± 0.012*	0.700 ± 0.065*
Group C	1.083 ± 0.090	0.346 ± 0.003	0.934 ± 0.076
	*p<0.001 A and B vs C	*p<0.001 A and B vs C	*p<0.001 B vs C
	**p<0.001 A vs B	**p<0.001 A vs B	**p<0.001 A vs B

All values are expressed as mean $\pm$ SD.

Table 6. Significant correlations between BMD and main clinical and biochemical parameters (r)

		BMI	Hormonal treatment duration	Ferritin
Group A	aBMD L2–L4	0.44*	0.32*	−0.31*
	vBMD L2–L4	0.43*	0.31*	−0.31*
	aBMD femoral neck	0.45*	0.42**	−0.30*
		*p=0.05	*p=0.05	*p=0.02
			**p=0.04	
Group B	aBMD L2–L4	0.43*	–	−0.30*
	vBMD L2–L4	0.42*	–	−0.31*
	aBMD femoral neck	0.42*	–	−0.32*
		*p=0.05		*p=0.01

level only in group B with respect to group C (Table 5). Correlations between BMD and different parameters are shown in Table 6. aBMD L2–L4, vBMD L2–L4 and aBMD of femoral neck were significantly correlated with BMI and ferritin both in group A and in group B. A significant correlation was also found between BMD values and hormonal treatment duration in group A patients. No relationship was noticed with the other parameters considered.

Discussion

Our study shows that thalassemic patients exhibit a significant loss of cortical and trabecular bone, as determined by DXA measurement of the femur and the lumbar spine. Moreover, it demonstrates that beta-thalassemic patients have different degrees of osteopenia: in treated patients without evidence of hypogonadism due to HRT, osteoporosis is observed only at the lumbar level, while in untreated patients, with evidence of hypogonadism, it also involves the femoral neck. Red marrow overstimulation and hyperplasia due to ineffective erythropoiesis, which produce medullary surface widening and cortical thinning, are known to be the major pathogenetic factors of osteoporosis in thalassemic patients, even if data on this subject are contradictory [27]. Increased iron deposition in bone could play a role in abnormal bone remodeling, as shown by de Vernejoul et al. [28] in pigs treated with parenteral iron, in which morphometric measurements demonstrated that osteoblast recruitment and the collagen synthesis rate fell. The probable role played by iron in the pathogenesis of osteopenia in thalassemics is confirmed in the present paper by the negative relationship found between serum ferritin levels and BMD in the patients of both groups.

Low plasma vitamin D concentrations, diabetes and hypothyroidism may constitute additional mechanisms. Hypogonadism, which is often observed in these patients, can also play an important role. It is known that sex steroid replacement therapy stimulates bone formation and breakdown, the effects of which are particularly pronounced during the last years of puberty and in states of gonadal steroid deficiency [17,19]. HRT has been shown to be associated in normal young men and women, and in thalassemic patients, with increases in bone turnover [29] and BMD [30].

Thalassemics have an unbalanced bone turnover with an increased resorption phase, as shown by high levels of HOP and pyridinium crosslinks. On the other hand, as shown by the very slight increase in alkaline phosphatase and osteocalcin levels, the neoformation phase does not follow this trend. This impairment induces a negative bone balance, which is more evident in hypogonadic patients. These data support the use of potent anti-resorptive drugs such as bisphosphonates in the treatment of osteoporosis in thalassemic patients [31].

The reduced BMD observed at the lumbar level in both treated and untreated thalassemics suggests that these patients are generally exposed to the risk of osteopenia, probably as a consequence of iron overload and bone marrow expansion. Hypogonadism, however, produces a more severe bone mass loss, as shown by the more severe degree of osteopenia found at the femoral level in untreated patients and by the significant positive correlation between BMD values and hormonal treatment duration.

Our data would seem to confirm that early HRT is able to improve the pattern of bone loss in these patients as also suggested by other authors who evaluated the contribution of hypogonadism to the development of osteoporosis in thalassemics and assessed the efficacy of sex hormone therapy in the improvement of bone density parameters [16].

In conclusion, our data demonstrate the important role played by hypogonadism in the development and deterioration of osteopenia/osteoporosis in thalassemia major. Consequently sex hormone replacement therapy

represents an appropriate tool in the prevention and treatment of osteoporosis in thalassemics, probably together with bisphosphonates in cases with severely increased bone resorption.

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