Significant Inverse Correlation of Serum Levels of Osteoprotegerin (OPG) and Transferrin Saturation in Thalassemia Dependent Transfusion (TDT) Patients

*Indra Wijaya[1], M Lucky Nurdiansyah Prameswara[2], Dimmy Prasetya[1], Laniyati Hamijoyo[3], Bachti Alisjahbana[4], Andri Reza Rahmadi[3]**

[1]Division of Hematology-Medical Oncology, Department of Internal Medicine, Faculty of Medicine Universitas Padjajaran - Dr. Hasan Sadikin Hospital, Bandung, Indonesia.
[2]Department of Internal Medicine, Faculty of Medicine Universitas Padjajaran - Dr. Hasan Sadikin General Hospital, Bandung, Indonesia.
[3]Division of Rheumatology, Department of Internal Medicine, Faculty of Medicine Universitas Padjajaran - Dr. Hasan Sadikin General Hospital, Bandung, Indonesia.
[4]Division of Infectious and Tropical Diseases, Department of Internal Medicine, Faculty of Medicine Universitas Padjajaran - Dr. Hasan Sadikin General Hospital, Bandung, Indonesia.

Corresponding Author:
Andri Reza Rahmadi, MD. Division of Rheumatology, Department of Internal Medicine, Faculty of Medicine Universitas Padjajaran - Dr. Hasan Sadikin Hospital. Jl. Pasteur no. 38 Bandung, Indonesia.
Email: muhamadluckynurdiansyah@gmail.com.

ABSTRACT

Background: *Osteoporosis is a major problem in transfusion-dependent thalassemia patients (TDT) patients. Osteoprotegerin (OPG) is one of several bone markers that are closely associated with osteoporosis in TDT patients. OPG is a glycoprotein that functions as a feedback receptor for the Receptor Activator of Nuclear Factor kappa B Ligand (RANKL), which is an alpha tumor necrosis factor receptor. One of the causes of decreased bone mass density is iron toxicity, which can be identified by showing elevated transferrin saturation. Bone mass dual X-ray absorptiometry (DEXA) is a gold standard for the diagnosis of osteoporosis, these procedures are not commonly available in Indonesia. This study was conducted to analyze the correlation between serum levels of OPG and transferrin saturation in TDT patients.* ***Methods:*** *A correlational study with a cross-sectional approach analyzed data from TDT patients at Hemato-Oncology Medic Outpatient Clinic, Hasan Sadikin General Hospital, Bandung, Indonesia. Primary data were obtained through blood sampling and anthropometry measurement while secondary data were obtained from the patient's medical records. OPG and transferrin saturation levels were assessed using the ELISA method. Research data were analyzed using the rank Spearman correlation test.* ***Results:*** *Data were collected from 51 research subjects (30 women dan 21 men). The median OPG level was 380 (170-1230) pg/mL and the median transferrin saturation level was 89.4 (66.7 – 96.2)%. Analysis of correlation showed a significant correlation between and transferrin saturation level with a coefficient value of r -0.539 and p-value <0.001.* ***Conclusion:*** *There was a significant inverse correlation between OPG with transferrin saturation in TDT patients.*

Keywords**:** *OPG, transferrin saturation, osteoporosis, transfusion-dependent thalassemia.*

INTRODUCTION

Osteoporosis is the most common complication associated with transfusion-dependent thalassemia (TDT) patients.[1] The pathogenesis is multifactorial and mainly includes bone marrow expansion, endocrine dysfunction, and iron overload.[2] The exact mechanisms by which these variables cause bone loss are still unclear.[3,4] A meta-analysis by Charoenngam et al. reported that the pooled prevalence of fracture among patients with thalassemia was 16%, with subgroup analysis describing prevalences of 18% and 7% in patients with TDT and NTDT, respectively.[5] Between 28% and 32% of people in Indonesia have osteoporosis or low bone density.[6] In the meantime, thalassemia's bone problem has a detrimental psychological effect, reduces the quality of life, and raises treatment costs.[7]

Thalassemia bone disease (TBD) is a unique bone disease that can impact all elements of bone structure and quality, as well as mineral density. Osteoporosis, fractures, spinal abnormalities, compression of the nerves, and pain are among the worst morbidities associated with TBD. It has become a major challenge to control the rising morbidity burden brought on by the severe thalassemia phenotype.[8] The hypothalamic-pituitary-gonadal axis malfunction, growth hormone effects on the parathyroid gland, diabetes, hypothyroidism, inefficient hemopoiesis, and direct iron toxicity to osteoblasts are a few of the aetiological causes for thalassemic osteoporosis. Because iron chelation treatments affect cartilage tissue, they may potentially contribute to osteopenia and osteoporosis.[8,9] Osteoprotegerin (OPG) and the receptor activator of nuclear factor-kappa β (RANK)/receptor activator of nuclear factor-kappa β ligand (RANKL) are the major cytokines related to the regulation of bone resorption.[10] The final major modulator of osteoclast activation and proliferation has been identified as the receptor activator of the nuclear factor-kappa β (RANK)/RANKL/OPG pathway.[10-12] Regular blood transfusions have been demonstrated to improve the survival of patients with thalassemia; at this point, they also raise the risk of iron excess and iron toxicity. One of the elements that lead to organ damage in thalassemia is non-transferrin-bound iron (NTBI), a free radical that mediates iron toxicity.[9,13] NTBI levels are only tested at a few research institutions abroad, therefore, surrogate markers such as serum ferritin and saturation transferrin have been used to measure iron toxicity.[7,9,14,15]

The measurement of bone mass dual X-ray absorptiometry (DEXA) in the lumbar area, femoral neck, and forearm is a non-invasive examination that accurately assesses bone density, however, these procedures are not widely available in Indonesia. Screening TDT patients with DEXA is necessary for detecting osteoporosis and improving bone health. Consequently, a need for bone markers exists.[7,10,16,17]

The biological effects of transferrin saturation and the OPG/RANKL system on a variety of metabolic bone diseases have been a focus of several developing investigations; nonetheless, the pathophysiology of bone disease in thalassemia remains entirely unclear.[9] These biomarkers are useful to provide the early assessment of osteoporosis when the BMD measurement of DEXA does not offer enough information to make the diagnosis.[7,16-17] Thus, the objective of the current study was to explore the serum levels of OPG and to detect their relations with transferrin saturation, as possible early predictors of the skeletal changes in TDT patients.

METHODS

This is a retrospective analysis. We collected the data from the medical records from TDT patients at the Hasan Sadikin Hospital Hematology-Medical Oncology Clinic Bandung, Indonesia from July to September 2023. This study was approved by the Health Research Ethics Committee, Faculty of Medicine Padjajaran University, and Dr. Hasan Sadikin Hospital Research Ethics Committee (No. DP.04.03/D. XIV.2.2.1/19547/2023).

The inclusion criteria were: (1) Age 18 years and over, (2) TDT patients who have medical record data. Patients with the following conditions were excluded from the study: (1) TDT patients including those who have never received iron chelation therapy, (2) TDT patients who had undergone a splenectomy procedure,

received steroid therapy with an equivalent dose of prednisolone 5mg during for more than 3 months. TDT patients with other comorbidities such as (1) infection disease, (2) liver disease, (3) hematological disorders, (4) diabetes mellitus, (5) chronic kidney disease, (6) bone disease, (7) hormonal disorders, (8) malnutrition, (9) vitamin D deficiency.

The blood samples were drawn from the vein by sterilized synergies with 5 milliliters. The sample was put in the labeled tube for blood to be used for preparing serum for the following biochemical and biomarkers. Blood was left at room temperature for 10 minutes for clotting, centrifuged at 6000 rpm for 10 minutes, and then serum was separated and frozen at -80 ºC until time for the laboratory analysis for the study.

The variables were BMI (Body Mass Index), the electronic balance and height device, were used for calculating the weight and height and applied the equation below: BMI = weight (kg)/height (m2). Biomarker measurement estimation human OPG the specific kit for measuring human OPG, and transferrin saturation levels in serum were supplied by (Elabscience-USA). The measurement of the human OPG ELISA (enzyme-linked immunosorbent assay-automated microtiter plate) kit was performed by (Elabscience-USA) sandwich immunoassay technique with a normal range between 160 -10.000 pg/ml.[18]

The characteristics of patients, for categorical data presented in frequency and proportion, and for numerical data, a normality test will be carried out using the Kolmogorov Smirnov test. Comparisons of categorical variables, expressed as proportions, correlations were evaluated using Rank Spearman's correlation coefficient. All statistical analyses were conducted using SPSS version 23.0 (SPSS, Chicago, IL).

RESULTS

There were 147 TDT patients at the Hemato-Oncology Medic Clinic Hasan Sadikin General Hospital from June 2021 to June 2022. A total of 51 patients were enrolled in this study (Figure 1), which consisted of 21 males (41,2%) and 30 females (58,8%) their median age was 22 which ranged from 19 to 44 years. The characteristics of the patients are presented in **Table 1**.

Based on **Table 1**, the median transferrin saturation was 89,4% with the lowest value of 66,7% and the highest value of 96,2%. These results showed that the serum OPG level with TDT was 380 pg/ml with the lowest value of 170 pg/ml and the highest of 1230 pg/ml (**Table 1**).

Table 2 shows a significantly negative correlation ($p<0.001$) between OPG and transferrin saturation ($r = -0.539$). The lower the OPG the higher the transferrin saturation measurement.

DISCUSSION

The result of this study indicated a significant negative correlation ($r = -0.539$) and ($p < 0.001$) between osteoprotegrin concentration with transferrin saturation in TDT patients which are shown in **Table 2**. Several author's studies indicated a significant decrease in the OPG concentration in a group of thalassemia major patients in comparison with the healthy group.[19-21] A Study by Alfaqih et al. showed

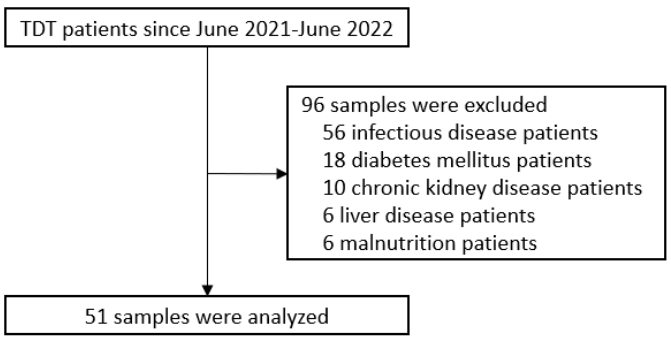

Figure 1. Flow diagram for transfusion-dependent thalassemia patients recruitment

Table 1. Characteristics of transfusion-dependent thalassemia patients.

Characteristics	N=51
Age, years (median)	22 (19 – 44)
Female, n (%)	30 (58.8)
Duration of thalassemia, years (median)	18 (10 – 35)
Frequency of blood transfusion median (min-max)	17 (6 – 38)
Blood transfusion volume (mL/kg/years) median (min-max)	168 (68 - 574)
BMI (kg/m^2)[a]	19.2 ± 2.6
Laboratory values	
Pre-transfusion hemoglobin (mg/dl)[a]	7,2 ± 0,8
Random blood sugar level (mg/dl), median (min-max)	114 (69-190)
Iron Status	
Fe serum (µg/dL), median (min-max)	216 (93 – 631)
TIBC (µg/dL), median (min-max)	247 (122 – 656)
Transferrin saturation (%), median (min-max)	89.4 (66.7 – 96.2)
Biochemical of bone marker	
OPG (pg/mL), median (min-max)	380 (170 – 1230)

Abbreviations: BMI, Body Mass Index; TIBC, total iron binding capacity; OPG, Osteoprotegerin
[a] Data are presented as the mean ± standard deviation

Table 2. Correlation of serum levels OPG and transferrin saturation in transfusion-dependent thalassemia.

Variables	Transferrin Saturation (%)		
	Coefficient r	Coefficient determinant r^2	P Value
OPG (pg/mL)	-0.539	0,29	<0.001*

Abbreviations: OPG, Osteoprotegerin.

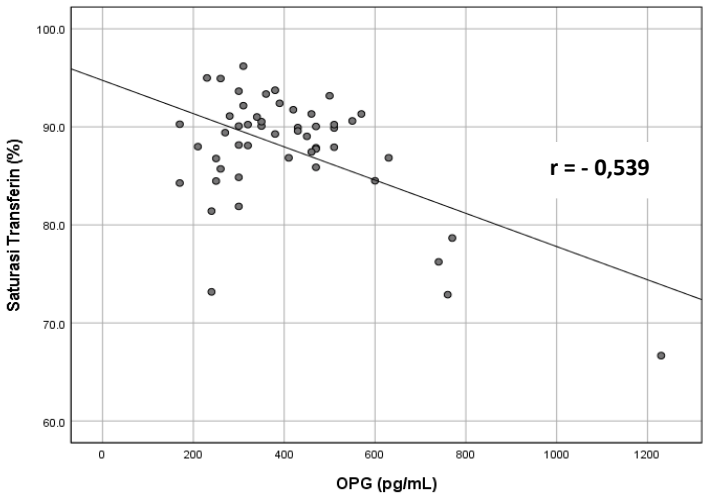

Figure 2. Scatterplot of serum levels OPG and transferrin saturation in TDT patients.

that RANKL concentrations were significantly higher in patients with thalassemia compared to the controls, but OPG concentrations were significantly lower in thalassemia patients. These results demonstrate that a dysregulated balance between the rate of bone resorption and bone production may be the cause of the dysregulated bone remodeling that occurs in thalassemia patients. It suggests that thalassemia patients have a net loss of bone due to a decrease in the rate of bone development correlated with an increase in the rate of bone resorption.[22] Several

studies have reported that in patients with thalassemia, endocrine effects, iron deposition, iron chelation therapy, or vitamin D therapy down-regulate osteoblasts, which are the primary generators of OPG. This results in an increase in RANKL and a decrease in OPG, which increases the risk of osteoporosis.[1-4] In contrast, another study indicated a significant increase in the OPG concentration in a group of TDT patients in comparison with a healthy group.[23] A Study by Hashemieh et al suggested that two cytokines, OPG and RANKL, have recently been recognized as important pacemakers in the pathogenesis of osteoporosis in TDT patients.[24] Many studies have demonstrated that NTDT patients have a significantly higher incidence of osteoporosis (81.6%) compared to TDT patients. This is related to a reduction or disruption of the bone architecture, which increases the risk of fractures, bone resorptions, decreased bone formation, and decreased bone mineral density (BMD).[25] Osteoporosis is an important problem for TDT patients. OPG was a bone marker that played a crucial role in the development of osteoporosis in TDT patients.[19-21]

TDT patients need frequent blood transfusions to control their chronic anemia. Iron overload is caused by repeated blood transfusions, increased hemolysis of red blood cells, and increased iron absorption in the gastrointestinal tract.[9] All body cells are very toxic to excess iron, which may result in major and permanent organic damage such as cirrhosis, diabetes, heart disease, hypogonadism, and osteoporosis. If untreated, these conditions can cause severe morbidity and death in TDT patients.[9] Sagare et al's study showed that transferrin saturation increased in both homozygous & heterozygous forms of thalassemia significantly as compared to control. This study showed that transferrin saturation may be the best way to utilize the information about iron overload & development of complications like oxidative stress due to non-bound iron form.[15] This study analyzed the correlation between OPG and transferrin saturation, the result showed that there was a negative and significant correlation between OPG with transferrin saturation in TDT patients. This is the first study to obtain a significant correlation between OPG and transferrin saturation in adult TDT patients. Atmakusuma et al's study showed that there was a significant correlation between bone mass density and transferrin saturation in adult patients with thalassemia. Although the correlation was weak, it was statistically significant.[7]

The pathogenesis of TBD is multifactorial, including age, gender, childhood growth retardation, bone marrow expansion, iron and chelator toxicity, physical inactivity, low body weight, nutritional deficiencies, and hormonal deficiencies, which are still not fully understood.[4] This study included 58.8% female TDT patients as participants. This was consistent with studies by Thavonlun et al and Koohmanaee et al who found that the incidence of thalassemia bone disease is more common in females.[3,4,19] Thavonlun et al's study showed that women tended to have fractures more than men. Thalassemia patients exhibit varying degrees of inefficient erythropoiesis, which are strongly correlated with the severity of genotype and clinical phenotype. Female sex was associated with an increased risk of osteoporosis.[3,4,19] The ages of the subjects in this study ranged from 19 years to 44 years with a median of 22 years. Wong et al study showed that the patient's comparatively young ages (mostly in their 20s and 30s), the variety of risk factors for bone loss (many specific to thalassemia patients), the frequency of fractures, and the reaction to therapy with bone-preserving medications are the aspects that differentiate this case. Adolescence is a crucial time for bone accretion, during which endocrinopathies (such as GH and sex hormones) and lifestyle factors—such as diet and exercise—play a crucial role in maximizing bone development and maintaining optimal bone health. The peak bone mass attained and the rate of bone loss thereafter are the primary determinants of BMD in young adults and beyond. TDT patients have a reduced ability to reach maximal bone mass.[26]

This study had limitations that could have an impact on the findings. Firstly, there was no hormonal assessment and its relation to bone mass density. Secondly, this study did not perform a BMD DEXA examination as a gold

standard examination. Lastly, this study was limited to a single center.

CONCLUSION

Based on the results, there was a significant inverse correlation between OPG with transferrin saturation in TDT patients.

REFERENCES

1. Stefanopoulos D, Papaioannou NA, Papavassiliou AG, et al. A contemporary therapeutic approach to bone disease in beta-thalassemia-a review. J Frailty Sarcopenia Falls. 2018;3(1):13.
2. Gaudio A, Xourafa A, Rapisarda R, Zanoli L, Signorelli SS, Castellino P. Hematological diseases and osteoporosis. Int J Mol Sci. 2020;21(10):3538.
3. De Sanctis V, Soliman AT, Elsefdy H, et al. Bone disease in β thalassemia patients: past, present and future perspectives. Metabolism. 2018;80:66-79.
4. Thavonlun S, Houngngam N, Kingpetch K, et al. Association of osteoporosis and sarcopenia with fracture risk in transfusion-dependent thalassemia. Sci Rep. 2023;13(1):16413.
5. Charoenngam N, Rittiphairoj T, Ponvilawan B. Fracture prevalence in thalassemia: A systematic review and meta-analysis. Arch Osteoporos. 2021;16, 171.
6. Tirtarahardja G, Setyohadi B, Weynand LS, Zhou Q. Bone mineral density reference values for Indonesian men and women; 2006. Available from: https://www.researchgate.net/publication/ 297712953. Accessed Oct 10, 2023.
7. Atmakusuma TD, Tenggara JB. Correlation of transferrin saturation and serum ferritin with bone mass density in adult transfusion-dependent beta-thalassemia patients. J Blood Med. 2021:827-32.
8. Piga A. Impact of bone disease and pain in thalassemia. Hematology Am Soc Hematol Educ Program. 2017;2017(1):272-7.
9. Leecharoenkiat K, Lithanatudom P, Sornjai W, Smith DR. Iron dysregulation in beta-thalassemia. Asian Pac J Trop Med. 2016;9(11):1035-43.
10. Kuo TR, Chen CH. Bone biomarker for the clinical assessment of osteoporosis: recent developments and future perspectives. Biomarker research. 2017;5(1):1-9.
11. Çelik T, Sangün Ö, Ünal Ş. Assessment of biochemical bone markers of osteoporosis in children with thalassemia major. Ital J Pediatr. 2022;105.
12. Sanchez VM, Blanquer M, Moraleda JM, Salido EJ, Perez O. New Insights Into Pathophysiology of β -Thalassemia. Front Med. 2022;9.
13. Shah FT, Sayani F, Trompeter S, Drasar E, Piga A. Challenges of blood transfusions in β-thalassemia. Blood Reviews. 2019;37.
14. Rivera LMJ. Iron Overload. Medicina Universitaria. 2015;17(69):240-2.
15. Sagare A, Trivedi D. Assessment of transferrin saturation as an indicator of iron overload in homozygous & heterozygous form of thalassemia. JPCBS. 2014;5:668-73.
16. Jeremiah MP, Unwin BK, Greenawald MH, Casiano VE. Diagnosis and management of osteoporosis. Am Fam Physician. 2015;92(4):261-8.
17. Gregson CL, Armstrong DJ, Bowden J, et al. UK clinical guideline for the prevention and treatment of osteoporosis. Arch Osteoporos. 2022;17(1):58.
18. Elabscience. RANKL, OPG ELISA Kit. EB. 2021.
19. Koohmanaee S, Dabandi B, Baghersalimi A, et al. The probability of indicating Osteoprotegerin as a biomarker for osteoporosis in patients with thalassemia major. Iran J Ped Hematol Oncol. 2021;11(1):18-23.
20. Wong P, Fuller PJ, Gillespie MT, Milat F. Bone disease in thalassemia: a molecular and clinical overview. Endocrine Reviews. 2016;37(4):320-46.
21. Abd Allah N, Galal A, Hamed HM, et al. The roles of Osteoprotegerin and rankl in pathogenesis of Osteoporosis in Egyptian beta-thalassemia major patients. J Appl Sci Res. 2010;6(8):937-42.
22. Abd El-Moneim ES, Zolaly MA, Al-Hawsawi ZM, Abdelmoneim AA, Abosdera MM. Age-related changes in biochemical bone profile in thalassemic children. Pediatr Neonatol. 2018;59(2):189-97.
23. Maryam KA, Arshad NA; Estimation of osteoprotgrin level in B thalassemia patient. AIP Conference Proceedings. 2019;2144 (1).
24. Alfaqih MA, Bashir N, Saadeh R, Khader Y, Barqawi M, Alqudah, S. Dysregulation of the RANKL/RANK/OPG axis in thalassemia intermedia patients. BMC research notes. 2018;11(1):5.
25. Hashemieh M, Azarkeivan A, Radfar M, et al. Prevalence of Osteoporosis among thalassemia patients from Zafar adult thalassemia clinic, Iran. Iranian Journal of Blood and Cancer. 2014; 6(3):143-8.
26. Asadov C, Gafarova S, Aliyeva G. Bone abnormalities in B-Thalassemia. Intermedia Blood Res Transfus J. 2017;1(1):1-3.