Ann Hematol (2016) 95:1329–1332
DOI 10.1007/s00277-016-2708-9

ORIGINAL ARTICLE

Evaluation of bone mineral density in patients with hemoglobin H disease

Tahereh Zarei[1] · Sezaneh Haghpanah[1] · Shirin Parand[1] · Hossein Moravej[2] · Mohammad Hossein Dabbaghmanesh[3] · Gholamhossein Ranjbar Omrani[3] · Mehran Karimi[1]

Received: 15 February 2016 / Accepted: 23 May 2016 / Published online: 3 June 2016

Abstract
Objectives This study was conducted to assess bone mineral density (BMD) and bone mineral content (BMC) of patients with hemoglobin H (HbH) disease.
Methods BMD and BMC were measured by dual energy X-ray absorptiometry of the lumbar spines and femur neck in 21 patients with Hb H disease over the age of 10 years.

An association of BMD with sex, age, hemoglobin, calcium, phosphorus, and serum ferritin level was also evaluated.
Results Prevalence of BMD below the expected range for age in the lumbar spine and femur neck region in patients with HbH disease were 33.3 and 14.3 %, respectively. Lumbar BMD was significantly lower in the patients compared to healthy individuals (median (min-max) 0.725 (0.595–0.924) vs. 1.061 (0.645–1.238), $P < 0.001$)). There was no significant relationship between BMD in the lumbar and femur neck with any of the evaluated variables (P value >0.05).
Conclusion Data regarding bone density in HbH disease is limited; osteoporosis as a common complication of β-thalassemia intermedia syndrome should be considered even in HbH which shows its prevalence is less than β-thalassemia intermedia.

Keywords Evaluation · Hemoglobin H · Bone mineral density

✉ Mehran Karimi
karimim@sums.ac.ir

[1] Hematology Research Center, Shiraz University of Medical Science, Shiraz, Iran

[2] Department of Pediatrics, School of Medicine, Shiraz University of Medical Science, Shiraz, Iran

[3] Endocrine and Metabolism Research Center, Nemazee Hospital, Shiraz University of Medical Sciences, Shiraz, IR Iran

Introduction

The α-thalassemias are the most common inherited disorders of hemoglobin synthesis. It caused by mutations in one or more of the four α-globin genes, leading to decreased or absent α-globin chain production [1].

Hemoglobin H (HbH) disease is caused by deletion or dysfunction of three of four α-globin alleles. The people with α-thalassemia intermedia or HbH disease usually have moderate anemia. This causes a phenotype of mild to moderate chronic hemolytic anemia. Hemoglobin H patients may require intermittent transfusion therapy especially during hemolytic or aplastic crises. Hemoglobin H disease is characterized by the presence of hepatosplenomegaly, microcytic hypochromic anemia, intraerythrocytic inclusion bodies. Other complications include infections, leg ulcers, gall stones, folic acid deficiency, and sometimes mild to moderate thalassemia-like bone changes that mainly affect the facial features [2–6].

Osteoporosis is a skeletal disease characterized by a generalized reduction in bone mineral density (BMD), microarchitectural deterioration of bone tissue, leading to increased bone fragility and significantly higher frequency of fractures [7–9].

Osteopenia and osteoporosis are important causes of morbidity in adult patients of both genders with β-thalassemia major (TM) or intermedia (TI). Osteoporosis is a multifactorial disease, thought to be mediated by an interaction between environmental factors as well as genetic factors. It is accepted that multiple acquired factors including liver disease, aging, genetic disorders of osteogenesis, endocrine disorders, and delay in sexual maturation are involved in the pathogenesis of osteoporosis in thalassemia [10–12].

Several studies have shown reduced bone mass and high rates of fracture in patients with TM and TI [13–15]. Based on our knowledge, data regarding bone density in HbH disease is

limited; on the other hand, α-thalassemia and HbH disease is not rare in our population [16].

Our aim was to investigate the status of BMD and possibly related factors in patients with HbH disease.

Material and methods

In this cross sectional study, we evaluated BMD and bone mineral content (BMC) in 21 patients over age 10 out of 37 Iranian patients with HbH disease, from November 2014 to August 2015, who were followed at an outpatient Thalassemia Clinic in Shiraz, Southern Iran. Also, we selected a sex- and age-matched control group ($n=21$) for comparison of BMD and BMC.

Exclusion criteria were other forms of thalassemia intermedia syndrome including β-thalassemia intermedia, sickle thalassemia, and HbE-thalassemia. Inclusion criterion was HbH disease in patients over 10 years old.

An informed written consent form was filled by those who accepted to take part in the study. The study was approved by the Ethics Committee of Shiraz University of Medical Sciences.

Diagnosis of HbH disease was based on complete blood cell (CBC), Hb electrophoresis and peripheral smear by checking inclusion bodies and gene analysis in some of them. The results of alpha globin gene analysis in patient number 1 showed –Med and −α3.7 (−−Med/-α3.7) and in patient number 12 showed α-5 nt α/–Med (double heterozygous α+ and α0).

All patients were non-transfusion dependent and took folic acid 5 mg/kg orally once a day.

We measured fasting serum calcium (Ca) and phosphate (P) with enzymatic colorimetric method by Hitachi 911 (Japan). 25-Hydroxy vitamin D (25OH-D) was measured with enzyme-linked immunosorbent assay (ELISA) method with DLD diagnostika kit (Germany) in all included patients. Fasting serum ferritin was measured with enzyme-linked fluorescent assay (ELFA) technology by mini Vidas analyzer (Biomerieux, France).

According to the International Society for Clinical Densitometry (ISCD) Official Position definition, a Z-score of −2.0 or lower was defined as "below the expected range for age," and a Z-score above −2.0 was regarded as "within the expected range for age" [17]. At the time of the study, no patient received any treatment such as bisphosphonate, following our results we referred our patients to endocrinologist if they needed treatment by bisphosphonate.

Lumbar spines (L1–L4) and right femur neck BMD and BMC were measured by means of dual energy X-ray absorptiometry (DXA) method by Lunar DPX-IQ densitometer, and the results were expressed as Z-score which are units of standard deviation (SD). Z-score is defined as the number of SDs above or below the mean for age- and sex-matched subjects. The BMD and BMC results were, respectively, expressed as mean values (g/cm^2) ± SD and (g) ± SD.

Statistical analysis

Data analysis was done by SPSS software version 17 (SPSS Inc, Chicago IL, USA). Comparison of quantitative variables between the two groups was done by Mann-Whitney test. Qualitative variables were compared by Chi-square test between the two groups. P value less than 0.05 was considered statistically significant.

Results

The demographic and paraclinical data of the patients are illustrated in Table 1.

Mean age of the patients was 20.23 ± 9.87 years and ranged from 11–56 years old.

Prevalence of patients with BMD in the lumbar spine below the expected range for age was 33.3 %. Also, 14.3 % of our patients suffered from BMD with below the expected range for age in the femur neck region.

We compared different variables including BMI, Ca, P, ferritin, Hb, HbF, T4, TSH, and 25OH-D levels in patients with BMD below the expected range for age. There was no significant association in any of the evaluated variables with BMD in the lumbar and femur neck (P value >0.05). A total of five (13.5 %) patients were splenectomized. The hypothyroidism was seen only in one patient with normal Z-score of BMD within the expected range for age.

Bone mineral density showed no significant association with age, sex and splenectomy (P value >0.05).

Table 2 shows comparison of BMD and BMC values between the patient and control groups. Lumbar BMD was significantly lower in the patients compared to controls (median (min-max) 0.725 (0.595–0.924) vs. 1.061 (0.645–1.238), $P<0.001$)).

Discussion

Our study shows a prevalence of 33.3 % of patients with BMD in the lumbar spine below the expected range for age. In addition, 14.3 % of our patients suffered from BMD with below the expected range for age in the femur neck region. Moreover, value of lumbar BMD was significantly lower in our patients in comparison with healthy individuals.

Some studies showed that fractures occurred more frequently among patients with TM and TI compared to the other thalassemia syndromes [18]. In one study, Jeans et al. showed osteoporosis or osteopenia in 96 % of patients with TM [19]. It seems that osteoporosis prevalence in HbH disease is less in

Table 1 Demographic, clinical, and paraclinical characteristics of the study population with hemoglobin H disease

Patients (number)	Age (Year)	Sex	Hb (g/dl)	HbF (%)	Ferritin (ng/ml)	BMI (kg/m^2)	Ca (mg/dL)	P (mg/dL)	T_4 (Mic gr/ml	TSH (IU/ml)	1,25 Vitamin OH-D (ng/ml)
1	13	Male	9.2	0.5	61.21	16.76	9.8	6.2	9	3	18
2	13	Female	9.8	11.6	215	13.53	10	3.8	7.5	2.3	21
3	13	Female	8.7	2.9	120.5	18.37	9.1	4.5	7.2	3.1	19.5
4	32	Female	8.1	1.2	201	22.72	9	4	7.5	3	20.3
5	19	Male	12.3	0.6	278	19.71	9.3	4.2	8.9	5	18.7
6	11	Male	8.2	0.3	313.2	16.51	10	5.3	8.4	6	22
7	56	Male	8.1	2.9	177.6	20.38	9.1	4.5	7.5	0.2	20.5
8	17	Male	8.7	8.57	136.6	19.84	9	4.5	7.3	2.7	22
9	12	Female	8.7	2	238	14.80	8.9	3.9	8.1	3	19.7
10	21	Male	10.3	23.1	210	19.49	8.9	5.7	9.2	3.3	20.5
11	21	Female	13	0.78	136.6	24.65	9	3.8	9.9	3	10
12	13	Female	8.5	0.3	238	19.34	8.7	4.6	6.5	4.3	21
13	23	Female	9.9	20.8	210	20.34	9.2	4.5	1.2	12.5	19
14	19	Male	11.3	0.4	439.5	19.43	10	2.8	7.1	2.6	21
15	26	Male	8.2	1.5	314	21.13	9.2	4.6	8.2	3.1	19
16	23	Female	9.7	1.77	510	27.48	8.3	4.2	7.5	4.4	16.3
17	15	Female	9.7	6.9	452	17.58	9.8	3.5	8.4	1.31	9
18	23	Female	8.7	1.22	181.7	37.40	9.2	3.7	9.3	2.8	21
19	13	Female	8.7	1.22	114.9	16.13	8.7	3.8	7.2	1.6	20.3
20	24	Male	11.2	0.6	81.9	18.41	8.9	3.4	7.6	1.91	36.22
21	18	Male	10.2	6.3	264	24.66	7.6	1.3	6.7	6.11	42.31

Normal range. Hb: male 13.8–17.2, female 12–15.6; HbF <2%; ferritin: male 20–250, female 10–120; BMI 18–24; Ca 8.3–10.6; P 2.5–5.8; T_4: 4.4–11.7; TSH 0.36–6.3; 1,25 vitamin OH-D >30

comparison with other thalassemia intermediate syndromes. However, further studies with larger groups of patients are recommended.

In our study, no association was found between any of the evaluated variables with BMD in the lumbar and femur neck.

Several studies have shown reduced bone mass in osteoporotic patients with thalassemia [20–22]. Additional studies found that osteopenia and osteoporosis are emerging as important causes of morbidity in patients of both genders with thalassemia [14, 19, 23–25].

Taher et al. evaluated 548 patients with β-TI showed osteoporosis as the most common disease-related complication (22.9 %) [26]. In HbH disease, ineffective erythropoiesis is less common than β-TI. It seems that osteopenia and osteoporosis in HbH is less common than in β-TI which was confirmed in our study.

In conclusion, based on our results, it seems to be rational to evaluate BMD in patients with HbH after age of ten and treat them if necessary despite its frequency is less than in TM and TI beta thalassemia patients.

Table 2 Bone mineral density and bone mineral content values of patients with hemoglobin H and controls

	BMD L1–L4	BMD femoral neck	BMC L1–L4	BMC femoral neck
Hemoglobin H				
Mean ± SD	0.739 ± 0.102	0.726 ± 0.156	44.353 ± 13.703	4.078 ± 1.334
Median (min-max)	0.725 (0.595–0.924)	0.690 (0.484–1.041)	42.100 (26.78–73.29)	3.950 (2.43–7.48)
Controls				
Mean ± SD	1.005 ± 0.174	0.844 ± 0.172	47.079 ± 10.357	4.261 ± 1.140
Median (min-max)	1.061 (0.645–1.238)	0.816 (0.510–1.267)	46.360 (27.55–64.18)	4.030 (2.26–6.97)
P value	<0.001*	0.051	0.354	0.514

BMD bone mineral density (g/cm^2), *BMC* bone mineral content (g)

* statistically significant

Acknowledgments This study was supported by The Shiraz University of Medical Sciences with grant number 93-01-32-8943. Also, I would like to thank Forough Saki for cooperating in the data collection.

Compliance with ethical standards

Conflict of interests The authors declare that they have no conflict of interest.

References

1. Steinberg MH, Forget BG, Higgs D R, Nagel RL (2001) Molecular mechanisms of a-thalassemia. Disorders of hemoglobin: Genetics, pathophysiology, and clinical management. Cambridge University Press 405–430
2. Laosombat V, Viprakasit V, Chotsampancharoen T, Wongchanchailert M, Khodchawan S, Chinchang W, Sattayasevana B (2009) Clinical features and molecular analysis in Thai patients with HbH disease. Ann Hematol 88(12):1185–1192
3. Fucharoen S, Winichagoon P (2012) New updating into hemoglobinopathies. Int J Lab Hematol 34(6):559–565
4. Fucharoen S, Viprakasit V (2009) Hb H disease: clinical course and disease modifiers. ASH Education Program Book 2009(1):26–34
5. Chui DH, Fucharoen S, Chan V (2003) Hemoglobin H disease: not necessarily a benign disorder. Blood 101(3):791–800
6. Higgs DR, Wood WG, Barton C, Weatherall DJ (1983) Clinical features and molecular analysis of acquired hemoglobin H disease. Am J Med 75(2):181–191
7. Organization WH (1994) Assessment of fracture risk and its application to screening for postmenopausal osteoporosis: report of a WHO study group [meeting held in Rome from 22 to 25 June 1992]. World Health Organization, Geneva
8. Larijani B, Resch H, Bonjour J, Meybodi HA, Tehrani MM (2007) Osteoporosis in Iran, overview and management. Supplementary issue on osteoporosis, Iranian Journal of Public Health:1–13
9. De Sanctis V, Soliman AT, Elsedfy H, Yassin M, Canatan D, Kilinc Y, Sobti P, Skordis N, Karimi M, Raiola G (2013) Osteoporosis in thalassemia major: an update and the I-CET 2013 recommendations for surveillance and treatment. Pediatr Endocrinol Rev 11(2):167–180
10. Stewart T, Ralston S (2000) Role of genetic factors in the pathogenesis of osteoporosis. J Endocrinol 166(2):235–245
11. Di Stefano M, Chiabotto P, Roggia C, Garofalo F, Lala R, Piga A, Isaia GC (2004) Bone mass and metabolism in thalassemic children and adolescents treated with different iron-chelating drugs. J Bone Miner Metab 22(1):53–57
12. Toumba M, Skordis N (2010) Osteoporosis syndrome in thalassaemia major: an overview. J Osteoporos 2010:537673
13. Dines D, Canale V, Arnold W (1976) Fractures in thalassemia. J Bone Joint Surg 58(5):662–666
14. Karimi M, Ghiam AF, Hashemi A, Alinejad S, Soweid M, Kashef S (2007) Bone mineral density in beta-thalassemia major and intermedia. Indian Pediatr 44(1):29
15. Pollak RD, Rachmilewitz E, Blumenfeld A, Idelson M, Goldfarb AW (2000) Bone mineral metabolism in adults with β-thalassaemia major and intermedia. Br J Haematol 111(3):902–907
16. Dehbozorgian J, Moghadam M, Daryanoush S, Haghpanah S, Imani fard J, Aramesh A, Shahsavani A, Karimi M (2015) Distribution of alpha-thalassemia mutations in Iranian population. Hematology 20(6):359–62
17. Crabtree NJ, Arabi A, Bachrach LK, Fewtrell M, Fuleihan GE-H, Kecskemethy HH, Jaworski M, Gordon CM (2014) Dual-energy X-ray absorptiometry interpretation and reporting in children and adolescents: the revised 2013 ISCD Pediatric Official Positions. J Clin Densitom 17(2):225–242
18. Vogiatzi M, Macklin E, Fung E, Vichinsky E, Olivieri N, Kwiatkowski J, Cohen A, Neufeld E, Giardina P (2006) Prevalence of fractures among the Thalassemia syndromes in North America. Bone 38(4): 571–575
19. Jensen C, Tuck S, Agnew J, Koneru S, Morris R, Yardumian A, Prescott E, Hoffbrand A, Wonke B (1998) High incidence of osteoporosis in thalassaemia major. J Pediatr Endocrinol Metab 11 Suppl 3:975–7
20. Voskaridou E, Terpos E, Spina G, Palermos J, Rahemtulla A, Loutradi A, Loukopoulos D (2003) Pamidronate is an effective treatment for osteoporosis in patients with beta-thalassaemia. Br J Haematol 123(4):730–737
21. Chan Y-L, Pang L-M, Chik K-W, Cheng JC, Li C-K (2002) Patterns of bone diseases in transfusion-dependent homozygous thalassaemia major: predominance of osteoporosis and desferrioxamine-induced bone dysplasia. Pediatr Radiol 32(7):492–497
22. Molyvda-Athanasopoulou E, Sioundas A, Karatzas N, Aggellaki M, Pazaitou K, Vainas I (1999) Bone mineral density of patients with thalassemia major: four-year follow-up. Calcif Tissue Int 64(6):481–484
23. Vogiatzi MG, Autio KA, Mait JE, Schneider R, Lesser M, Giardina PJ (2005) Low bone mineral density in adolescents with β-thalassemia. Ann N Y Acad Sci 1054(1):462–466
24. Vogiatzi MG, Macklin EA, Fung EB, Cheung AM, Vichinsky E, Olivieri N, Kirby M, Kwiatkowski JL, Cunningham M, Holm IA (2009) Bone disease in thalassemia: a frequent and still unresolved problem. J Bone Miner Res 24(3):543–557
25. Morabito N, Lasco A, Gaudio A, Crisafulli A, Di Pietro C, Meo A, Frisina N (2002) Bisphosphonates in the treatment of thalassemia-induced osteoporosis. Osteoporos Int 13(8):644–649
26. Taher AT, Musallam KM, Karimi M, El-Beshlawy A, Belhoul K, Daar S, Saned M-S, El-Chafic A-H, Fasulo MR, Cappellini MD (2010) Overview on practices in thalassemia intermedia management aiming for lowering complication rates across a region of endemicity: the OPTIMAL CARE study. Blood 115 (10):1886-1892