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Correlation of OPG/RANKL in patients with thalassemia major at the center of Haemoglobinopathy Lushnje, Albania

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Abstract

Osteoporosis is an important cause of morbidity in hemoglobinopathy patients. It is characterized by low bone mass and disruption of bone architecture, resulting in reduced bone strength and increased risk of fractures. Osteoprotegerin (OPG) and receptor activator of NF-kappa-B ligand (RANKL) have recently been implicated in the pathogenesis of various types of osteoporosis. Our study aimed to determine the correlation between OPG/RANKL and patients affected by thalassemia major at the Center of Hemoglobinopathy in Lushnje. Methods: We measured serum OPG and RANKL levels in 70 patients with Thalassemia major and 67 healthy controls, determining correlations with BMD. Results: Serum OPG levels were significantly lower in thalassemic patients compared to the control group. Serum RANKL levels were higher in β-thalassemia patients compared to controls. 31.1% of our patients with Thalassemia major had osteoporosis and 21.6 % had osteopenia. We found a correlation between OPG-BMD (r=-0.768, p=0.000, and RANKL-BMD (r=0.468; p=0.000). Conclusion: OPG and RANKL in Thalassemia major patients should be considered as the main factors responsible for osteoclast activation.

Keywords: Thalassemia, osteoporosis, OPG/RANKL

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1. Introduction

Our study aimed to determine if there is any correlation between OPG/RANKL and the patients affected by thalassemia major in the Center of Haemoglobinopathy in Lushnje, Albania. Lushnja is a town in the west of Albania and it's very well known for thalassemia. The data obtained in 2006, on the screening of thalassemia carriers in the high school of the Lushnja district, showed that the transferability of thalassemia was quite high in the entire district. The prevalence of thalassemia was higher in plain and coastal areas 10-11%. (Refatllari et al 2008).

Beta thalassemia syndromes are mostly autosomal recessive disorders characterized by beta-globin chain synthesis genetic deficiency. (Oğuz et al., 2023) Osteoporosis is an important cause of morbidity in thalassemia patients. (Oğuz et al., 2023) It is characterized by low bone mass and disruption of bone architecture, resulting in reduced bone strength and increased risk of fractures. Over the last decade, studies have described a high prevalence of osteopenia and osteoporosis in well-treated thalassaemias, reaching up to 50%. (Tari et al., 2018) Osteoprotegerin (OPG) and receptor activator of NF-kappa-B ligand (RANKL) have recently been implicated in the pathogenesis of various types of osteoporosis. (Schündeln et al., 2014)(Yu et al., 2019)

1.1. Methods:

We measured serum OPG and RANKL levels in 70 patients(35 male and 35 female, mean age 28.3 ± 13.6 years)with Thalassemia major and in 67 healthy controls, determining correlations with BMD. Patients presented at the Thalassemia Center every 21 days to receive transfusions. They were diagnosed with thalassemia by electrophoresis of hemoglobin when they were 2 years old. The control group consisted of 67 patients admitted to the hospital for the routine control (38 female and 29 male) with a mean age of 32 ±14 years. Five milliliters of fasting pre-transfusion venous blood was collected and serum was stored at −20°C after separation. BMD (bone mineral density) was determined by dual-energy X-ray absorptiometry (DEXA). We measured biochemical markers of bone metabolism (calcium, phosphorus, ALP-DEA, osteocalcin,β-CrossLaps, vitamin D, PTH), hemoglobin and ferritin.

1.2. Data analysis:

BMD values were compared with reference values from healthy people with similar age, sex, and ethnicity to calculate a Z score, the number of SDs from the expected mean. Z scores lower than −2.5 were accepted as "low bone mineral density" or osteoporosis. Z-scores <2.5 were accepted as osteopenia and z-score -1 to +1 were accepted as normal.

Osteoprotegerin (OPG) and the receptor activator of nuclear factor-kappa B (RANK)/receptor activator of nuclear factor-kappa B ligand

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(RANKL) are the major cytokines related to the regulation of bone resorption (Jafari et al., 2019). The receptor activator of the nuclear factor-kappa B (RANK)/RANK ligand (RANKL)/osteoprotegerin (OPG) pathway has been recently recognized as the final, dominant mediator of osteoclast proliferation and activation (Koohmanaee et al., 2021) (AbdAllah et al., 2010).

A commercially available kit measured serum osteoprotegerin(OPG) (Biovendor ELISA, version 17131204). Intra-assay (n=5) ≤5% as provided by the manufacturer. The assay for measuring OPG in serum used the sandwich method in ELISSA format with a monoclonal capture and polyclonal antibody detection. The median value, according to the company Biomedical, Vienna is 2.7 pmol/L. OPG is stable at -20 0 in serum and EDTA, citrate, and heparin plasma at +4 0 for 14 days. The assay for measuring RANKL in serum uses the sandwich method in Elissa format with a monoclonal capture and polyclonal antibody detection (Biomedical). The lower limit of detection for this assay is 0.08 pmol/L. Accuracy for RANKL (Biomedical) is intraassay CV from 3% (at 3.2 pmol/L) to 5% (at 1 pmol/L) and interassay CV from 6% (at 1.78 pmol/L) to 9% (at 0.8 pmol/L). The Statistical Package for the Social Sciences (SPSS) 22 program was used for statistical analysis. Variables were found to be statistically significant at $p<0.0$

2. Results:

The study group included 70 β-TM patients (35 female, 35 male) and 67 controls (38 female and 29 male). The mean age of β-TM group was 25± 13.1(18-64) years, while in control group was 32.3 ±14(19-65) years. The mean age and sex distribution of the groups were not significantly different ($p>0.05$). Serum calcium, phosphorus, PTH, and Vitamin D levels were within normal limits and didn't differ between patients and control groups. Comparisons of major cytokines (OPG/RANKL) related to the regulation of bone resorption between patients with thalassemia major and healthy controls were presented in Table 1.

Table 1. Comparisons of OPG/RANKL levels between Thalassemia major and controls

	β-TM(n=70)	Control(n=67)	p
age(years) (mean± SD)	25 ±13.1	32.3 ±14	>0.05
OPG(pmol/L) (mean± SD)	3.2± 1.48	10.2 ±7.5	<0.01
RANKL(pmol/L) (mean± SD)	0.26 ±0.17	0.11± 0.89	<0.01

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Serum OPG was significantly lower in thalassemic patients compared to the control group. Serum RANKL was higher in β-thalassemia compared to controls.

Table 2. Correlation OPG/RANKL levels and BMD

Variable		OPG	RANKL	BMD
OPG	Coefficient of correlation significance	1	-0.491** 0.000	-0.768** 0.000
RANKL	Coefficient of correlation significance	-0.491** 0.000	1	0.468** 0.000
BMD	Coefficient of correlation significance	-.0768** 0.000	0.468** 0.000	1

**. Correlation is significant at level 0.01

Table 2 shows a strong correlation between OPG-BMD (r=-0.768; p=0.000) and OPG-RANKL (r=-0.491; p=0.000).

Table 3, presents the distribution of BMD between thalassemic patients and the control group. We found that 31.1% (22 from 70) of our patients with Thalassemia major had osteoporosis 21.6 % (15 from 70) had osteopenia and 48% (33 from 70) presented normal BMD. The normal group had 5% (3 from 67) osteoporosis,12% (8 from 67) osteopenia, and 83% (49 from 67) had normal BMD.

Table 3. Distribution of BMD between thalassemic patients and control group

	Osteoporosis	Osteopenia	Normal
Thalassemic patients (70 patients)	22	15	33
Control group (67 person)	3	8	49

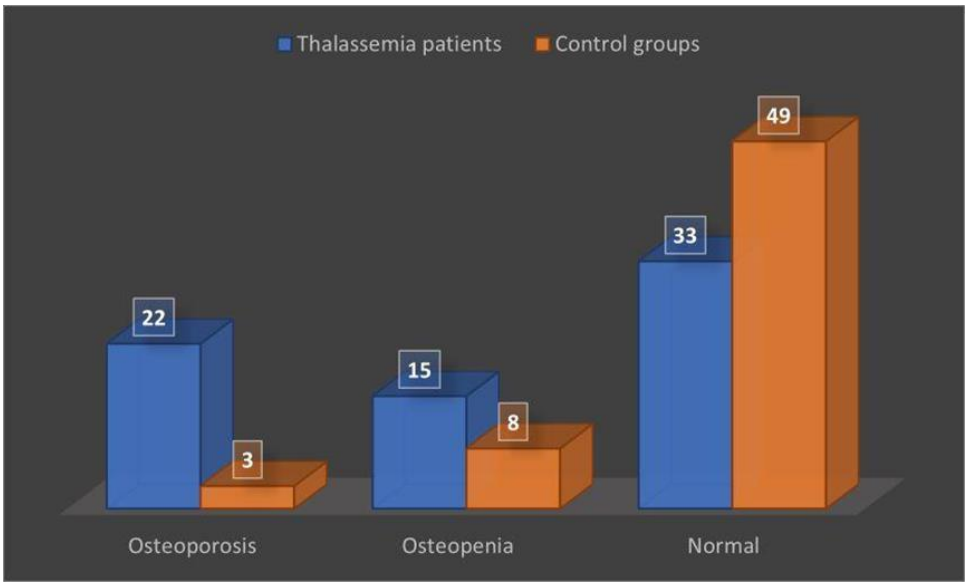

Figure 1: BMD in thalassemic patients and control group

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3. Discussion:

Hemoglobinopathies are a common cause of skeletal morbidity and increased bone fracture risk in hemoglobinopathies patients(Tombak et al., 2020). Its pathogenesis is multifactorial and mainly includes bone marrow expansion, endocrine dysfunction, and iron overload (Pietrapertosa et al., 2009). In this study, we investigated the OPG and RANKL values of our patients diagnosed with β-TM. We compared these values with BMD (bone mineral density) to observe whether there was a correlation between them. The OPG/RANKL system plays an important role in the activation and proliferation of osteoclast precursors. Upon analyzing our data, the levels of OPG and RANKL cytokines were compared through the analysis of variance ANOVA. We noticed that we had a lower mean value of OPG in thalassemia compared to the control group. (OPG in TM 3.2 ±1.48 pmol/L: control 10.2± 7.5 pmol/L and a higher level of RANKL in TM 0.26±0.17; control 0.11±0.89). In our study, a negative correlation was noted between OPG and BMD (r =-0.768; p=0.000) and a positive correlation between OPG and T-score (r=0.729; p=0.000), as in the study of (Rogers & Eastell, 2005). In our study, it was observed that patients with hemoglobinopathy associated with osteopenia/osteoporosis had lower OPG levels and a lower OPG/RANKL ratio compared to patients with normal BMD. In the study of (Morabito et al., 2004) as far as the OPG/RANKL system is concerned, thalassemic patients showed no differences in plasma levels of OPG compared with controls that are not the same as our study and significantly higher plasma levels of RANKL, as we found in ours. (Tsartsalis et al., 2019)showed that sixty-four patients with TM (32 men and 32 women) participated in the study, almost the same number of patients were in our study (35 men and 35 women). The statistical analysis of the biochemical markers of bone metabolism revealed overall significant differences between the three groups only for RANKL and OPG/RANKL (p=0.049 and p=0.009). RANKL was higher and OPG/RANKL was lower in TM patients compared to the osteoporosis group. In our study, RANKL was higher too. (Hamidpour et al., 2022) showed the biochemical parameters in the (patients/ controls) including calcium and alkaline phosphatase (ALK) 9.1/10.2 mg/dL and 171.1/310 IU, respectively indicating a significant decrease (P< 0.05) compared to the controls. In our study, the level of calcium was 8.8mg/dl, and alkaline phosphatase (ALP-DEA) was 180IU/ml, the same as in our study. On the contrary, the mean levels of Ferritin and Zinc were 1914.18 µg/L and 113.92 mg/mL, respectively which were significantly increased (P= 0.015 and P=0.045, respectively). We measured the level of ferritin, which was high too.(Koohmanaee et al., 2021) showed 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. Our study showed that 48% of patients were normal,

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21% had osteopenia and 31% had osteoporosis. 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 in patients with osteoporosis. We had more females than males with osteopenia and osteoporosis too. 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). (AbdAllah et al., 2010) showed that β-TM patients presented an altered bone turnover, with an increased resorption phase. The thalassemic patients showed significantly lower serum levels of OPG (P=0.0001). In contrast, RANKL levels were significantly higher in β-TM patients (P=0.001), who consequently showed a lower OPG/RANKL ratio (P=0.001), the same as in our study. (Pietrapertosa et al., 2009) showed that all the thalassemic patients had reduced BMD, and 35.5% presented osteoporosis. The thalassemic patients had significantly higher serum levels of OPG than the controls, while their higher RANKL levels were at the threshold of significance. The OPG/RANKL ratio showed a higher level of respect for the controls. No statistically significant correlation was observed between the T-score and RANKL neither between the T-score and OPG nor between T-score and OPG/RANKL ratio. In our study, 31.1 % presented osteoporosis OPG was lower than in the control group and RANKL was higher. (Schündeln et al., 2014) showed vitamin D deficiency with 25 OH-vitamin D serum levels below 20 ng/ml was a common finding (80.5%) in this cohort, we met the same results in our study. Analysis of RANKL, osteoprotegerin (OPG), and osteocalcin levels indicated an alteration in bone modeling with significantly elevated RANKL/OPG ratios (control: 0.08+0.07; patients: 0.26+0.2, P = 0.0007). Our study showed (control:0.01+0.12; patients 0.08+0.11, P=0.002). (Youssry et al., 2022) showed the mean of spine dual-energy X-ray absorptiometry (DXA) Z-score in patients was −1.66 ± 1.02 standard deviation (SD). Twenty-four of them had low spine DXA Z-scores. The patients showed significantly lower OPG levels and OPG/RANKLs ratios than the control group (3.28 ± 9.11 ng/ml and 11.38 ± 14.93 ng/ml, and 0.01 ± 0.03 and 0.07 ± 0.09, respectively), in our study we had lower OPG levels too compared to the control group (3.2 ±1.48pmol/L and 10.2 ±7.5 pmol/L). (Çelik et al., 2022) showed serum OPG levels were significantly lower in thalassemic than in controls. The mean ratio of RANKL/OPG was significantly higher in the thalassemic patients than in the control group, which was the same in our study. Osteoporosis was detected in 10 (3 female and 7 male) of 38 patients (26.3%) according to the femur Z score and in 6 of them (4 male and 2 female) (15.8%) according to the spine Z score.

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Conclusions

Serum OPG/RANKL concentrations can be used as a biochemical marker in screening patients with hemoglobinopathy for the development of osteoporosis. Osteoporosis is a multifactorial disease and may occur early, especially in chronic diseases such as thalassemia. Because of the difficulties in diagnosis and follow-up, screening with DEXA and measuring ferritin level and RANKL/OPG ratios regularly is essential. It should be kept in mind that osteoporosis may develop with advancing age in both sexes. According to the 2021 guidelines for the management of transfusion-dependent thalassemia by the Thalassemia International Federation (TIF)(Farmakis et al., 2022), assessment of BMD by dual-energy X-ray absorptiometry (DXA) should be performed every 24 months after the age of 10 years, accompanied by vertebral fracture assessment. In addition, annual assessment of bone health should include measurement of serum calcium, phosphate, alkaline phosphatase, 25 (OH) vitamin D, PTH (parathyroid hormone), and, ideally, one marker of bone formation, and one marker of bone resorption.

Conflict of Interest: The authors reported no conflict of interest.

Data Availability: All data are included in the content of the paper.

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Declaration for Human Participants: This study has been approved by the Ethical Board of Alexander Moisiu University Durres and the principles of the Helsinki Declaration were followed.

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