Original Article

Changes of Bone Density in Pediatric Patients with β-thalassemia Major after Allogenic Hematopoietic Stem Cell Transplantation

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Abstract

Background: Thalassemia major and its treatment by stem cell transplantation can have deleterious effects on bone integrity. This study assesses the adverse effects of transplantation on growing bones of pediatric thalassemic patients.

Methods: Bone mineral density (BMD) of 20 patients from three thalassemia classes whose mean (SD) age was 7.4 (3.8) years were tested with a Norland XR-46 device at baseline (before transplantation), 6 and 12 months after transplantation.

Results: At 6 and 12 months after transplantation we observed no significant changes in mean BMD. There were no Z-scores less than -2 among patients. Class 3 thalassemia did not negatively impact BMD. Calcium (Ca), phosphorous (P) and ferritin levels were not significantly related to patients' BMD scores. Transfusion duration and chelation therapy showed positive significant relationships to BMD (g/cm^2), but no significant relation with the BMD Z-score. The deleterious relation between corticosteroid use and changes in BMD was not significant. In contrast, patients who developed acute graft versus host disease (aGVHD) after transplantation showed significant adverse effects on BMD of their femur ($P = 0.020$) and spine ($P = 0.027$).

Conclusion: Stem cell transplantation in pediatric thalassemic patients who do not develop aGVHD does not appear to have any significant positive or negative effects on BMD.

Keywords: Acute graft versus host disease, β-thalassemia major, bone mineral density, hematopoietic stem cell transplantation

Cite the article as: Hamidieh AA, Hamidi Z, Nedaeifard L, Heshmat R, Alimoghaddam K, Larijani B, Ghavamzadeh A, Mohajeri-Tehrani MR. Changes of Bone Density in Pediatric Patients with β-thalassemia Major after Allogenic Hematopoietic Stem Cell Transplantation. *Arch Iran Med*. 2013; **16(2):** 88 – 92.

Introduction

In β-thalassemia major, red blood cell hemolysis accompanied by ineffective erythropoiesis leads to extreme bone marrow hyperplasia, extramedullary hematopoiesis, growth retardation, jaundice, pallor, skeletal abnormalities and progressive hepatosplenomegaly which develop in early childhood and necessitate life-long blood transfusions.[1]

Patients with thalassemia also show a variety of bone disorders that include bone deformities, osteopenia and osteoporosis, growth failure, and spinal deformities.[2] Osteoporosis characterized by low bone mass remains a common problem even in well-treated patients and can cause pathologic fractures and further complications in the course of the disease.[3]

The goal of long-term hypertransfusional support in thalassemia major patients is to treat the anemia caused by the combination of red cell destruction and ineffective erythropoiesis, thus improving the patient's sense of well-being while simultaneously suppressing enhanced endogenous erythropoiesis. This strategy not only treats the anemia, but also suppresses endogenous erythropoiesis so that the extramedullary hematopoiesis and skeletal changes may be suppressed Although improvements in hypertransfusion therapy have enhanced the prognosis of thalassemia major, hematopoietic stem cell transplantation (HSCT) remains the only definitive cure for affected patients.[4,5]

A classification system introduced by Lucarelli is used for patients who are potential HSCT candidates. This classification assesses risk factors predictive of outcome and prognosis.[6] Transplant recipients are at increased risk of accelerated bone loss and osteoporosis. Multiple factors including conditioning regimens, cyclosporine and high corticosteroid use for graft versus host disease (GVHD), gonadal failure, vitamin D deficiency prolonged immobility, decreased osteoprogenitor cells and secondary hyperparathyroidism may contribute to bone loss.[7] Some of these risk factors exist prior to transplantation.[8]

Contradicting reports exist regarding the positive or negative effects of HSCT on bone density in thalassemic patients. Klopfenstein et al.,[9] reported the results of a study by Petryk et al. that found the incidence of pre-existing osteopenia to be 18% which increased to 33% one year after transplantation. They reported also, a 16% incidence of osteoporosis, which increased to 18% one year after transplantation.[9] Other investigators have indicated that there may be positive changes in bone mineral density (BMD) after transplantation.[10]

Second to Italy, Iran has the next largest population of transplanted thalassemic patients.[11] There is insufficient information about bone status of thalassemic patients before and after transplantation. This study attempts to assess the changes of bone den-

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Accepted for publication: 12 November 2012

sity in transplanted pediatric thalassemic patients with the goal of monitoring and improving their quality of life.

Patients and Methods

Participants

This study included 26 patients (age <15 years) diagnosed with β-thalassemia major who were candidates for HSCT. Desferal, an iron-chelating agent, was used by all patients. Of 26 patients enrolled, only 20 completed all three phases of the study. During the study, two patients died and four (one from abroad) withdrew because they resided a long distance from the study location. All patients received the standard monitoring program recommended for patients after HSCT.

All patients were pre-pubertal and they remained pre-pubertal one year after HSCT. There were no cases with diabetes or thyroid disorders in this study.

The study was approved by the local Institutional Review Board. Patients and their parents provided signed informed consent as part of the inclusion criteria. The female to male ratio was 1:1. This study contained patients from all three classes of thalassemia according to the Lucarelli classification (Table 1). In all donor-recipient pairs, histocompatibility was determined by serology or low resolution molecular typing for HLA-A and B antigens and by allelic typing for-DRB1. The stem cell source cell was bone marrow in 8 patients and peripheral blood stem cells in 12 patients.

Patient characteristics at HSCT

This study recruited all β-thalassemia patients who were transplanted in the Pediatric Unit of the Hematology, Oncology and Stem Cell Transplantation Research Center at Shariati Hospital, Tehran, Iran. The medical history and details regarding disease control were obtained from chart reviews. Our center's policy is to transplant all transfusion-dependent β-thalassemia major patients who have HLA-identical related donors, regardless of their disease severity. Class 1 and 2 patients were prepared for transplantation with a combination of busulfan 3.5 mg/kg PO daily in divided doses for four (-8 to -5) days, cyclophosphamide 50 mg/kg once daily IV for four (-4 to -1) days and horse antithymocyte globulin 5 mg/kg daily IV for two (-2 to -1) days. HSCT conditioning for class 3 patients included busulphan 3.5 mg/kg PO in daily divided doses for four (-8 to -5) days and cyclophosphamide 40 mg/kg once daily IV for four (-4 to -1) days. No one in the study received total body irradiation therapy. Prophylaxis for GVHD included cyclosporine (1.5 mg/kg/IV from day -2 until day +7 and 3 mg/kg/IV from day +7) and methotrexate (10 mg/m² on day +1 and 6 mg/m² on days +3 and +6). All 20 patients in the study who completed all three study phases did not need any transfusion for at least one year after HSCT. After hospital discharge, we followed patients in our post-HSCT clinic weekly during the first month, then every two weeks until day +100 and thereafter according to the condition of each patient. The follow up schedule was continued until one year. Anthropometric factors such as age, sex, weight, and height were also recorded.

Treatment related adverse events were graded (NCI, 1998), using the National Cancer Institute (NCI) Common Toxicity Criteria (CTC). Acute GVHD (aGVHD) and chronic GVHD (cGVHD) were graded according to standard criteria.[12,13]

BMD measurement

DXA (Norland, XR-46) was used to measure BMD at the lumbar spine (L2-L4; anteroposterior view) and femoral neck. All BMD measurements were performed by one machine in the Special Medical Center (SMC), the Medical Branch of the Charity Foundation for Special Diseases. The CV of the machine was generally 1% in the spine and 1.5% in the femur area. Results were analyzed by pediatric specific software. DXA (Norland, XR-46) reports the results of pediatric BMD with Z-score and matched by age and sex. The reference data used in the current study was obtained from the American population. Patients were scanned by the DXA method at baseline before HSCT, at 6 months (mean 181 days after transplantation), and 12 months (mean 356 days) after HSCT. For the definition of osteoporosis and osteopenia, we used the criteria adopted by the World Health Organization which was mostly applicable to adults.[10] We used the term "low bone mineral density for age" as described by the 2007 ISCD Pediatric Position Development Conference for a child with a Z-score below -2.0.[14]

Statistical analysis

Statistical analysis was performed using SPSS version 12. Absolute BMD value changes were tested by ANOVA among results of three separate BMD measurements (before HSCT and at 6 and 12 months after HSCT). The correlations among various parameters and BMD results of the lumbar spine or hip were analyzed using Pearson's coefficients. All comparisons were made two-tailed, and statistical significance was set at 5%.

Table 1. Baseline characteristics of participants.

Parameters	Mean ± SD
Age (years)	7.4 ± 3.8 (range: 3–14)
Male/Female	10/10
Thalassemia class	
1	6
2	12
3	2
Range of Z-score of the femur	-0.73–0.94
Range of Z-score of the spine	-1.28–0.01
Serum Ca (mg/dL)	9.1 ± 0.34
Serum P (mg/dL)	5.1 ± 0.9
Serum alk-ph (u/L)	392.1 ± 134.5
Ferritin (ng/mL)	1347.8 ± 648.5
Transfusion duration (months)	69.7 ± 49.7
Chelating therapy duration (months)	46.7 ± 38.6

C = calcium; P = phosphorous; Alk-ph = alkaline phosphatase.

Table 2. Characteristics of HSCT in patients.

Patient number	Use of corticosteroids	Corticosteroids (days taken)	Cyclosporine (days taken)	Chronic GVHD	Acute GVHD grades
1	Yes	90	365	No	1
2	No	0	239	No	0
3	Yes	112	259	No	2
4	No	0	250	No	0
5	No	0	284	No	0
6	No	0	294	No	0
7	Yes	88	200	No	1
8	Yes	96	227	No	2
9	Yes	180	365	No	1
10	Yes	95	180	Yes	4
11	No	0	237	No	0
12	Yes	120	210	No	2
13	Yes	90	365	No	3
14	Yes	200	270	Yes	3
15	Yes	330	365	Yes	4
16	No	0	227	No	0
17	Yes	270	365	No	2
18	Yes	40	230	No	2
19	Yes	330	330	Yes	2
20	Yes	90	210	No	1

HSCT = hematopoietic stem cell transplantation; GVHD = graft versus host disease.

Table 3. Results of BMD study in patients.

BMD	Before HSCT (mean ± SD)	6 months after HSCT (mean ± SD)	12 months after HSCT (mean ± SD)	*P*-values of ANOVA test
Femoral region (g/cm^2)	0.599 ± 0.116	0.607 ± 0.119	0.616 ± 0.111	0.902
Spinal region (g/cm^2)	0.461 ± 0.086	0.479 ± 0.080	0.501 ± 0.093	0.346

BMD = bone mineral density; HSCT = hematopoietic stem cell transplantation.

Table 4. Results of Z-scores in patients.

Z-score	Before HSCT (mean ± SD)	6 months after HSCT (mean ± SD)	12 months after HSCT (mean ± SD)	*P*-values of ANOVA test
Femoral region	-0.2432 ± 0.4819	-0.3940 ± 0.7894	-0.4365 ± 0.7019	0.646
Spinal region	-0.5438 ± 0.3687	-0.5390 ± 0.4700	-0.4990 ± 0.4666	0.941

HSCT = hematopoietic stem cell transplantation

Results

The mean (SD) age of patients was 7.4 (3.8) years. Only 14 out of 20 had a history of corticosteroid therapy after HSCT. The mean (SD) duration of corticosteroid use was 152 (95) days and the mean (SD) duration of cyclosporine use was 273 (63.7) days. At the beginning of the study no participants had Z-scores below -2.0, thus there was no child with "low bone mineral density for age" as described by the 2007 ISCD Pediatric Position Development Conference and no one reached this low density level as the study progressed. Characteristics of patients who underwent HSCT are shown in Table 2. The results of pediatric BMD scores are adjusted for age and sex and reported as Z-scores. We calculated Z-scores of height and weight of patients and compared the mean of Z-scores of these parameters with mean of BMD of patients' femur and spine (g/cm^2) scores. We used the paired *t*-test for these comparisons. There was no significant difference among means of Z-scores of height, weight and BMD in patients.

The means of calcium (Ca), phosphorous (P) and alkaline phosphase (Alk-ph) in patients before transplantation were normal. BMD (g/cm^2) and Z-score were not significantly related with Ca, P, Alk-ph, and ferritin levels before transplantation. Ferritin level after transplantation had no significant relation with BMD. Transfusion duration and chelation therapy had positive significant relations with BMD (g/cm^2), but no significant relation with BMD Z-scores was noted.

Class 3 thalassemia had no negative effects on BMD and Z-score results before and after HSCT. Corticosteroid and cyclosporine use after transplantation and duration of use had no significant effect on BMD and Z-score of patients in different phases of the study. There were 14 (70%) who developed the following aGVHD grades: grade I (4); grade II (6); grade III (2), and grade IV (2). There were 4 (20%) who experienced limited cGVHD. cGVHD did not have any considerable side effect on the BMD femur results after 6 (P = 0.227) and 12 (P = 0.754) months, neither was there a considerable effect on spine BMD results after 6 (P = 0.197) and 12 months (P = 0.523). In contrast, aGVHD showed a significant adverse effect on BMD of the femur (P = 0.020) and spine (P = 0.027) after 12 months. BMD and Z-score results for the femoral and spinal regions at 6 and 12 months after HSCT showed no significant changes after HSCT (Tables 3 and 4).

Discussion

The results of our study showed no significant effect of HSCT on BMD in pediatric thalassemia patients. The use of corticosteroids and class of thalassemia also had no significant negative effect on BMD. aGvHD was an effective parameter on the BMD of transplanted children at one year following transplantation.

β-thalassemia major is a hemoglobin disorder with devastating adverse effects and high risk of death if untreated. Patients require lifelong blood transfusions and extensive use of chelating agents.[1] Even those with regular state of the art treatments generally experience numerous complications such as growth retardation, skeletal abnormalities, low bone mass and osteoporosis.[2] HSCT has opened a new window of hope to induce cure and provide disease-free survival for these patients. However, HSCT has risks and complications for treated patients.[5] One complication is low bone density which is a common problem among all patients, irrespective of disease type, who are treated by HSCT.[7] Low bone mass and osteoporosis can cause pathologic fracture which complicates the course of the disease and results in significant morbidity and mortality. DXA is the gold standard of bone mineral densitometry. Although there are other available methods for this purpose, the DXA still remains the best technique for determining low bone mass in both adults and children.[15] We have observed no significant differences among the means of Z-scores of height, weight and BMD in patients. Possibly, patients' BMD is unaffected by short stature that is common in thalassemic patients.

At present, HSCT is the only curative treatment for β-thalassemia major, and many treated patients achieve a disease-free life after HSCT.[5] We hope that following curative treatment with HSCT, its complications such as low bone mass will improve. However, among our pediatric patients there was no significant worsening or improvement in the BMD after transplantation. In addition, corticosteroid use in our patients did not significantly affect BMD. Since Class III thalassemia patients had more debilitating disease compared to the other classes and generally have poorer prognosis after HSCT, we anticipated lower BMD in this group both before and after transplantation. However, despite our expectation, the class III patients in our study group had significantly better BMD before and after transplantation. One explanation for these unexpected results could have been the low sample size. Similarly, the low sample size of our study might have been the reason that cyclosporine and corticosteroids had no significant effect on BMD. Investigators such as D'Souza et al.[7] and Schulte et al.[16] have reported a significant lowering effect of corticosteroids on BMD of transplanted patients. However, their studies did not specifically address pediatric patients with thalassemia. On the other hand, in a study conducted by Daniels et al.[17] no statistically significant correlation between glucocorticoid exposure and BMD in transplanted children was found. In some post-transplant studies, the non-significant effect of concomitant corticosteroid and cyclosporine use on BMD has been explained by the fact that cyclosporine exposure has demonstrated high-turnover bone loss in animal studies.[18,19] A similar effect has been suggested but not yet proven to occur in humans.[20,21] It has been proposed that co-administration of cyclosporine and glucocorticoids may offset the individual effect of each agent on bone turnover. Thus, in the initial post-transplant period when doses of glucocorticoids are high, the steroid effect may predominate, leading to a low bone turnover state. Later, the lower dosing of maintenance glucocorticoid may "unmask" the high turnover state induced by cyclosporine.[22] Such findings closely match the current study results, where the mean duration of cyclosporine use was longer than the mean duration of corticosteroid use.

There are conflicting reports regarding unfavorable effects of GVHD on BMD. In some studies, GVHD has been identified as a risk factor for low bone mass after HSCT.[16,23] On the other hand, in a report by Kaste et al., GVHD was not associated with significantly lower BMD.[24] In the current study, aGVHD was significantly related with BMD of patients at 12 months after transplantation. GVHD grade IV patients had lower BMD than other aGVHD patients in all areas. This might be attributed to the high dose of corticosteroid administration in these patients.

In our study, we found that the use and duration of corticosteroids did not affect patient BMD, while aGVHD found effective. This might be the result of a shorter duration of corticosteroid administration in HSCT compared with other types of transplantation. On the other hand, aGVHD is a major inflammatory process of which factors such as inflammation, nutrition and activity may account for BMD changes in these patients.

Although transfusion duration and chelation therapy positively affected absolute BMD, this significant effect was undermined by inability to affect the Z-score. It can mean that higher absolute BMD (g/cm^2) in patients with more transfusion and chelation therapy would be the effect of increasing age and increasing bone size in children because this effect (increasing BMD) disappears when BMD is adjusted for patient age and sex (Z-score of the spinal and femoral regions).

We found no significant differences between BMD results in all three phases of the study (before HSCT, and at 6 and 12 months after HSCT). In contrast, Schulte et al. in a long-term follow-up of transplant recipients have reported that the lowest BMD of the femoral neck was observed 24 months after transplantation.[16] In retrospect, if this study had a longer follow-up period we might have also unveiled significant changes. Of note, at the beginning of this study, our patients were not very osteopenic and no case of "low bone mineral density for age" child was found. We believe that the younger age of our patients and therefore shorter duration of thalassemia prior to transplantation played a positive role on BMD. Generally, the mean age of participants in studies that reported a significant rate of low BMD were higher than this study.[2,10,25–27] Vogiatzi et al. found that adolescence was a critical period for the occurrence of low bone mass in thalassemia and suggested that bone turnover played a central role in this process.[27] This could explain why the bone mass of our patients who had not yet reached adolescence were unaffected by their disease (thalassemia), nor by HSCT treatment. The favorable BMD status of our patients might be the result of successful progress towards the implementation of an active 'health network service' that was formed in response to a large population of thalassemic patients in Iran.

In summary, the results of this study showed that HSCT did not have a negative effect on bone density in our pediatric patients with thalassemia. Cure of the disease by HSCT also had no significant favorable effect on BMD. To clarify the contradictory findings between our results and those of other investigators, we have suggested a study with larger sample size and a longer follow-up period after transplantation. Comparison of the results with healthy control groups and non-transplanted thalassemic patients would be a meaningful attempt in understanding the changes in

BMD in the course of this disease. Matching a patient's BMD changes with their pubertal status, the results of their gonadal and hormonal assessments, and bone marker changes can make the pathophysiology of this disease more clear. We hope that future studies will be helpful to provide a better quality of life for all patients with thalassemia.

Acknowledgments

The authors express their appreciation to the Special Medical Center of the Charity Foundation for Special Disease of Iran, in addition to Dr. F. Mohseni and Mrs. A. Oojaghi for their valuable assistance in this study. We extend our gratitude to Dr. Amir Hamdi and Mrs. Nooshin Naemi for their helping in editing the manuscript.

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