Osteoporosis and osteosclerosis in sickle cell/β-thalassemia: the role of the RANKL/osteoprotegerin axis

Ersi Voskaridou
Eleni Stoupa
Lito Antoniadou
Evangelos Premetis
Kostas Konstantopoulos
Ioannis Papassotiriou
Evangelos Terpos

Bone mineral density (BMD) was evaluated in 52 patients with HbS/β-thalassemia. Seventeen (32%) patients had osteopenia/osteoporosis and 30 (57%) had osteosclerosis. Bone resorption was diminished in patients with osteosclerosis and increased in those with osteopenia/osteoporosis. The sRANKL/osteoprotegerin ratio was elevated in the osteosclerotic group. Osteoporosis patients had mild renal impairment and their BMD correlated with osteoprotegerin, and bone resorption markers. Osteosclerosis patients had multiple infarctions in the studied bones that led to reduced osteoclast activity and increased BMD. In conclusion, HbS/β-thalassemia patients may develop osteopenia/osteoporosis mainly due to marrow expansion or osteosclerosis because of ischemia after a vaso-occlusive crisis. The RANKL/ osteoprotegerin axis participates in these phenomena.

Key words: osteoporosis, osteosclerosis, sickle cell disease, thalassemia, bone mineral density.

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Froom the Thalassemia Center, Laikon General Hospital, Athens, Greece (EV); Department of Medical Research, General Airforce Hospital, Athens, Greece (ES, ET); Bioiatriki Medical Centre, Athens, Greece (LA); Departments of Laboratory Hematology (EP) and Clinical Biochemistry (IP), "Aghia Sophia" Children's Hospital, Athens, Greece; First Department of Internal Medicine, University of Athens School of Medicine, Laikon General Hospital, Athens, Greece (KK).

Correspondence:
Evangelos Terpos, MD, PhD,
Department of Medical Research,
General Airforce Hospital,
Kanellopoulou street 3,
GR-11525, Athens, Greece.
E-mail: eterpos@hotmail.com/
e.terpos@imperial.ac.uk

Bone involvement is the commonest clinical manifestation of sickle cell disease.[1] The infarction of vertebral bone marrow and the presence of osteoporosis may produce collapse of the vertebrae with the typical *fish mouth* appearance.[2] Marrow hyperplasia seems to be the most important pathogenetic factor for bone loss in patients with sickle cell disease.[3] However, there is no information in the literature on the prevalence and etiology of osteoporosis in patients with double heterozygosity of sickle cell disease and β-thalassemia (HbS/β-thalassemia). The human skeleton is remodeled continuously through life. The receptor activator of nuclear factor-κB ligand (RANKL) is a potent enhancer of osteoclastogenesis, while osteoprotegerin (OPG) is the decoy receptor of RANKL.[4,5] An imbalance of the RANKL/OPG ratio has been described in both postmenopausal and β-thalassemia major-induced osteoporosis.[6-8] The aim of the present study was to evaluate the bone mineral density (BMD) of HbS/β-thalassemia patients in parallel with markers of bone remodeling in an attempt to better understand the pathophysiology of bone disease in these patients. To our knowledge, such studies are not available in the literature.

Design and Methods

We studied 52 patients with HbS/β-thalassemia (23M/29F; median age 40 years, range: 23-70 years). All patients were in a stable phase of their disease and were transfused sporadically. No patient received agents known to affect bone metabolism, such as bisphosphonates, calcium, and vitamin D, and only one woman was postmenopausal. All patients had undergone radiography of the lumbar spine and both femoral necks and none had vertebral collapse. The BMD of the lumbar spine (L1-L4) and the left femoral neck was determined by dual energy X-ray absorptiometry (DEXA; LUNAR, Madison, WI, USA) in all patients. In cases of known infarctions in the left femoral neck, the BMD of the right femoral neck was used. The following series of bone remodeling indices was evaluated in the serum of both patients and controls (10 males/15 females; median age: 40 years, range: 24-64 years): i) osteoclast-stimulating factors [soluble RANKL (sRANKL), OPG], ii) bone resorption markers [C-telopeptide of type-I collagen (CTX), tartrate-resistant acid phosphatase isoform-5b (TRACP-5b)], and iii) bone formation markers [bone-alkaline phosphatase (bALP), osteocalcin, C-terminal propeptide of collagen type-I (CICP)], as previously described.[9] Furthermore, each control was examined to ensure that there was no evidence of bone disease (osteoporosis or osteoarthritis) and no receipt of medication that could alter bone turnover during the preceding 6 months. Patients and controls also had a thorough evaluation of their renal function with measurements of creatinine clearance and serum cystatin-C, as previously described.[10] Bone marrow expansion (serum soluble transferrin receptor, sTfR), serum erythropoietin and intact parathyroid hormone levels were also determined using standard methodology. Informed consent was obtained from all

Table 1. **Median values (range) of the studied parameters in HbS/β-thalassemia patients with osteopenia/osteoporosis and osteosclerosis.**

	HbS/β-thal with osteoporosis (n=17)	*HbS/β-thal with osteosclerosis (n=30)*	*p-value*	*Controls (n=25)*	*p (osteoporosis vs. controls)*	*p (osteosclerosis vs. controls)*
Age (years)	45 (23-70)	40 (32-60)	0.57	40 (24-64)	0.88	0.64
Interacting β^0-thalassemia genes (n)	16	21	0.19			
Days of hospitalization due to painful crises	13.6 (2-20)	12.8 (3-17)	0.43			
BMD L1-L4 (g/cm²)	0.92 (0.44-1.04)	1.62 (1.02-2.19)	<0.0001			
T-score	-2.1 (-1.11 to -5.2)	+3.63 (+1.42 to +7.9)				
BMD-femoral neck (g/cm²)	0.73 (0.45-0.86)	1.28 (0.85-2.1)	<0.0001			
T-score	-1.8 (-1.1 to 3.04)	+1.81 (-0.8 to +8.5)				
sRANKL (pmol/L)	0.24 (0-1.85)	0.45 (0-1.94)	0.39	0.12 (0-2.32)	0.86	0.32
OPG (pmol/L)	5.96 (3.93-14.16)	5.67 (3.12-13.73)	0.26	4.68 (2.35-9.44)	<0.001	<0.02
sRANKL/OPG ($\times 10^{-2}$)	4.54 (0-32.99)	10.48 (0-59.48)	0.05	3.70 (0-67.28)	0.81	0.49
CTX (ng/mL)	0.91 (0.09-1.82)	0.45 (0.04-1.29)	0.01	0.60 (0.2-2.03)	0.04	<0.0001
TRACP-5b (U/L)	2.25 (0.52-4.34)	2.06 (0.41-4.15)	0.11	2.03 (0.85-3.36)	0.52	0.67
bALP (U/L)	32.83 (17.25-61.43)	24.56 (11.42-55.84)	<0.01	20.99 (9.45-27.51)	<0.0001	<0.02
Osteocalcin (ng/mL)	10.93 (1.04-43.24)	9.97 (0.31-19.82)	0.28	12.57 (2.48-30.19)	0.28	<0.01
CICP (ng/mL)	135.73 (39.93-246.1)	103.32 (56.72-197.4)	0.33	45.98 (5.04-124)	<0.001	<0.001
Hemoglobin (g/dL)	8.5 (7.8-9.1)	9.3 (8.1-10.0)	0.11	13.8 (11.8-15.6)	<0.0001	<0.0001
Cystatin-C (mg/L)	0.9 (0.51-2.27)	0.89 (0.41-1.46)	0.17	0.81 (0.62-1.01)	0.02	0.1
Creatinine clearance (mL/min/1.73 m²)	84 (50-136)	90 (64-152)	0.13	107 (80-127)	0.029	0.117
Parathyroid hormone (pg/mL)	21.89 (11.63-74.37)	20.37 (7.66-49.73)	0.028	22.9 (15.6-27.3)	0.234	0.118
Calcium (mg/dL)	9.2 (8.3-11)	9.1 (8-10.2)	0.77	9.4 (8.8-10)	0.467	0.321
Erythropoietin (U/L)	82.3 (26.3-585.6)	126.1 (36.4-680.6)	0.26	8.2 (2.1-23.7)	<0.0001	<0.0001
sTfR (nmol/L)	110.4 (50.2-126.9)	104.7 (27.9-304.4)	0.22	21.8 (11.2-30.6)	<0.0001	<0.0001

patients prior to inclusion in the study. The study was conducted with the approval of the hospitals' ethical committees in keeping with the guidelines of the Declaration of Helsinki. Wilcoxon's signed rank sum test and Student's t-test were applied to evaluate the differences between patients and controls, while the Mann-Whitney test was used for estimating differences between groups of patients. The correlation between various parameters and BMD was evaluated with the Spearman's (r_s) correlation coefficient.

Results and Discussion

Based on BMD results patients were divided into three groups. According to WHO criteria (T-score of below -1), 17 patients (32.6%; 8M/9F) had osteopenia or osteoporosis in either L1-L4 or the femoral neck. In contrast, 30 patients (57.6%; 12M/18F) had osteosclerosis, while only five patients had normal BMD in both studied sites. We defined osteosclerotic patients as those who had a BMD T-score of above +1 in either L1-L4 or the femoral neck and whose results of plain radiography of the same area were compatible with osteosclerosis (Table 1). HbS/β-thalassemia patients had higher levels of serum OPG, bALP, CICP, erythropoietin and sTfR compared with controls, independently of their BMD status. The sRANKL/OPG ratio in patients with osteopenia/osteoporosis was lower than that in osteosclerosis patients. CTX levels were significantly lower in osteosclerotic patients than in either controls or osteopenic/osteoporotic patients, while CTX levels were higher in the osteopenia/osteoporosis group than in the controls. Moreover, bALP serum levels were higher in patients with osteopenia/osteoposis than in patients with osteosclerosis (Table 1). Compared to con-

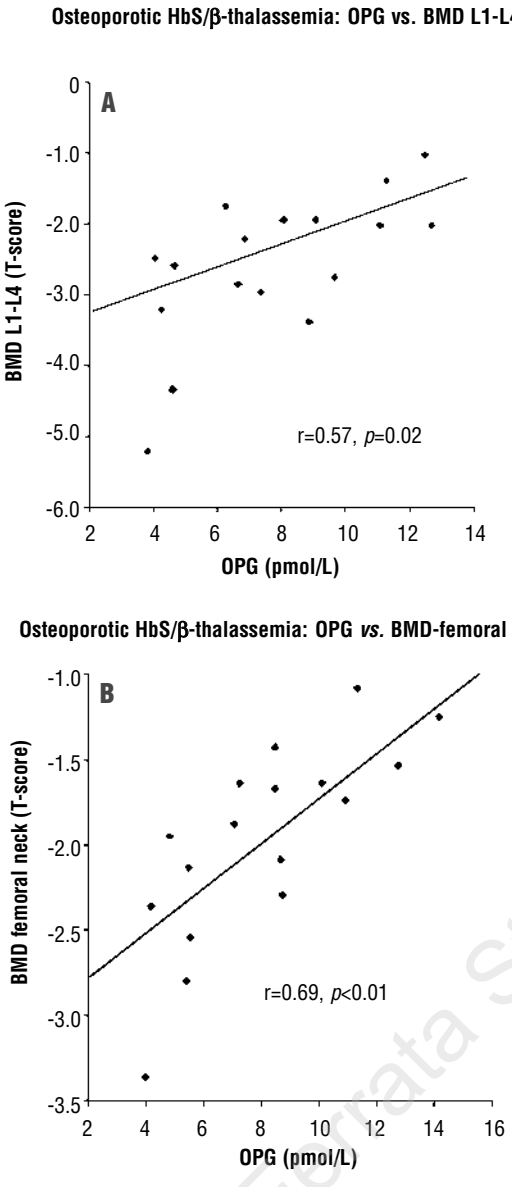

Figure 1. In HbS/β-thalassemia patients with osteopenia/osteoporosis, OPG serum levels strongly correlated with BMD of both L1-L4 (A) and the femoral neck (B), possibly representing a compensatory effect to increased bone resorption.

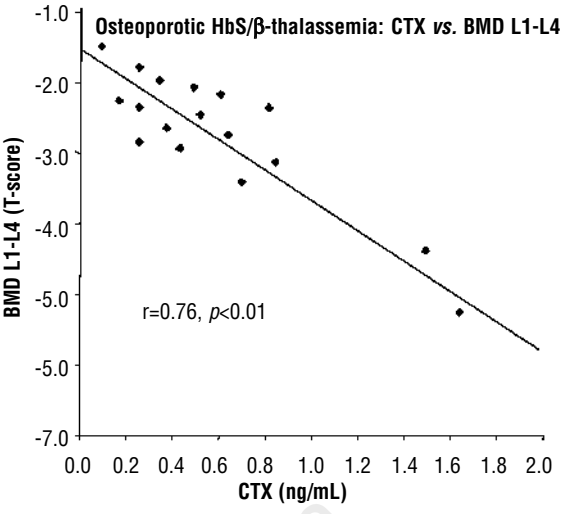

Figure 2. The strong negative correlation between CTX and BMD of L1-L4 suggests that increased bone resorption is present in HbS/β-thalassemia patients with osteopenia/oteoporosis.

trols, patients with HbS/β-thalassemia had lower values of creatine clearance and higher levels of serum cystatin C. However, there was no difference in either creatinine clearance or cystatin C between HbS/β-thalassemia patients with osteopenia/osteoporosis or osteosclerosis. Patients with osteopenia/osteoporosis had higher intact parathyroid hormone levels than had osteosclerosis patients, although all values were within normal limits.

In HbS/β-thalassemia patients with osteopenia/osteoporosis, the BMD of both L1-L4 and the femoral neck correlated with OPG levels (r=0.57, p=0.02; r=0.69, p<0.01, respectively; Figure 1). Furthermore, L1-L4 BMD displayed a strong correlation with CTX (r=-0.76, p<0.01; Figure 2), while CTX was significantly correlated with both erythropoietin (r=0.52, p=0.02) and sTfR (r=0.51, p=0.04). OPG correlated significantly with bALP (r=0.56, p=0.03), TRACP-5b (r=0.55, p=0.04), sRANKL (r=-0.56, p=0.03), and age (r=0.61, p=0.01). No correlation was observed between BMD and age. Among the HbS/β-thalassemia patients with osteosclerosis, a strong positive correlation was observed between BMD of the L1-L4 and femoral neck (r=0.46, p=0.02). OPG and sRANKL/OPG values correlated with TRACP-5b (r=0.5, p=0.01; r=-0.62, p=0.002). Cystatin-C serum levels were strongly correlated with OPG (r=0.55, p=0.007), sRANKL/OPG ratio (r=-0.44, p=0.03), and TRACP-5b (r=-0.46, p=0.02). Osteopenia/osteoporosis is a well-known complication of both sickle cell disease and thalassemia major, but there is very limited information in the literature about the pathophysiology of bone disease in HbS/β-thalassemia.[1] Almost one third of our HbS/β-thalassemia patients had osteopenia/osteoporosis with increased resorptive phase as assessed by the elevated serum levels of CTX, which correlated with BMD of L1-L4. In this cohort of patients, OPG serum levels were elevated and correlated with BMD of both L1-L4 and the femoral neck; this finding has also been described in post-menopausal osteoporosis.[6,11] The increased OPG levels may represent a compensatory response to enhanced bone resorption, a phenomenon which is not observed in patients with thalassemia major-induced osteoporosis in whom OPG levels are either reduced or within normal values, while bone resorption is increased.[7,8] Schett *et al.* showed that low levels of serum sRANKL and high levels of serum OPG were associated with an increased incidence of non-traumatic fracture.[12] According to this observation, our patients who have increased OPG values and normal sRANKL seem to be at high risk of fractures and suitable treatment may be considered. Bones are affected by both hemolytic and vaso-occlusive processes in sickle cell disease.[1] In our study, we found increased erythropoietic activity (assessed by increased

sTfR and erythropoietin serum levels) which showed a strong correlation with CTX, thus confirming that bone marrow expansion is one of the major reasons for bone destruction in HbS/β-thalassemia. Patients with osteopenia/osteoporosis also had mild renal impairment, which might be implicated in the pathogenesis of bone loss in these patients as it is well-known that patients with renal dysfunction have increased bone resorption[13] and may develop osteoporosis.[14] However, there was no evidence of hyperparathyroidism in these patients and therefore it is very difficult to support the hypothesis that this renal impairment was affecting bone loss. Interestingly, we observed that the majority of patients had increased BMD, a finding that has not been described previously in patients with either thalassemia major or sickle cell disease. These patients also had osteosclerosis, as shown by plain radiography. In this group, bone resorption assessed by CTX levels was diminished. Ten of these patients underwent computed tomography scanning of both L1-L4 and the femoral neck, which revealed the presence of multiple infarctions in different areas of the studied bones alternated with resorbing areas. Experimental studies suggest that the ratio of normal osteocytes decreases significantly, while pyknotic osteocytes increase after the second day of ischemia.[15] In our cohort of patients with osteosclerosis the diminished bone resorption was probably due to the lack of osteoclasts because of ischemia. Furthermore the increased levels of OPG, bALP and CICP demonstrated increased osteoblast function, which may be compensatory due to the possible lack of osteoclasts in ischemia areas. This increased osteoblast function overbalances the increased bone loss due to the enhanced erythropoietic activity, thus leading to elevated BMD values in both L1-L4 and the femoral neck. The subsequent increase in sRANKL and sRANKL/OPG levels, even if not significantly higher than levels in controls, may indicate increased production of RANKL by stromal cells in an attempt to enhance osteoclast differentiation. This hypothesis would also explain the presence of resorbing areas near the osteosclerotic ones in computed tomography scans of osteosclerotic patients with HbS/β-thalassemia. These results are consistent with those observed in cases of osteosclerosis of different etiology.[16,17]

In conclusion our results show that patients with HbS/β-thalassemia may develop osteopenia/osteoporosis or osteosclerosis during the course of their disease. Increased bone resorption mainly due to bone marrow expansion leads to bone loss, while diminished bone resorption due to ischemia after vaso-occlusive crises may be responsible for the development of osteosclerosis. The RANKL/OPG axis seems to participate in these phenomena. Novel agents targeting this system may be useful for the management of these important complications in HbS/β-thalassemia.

EV and ET designed the study, interpreted the data, and wrote the paper; ES, LA, EP, IP, and ET performed all laboratories tests; IP, KK and ET interpreted the data and the statistical analysis.

All authors carefully reviewed and agreed with the submission of this brief report. The authors declare that they have no potential conflicts of interest.

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