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Correlation of OPG/RANKL in Patients with Thalassemia Major in 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 been recently implicated in the pathogenesis of various types of osteoporosis. The aim of our study was to determine if there was any correlation between OPG/RANKL and the patients affected by thalassemia major in the Center of Haemoglobinopathy in Lushnje. Methods: We measured in 70 patients with Thalassemia major and in 67 healthy control serum OPG and RANKL levels and determined correlations with BMD. We measured T-score and BMD too. Results: 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). OPG-T-score (r=0.729. p=0.000) and Rankl-T-score r=-0.409; p=0.000). Conclusion: OPG and RANKL in Thalassemia major patients should be consider as a main factor responsible for osteoclast activation.

Keywords: Thalassemia,osteoporosis, OPG/RANKL

Introduction

The aim of our study was 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 south west of Albania and it's very known for thalassemia. From the data obtained in 2006, on the screening of thalassemia carriers in the high school of Lushnja district, it resulted that the transferability of thalassemia was quite high in the entire district. The prevalence of thalassemia was higher on plain and costal areas 10-11%. (Refatllari E.et al, 2008)

Osteoporosis is an important cause of morbidity in thalassemia patients. (S. H. 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. During the last decade, the presence of osteopenia and osteoporosis in well-treated thalassaemics has been described in different studies with high prevalence up to 50%(K. Tari et al 2013). Osteoprotegerin (OPG) and receptor activator of NF-kappa-B ligand (RANKL) have been recently implicated in the pathogenesis of various types of osteoporosis. (M. M. Schündeln et al [2014 and] U. Yu et al2019)

Methods:

We studied serum OPG/RANKL levels in a total of 90 patients affecting by thalassemia major admitted to the Center of Hemoglobinopathy Lushnje, Albania, 45 male and 45 female, mean age 28.3 ± 13.6 years. Patients presented at the Thalassemia Center every 21 days to receive transfusions. The control group consisted of 67 patients admitted to the hospital for the routine control (38 female and 29 male) with mean age 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 (serum calcium, phosphorus,ALP-DEA,osteocalcin,β-CrossLaps,vitamin D,PTH),hemoglobin and ferritin.

Date 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-score<2.5 were accepted as osteopenia and z-score -1 to +1 were accepted normal.

Osteoprotegerin (OPG) and the receptor activator of nuclear factor-kappa B (RANK)/receptor activator of nuclear factor-kappa B ligand (RANKL) are the major cytokines related to the regulation of bone

resorption [7]). The receptor activator of the nuclear factor-kappa B (RANK)/RANK ligand (RANKL)/osteoprotegerin (OPG) pathway has been recently recognised as the final, dominant mediator of osteoclast proliferation and activation [6.8]

Serum osteoprotegerim (OPG) was measured by a commercially available kit (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 Biomedica, Vienna is 2.7 pmol/L. OPG is stable at -20 0 in serum and EDTA, citrate, heparin plasma and is stable 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 (Biomedica). The lower limit of detection for this assay is 0.08 pmol/L. Accuracy for RANKL (Biomedica) 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 Socila Sciences (SPSS) 22 programme was used for statistical analysis. Variables were found to be statistically significant at $p<0.05$

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 related to the regulation of bone resorption between patients with thalassemia major and healthy controls were presented in table1.

Table 1. Comparisons OPG/RANKL 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

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

Table 2. Correlation OPG/RANKL 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

In table 2, we found a strong correlation between OPG-BMD (r=-0.768;p=0.000) and OPG-RANKL(r=-0.491; p=0.000).

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

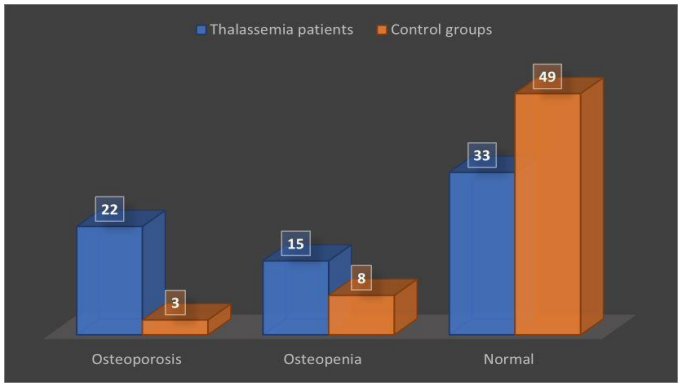

Figure 1. BMD in thalassemic patients and control group

Discussion:

Haemoglobinopathies are a common cause of skeletal morbidity and increased bone fracture risk in haemoglobinopathies patients (A. Tombak et al 2020). Its pathogenesis is multifactorial and mainly includes bone marrow expansion, endocrine dysfunction and iron overload(M. M. Schündeln et al 2012 and A. C. Pietrapertosa 2009). In this study, we investigated 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 activation and proliferation of osteoclast precursors. Analyzing our dates, the value of OPG and RANKL cytokines, through the analysis of variance ANOVA, we noticed that we had 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 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 et al 2005. In our study, it was observed that patients with thalassemia major associated with osteopenia/osteoporosis had lower OPG levels and a lower OPG/RANKL ration compared to patients with normal BMD. In the study of Morabiot 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 is not the same with our study and significantly higher plasma levels of RANKL, as we found in ours. In the study of Pietrapertosa 2009. The thalassemic patients had significantly higher serum levels of OPG than the controls, not the same in ours, while their higher RANKL levels, were at the threshold of significance as in ours. We found correlation with the study of Celik et al 2022, where he said that serum OPG levels were significantly lower in thalassemic children than in controls. We found correlation between OPG/RANKL-BMD, but that did not agree with different studies from Italy and Greece (M. Toumba and N. Skordis 2010)

In the study of O. E. Amer et al 2022,bone turnover was significantly increased in thalassemic patients compared to controls, but OPG was significantly higher in healthy subjects, the same was found in our study too. BMD values did not correlate with OPG/sRANKL system; in our study, it correlated.

A. N. Tsartsalis et al in 2019, showed that sixty-four patients with TM (32 men and 32 women) participated in the study,almost the same number of patients was 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 osteoporosis group. In our study RANKL was higher too.

M. Hamidpour et al in 2022, showed the biochemical parameters in the (patients/ controls) including calcium and alkaline phosphatase (ALK) were 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, level of calcium was 8.8mg/dl and alkaline phosphatase (ALP-DEA) was 180IU/ml, the same as in our study too. 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 level of ferritin, which was high too.

S. Koohmanaee et al in 2021showed 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 48% of patients normal, 21% osteopenia and 31% 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 patients with osteoporosis. We had more female than male 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).

N. AbdAllah et al in 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), whereas 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.

A. C. Pietrapertosa et al in 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 higher level respect to 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 and OPG was lower than in control group and RANKL was higher.

I. Youssry et al in 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 control group (3.2 ±1.48pmol/L and 10.2 ±7.5 pmol/L)

T. 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, 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.

O. E. Amer et al 2022, showed the results suggested that OPG was significantly and positively correlated with age in the osteoporosis group ($r = 0.29$, $p < 0.05$), while it was inversely correlated with BMD femoral neck left ($r = -0.56$, $p < 0.001$) and BMD femoral neck right ($r = -0.37$, $p < 0.05$) in the same group. Furthermore, the RANKL/OPG ratio had a positive and significant correlation with BMI ($r = 0.34$, $p < 0.05$), BMD femoral neck left ($r = 0.36$, $p < 0.05$) and BMD femoral neck right ($r = 0.35$, $p < 0.05$) in the osteopenia group. Multiple regression analysis showed that OPG contributes to BMD variations in the osteopenia group ($p = 0.03$). In our study, we found correlation between OPG and age of patients with osteoporosis ($r=0.27$ $p<0.05$)

Conclusion

Serum OPG/RANKL concentrations can be used as a biochemical marker in screening patients with haemoglobinopathy 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 on a regular basis 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) [17], 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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