Original Article *Iran J Ped Hematol Oncol. 2021, Vol 11, No 1, 18-23*

The probability of indicating Osteoprotegrin as a biomarker for osteoporosis in patient with thalassemia major

Shahin Koohmanaee MD[1], Bahram Dabandi MD[1], Adel Baghersalimi MD[1], Roghayeh Zare PhD[1], Mohammad Aghaeizadeh Zoroufi MD[1], Seyyedeh Golnaz MirMonsef MD[1], Afagh Hassanzadeh Rad PhD[1], Arian Akhavan MD[1], Kioomars Golshekan MD[1], Farzaneh Moamer MD[1], Abdolreza Medghalchi MD[2], Setila Dlili MD[1,*]

1. Pediatric Diseases Research Center, Guilan University of Medical Sciences, Rasht, Iran.
2. Eye Research Center, Guilan University of Medical Sciences, Rasht, Iran
*Corresponding author: Dr Setila Dlili, Pediatric Diseases Research Center, Guilan University of Medical Sciences, Rasht, Iran. E-mail: setiladalili1346@yahoo.com. ORCID ID: 0000-0001-9591-0821

Received: 01 April 2020 **Accepted:** 22 October 2020

Abstract

Background: Osteoporosis is one of the main causes of morbidity in patients with thalassemia major. Osteoprotegerin (OPG) is secreted by osteoblasts and osteogenic stromal stem cells and protects the skeleton from excessive bone reabsorption. In this study, the authors aimed to assess the relationship between OPG with osteoporosis and osteopenia in patients with thalassemia major.

Materials and Methods: In this analytic cross-sectional study, 37 patients aged 8-18 years, with thalassemia major were enrolled. Biochemical markers including hemoglobin, ferritin, calcium, phosphorus levels, and MRI T2* heart and liver were assessed. A bone mineral densitometry (BMD) was performed as well. Statistical analysis was performed by the independent T-test and Chi-Square test using the SPSS 20. The Multiple linear regression analysis was used to investigate the association between the BMD Z-score and OPG by the effect modification.

Results: The mean age of patients was 14.86±3.72 years. Normal bone density, osteopenia, and osteoporosis were noted in 2 (5.4%), 21 (56.8%), and 14 (37.08%) patients, respectively. The number of girls (P=0.042), mean age (P=0.045), and MRI T2* heart (P=0.033) in patients with osteopenia was significantly higher than patients with osteoporosis. The BMD Z-score was not significantly associated with OPG regarding the total number of participants, whereas in patients with osteoporosis, this association was significant (P=0.001). In all effect modified models, BMD remained statistically non-significant except for body mass index modification (P=0.046).

Conclusion: Based on the results, it seems that further complicated studies are needed to be performed on this issue.

Keywords: Beta-Thalassemia, Child, Osteoprotegerin

Introduction

Thalassemia major (TM) is an inherited disease with a reduced quantity of globin chains, hemolysis, and increased red blood cells (1). Osteoporosis in patients with TM is the main reason for morbidity. The etiopathogenesis of osteoporosis in these patients is multifactorial. It includes endocrinological conditions, iron overload, bone marrow expansion, and genetic factors. Considering the pathogenesis of osteoporosis, osteoprotegerin (OPG) and receptor activator of nuclear factor kappa B ligand (RANKL) are essential mediators (2).

During life, constant remodeling of bones occurs. For this remodeling, two main types of cells are mandatory cell types. Osteoblasts are cells that synthesize new bone tissue and are the cells used for bone-building. Osteoclasts absorb the matrix of bones and break them. There is a tight balance between these two types of cells. RANKL/RANK controls the development of osteoclasts by regulating the physiology of bone. Besides, OPG prevents osteoclasts' development (3).

The skeleton can be protected from excessive bone reabsorption by the secretion of OPG from osteoblasts and osteogenic stem cells and its binding to RANKL to inhibit the relations with RANK. Therefore, the RANKL/OPG ratio in bone marrow is thus an important

determinant of bone mass in normal and disease states. OPG inhibits bone loss by acting as a decoy receptor to RANKL and preventing it from binding to RANK (1). Despite reports that mentioned a role for the RANKL/RANK/OPG axis in determining bone loss in TM (2, 3), in patients with TM compared to the osteoporosis group, RANKL is higher and OPG/RANKL is lower (4). Bone mineral density (BMD) is a method of choice for assessing bone status in adults. Assessing BMD in children has some shortages like the lack of a normal BMD for childhood and lack of cooperation during bone density assessment. Z-score is the current method for assessing BMD in childhood that is designed based on the normal childhood population and not yet fully standardized. However, performing BMD may be time-consuming, expensive, and inaccessible in some medical centers. As finding an alternative method can be helpful and regarding the probable relation between OPG and BMD, this study aimed to assess the relationship between OPG and osteoporosis and osteopenia in patients with TM.

Materials and Methods

This study was a cross-sectional study with This was an analytic cross-sectional study that was conducted on patients with TM aged 8-18 years. They had regular visits in 17 Shahrivar children's hospital from Jan 2017 to Jan 2018. Participants had no chronic bone diseases such as osteomalacia and osteogenic imperfecta. All patients with the diagnosis of TM who regularly transfused at the thalassemia ward were included. For all patients, OPG and bone densitometry were performed. BMD of the lumbar spine (L1-L4) and total hip were measured on dual X-ray energy absorptiometry (DXA; Hologic, Inc., Bedford, MA, USA)

The BMD Z-scores at the hip and lumbar spine were categorized into three subgroups: Normal subgroup was indicated with the Z score > -1. Osteopenia was addressed by the Z score between -1 and -2.5 and osteoporosis was indicated by the Z score < -2.5 (1). Data were gathered by a checklist including age, sex, body mass index (BMI), mean hemoglobin and ferritin levels in the last 6 months, heart and liver MRI T2* mode, calcium, and phosphor levels.

Statistical analysis

Data were reported by descriptive statistics including mean, standard deviation, number, and percent. The Shapiro-Wilks test was used to assess the normal distribution of quantitative data. As the distribution of all variables was normal, for comparing the quantitative demographic and clinical characteristics between the two groups, the independent T-test was used. Comparing qualitative variables was performed by the Chi-Square/Fisher's exact tests. Associations between the quantitative variables were explored using the ordinary linear regression analysis. The Multiple linear regression model was used to investigate the association between the BMD Z-score and OPG by the effect modification. The $P < 0.05$ was noted as statistical significance in SPSS version 20.

Ethical consideration

Ethical approval was obtained from the Ethics committee of Guilan University of Medical Sciences (Code: IR.GUMS.REC.1398.299, Date: 2019-09-07) and informed consent letters were obtained from parents.

Results

In this study, 37 patients with the mean age of 14.86 ± 3.72 years were enrolled. Most of them were female (n=21, 56.8%). The mean BMI (kg/m2) and BMD were 18.25±0.86, and -2.24±1.02, respectively. Results showed that the mean level of hemoglobin (g/dL), ferritin (ng/mL), calcium (mg/dL), and phosphorus (mg/dL) during previous six months were 9.56±0.92, 1525.60±1164.94, 9.4±0.40 and, 4.38±0.97, respectively (Table I).

Results showed that 2 (5.4%) patients had normal and the remained 35 (94.6%) patients had abnormal bone density

including 21 (56.8%) osteopenia and 14 (37.08%) osteoporosis. Due to the small sample size in the group with normal bone density, from this point, the comparison between groups was restricted to patients with osteopenia and osteoporosis. The frequency of osteopenia in girls was significantly higher (P=0.042). The mean age in patients with osteopenia was significantly higher than in patients with osteoporosis (P=0.045). Table I shows that there was no significant difference between osteopenia and osteoporotic groups in terms of BMI, mean serum level of ferritin, OPG, calcium, and phosphorus levels (p>0.05). The mean of heart MRI T2* results in patients with osteopenia was significantly higher than patients with osteoporosis (P=0.033) but there was no significant difference in terms of liver T2* results (Table I).

Results in Table II revealed that BMD Z-score was not significantly associated with OPG level in all participants. After stratifying groups based on the BMD, it was observed that in patients with osteoporosis, one unit increase in BMD Z-score was significantly associated with a 4.51 unit decrease in OPG level (P=0.001). Figure 1 shows a scatter plot for the association between BMD Z-score and OPG in patients with osteopenia and osteoporosis.

The multiple linear regression models were used to assess whether the association between the BMD Z-score was statistically significant after accounting for each of the demographic characteristics and biochemical bone markers. None of the demographic characteristics and biochemical bone markers reached statistical significance (P>0.05). In all models, BMD remained statistically non-significant except for BMI modification (P=0.46). Holding BMI, one unit increase in BMD was associated with a 1.22 unit decrease in OPG level (Table II).

Table I: Comparison of demographic characteristics and biochemical bone markers among patients with osteopenia and osteoporosis BMD Z-scores in thalassemic children.

	Osteoporosis	Osteopenia	Normal	Total	P-value
Total n(%)	14 (37.8)	21(56.8)	2 (5.4)	37 (100.0)	-
Sex (Girl/Boy)	5(23.8)/9(64.3)	14(66.7)/7(33.3)	2(9.5)/0(0.0)	21(56.8)/16(43.2)	0.042[a]
Age (years)	13.85±4.25	16.09±2.73	10.3±1.24	14.86±3.72	0.045[b]
BMI (kg/m^2)	18.24±0.69	18.27±1.00	18.16±0.54	18.25±0.86	0.928[b]
OPG (pmol/L)	4.18±3.88	4.50±4.81	2.03±1.06	4.22±4.32	0.840[b]
Hemoglobin (g/dL)	9.51±0.69	9.65±1.07	8.96±0.42	9.56±0.92	0.668[b]
Ferritin (ng/mL)	1784.18±1215.22	1470.32±1132.11	453.12±254.3	1525.60±1164.94	0.441[b]
Calcium (mg/dL)	9.47±0.42	9.39±0.40	9.20±0.38	9.4±0.40	0.572[b]
Phosphorus (mg/dL)	4.70±1.20	4.35±0.59	3.10±0.41	4.38±0.97	0.263[b]
Heart MRI T^{2*} (ms)	26.03±7.38	30.92±6.42	23.56±0.01	28.74±7.02	0.033[b]
liver MRI T^{2*} (ms)	9.41±7.55	10.16±6.30	23.14±0.01	10.61±7.20	0.969[b]

Table II: Effect modification of the association between BMD and OPG by demographic characteristics and biochemical bone markers

	BMD			OPG		
	P-value [a]	Sd	β	P-value [a]	Sd	β
Total	0.106	0.69	-1.15			
Osteopenia	0.906	2.95	-0.35			
Osteoporosis	0.001	0.88	-4.51			
Sex	0.114	0.82	-1.33	0.317	1.54	1.57
Age (years)	0.112	0.78	-1.28	0.154	0.21	0.31
BMI (kg/m^2)	0.046	0.69	-1.22	0.939	0.79	0.06
Hemoglobin (g/dL)	0.175	0.79	-1.10	0.513	0.81	-0.53
Ferritin (ng/mL)	0.175	0.79	-1.10	0.693	0.01	0.01
Calcium (mg/dL)	0.165	0.843	-1.20	0.690	1.98	-0.79
Phosphorus (mg/dL)	0.137	0.79	-1.20	0.297	0.84	-0.89
Heart MRI T^{2*} (ms)	0.190	0.90	-1.21	0.837	0.12	0.03
liver MRI T^{2*} (ms)	0.303	0.87	-0.92	0.419	0.12	-0.10

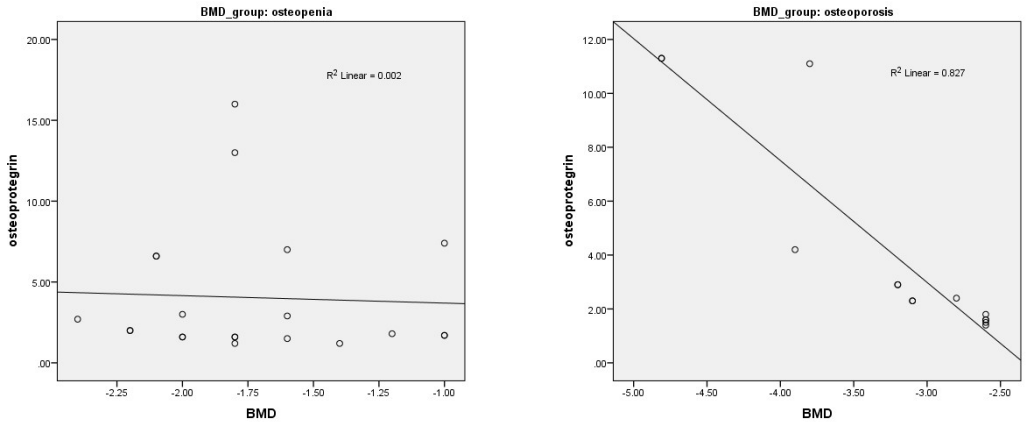

Figure 1. Scatter plot for the association between BMD Z-score and OPG in patients with osteopenia and osteoporosis.

Discussion

Osteoporosis is a multifactorial disease that leads to significant morbidity in TM. Based on the difficulty of interoperating z-score results as the common tool for evaluating BMD in childhood, the authors decided to assess OPG level as a probable practical tool. Results showed that the frequency of osteoporosis and osteopenia was much higher than previous studies (2-4). It seems that the higher frequency of bone abnormalities compared to previous investigations may be occurred due to the low mean of BMI in this study. As only two patients had normal BMD, comparing OPG levels in the osteoporotic and osteopenic patients was performed. Results showed that OPG was not a predictor for both

osteoporosis and osteopenia in the existence of BMI. Because based on the previous results, BMI was attributed to BMD. However, after adjusting the model and removing BMI as an effective variable, results showed a significant relation between OPG levels and osteoporosis and osteopenia.

The results of the current study were consistent with the previous studies by Dogan et al. (9), Salamat et al. (10), Lloyd et al. (11) that showed the relation between BMI and BMD. Although Hoxha et al. (12) showed consistent results, they mentioned a positive correlation between weight and BMI with both femur neck BMD and total hip BMD in all-male subjects. However, Greco et al. (13) demonstrated that obese patients had low lumbar BMD. Besides, Emaus et al. (14) showed a linear correlation between BMI and BMD. They noted that by each unit increase in BMI, an increase of 0.008 g/cm2 in L1-L4 BMD, 0.017 g/cm2 in the femur neck BMD and 0.018 g/cm2 in the total hip BMD was observed. In patients with osteopenia, there was no significant relation between BMD and OPG. However, this relation was significant in patients with osteoporosis, Pietrapertosa et al. noted that with a unit increase in bone mineral density, the mean OPG decreased by 4.51 units. Another goal of this study was to compare the normal and osteoporotic groups that were not possible because of the limitation in sample size (2). The mean age of participants was 14.86 ± 3.73 years. However, the mean age of patients with osteoporosis was significantly lower than those who had Osteopenia (P = 0.045), that may be due to the early treatment of thalassemia patients even without fracture or the increase of BMD with aging and reaching puberty. Results showed that the majority of girls had osteopenia (66.7%) and the remained 35.7% had osteoporosis. However, the occurrence of osteoporosis in boys was higher than in osteopenia. This may be noted as a result of sooner puberty in girls and early attention to their development. Besides, hormones can affect BMD (5, 6).

Comparing osteopenic and osteoporotic groups regarding hemoglobin, ferritin, calcium, and phosphorus levels were not significantly different. It seems that as it was expected, this may be due to the exact pathophysiology of the disease. By moderating the effect of variables such as age, sex, cardiac iron load, hepatic iron load, hemoglobin, calcium, and phosphorus, the relationship between BMD and OPG was not significant except for BMI. For this reason, the authors recommend further studies to be performed in specific BMI groups.

Conclusion

Given the scarcity of these findings as a clue for further investigations, the low sample size, and the importance of assessing patients with thalassemia major, it seems that further complicated studies are needed to be performed on this issue.

Acknowledgments

This investigation was based on the thesis submitted by the fourth author (Dr. Seyyed Mohammad Aghaeezadeh Zoroufi) to the Guilan University of Medical Sciences.

Conflict of interest

The authors declare no conflict of interest.

References

1. Gaudio A, Morabito N, Catalano A, Rapisarda R, Xourafa A, Lasco A. Pathogenesis of Thalassemia Major-associated Osteoporosis: A Review with Insights from Clinical Experience. J Clin Res Pediatr Endocrinol 2019; 11(2): 110-117.

2. Pietrapertosa A, Minenna G, Colella S, Santeramo T, Renni R, D'Amore M. Osteoprotegerin and RANKL in the pathogenesis of osteoporosis in patients with thalassaemia major. Panminerva Med 2009; 51(1):17-23.

3. Rao S, Cronin SJ, Sigl V, Penninger JM. RANKL and RANK: from mammalian physiology to cancer treatment. Trends Cell Biol 2018; 28(3): 213-223.
4. Ikebuchi Y, Aoki S, Honma M, Hayashi M, Sugamori Y, Khan M, et al. Coupling of bone resorption and formation by RANKL reverse signalling. Nature 2018; 561(7722):195-200.
5. Boyce BF, Xing L. The Rankl/Rank/Opg pathway. Curr Osteoporos Rep 2007; 5(3): 98-104.
6. Shamshirsaz A, Bekheirnia M, Kamgar M, Pakbaz Z, Tabatabaie S, Bouzari N, et al. Bone mineral density in iranian adolescents and young adults with β-thalassemia major. Pediatr Hemat Oncol 2007; 24(7): 469-479.
7. Leung T, Hung EC, Lam CW, Li C, Chu Y, Chik K, et al. Bone mineral density in children with thalassaemia major: determining factors and effects of bone marrow transplantation. Bone Marrow Transplant 2005; 36(4): 331-336.
8. Tzoulis P, Ang AL, Shah FT, Berovic M, Prescott E, Jones R, et al. Prevalence of low bone mass and vitamin D deficiency in β-thalassemia major. Hemoglobin 2014; 38 (3): 173-178.
9. Doğan A, Nakipoğlu-Yüzer GF, Yıldızgören MT, Özgirgin N. Is age or the body mass index (BMI) more determinant of the bone mineral density (BMD) in geriatric women and men? Arch Gerontol Geriatr 2010; 51(3):338-341.
10. Salamat MR, Salamat AH, Abedi I, Janghorbani M. Relationship between weight, body mass index, and bone mineral density in men referred for dual-energy X-ray absorptiometry scan in Isfahan, Iran. J. Osteoporos 2013; 2013(205963): 1-7.
11. Lloyd JT, Alley DE, Hawkes WG, Hochberg MC, Waldstein SR, Orwig DL. Body mass index is positively associated with bone mineral density in US older adults. Arch. Osteoporos 2014; 9(1): 175.
12. Hoxha R, Islami H, Qorraj-Bytyqi H, Thaçi S, Bahtiri E. Relationship of weight and body mass index with bone mineral density in adult men from Kosovo. Materia Socio Medica 2014; 26 (5): 306-308.
13. Greco EA, Fornari R, Rossi F, Santiemma V, Prossomariti G, Annoscia C. Is obesity protective for osteoporosis? Evaluation of bone mineral density in individuals with high body mass index. Int J Clin Pract 2010;64(6):817-820.
14. Emaus N, Wilsgaard T, Ahmed LA. Impacts of body mass index, physical activity, and smoking on femoral bone loss: the Tromsø study. J Bone Miner Res 2014; 29(9):2080-2089.