1. INTRODUCTION

β-thalassemia is a hereditary disorder caused by defective globin production, resulting in abnormal as well as decreased quantity of globin chains. Low bone mineral density (BMD) is one of the most frequent and important problems in patients with thalassemia (1-3). Some factors leading to low BMD in these patients include deficiencies in vitamin C, vitamin D, and zinc along with increased osteoclast (and decreased osteoblast) activity. Additionally, augmented bone turnover leads to lower BMD in individuals with this disorder (4, 5). Thalassemia-induced osteoporosis is responsible for skeletal pain and in severe forms for pathologic fractures (6). Patients with β-thalassemia may also have spinal deformities, scoliosis, and nerve compression (7, 8). Prevalence of thalassemia-associated osteoporosis in patients with β-thalassemia is approximately 40–50% (7). Bisphosphonates such as zoledronic acid are the primary class of medication used among the different medications for thalassemia-associated osteoporosis, with varying success rates (9). Zoledronic acid is a potent inhibitor of bone resorption, and likely improves bone density through direct inhibition of osteoclasts by interfering with protein prenylation (10). Zoledronic acid has been tested with different dosing regimens (4 mg IV every 3 months or 4 mg IV every 6 months) for osteoporosis management in thalassemia.

Administration of Intravenous Zoledronic Acid Every 3 Months vs. Annually in β-thalassemia Patients with Low Bone Mineral Density: a Retrospective Comparison of Efficacy

Hadi Darvishi-Khezri1, Mehrnoush Kosaryan2, Rosseta Akbarzadeh2, Aily Aliasgharian3, Mehran Fazli5

ABSTRACT

Introduction: The benefit of annual administration of zoledronic acid in the management of thalassemia-associated osteoporosis is unknown. Aim: The aims of this study were to evaluate the efficacy of treatment with two different dosing regimens of IV zoledronic acid (annually versus every 3 months) for increasing low bone mineral density (BMD) in patients with osteoporosis associated with β-thalassemia as annually and 3-monthly on bone density in patients. Materials and Methods: This retrospective, single-center study analyzed patients’ clinical records and bone density measurements. Those enrolled in the study were 14 to 53 years of age, had documented β-thalassemia, and were treated with IV zoledronic acid on either an annual or every 3 months dosing regimen. Dual-energy X-ray absorptiometry was used to obtain the z-score for BMD in the lumbar spine and femoral neck. Results: Thirty-four patients were enrolled in the study; 15 (44.1%) had been treated annually, and 19 (55.9%) had been treated every month. In patients receiving treatment with the once-yearly dose of zoledronic acid, significant increases were observed in the lumbar spine BMD z-score, from -2.45 ± 0.69 to -1.97 ± 0.82 (P=0.02). When comparing BMD across the two treatment regimens, the mean lumbar spine BMD was 0.82 greater (95% CI 0.31, 1.33, P=0.003) and the mean femoral neck BMD 0.37 greater (95% CI -0.15, 0.87, P=0.1) in the group receiving annual zoledronic acid treatment. Conclusions: In patients with thalassemia-associated osteopenia, annual treatment with zoledronic acid increases lumbar spine bone density while being more effective, less expensive, and associated with fewer adverse events than dosing every 3 months.

Keywords: Beta thalassemia, Thalassemia major, Thalassemia intermedia, Osteoporosis, Zoledronic acid, Bone Mineral density.
patients (11). Zoledronic acid 4 mg IV every 3 months is one of the most promising protocols (8-9, 12) but evidence is lacking with respect to other potential doses (such as IV zoledronic acid administered annually) for the treatment of low BMD associated with thalassemia (13-15). Studies have shown that 5-mg IV zoledronic acid administered once annually can lead to increased BMD and reduced bone resorption and formation in women with postmenopausal osteoporosis (16). Regarding the complex etiology of thalassemia-associated osteoporosis, all bisphosphonates must be administered in higher doses (sum of dose during a year) in patients with thalassemia-associated osteoporosis than in women with post-menopausal osteoporosis to ensure similar effectiveness (17). A 3- to 5-year treatment period should be considered for management of osteoporosis with bisphosphonates (18, 19); longer treatment periods or doses >5 mg annually are not advisable considering the short-term exposure and the unknown safety (and potential side effects) of other potential regimens (11).

At our center, we have observed cases in which BMD increased with annual dosing regimens of zoledronic acid. However, the benefit of annual administration of zoledronic acid in the management of thalassemia-associated osteoporosis is unknown. The aim of this study was to compare the efficacy of annual dosing versus dosing every 3 months for zoledronic acid on the BMD of patients with β-thalassemia.

### 2. MATERIALS AND METHODS

Patients were enrolled in this retrospective study in 2016 after review of patients’ medical records at the Thalassemia Research Center (TRC), Iran. The study was approved, and consent for the use of patient records was given by the Research Ethics Committee, Mazandaran University of Medical Science (MUMS), Iran. The study group consisted of patients with low BMD who were or who had been previously being monitored in Bu-Ali Sina Hospital at some point in time between 2011 and 2016. The inclusion criteria were a) thalassemia major or intermedia; b) BMD z-score ≤ -2.5 (lumbar spine or femoral neck); c) current and previous treatment with zoledronic acid, either annually or every 3 months; and d) availability of BMD data before and after treatment. Patients who were treated with other types of bisphosphonates were not included in the study. Exclusion criteria included previous use of another bisphosphonate. At Bu-Ali Sina Hospital, BMD has been assessed using dual-energy X-ray absorptiometry (DEXA) annually in all adult patients with thalassemia. All patients used 500 to 1000 mg of calcium plus vitamin D supplements according to recommendations of treatment of vitamin D deficiency in thalassemic patients with considering their vitamin D level (20). Zoledronic acid (Zolena 4-mg ampules; Ronak Daru, Tehran, Iran) was administered as a 4-mg IV dose in 500 ml of normal saline. Zoledronic acid was administered every 3 months in patients with osteoporosis. This 4-mg dose was administered every 3 months according to current osteoporosis management guidelines (15). Patients who were unable to continue this course of treatment (often due to adverse events) were given an annual IV dose in its place. During the case enrollment process, patient files were reviewed to obtain data on the following: lumbar spine and femoral neck z-scores at annual BMD measurements, age, gender, dependent/not dependent on blood transfusions, duration of blood transfusion(s), units of transfused blood per year, received iron via blood transfusion, iron-chelating therapy data, mean serum ferritin and hemoglobin (measured during the previous five years), mean ca (mg/dL) and vitamin D (ng/ml) levels during treatment with zoledronic acid, history of splenectomy, and history of diabetes mellitus. Each PRBC (packed red blood cell) packed contains 250 mg of iron; so, the iron intake from blood transfusions in mg/year was calculated as: 250 × the number of units given during one year (21). All patients were on a steady dose of iron-chelators during treatment with zoledronic acid [desferrioxamine (20-40 mg/kg/day), deferasirox (20-40 mg/kg/day), and deferriprone (50-100 mg/kg/day)].

### 3. RESULTS

Thirty-four patients were enrolled who had used zoledronic acid; of these patients, 15 (44.1%) were on the once-yearly dosing regimen and 19 (55.9%) received quarterly (every 3 months) IV doses. Table 1 shows the basic and clinical characteristics of patients with β-thalassemia receiving treatment with zoledronic acid. No significant differences were found among the two groups in the different baseline demographic and clinical characteristics described in the materials and methods (Table 1). The results of our study show that BMD z-score of lumbar spine significantly increased from -2.45 ± 0.69 to -1.97 ± 0.82 in patients being treated with the once-annual zoledronic acid regimen (P=0.02). The femoral neck BMD z-score increased after zoledronic acid therapy as annually; however, this increase was not significant (from -1.18 ± 0.54 to -0.96 ± 0.75; P=0.2). The BMD z-score of lumbar spine in thalassemia patients treated with 3-monthly zoledronic acid was -2.52 ± 0.75, which decreased to -2.79 ± 0.63 after treatment (P=0.1). Before and after femoral neck BMD z-scores in patients who received zoledronic acid every 3 months were -1.39 ± 0.89 and -1.36 ± 0.65, respectively (P=0.8). There was no statistically significant difference between BMDs that was obtained at baseline for thalassemia patients treated with zoledronic acid every 3 months versus annually at the lumbar spine and femoral neck (P=0.9, P=0.4, respectively). Average lumbar spine BMD was 0.82 greater (95% CI 0.31, 1.33, P=0.003) and average femoral neck BMD 0.37 greater (95% CI -0.15, 0.87, P=0.1) in the group treated annually versus every 3 months with zoledronic acid.
Administration of Intravenous Zoledronic Acid Every 3 Months vs. Annually in β-thalassemia Patients with Low Bone Mineral Density

| Variables                          | Zoledronic acid | P value |
|------------------------------------|-----------------|---------|
|                                    | Annually (n=15) | 3-monthly (n=19) |
| Age (year)                         | 30.6±6.9        | 33.7±2.2 | 0.1 |
| Gender (F/M)                       | 10/5            | 12/7    | 0.5 |
| Transfusion dependent              | 3 (20)          | 4 (21.1) | 0.5 |
| Transfused blood (unit/year)       | 26.14±13.83     | 28.01±8.99 | 0.7 |
| Duration of blood transfusion (year)| 18.8±12.9      | 17.5±9.3 | 0.8 |
| Received iron via blood transfusion (mg/year) | 6535±3459 | 7000±2248 | 0.7 |
| Type of iron-chelating therapy     | DFO 2 (13.4)    | 1 (5.3)  | 0.5 |
|                                    | DFX 2 (13.4)    | 5 (26.4) |       |
|                                    | Combination therapy with DFO and DFP 1 (5.3) |         |
| Duration of treatment with DFO (year) | 4.71±5.15    | 5.44±3.32 | 0.4 a |
| Duration of treatment with DFX (year) | 2.34±1.53    | 2.65±1.19 |       |
| Duration of combination therapy with DFO and DFP (year) | 4.66±3.05 | 6.5±3.2 | 0.4 |
| Duration of iron-chelating therapy (year) | 24.1±4.3  | 16.2±9.9 | 0.1 |
| Ferritin (5-year mean; mg/dL)      | 1240±198.8      | 980±519.2 | 0.3 |
| Hb (5-year mean; gr/dl)            | 9.67±1.14       | 9.04±1.05 | 0.1 |
| Ca level (mg/dL)                   | 9.33±0.79       | 9.07±0.62 | 0.3 |
| Vit D level (ng/mL)                | 28.96±20.42     | 24.44±9.75 | 0.5 |
| Splenectomy                        | 3 (20)          | 4 (21.1) | 0.6 |
| Diabetes mellitus                  | 1 (6.7)         | -        | 0.3 |

Table 1. Demographic and clinical characteristics of patients with β-thalassemia treated with zoledronic acid as annually and 3-monthly.

Figure 1. Z-scores for lumbar spine and femoral neck BMD before and after treatment with zoledronic acid.

Thirty percent of patients experienced flu-like symptoms and bone pain after treatment. Figure 1 shows changes in BMD z-scores for lumbar spine and the proximal femoral neck before and after treatment with zoledronic acid annually versus every 3 months in patients with thalassemia.

4. DISCUSSION

The pathogenesis of thalassemia-related osteoporosis is multifactorial and dependent on genetic variants, bone marrow expansion, iron burden, iron chelators, renal involvement, and endocrine condition; therefore, osteoporosis management is difficult in these patients (11). This study showed that single-dose zoledronic acid therapy over a year (4-mg, single dose once yearly) was effective in improving lumbar spine BMD in the treatment of osteoporosis in patients with thalassemia.

Several studies have shown the positive effects of IV infusion of zoledronic acid either every 3 months or every 6 months at a dose of 4 mg (annual total exposure, 8 or 16 mg) for osteoporosis management in patients with thalassemia. However, evidence related to the annual IV administration of zoledronic acid is, to our knowledge, currently lacking. Voskaridou et al., carried out studies that assessed the efficacy of dosing every 3 months or every 6 months (22). In one of these studies, the mean % change of lumbar spine and femoral neck BMD was 5.8% and 4.8%, respectively, with dosing every 6 months, and 15.2% and 11.3%, respectively, for dosing every 3 months; these increases were not significant (P>0.05) (22). In another study, 18 patients with β-thalassemia who received 4 mg of zoledronic acid every 3 months for one year were compared to a control group (n=10) (23). All patients received 500 mg of calcium and 400 IU of vitamin D as daily supplements. This study showed statistically significant improvement in BMD for the lumbar spine, femoral neck, and trochanter/total hip. They also performed laboratory tests for levels of osteocalcin, 25(OH) vitamin D, 1,25(OH)2 vitamin D, bone alkaline phosphatase, parathyroid hormone (PTH), urine creatinine, and urinary deoxypyrlandilone at baseline and at 3-, 6-, and 9-month intervals. Apart from PTH, all other measured changes were statistically significant throughout the course of follow-up. Osteocalcin and bone alkaline phosphatase decreased, while vitamin D metabolites increased. In our study, we did not find a positive change in the lumbar spine or femoral neck BMD after treatment with zoledronic acid at a dosage of every 3 months.

In another study, Gilfillan et al., recruited 23 subjects with β-thalassemia major in Australia for a paired, matched randomization trial (5). Twelve patients received zoledronic acid 4 mg every 3 months and 11 received placebo (5); the follow-up period was 2 years. These researchers used T-score and BMD as g/cm² values in lumbar spine, femoral neck, total hip, and for the total body. Lumbar spine had 10.2 ± 2.3% BMD improvements in the first year of treatment, then remained stable in the second year. The same effect was found at all other measured areas; the best response, overall, was found during the first year of treatment (24). To summarize, in the current study, we compared the effects of 4-mg zoledronic acid administered once yearly with its administration every 3 months (accumulated dose of 16 mg) on annual BMD. Treatment with zoledronic acid as a single dose of 4 mg over a year had better outcomes with re-
spect to reversing bone loss; moreover, the cost was lower, with reduced incidence of adverse events. Perifanis et al., performed a study on 29 patients with β-thalassemia major and 20 age- and sex-matched healthy blood donors. Patients received 1 mg of zoledronic acid as an intravenous infusion every 3 months. Osteoprotegerin, N-terminal cross-linking telopeptide of type I collagen (NTX), osteocalcin (OC), and insulin-like growth factor 1 (IGF-1) were measured before and after each zoledronic acid infusion, noting no difference in baseline serum OPG between the thalassemia and the control group. Serum NTX was higher in the thalassemia group, but the difference was not statistically significant; serum OC was significantly higher in the thalassemia group, while IGF-1 values were lower (P<0.001). The effect of zoledronic acid on these parameters showed a very slight increase in serum OPG (+0.2 pmol/L = 7%), a 28% decrease in NTX (P<0.05), >70% decrease in OC, and a significant decrease in IGF-1 (P<0.05). The BMD T-score was -2.87 ± 0.71 before and -1.32 ± 0.80 after 4 zoledronic acid infusions (p<0.05). The slight increase in OPG can be attributed to the effect of osteoblasts in indirectly affecting bone resorption. The researchers concluded that zoledronic acid is the most potent agent for inhibition of bone resorption (by decrease in OC). The decrease in IGF-1 had not been reported previously (24). The major limitation of this study was the small sample size, as well as enrollment of heterogeneous group of thalassemic patients. Other potentially confounding factors related to osteoporosis could not be considered in this work. As once-yearly administration is less expensive and more convenient - along with being better tolerated - we recommend further research to see if this protocol is superior to reversing bone loss; moreover, the cost was low-

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5. CONCLUSION

Treatment with zoledronic acid as an annual infusion effectively increases lumbar spine BMD and is a more efficacious, less expensive and well-tolerated option to consider in place of treatment with IV infusion every 3 months for thalassemia-associated osteoporosis.

- **Author’s contributions:** Study conception and design: HDK, MK, RA, AA and MF. Acquisition of data: RA, AA and MF. Statistical analysis and interpretation of data: HDK. Drafting of the manuscript: HDK, MK, RA, AA and MF. Critical revision of the manuscript for important intellectual content: HDK, MK, RA, AA and MF.

- **Conflict of interest:** The authors declare no conflicts of interest.

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