Effect of cyclic pamidronate administration on osteoporosis in children with β-thalassemia major: A single-center study

Running Title: Pamidronate in β-thalassemia major children

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Abstract

Background: Osteopenia and osteoporosis represent a prominent cause of morbidity in children with thalassemia. Multiple factors are responsible for the pathogenesis of bone loss in thalassemia, including diabetes, hypothyroidism, parathyroid gland dysfunction, accelerated hemopoiesis, direct iron toxicity of osteoblasts, iron chelators, and deficiencies of growth hormone or insulin growth factors.

Purpose: To assess the effect of pamidronate administration on β-thalassemia major–induced osteoporosis in children.

Methods: This study assessed the effects of different treatments (calcium and vitamin D versus calcium, vitamin D, and pamidronate) on patients with β-thalassemia major and osteoporosis. Bone mineral density (BMD) and Z-scores were measured at baseline and after 1 year of treatment using dual-energy X-ray absorptiometry.

Results: The mean baseline BMD values of the lumbar spine were $0.71 \pm 0.07$ (g/cm²) and $0.74 \pm 0.07$ (g/cm²), respectively, while those at the end of the study were $0.81 \pm 0.07$ (g/cm²) ($P < 0.001$) and $0.78 \pm 0.07$ (g/cm²) ($P > 0.05$), respectively. The mean baseline Z-scores of the lumbar spine were $-3.53 \pm 0.55$ and $-3.17 \pm 0.61$, while those after treatment were $-2.1 \pm 0.32$ ($P = 0.001$) and $-3.11 \pm 0.67$ ($P > 0.05$), respectively. The baseline alkaline phosphatase levels were $351.5 \pm 86.07$ µg/dL and $357.6 \pm 89.7$ µg/dL, while those after treatment were $220.4 \pm 59.26.07$ µg/dL ($P < 0.001$) and $320.3 \pm 83.99$ µg/dL ($P > 0.05$), respectively.

Conclusion: Pamidronate administration effectively increased the BMD and Z-scores of children with β-thalassemia major. Pamidronate had a favorable safety profile with no related serious adverse events during the study period.
Keywords: β-thalassemia major, BMD, Bone mineral density, Osteoporosis, Pamidronate

Key message
Question: What is the effect of cyclic pamidronate administration on osteoporosis in children with β-thalassemia major?
Finding: The dual-energy X-ray absorptiometry scan findings of children with β-thalassemia major and osteoporosis were improved after pamidronate administration.
Meaning: Cyclic pamidronate effectively treated osteoporosis in children with β-thalassemia major.
Introduction

Thalassemia major is an inherited hemoglobinopathy due to defect in the ability in the synthesis of beta-globin chains [1]. Thalassemia causes a defect in bone metabolism as a chronic hematological disorder due to the disturbance in the balance between osteoblasts and osteoclasts activities. [2] Osteoporosis is a major cause of morbidity in beta-thalassemia cases. [3] The pathogenesis of osteoporosis in thalassemia is complicated and several genetic and acquired factors are implicated in the incidence of bone diseases, including hypogonadism, hypothyroidism, growth hormone (GH) and insulin growth factor-(IGF)-1 deficiency, ineffective haemopoiesis with progressive marrow expansion and direct iron toxicity on osteoblasts. [4]

Bisphosphonates reported being the major drugs in the therapeutic arsenal of osteoporosis. They have powerful anti-resorptive actions. Their diversity in action and anti-fracture efficacy may be clinically varied depending on the strength of connection and detachment to the bone tissue [5]. Bisphosphonates were effective in increasing bone mineral density (BMD) and prevention of bone fractures in patients with osteoporosis. [6] Pamidronate, a second generation of amino bisphosphonates, was used intravenously with minimal side effects for the management of osteoporosis. [7] The administration of Pamidronate in the pediatric patients with beta-thalassemia is not widely used and its effects on osteoporosis have not been properly evaluated. [8]

In this study, BMDs of children with beta-thalassemia major were reported. Changes in BMDs due to administration of calcium/vitamin D alone or in combination with Pamidronate during a one-year follow-up were also reported.

Methods
Study population:

This study was conducted on 68 Egyptian patients with β-thalassemia major disease during March 2018 to April 2019. Written consent was obtained from each participant and the study was approved by the medical ethics committee of Menoufia University Hospital, Egypt.

A full history was taken from all patients regarding demographic data and previous medical history including age, gender, height, weight, duration and frequency of blood transfusion. In addition, all participants were questioned about any symptoms like bone pain and life quality changes during clinical examinations every two months. Selected patients were under regular blood transfusion once about every 3 to 4 weeks and all were on chelation therapy by standard protocol. Patients were divided into two groups according to treatment protocols:

**Group 1:** patients regularly received a three-hour intravenous (IV) infusion of Pamidronate in a dose of 15 mg/dose, every 3 months for 1 year [9] and also received calcium in dose of 40 mg/kg and vitamin D in dose of 2000 IU of vitamin D3 daily for 8 weeks supplementary treatment.

**Group 2:** patients received calcium and vitamin D supplementary treatment only.

During the entire period of the study, patients were regularly checked by physical examination and also by hematological (CBC) and biochemistry (Alkaline phosphatase, ferritin and serum iron) laboratory analysis. For evaluation of treatment efficacy, BMD and Z-score of patients’ lumber region were measured at the beginning and at the end of the study. The Z-score is the number of standard deviations above or below the average for age- and sex-matched control subjects where control group consisted of
healthy children; non-hospitalized with no pathological findings had been recorded in their physical examinations and being within the same range of age recruited from outpatient general pediatric clinic after being treated for simple infections. They did not receive Vitamin D and Calcium.

Inclusion and exclusion criteria

Inclusion criteria for the present study were having B-thalassemia major, BMD < -2.5, serum ferritin level > 1000 mg/dl, more than 10 sessions of blood transfusion or having received more than 100 cc/kg of blood up to the study time. Selected patients were under regular blood transfusion once about every 3 to 4 weeks and all were on chelation therapy by standard protocol. Exclusion criteria were a history of bone diseases, leukemia or other neoplastic disorders, gastrointestinal disorders or inflammatory conditions during the study.

Bone mineral density:

BMD evaluation was done using dual energy X-ray absorptiometry (DXA) (Norland–XR-46, USA, version 3.9.6/2.3.1) at lumbar spines (LS) (L1–L4). The BMD results were converted to age- and gender-specific Z-scores based on the normative reference data for BMD in Egyptian children. Patients with BMD of less than -2.5, at the beginning of study, were considered osteoporotic.

Growth and puberty

11 children of group I were in prepubertal stage (stage 1), 23 children were in pubertal stage where (10 children in stage 2 and 13 children in stage 3). 10 children of group 2
were in prepubertal stage (stage 1), 24 children were in pubertal stage where (11 children in stage 2 and 13 children in stage 3) according to Tanner staging.

Follow-up

Each patient was examined every 2 months including clinical examination in regard to their weight; height and pubertal staging according to Tanner. They were measured serially and biochemical measurements were performed at every visit and X-ray. ECHO was done every 4 months for cardiac assessment and also abdominal sonography done every 4 months for liver assessment.

Biochemistry

Serum total calcium, phosphate, creatinine and alkaline phosphatase activity were measured using colorimetric methods.

Bone densitometry

BMD evaluation was done using dual energy X-ray absorptiometry (DEXA) at lumbar spines (LS) (L1–L4) after one year from treatment.

Radiographic evaluation

For quantitative evaluation of osteoporotic lesions, we compared x-rays on lumbar spines that were obtained at the start of treatment to those from the last follow-up visit. Radiographs were screened for lesions that were sufficiently well delimited to allow for measurement of lesion size.

Statistical analyses

Data was collected during the entire period of the study and analyzed by SPSS statistical software. Continuous variables were presented as mean ± SD, while for categorical
variables; numbers (%) were used. Chi-square ($\chi^2$) test was used for comparison of the categorical variables. Student’s t and ANOVA tests were used to compare continuous parametric variables in two and more than two groups, respectively. Statistical significance was based on two-sided design-based tests evaluated at the 0.05 level of significance.

**Results**

Thirty-four $\beta$-thalassemia patients on monthly Pamidronate plus standard treatments (Group 1), thirty-four patients on standard treatments only (Group 2), and thirty-four cases as controls were recruited. Table 1 summarizes their demographic characteristics and controls. Demographic characteristics included age, sex, body weight, height, and body mass index. There were highly statistical significance ($P<0.001$) as regards weight, statistical significance ($P<0.01$) as regards height and no statistical significance as regards age, sex, and BMI.

In all patients, the values of Hb, serum calcium, serum phosphorous, ALP, serum 1,25 hydroxy cholecalciferol, ferritin, iron, and TIBC were clearly lower than those of the controls ($P<0.001$) (Table 1). The above parameters did not display any significant differences between the two groups. The mean baseline BMD of the lumbar spine was $0.71\pm0.07$ (gr/cm$^2$). After one year of treatment with Pamidronate, it reached $0.81/0\pm0.07$ (gr/cm$^2$) in Group 1 ($P<0.001$).The baseline mean of the Z-score for the lumbar region was $-3.53\pm0.55$. At the end of the study period it reached $-2.1\pm0.32$ ($P<0.001$) in Group 1 (Table 2). The baseline alkaline phosphatase was $351.5\pm68.07$ $\mu$g/dl. After treatment, this value decreased to $220.4\pm59.26\mu$g/dL ($P<0.001$) in Group 1 (Table 2).
The mean baseline BMD of lumbar spine in Group 2 was 0.74±0.07 (gr/cm²). After one year of treatment with standard treatment it became 0.78±0.07 (gr/cm²) (P>0.05). The baseline mean of the Z score for the lumbar region in Group 2 was -3.17±0.61. At the end of the study period it became -3.11±0.6 (P>0.05). The baseline alkaline phosphatase in Group 2 was 357.6±89.7 µg/dl. After treatment, this value decreased to 320.3±83.99 µg/dl (P>0.05) in Group 2.

Table (3) illustrates the changes in BMD and Z-scores at the lumbar spine in two patients groups. The BMD and Z-scores significantly improved in patients with standard plus Pamidronate treatment [median (interquartile range, IQR): -3.01 (−2.63 to −3.89) at baseline and −2.12 (−2.06 to −3.72) at the end of the study; P = 0.018]. On the other hand, these changes were not significant in patients with standard treatments (P = 0.593).

The BMD and Z-score at the lumbar spine were increased in both group but in patients who received the standard treatment; this increase was not significant while a patient who received standard treatment plus Pamidronate showed a highly significant increase (Table 4).
Discussion

The management of thalassemia children by optimized transfusion programs and chelating therapy has improved markedly over the past few years leading to prolongation of the life expectancy. Osteoporosis and osteopenia are a prominent cause of morbidity in children with thalassemia. [10] These changes are due to increased marrow erythropoiesis and excess iron deposition resulting in expansion of bone marrow cavities and reduced trabecular bone volume.[11] Chelation therapy, deficiency of vitamins and minerals like vitamin D and zinc and presence of endocrinopathies like hypoparathyroidism, hypothyroidism and diabetes mellitus also contributes to developing the bone diseases.[12]

In this study, we evaluated the changes in BMDs due to the administration of calcium/vitamin D alone or in combination with Pamidronate during a one-year follow-up. Few similar studies have assessed the effect of Pamidronate on the improvement of osteoporosis in children with thalassemia.

In this study, the mean weight and height of patients with thalassemia were significantly lower than controls while BMI of patients was lower though the difference was not statistically significant which is in accordance with previous studies suggesting that chronic illness and endocrinial changes due to iron overload are the main causes. [13]

Hypocalcemia and vitamin D deficiency were observed in almost all the patients of thalassemia in this study. Also, there was a significant high level of phosphorus in patients with thalassemia. This could be due to hypoparathyroidism due to iron deposition in the parathyroid gland evidenced by elevated bone alkaline phosphatase in these patients .[14] Other factors also play a role including deficient calcium intake,
IGF-I deficiency, delayed puberty and hypogonadism and decreased synthesis of 25-
OH-D, due to hepatic siderosis.[11]

This study revealed a highly significant increase in BMD and Z-score in thalassemic
children after administration of Pamidronate plus Calcium /Vitamin D while
administration of Calcium /Vitamin D alone has led to a significant increase in BMD
and Z-score in thalassemic children. Naderi et al. [15] concluded that Pamidronate was
effective in increasing the bone mineral density and improving the osteoporosis
condition in adult patients with β-thalassemia major.

Viereck et al. [16] demonstrated that pamidronate can enhance the production of
OPG (osteoprotegerin) by primary human osteoblasts, thus antagonizing the
osteoclast genetic activity of RANKL and finally increase the BMD. Voskaridou et
al. [17] indicated that pamidronate increased the BMD and diminished the markers
of osteoclast function including tartrate-resistant acid phosphatase isoform 5b
(TRACP-5b), N-telopeptides of collagen type I (NTX), OPG, and RANKL in
thalassemia major-induced osteoporosis and postmenopausal women.

In this study, measurement of serum alkaline phosphatase also revealed a highly
significant decrease after administration of Pamidronate plus Calcium /Vitamin D and
to a significant decrease administration of Calcium /Vitamin D alone that itself was an
indicator of osteoporosis improvement as mentioned by Naderi et al. [15] In
thalassemia major patients had increase in serum alkaline phosphatase as a marker of
bone resorption and seemed to account for increased osteoclastic activity, diminished
bone mineral density. Consequently, bone diseases were observed. [17]
Many factors are responsible for increasing bone turnover in patients with thalassemia, but bisphosphonates such as Pamidronate, are effective in repairing the bone mineral density plus Calcium/Vitamin D administration.[18]

Footnotes

Author contribution

Mahmoud A. El Hawy: Study design, data collection and interpretation of data.

Nagwan Y. Saleh: Statistical analysis and paper writing.

Conflict of interest: none

Funding source: none

Conflicts of interest

No potential conflict of interest relevant to this article was reported.
References


Table 1. Baseline and laboratory characteristics of the studied children

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>Group 1</th>
<th>Group 2</th>
<th>P.value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N = 34</td>
<td>N = 34</td>
<td>N = 34</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>11.12±3.94</td>
<td>12.35±3.59</td>
<td>11.94±3.64</td>
<td>0.383</td>
</tr>
<tr>
<td>Sex male n (%)</td>
<td>16(47.1)</td>
<td>18(52.9)</td>
<td>23(67.6)</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Weight (Kg)</td>
<td>29.53±7.72</td>
<td>24.35±5.53</td>
<td>23.50±5.86</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>134.56±18.91</td>
<td>125.91±11.74</td>
<td>124.24±12.02</td>
<td>0.009</td>
</tr>
<tr>
<td>BMI (Kg/m²)</td>
<td>16.04±0.51</td>
<td>15.12±0.98</td>
<td>17.91±17.39</td>
<td>0.509</td>
</tr>
<tr>
<td>Hb (gm/dl)</td>
<td>11.24±1.03</td>
<td>8.66±0.46</td>
<td>8.94±0.41</td>
<td>P1&lt;0.001</td>
</tr>
<tr>
<td>Calcium (mg/dl)</td>
<td>9.48±0.42</td>
<td>7.99±0.81</td>
<td>7.93±1.05</td>
<td>P2 &gt;0.05</td>
</tr>
<tr>
<td>Phosphorus (mg/dl)</td>
<td>4.51±0.48</td>
<td>5.38±0.66</td>
<td>5.51±0.60</td>
<td>P1&lt;0.001</td>
</tr>
<tr>
<td>Vitamin D (ng/ml)</td>
<td>35.59±6.49</td>
<td>17.53±6.68</td>
<td>15.78±6.72</td>
<td>P1&lt;0.001</td>
</tr>
<tr>
<td>Alkaline phosphatase</td>
<td>158.21±12.33</td>
<td>351.47±68.07</td>
<td>357.62±89.72</td>
<td>P1&lt;0.001</td>
</tr>
<tr>
<td>Ferritin (ng/ml)</td>
<td>58.68±20.53</td>
<td>2773.24±1219.34</td>
<td>2553.82±1787.61</td>
<td>P1&lt;0.001</td>
</tr>
<tr>
<td>Iron (mcg/dl)</td>
<td>39.35±28.46</td>
<td>184.85±63.39</td>
<td>194.12±76.54</td>
<td>P1&lt;0.001</td>
</tr>
</tbody>
</table>

P1 < 0.001, P2 > 0.05
<table>
<thead>
<tr>
<th></th>
<th>Group 1 (mcg/dl)</th>
<th>Group 2 (mg/dl)</th>
<th>Group 3 (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TIBC</td>
<td>320.29±61.17</td>
<td>149.53±77.56</td>
<td>137.24±77.38</td>
</tr>
<tr>
<td>Creatinine</td>
<td>0.54±0.18</td>
<td>0.56±0.20</td>
<td>0.66±0.24</td>
</tr>
<tr>
<td>Urea</td>
<td>30.53±6.26</td>
<td>34.09±7.46</td>
<td>34.97±7.43</td>
</tr>
</tbody>
</table>

P1 < 0.001  
P2 > 0.05  
P1 > 0.05  
P2 > 0.05  
P1 > 0.05  
P2 > 0.05

BMI, body mass index; Hb, hemoglobin; TIBC, total iron-binding capacity

Group 1 patients received pamidronate; Group 2 patients received calcium and vitamin D

P1: comparison of the three groups; P2: comparison of Groups 1 and 2

P > 0.05, non-significant; P < 0.001, highly significant
Table 2. Comparison of alkaline phosphatase level and BMD of patients in Group 1 as markers of bone remodeling before versus after treatment.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group (1)</th>
<th>Change</th>
<th>p.value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before</td>
<td>After</td>
<td></td>
</tr>
<tr>
<td>Alkaline phosphatase(IU/L)</td>
<td>351.5±68.07</td>
<td>220.4±59.26</td>
<td>131.1</td>
</tr>
<tr>
<td>BMD (g/cm²)</td>
<td>0.71±0.07</td>
<td>0.81±0.07</td>
<td>-0.10</td>
</tr>
<tr>
<td>Z-score of BMD</td>
<td>-3.53±0.55</td>
<td>-2.1±0.32</td>
<td>-1.43</td>
</tr>
</tbody>
</table>

BMD, bone mineral density

Group 1 patients received pamidronate; Group 2 patients received calcium and vitamin D

P < 0.001, highly significant
Table 3. Comparison of alkaline phosphatase level and BMD of patients in Group 2 as markers of bone remodeling before versus after treatment.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group (2)</th>
<th>Change</th>
<th>p.value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before</td>
<td>After</td>
<td></td>
</tr>
<tr>
<td>Alkaline phosphatase(IU/L)</td>
<td>357.6±89.7</td>
<td>320.3±83.99</td>
<td>83.3</td>
</tr>
<tr>
<td>BMD(g/cm²)</td>
<td>0.74±0.07</td>
<td>0.78±0.07</td>
<td>-0.04</td>
</tr>
<tr>
<td>Z-score of BMD</td>
<td>-3.17±0.61</td>
<td>-3.11±0.6</td>
<td>-0.06</td>
</tr>
</tbody>
</table>

BMD, bone mineral density

Group 1 patients received pamidronate; Group 2 patients received calcium and vitamin D

P > 0.05, non-significant
Table 4. Comparison of BMD and Z-scores at the lumbar spine before versus after treatment by group.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group (1)</th>
<th>Group (2)</th>
<th>P.value</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMD before treatment (g/cm²)</td>
<td>0.71±0.07</td>
<td>0.74±0.07</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Z-score of BMD Before</td>
<td>-3.5±0.55</td>
<td>-3.16±0.61</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>BMD After treatment (g/cm²)</td>
<td>0.81±0.07</td>
<td>0.78±0.08</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Z-score of BMD After</td>
<td>-2.1±0.3</td>
<td>-3.11±0.6</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>P.value BMD</td>
<td>&lt;0.001</td>
<td>&gt;0.05</td>
<td></td>
</tr>
<tr>
<td>P.value Z-score</td>
<td>&lt;0.001</td>
<td>&gt;0.05</td>
<td></td>
</tr>
</tbody>
</table>

BMD, bone mineral density

Group 1 patients received pamidronate; Group 2 patients received calcium and vitamin D. P > 0.05, non-significant; P < 0.001, highly significant.