

# Comparison of Bone Mineral Density Changes in Pediatric Thalassaemic Patients With and Without Hematopoietic Stem Cell Transplant

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## Abstract

**Objectives:** Beta thalassemia major is a genetic hemoglobin disorder that affects bone density. The disease leads to deteriorating bone structure but can be treated with hematopoietic stem cell transplant. We aimed to assess bone mineral density changes in pediatric beta thalassemia major patients who had undergone a hematopoietic stem cell transplant compared with similarly affected patients who had not undergone a hematopoietic stem cell transplant.

**Materials and Methods:** Forty beta thalassemia major patients, 20 transplant and 20 nontransplant, younger than 16 years of age were enrolled. The mean age of transplant patients was 8.15 years and nontransplant patients was 9.5 years ( $P = .242$ ). The female:male ratio was 1:1 in both groups. None of the patients reached puberty during this study. Bone mineral density was evaluated in transplant patients before and 1 year after hematopoietic stem cell transplant. Bone mineral density of nontransplant patients also was evaluated 1 year after their initial bone mineral density test. A Norland XR-46 densitometer was used to make all bone mineral

density measurements. None of the patients had a z score  $< -2$ .

**Results:** Mean bone mineral density changes in the femur and spine during this study were  $0.008 \pm 0.075$  g/cm<sup>2</sup> and  $0.048 \pm 0.045$  g/cm<sup>2</sup> in transplant patients and  $0.045 \pm 0.072$  g/cm<sup>2</sup> and  $0.036 \pm 0.058$  g/cm<sup>2</sup> in nontransplant patients. No significant differences between bone mineral density changes in transplant and nontransplant patients were detected during the study.

**Conclusions:** No significant effects on bone mineral density were detected in hematopoietic stem cell transplant pediatric beta thalassemia major patients compared with similarly affected nontransplant patients. Studies of longer duration may be required to identify significant changes in bone mineral density in hematopoietic stem cell transplant patients.

**Key words:** Bone marrow transplantation, Children, Skeletal changes, Osteoporosis risk factors

## Introduction

Beta thalassemia is a hereditary red blood cell disorder caused by reduced or absent synthesis of hemoglobin beta globin chains. When synthesis is absent in 2 beta globin chain genes, beta thalassemia major occurs. Affected patients develop anemia, impaired growth, and skeletal anomalies during infancy.<sup>1</sup>

Ineffective erythropoiesis, bone expansion, and extramedullary hematopoiesis are accompanied by different types of bone disorders including bone pain, bone and spinal deformities, bone age delay, short stature, and low bone mineral density (BMD).<sup>2</sup> The common finding of low BMD has been reported, based on different bone densitometry methods,<sup>3</sup> some

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**Acknowledgements:** The authors declare that they have no sources of funding for this study, and they have no conflicts of interest to declare. The authors thank the Special Medical Center of Charity Foundation for Special Disease of Iran, and Mrs. Asieh Oojaghi and Miss Fatemeh Ghorbanali for their valuable assistance in this study. We extend our gratitude to Miss Hamideh Hesabgar, Dr. Sheema Rezaian, and Mr. Mohammad Rava for their help in editing the manuscript.

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*Experimental and Clinical Transplantation* (2015) 2: 173-178

of which are not generally in good agreement.<sup>4</sup> Several factors are addressed in the reduction of bone mass in thalassemic patients, including endocrinopathies, growth delay,<sup>5-6</sup> and liver disease.<sup>7</sup> On a molecular basis, an increase in the ratio of sRANKL/OPG is reported in thalassemic patients,<sup>8</sup> indicating enhanced osteoclastic bone resorption and bone loss.<sup>9</sup> A negative correlation has been found between 17-beta oestradiol in female thalassemics and free testosterone in male thalassemics and the sRANKL/OPG ratio indicates the role of the RANKL/OPG system as a modulator for sex steroid actions on bone remodeling.<sup>10</sup> It appears that vitamin D deficiency is another contributing factor to low bone mass associated with thalassemia; however, the prevalence of this problem as reported in different studies varies.<sup>5</sup> Gradual liver iron overloading (and deficiency in liver hydroxylation of vitamin D) and a defect in vitamin D absorption in older thalassemic patients, have been reported as disease mechanisms.<sup>6</sup>

As suggested previously, thalassemic patients need frequent blood transfusions during their life.<sup>1</sup> This regimen causes progressive iron overload. Osteoid maturation and mineralization, are interfered by iron. Iron binds into calcium hydroxyapatite crystals; thus, growth of hydroxyapatite crystals is affected and osteoid tissue increases in bone tissue as a result.<sup>8</sup> In addition, hemochromatosis-induced iron overload, is commonly associated with diabetes, hypogonadism, hypothyroidism, and hypoparathyroidism.<sup>11</sup> These problems also are risk factors for low BMD. Even the intensive use of chelating agents used to remove the excess iron<sup>12</sup> may cause growth failure<sup>5</sup> and low BMD.

Correction of the genetic defects with hematopoietic stem cell transplant (HSCT) is the only definitive cure for thalassemia.<sup>13</sup> However, many studies report vulnerability to bone loss and osteoporosis after any type of transplant.<sup>14-16</sup>

Several factors including gonadal failure, prolonged immobility, decreased osteoprogenitor cells, conditioning regimens, vitamin D deficiency, secondary hyperparathyroidism, cyclosporine, high corticosteroid use, and graft-versus-host disease (GVHD) have been reportedly involved in bone loss and osteoporosis after bone marrow transplants.<sup>17-18</sup> However, all studies did not identify bone density deteriorating effects for all of the factors mentioned.<sup>19</sup>

Because after HSCT, the background disease with deleterious effects on bone is completely cured, the

hypothesis emerges that bone density also improves. This hypothesis is strengthened by studies that report positive effects of HSCT on BMD.<sup>20</sup> However, some studies found no positive or negative effects on BMD,<sup>19</sup> and Petryk and associates found the incidence of preexisting osteopenia and osteoporosis increased 1 year after bone marrow transplants in patients with different background diseases.<sup>21</sup>

Because Iran has one of the largest populations of major thalassemic patients (nontransplanted and transplanted),<sup>22</sup> and these patients have various bone complications, we attempted to evaluate bone density changes in major thalassemic patients after HSCT compared with similarly afflicted patients who did not undergo HSCT.

## Materials and Methods

### Subjects

The original study population from which the participants of the present study were drawn, included 26 patients (age < 16 y) diagnosed with beta thalassemia major who were candidates for HSCT. Deferoxamine, an iron-chelating agent, was used by all patients. Of the 26 enrolled, only 20 completed all phases of the study. During the study, 2 patients died and 4 (1 from abroad) withdrew because the distance from their residences to the study site was too far. All patients underwent a standard monitoring program recommended for patients after HSCT. Because 20 transplanted patients ultimately completed the study, we chose 20 nontransplanted age- and sex-matched patients to compare results. Ultimately, 40 pediatric beta thalassemia major patients (20 transplant and 20 nontransplant), < 16 years of age were enrolled in this prospective study. The female:male ratio was 1:1 in both groups. None of the patients was pubertal. This study was approved by the local institutional review board and the parents of the enrolled patients signed the consent forms. The protocol conforms to the ethical guidelines of the 1975 Helsinki Declaration.

Osteoporosis risk factors in the medical records of patients were considered study exclusion criteria. Hematopoietic stem cell transplant procedures for thalassemia major patients were performed in the pediatric unit of the Hematology, Oncology, and Stem Cell Transplantation Research Center.

All transplant patients were assessed before and after HSCT. Nontransplant patients also were

assessed initially and 1 year later. The mean time intervals for bone mineral densitometry in transplant patients and nontransplant patients were 13 and 17.8 months. We expect nontransplant patients to not have any significant changes in BMD in less than 1 year<sup>10</sup>; therefore, we think statistical significant difference between time intervals of 2 BMDs in 2 group of patients was not clinically significant. Bone marrow density in all patients was determined with a Norland XR-46 densitometer (CooperSurgical, Inc. USA). No patients had a z score < -2.

### Patient characteristics for hematopoietic stem cell transplant

Histocompatibility was determined by low-resolution molecular typing for HLA-A and -B antigens and by allelic typing for -DRB1. All hematopoietic stem cells were provided by related donors.

Patients were categorized according to the criteria of Lucarelli.<sup>23</sup> Class 1 and class 2 patients were prepared for transplant with a combination of oral busulfan 3.5 mg/kg daily, in divided doses for 4 days (-8 to -5), cyclophosphamide 50 mg/kg once daily IV for 4 days (-4 to -1) and horse antithymocyte globulin 5 mg/kg daily IV for 2 days (-2 to -1); class 3 patients were conditioned using oral busulfan 3.5 mg/kg in divided doses daily for 4 days (-8 to -5) and cyclophosphamide 40 mg/kg once daily IV for 4 days (-4 to -1). No patients in this study received total body irradiation. 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/m<sup>2</sup> on day +1 and 6 mg/m<sup>2</sup> on days +3 and +6). All 20 patients who completed 3 phases of this study were transfusion-free up to 1 year after HSCT. During the first month after hospital discharge patients were followed-up in our post-HSCT clinic weekly, then every 2 weeks to day +100, and thereafter according to the condition of each patient, until 1 year. Anthropometric factors (eg, age, sex, weight, and height) also were recorded. Acute GVHD (aGvHD) and chronic GVHD (cGvHD) were graded according to standard criteria.<sup>24-25</sup>

### Bone mineral density measurement

Bone mineral density at the lumbar spine (L2-L4; anteroposterior view) and femoral neck of patients were measured by dual-energy X-ray absorptiometry with a Norland XR-46 densitometer. All measurements were made on the same machine at Tehran Special

Medical Center, the medical branch of Charity Foundation for Special Diseases). Results were analyzed using specific pediatric software.

World Health Organization osteoporosis and osteopenia criteria are mainly applicable to adults; therefore, we used the term low bone mineral density for age according to the "2007 ISCD Pediatric Position Development Conference, criterion" for a child with a z score < -2.0.<sup>26</sup>

### Statistical analyses

Continuous parameters are reported as means, standard deviations, and ranges. Categorical parameters are reported as simple percentages. For test of normality of distribution of our parameters (because our sample size was small (20 cases)), we used normality plots with tests (Shapiro-Wilk statistic is calculated when the weighted sample size lies between 3 and 50). The independent samples *t* test was used to compare the means of the 2 groups. A bivariate correlation procedure was used to measure correlations, that is how variables or rank orders were related. Pearson's correlation coefficient was used for normally distributed parameters and Spearman's rho was used for abnormally distributed parameters. Significance was determined as  $\alpha$  (or *P* value) = .05, and statistical analyses were performed with SPSS software (SPSS: An IBM Company, version 16.0, IBM Corporation, Armonk, NY, USA).

### Results

Patient characteristics are shown in Table 1. No participants had z scores < -2.0 in the beginning of study and none reached the level of "low density for age" during the progress of this study. The mean femur and spine BMD in the beginning of study in transplant patients was  $0.623 \pm 0.107$  g/cm<sup>2</sup> and  $0.472 \pm 0.082$  g/cm<sup>2</sup>. After HSCT, mean femur and spine BMD was  $0.632 \pm 0.108$  g/cm<sup>2</sup> and  $0.521 \pm 0.078$  g/cm<sup>2</sup>. Bone mineral density of femur and spine in the beginning of study in nontransplant patients was  $0.661 \pm 0.155$  g/cm<sup>2</sup> and  $0.520 \pm 0.123$  g/cm<sup>2</sup>. In the second phase of the study, in nontransplant patients, mean femur and spine BMD was  $0.706 \pm 0.143$  g/cm<sup>2</sup> and  $0.556 \pm 0.101$  g/cm<sup>2</sup>.

The relation between corticosteroid use and BMD changes was not significant. In contrast, chronic and acute GVHD after transplant, especially stage IV,

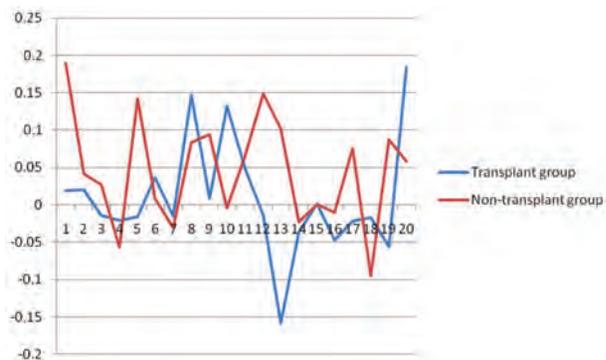
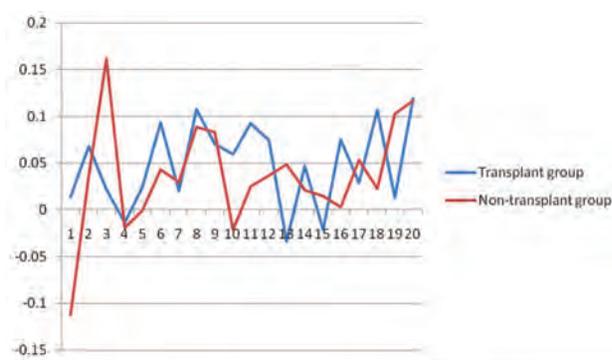
**Table 1.** Baseline Patient Characteristics \*

Parameters	Transplant candidates	Nontransplant candidates
Age (y)	8.1 ± 3.7 (3-14)	
Male/female	10/10	
BMD of femur (g/cm <sup>2</sup> )	0.623 ± 0.107 (0.450-0.810)	
BMD of spine (g/cm <sup>2</sup> )	0.472 ± 0.082 (0.350-0.699)	0.520 ± 0.123 (0.260-0.758)
Z score of femur	-0.29 ± 0.42 (-1.00-0.94)	-0.45 ± 0.61 (-1.46-0.58)
Z score of spine	-0.61 ± 0.36 (-1.28-0.04)	-0.67 ± 0.49 (-1.64-0.24)

Abbreviations: BMD, bone mineral density

showed a significant effect on BMD changes in femur ( $P = .011$  and  $.004$ ) and spine ( $P = .006$  and  $.021$ ).

During the study, mean BMD changes in the femur and spine of transplant patients was  $0.008 \pm 0.075$  g/cm<sup>2</sup> and  $0.048 \pm 0.045$  g/cm<sup>2</sup>. Mean BMD changes in the femur and spine in nontransplant patients were  $0.045 \pm 0.072$  g/cm<sup>2</sup> and  $0.036 \pm 0.058$  g/cm<sup>2</sup>. There were no significant differences in BMD changes between nontransplant and transplant patients for 1 year. Figures 1 and 2 show the patient results.

**Figure 1.** Changes in Bone Mineral Density of the Femurs**Figure 2.** Changes in Spinal Bone Mineral Density

## Discussion

Beta thalassemia major is an inherited hemoglobin disorder for which HSCT has opened a new window of hope for a cure. When transplant is not a choice

(especially in patients without a compatible donor), patients must be treated by regular monthly blood transfusions and chelation therapy. Both HSCT and hypertransfusion may lead to bone problems; thus, further research is warranted to compare the effects of treatments on bone density. To the best of our knowledge, to date there has been no other study focused on this area, particularly in children. Although we did not find any significant differences between BMD in transplanted and nontransplanted patients, these results are not surprising because we found no significant relation between corticosteroid use and BMD changes in HSCT cases.

These findings are in contrast with those of many investigators such as D'Souza and associates<sup>17</sup> and Schulte and associates,<sup>27</sup> who reported a significant lowering effect of corticosteroids on the BMD of transplant patients. Their studies did not specifically concern pediatric thalassemic patients. Studies such as the one by Daniels and associates<sup>28</sup> did not find a statistically significant correlation between corticosteroid use and BMD in children that received transplants. The nonsignificant effect of concomitant glucocorticoid and cyclosporine use on BMD in some animal studies can be explained by the interference of glucocorticoids and cyclosporine suppresses the individual effect of each agent on bone turnover. Thus, in the first posttransplant phase, when doses of corticosteroid are high, the steroid effect may predominate, leading to a low bone turnover state. Later, lowering the dosage of maintenance corticosteroids may "unmask" the high turnover state induced by cyclosporine.<sup>19</sup>

We also identified the deleterious effect of GVHD on BMD changes in HSCT patients. In some studies, GVHD was identified as a risk factor for low BMD after HSCT<sup>27,29</sup>; however, in another study, GVHD was not associated with a significantly lower BMD.<sup>30</sup> In our study, chronic GVHD showed a significant relation to bone changes, and GVHD grade IV patients after 1 year had a lower BMD than did all the other acute GVHD patients in all regions.

It may appear conflicting that we did not find a negative effect for corticosteroid use and its duration on BMD changes, while GVHD (associated with high dose corticosteroids use) had negative effects. However, it must not be forgotten that GVHD is a major inflammatory process in which other factors such as inflammation, activity, and nutrition also may affect BMD in these patients. Also, the low prevalence of these complications (4 cases of chronic GVHD and 2 cases of stage IV acute graft-versus-host disease) may have neutralized the adverse effects on mean BMD in the transplant patient group.

If the duration of our study was longer, we might have observed more significant changes, as Schulte and associates showed in their long-term study. They observed patients for 24 months after bone marrow transplants and found that femoral neck BMD was decreased.<sup>27</sup>

At the beginning of this study, our patients did not have severe osteopenia and no cases of "low bone mineral density for age" were identified, which could be due to the low mean age of our participants compared with other studies. Generally the mean age of participants in other studies was higher and the majority of patients had low BMD.<sup>11,20,31,33</sup> Vogiatzi and associates found that adolescence is a critical period for the augmentation of loss of bone mass in thalassemia and suggested that bone turnover plays an important role in this process.<sup>33</sup> These findings may better explain why our patients, who were all < 16 years of age, showed no significant changes in BMD. In our previous study of transplant patients we found that patients with a mean age of 7.4 years did not have z scores < -2 initially and 12 months after HSCT.<sup>34</sup> The positive bone mass results of our patients may be due to the developing health network that has formed resulting from the large population of thalassemic patients in our country. However, with a larger sample size, we could identify more significant BMD changes in patients in different phases of disease.

In summary, we found no negative or positive effects of HSCT on bone density in transplant pediatric beta thalassemic patients with to nontransplant patients. We believe that using healthy controls and the results of patients' gonadal, hormonal, and bone marker changes, can aid in identifying the pathogenesis of the disease.

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