The association of growth differentiation factor-15 levels and osteoporosis in patients with thalassemia

Nattiya Teawtrakul (nattiya@kku.ac.th)
Khon Kaen University
Siriyakorn Chansai
Khon Kaen University
Supawadee Yamsri
Khon Kaen University
Kanchana Chansung
Khon Kaen University
Chinadol wanitpongpun
Khon Kaen University
Theerin Lanamtieng
Khon Kaen University
Pisa Phiphitaporn
Khon Kaen University
Supan Fucharoen
Khon Kaen University
Chatlert Pongchaiyakul
Khon Kaen University

Research Article

Keywords: Growth differentiation factor 15, Osteoporosis, Thalassemia

Posted Date: February 28th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1352391/v1

Abstract

Introduction: Ineffective erythropoiesis is a significant risk factor for osteoporosis in individuals with thalassemia. Growth differentiation factor-15 (GDF15), a biomarker of ineffective erythropoiesis, has previously been found to be elevated in thalassemia patients. The purpose of this study was to examine the association between GDF15 levels and osteoporosis in patients with thalassemia.

Methods: A cross-sectional study was conducted in 130 adult patients with thalassemia at Srinagarind Hospital, Thailand. Bone mineral density (BMD) at the lumbar spine was evaluated by dual-energy X-ray absorptiometry (DXA), and with a Z-score of less than -2.0 SD was defined as osteoporosis. GDF-15 was measured using the enzyme-linked immunosorbent assay (ELISA). Logistic regression analysis was used to examine the associated factors with the development of osteoporosis. Receiver operator characteristic (ROC) curve analysis was used to estimate the threshold of GDF15 in predicting osteoporosis.

Results: Osteoporosis was detected in 55.4% (72/130) of the patients. Advanced age and high GDF15 levels were positively associated with osteoporosis, while an increased hemoglobin level was negatively associated with osteoporosis in patients with thalassemia. In this study, the GDF15 level's ROC demonstrated a good performance in predicting osteoporosis (AUC=0.77). Using a cut-off point of GDF15 ≥ 20,000 pg/mL, the sensitivity and specificity were 90.2% and 60.3%.

Conclusions: The prevalence of osteoporosis is high among adult thalassemia patients. Age and high GDF15 levels were significantly associated with osteoporosis in this study. A higher hemoglobin level is associated with a lower risk of osteoporosis. The findings of this study suggest that GDF15 could be used as a predictive biomarker for osteoporosis in patients with thalassemia. Adequate red blood cell transfusions and suppression of GDF15 function may be beneficial in preventing or delaying osteoporosis progression.

Introduction

Thalassemia bone disease (TBD) is a common disease-related complication in patients with thalassemia. Because of advances in thalassemia management, such as red blood cell (RBC) transfusion, iron chelation therapy, and novel treatments that have resulted in increased life expectancy, the prevalence of TBD appears to be increasing and affects the majority of adult thalassemia patients.

Even though TBD encompasses a wide range of bone complications, osteoporosis is the most common bone complication in thalassemia patients, with a prevalence of 40–60% of adult patients with thalassemia major worldwide increasing to 90% in elderly or inadequately treated individuals, and representing an important cause of morbidity in adult patients with thalassemia.[1–3] The pathogenesis of osteoporosis in thalassemia is multifactorial in nature. It includes bone marrow expansion, iron overload and iron chelation therapy, endocrinopathies, such as hypogonadism, hypothyroidism, and growth failure, etc., vitamin deficiency, nutritional imbalance and a lack of physical activity. [4,5] It is widely accepted, however, that ineffective erythropoiesis (IE) and erythroid expansion in the bone marrow resulting in

decreased trabecular bone tissue and cortical thinning, are directly involved in the pathophysiology of osteoporosis.[6–8]

Growth differentiating factor 15 (GDF15), a divergent member of the transforming growth factor-β (TGF-β) superfamily, has been identified as a hepcidin-suppression factor that is expressed at a high level in thalassemia patients with IE. [9,10] The connection between GDF15 levels and bone metabolism is still unclear. Hinoi *et al* found that GDF15 stimulated osteoclast differentiation resulted in bone loss in mice [11], as well as, Westhrin *et al* demonstrated that GDF15 inhibited osteoblast differentiation and decreased the expression of bone formation markers. [12] Wakchoure *et al* found that GDF15 stimulated osteoblast differentiation *in vitro.*[13] In addition, low hepcidin function contributes to iron overload-induced osteoporosis by inhibiting osteoblast formation and increasing osteoclast activity. [14]

There have been few clinical studies that have investigated the relationship between GDF15 and bone parameters. Despite this paucity, previous studies have found a negative relationship between GDF15 and BMD in postmenopausal Chinese and Korean women. [15] Furthermore, GDF15 levels were significantly higher in thalassemia patients compared to healthy individuals [9,16], and they were also related to clinical severity in patients with non-transfusion-dependent thalassemia. [6] Due to the scarcity of research on the effects of GDF15 on bone metabolisms in patients with thalassemia, the purpose of this study was to examine the relationship between the GDF15 levels and osteoporosis in adult thalassemia patients.

Patients And Methods

A cross-sectional study was conducted at Srinagarind Hospital, Khon Kaen University, in adult patients with thalassemia aged 18 years or older. Medical history taking and physical examinations were performed by hematologists. Clinical characteristics and laboratory data that were identified in the literature as potential risk factors for thalassemia-associated osteoporosis (TAO) were collected. Endocrinopathies were defined as having at least one of the following endocrine disorders: 1) diabetes mellitus, 2) hypothyroidism, 3) growth retardation, and 4) hypogonadism. Bone mineral density (BMD) at the lumbar spine was measured by dual-energy x-ray absorptiometry or DXA (Lunar prodigy model, GE Lunar). Osteoporosis was defined as a Z-score of less than − 2.0 SD in this study. All laboratory tests were performed before patients received a red blood cell transfusion. GDF-15 levels were measured using enzyme-linked immunosorbent assay (ELISA) kits; ab155432-GDF-15 Human ELISA (Abcam, Cambridge, UK).

All participants gave written informed consent to participate and publish the data. The research protocol was approved by the ethical committee of Khon Kaen University Thailand (HE641608) according to the Declaration of Helsinki.

Statistical analysis

Continuous results are presented as mean and standard deviations (SD). Categorical results are reported as numbers and percentages. Logistic regression analyses were used to investigate the relationship between variables and osteoporosis with a significance level of $P < 0.05$. The receiver-operating characteristics (ROC) curve was used to determine the area under the ROC curve and define the optimal cut-off point. The sensitivity and specificity were calculated using a 2x2 table. The STATA program version 10 was used for all statistical analyses (StataCorp, College Station, TX).

Results

A total of 130 thalassemia patients were enrolled, with a mean age of 33.2 years. The average hemoglobin (Hb) and serum ferritin levels were 7.5 g/dL and 1492.6 ng/mL. The most common thalassemia type in this cohort was hemoglobin E/Beta-thalassemia (87, 66.9%). Splenectomy was performed on nearly half of the patients (56 patients, 43.1%). Five patients (3.8%) were in premature menopause, and six patients (4.6%) smoked. The summary of baseline characteristics is shown in Table 1.

Table 1
Baseline characteristics of study patients

Characteristics	Patients (n = 130)
Mean age ± SD, years	33.2 ± 13.1
Mean hemoglobin ± SD, g/dL	7.5 ± 1.1
Mean serum ferritin ± SD, ng/mL	1492.6 ± 1282.9
Mean GDF15 level ± SD, pg/mL	42743.7 ± 34808.8
Gender, n (%)	
Female	78 (60)
Male	52 (40)
Osteoporosis, n (%)	
No	58 (44.6)
Yes	72 (55.4)
Splenectomy, n (%)	
No	74 (56.9)
Yes	56 (43.1)
Smoking, n (%)	
No	124 (95.4)
Yes	6 (4.6)
Menopause, n = 78 (%)	
No	73 (93.6)
Yes	5 (6.4)
Endocrinopathies, n (%)	
No	111 (85.4)
Yes	19 (14.6)
Transfusion dependent thalassemia, n (%)	
No	74 (56.9)
Yes	56 (43.1)
Thalassemia group, n (%)	
- β-thalassemia/Hb E	87 (66.9)

Characteristics	Patients (n = 130)
- Non-deletional Hb H disease	25 (19.2)
- Deletional- Hb H disease	18 (13.9)

Seventy-two patients (55.4%) had osteoporosis. More than half of the patients were non-transfusion-dependent thalassemia (74, 56.9%). GDF15 levels were significantly higher in thalassemic patients with osteoporosis compared to those without osteoporosis (Fig. 1). Univariate analysis is shown in Table 2. There were four risk factors that statistically associated with osteoporosis including; 1) age per 10-year increase (odds ratio [OR] = 1.5, 95%CI 1.1–2.1, p-value = 0.004), 2) GDF15 levels per 10,000 pg/mL increase (OR = 1.3, 95%CI 1.2–1.5, p-value < 0.005), 3) endocrine disorders (OR = 3.5, 95%CI 1.1–11.3, p-value = 0.03), and 4) hemoglobin levels (OR = 0.5, 95%CI 0.4–0.8, p-value = 0.001). Table 3 shows the multivariate analysis of risk factors for osteoporosis. Advanced age (per 10-year increase) and high GDF15 levels (per 10,000 pg/mL increase) were remained statistically significant increased risk of osteoporosis (adjusted odds ratio [AOR] = 1.6, 95%CI 1.1–2.2, p-value = 0.009 and AOR = 1.3, 95%CI 1.1–1.5, p-value = 0.001). On the contrary, high hemoglobin levels decreased the risk of osteoporosis (AOR = 0.6, 95% CI: 0.4–0.9, p-value = 0.015). The receiver-operating characteristics (ROC) curve of the GDF15 levels and osteoporosis is shown in Fig. 2. The area under the ROC curve was 0.77 (95% CI 0.6–0.8, p-value = 0.04). The ROC curve revealed that 20,000 pg/mL was an optimal cut-off point. Using this cut-off point, the sensitivity and specificity of the GDF15 for predicting osteoporosis were 90.2% and 60.3%.

Table 2
Univariate analysis of factors associated with osteoporosis

Variables	OR	95% CI of OR	p-value
Age (per 10-year increase)	1.5	1.1–2.1	0.004
Hemoglobin (g/dL)	0.5	0.4–0.8	0.001
GDF15 (per 10,000 pg/mL increase)	1.3	1.2–1.5	<0.005
Menopause	3.3	0.4–30.8	0.2
Endocrinopathies	3.5	1.1–11.3	0.03
Smoking	4.2	0.5–37.5	0.19
Abbreviation: GDF15 = growth differentiation factor-15, OR = odds ratio, 95% CI = 95% confidence interval			

Table 3
Multivariate analysis of factors associated with osteoporosis

Variables	AOR	95% CI of AOR	p-value
Age (per 10-year increase)	1.6	1.1–2.3	0.005
Hemoglobin (g/dL)	0.6	0.4–0.9	0.017
GDF15 (per 10,000 pg/mL increase)	1.3	1.1–1.5	0.001
Menopause	1.6	0.1–19.2	0.7
Endocrinopathies	2.7	0.7–10.6	0.1
Smoking	2.4	0.2–25.8	0.5
Abbreviation: GDF15 = growth differentiation factor-15, AOR = adjusted odds ratio, 95% CI = 95% confidence interval			

Discussion

More than half of the patients in this cohort had thalassemia-associated osteoporosis (55%). The prevalence of osteoporosis in this study was comparable to the previous studies in adult thalassemia patients.[17–19] Advanced age and high GDF15 levels were associated with an increased risk of osteoporosis, whereas high pre-transfusion hemoglobin levels were associated with a decreased risk of osteoporosis. Because osteoporosis is a time-dependent complication, aging is well established as a significant risk factor. This study discovered that a 10-year increase in age significantly increased the risk of osteoporosis, with an AOR of 1.6 (p-value = 0.005).

Growth differentiating factor-15 is a biomarker of ineffective erythropoiesis found in high levels in thalassemia patients. Furthermore, high GDF15 levels are also associated with clinical severity in NTDT patients. [6] GDF15 levels were found to be significantly higher in thalassemia patients with osteoporosis compared to those without osteoporosis. Moreover, high GDF15 levels (per 10,000 pg/mL increase) in these thalassemia patients were associated with an increased risk of osteoporosis (AOR = 1.3, p-value = 0.001). This finding could be correlated to high GDF15 levels in these patients, which suppresses hepcidin functions. Xu *et al.* found that hepcidin protects against iron overload-induced bone loss. Low hepcidin levels contribute to osteoporosis by inhibiting osteoblast functions and increasing osteoclast activity, resulting in low bone mineral density. [14] As a result, high GDF15 levels had a negative correlation with bone mass in thalassemia patients. High GDF15 levels may represent the underlying ineffective erythropoiesis in individual thalassemia patients. Ineffective erythropoiesis may be one of the primary causes of osteoporosis in thalassemia patients. The ROC in Fig. 2 demonstrated that GDF15 levels performed well in predicting the development of osteoporosis. Using the cut-off level of GDF15 ≥ 20,000 pg/mL, the sensitivity and specificity were 90.2% and 60.3% in predicting osteoporosis. This finding suggested that GDF15 levels in thalassemia patients could be used as one of the predictive biomarkers of osteoporosis.

Anti-GDF15 antibodies inhibit osteoclast activity, preventing bone loss, according to a preclinical study. [11] Sotatercept is a soluble activin-receptor type 2A (ActRIIa) IgG-Fc fusion protein. It works as a ligand trap for TGF-β superfamily ligands including GDF15. Sotatercept has been shown in clinical studies to increase biomarkers of bone formation and hemoglobin levels in postmenopausal women. [20,21] Similarly, Luspatercept, a soluble activin-receptor type 2B (ActRIIb) IgG-Fc fusion protein, acts as a ligand trap for TGF-β superfamily ligands, inhibiting the Smad-signaling pathway. A recent phase III study of Luspatercept in patients with transfusion-dependent beta-thalassemia showed that it can increase Hb levels while decreasing the red blood cell transfusion burden. [22] The activin-receptor ligand traps may not only raise Hb levels but also improve bone metabolism in thalassemia patients. Further research into the role of these agents in preventing or improving osteoporosis in thalassemia patients is needed.

Chronic anemia in thalassemia patients is caused by ineffective erythropoiesis, chronic hemolysis, and decreased globin production. As a result, chronic anemia causes bone marrow expansion and low bone mass in these patients. This study found that increasing Hb levels reduces the risk of osteoporosis in thalassemia patients (AOR = 0.6, p-value = 0.017). This finding suggests that adequate red blood cell transfusion may help thalassemia patients avoid bone loss and the development of osteoporosis.

Endocrine disorders are well established as a significant risk factor for osteoporosis and fractures in the individual with thalassemia.[3,23–25] The univariate analysis showed that endocrinopathies are strongly associated with osteoporosis (OR = 3.5, p-value = 0.03). In multivariate analysis, however, endocrine disorders did not show a statistically significant with osteoporosis. These findings may be due to the majority of the enrolled patients being young adults, and the number of endocrine disorders in this cohort was small. Other risk factors that have been indicated as risk factors for osteoporosis, i.e., menopause, and smoking were not significantly associated with osteoporosis in this study due to the limited number of events.

The current finding must be interpreted in the context of several potential strengths and weaknesses. Endocrine disorders were diagnosed in this study, primarily based on medical history and physical examinations, and completed investigations of hypogonadism and growth retardation were not performed in all cases. As a result, the prevalence of these endocrine disorders could be understated. To the best of the authors' knowledge, this was the first study to show the relationship between GDF15 levels and osteoporosis in adult thalassemia patients. This study enrolled a variety of thalassemia genotypes with different clinical severities. Further research in distinct genotypes of thalassemia, however, is needed to determine the utility of this biomarker in predicting osteoporosis.

Finally, thalassemia-associated osteoporosis is common in adult thalassemia patients. Advanced age and high GDF15 levels were both significant risk factors for osteoporosis. A higher hemoglobin level is associated with a lower risk of osteoporosis. These findings could have clinical implications for using the GDF15 levels as a predictive biomarker to assess the risk of osteoporosis in thalassemia patients. Furthermore, adequate RBC transfusion and GDF15 inhibition may prevent or improve this complication.

Declarations

Acknowledgements:

The authors would like to thank Emeritus Professor James A. Will, University of Wisconsin-Madison, for helping in preparing the manuscript via publication clinic of Khon Kaen University, Thailand. This work was supported by Khon Kaen University, Khon Kaen, Thailand. SF is a recipient of the Thailand Research Fund (TRF) Research Team Promotion Grant (RTA) of the Thailand Science Research and Innovation (TSRI), Thailand (Contract ID RTA6280005).

Authors' Contributions:

NT designed the study, collected clinical data, performed statistical analysis, wrote the first draft of the manuscript, and made the final revision of the manuscript; SC performed experimental assays; SY helped in sample preparation and experimental assays; KC, CW, TL, and PP collected clinical data; GF and SF generally supervised the study; CP generally supervised the study, and made the final revision of the manuscript. All authors approved the final version of the manuscript and the submission to Scientific Reports journal.

Funding:

This study received grant support from the Thailand Research Fund (TRF) Research Team Promotion Grant (RTA) of the Thailand Science Research and Innovation (TSRI), Thailand (Contract ID RTA6280005) and the Faculty of Medicine, Khon Kaen University.

Data availability:

The data that support the findings of this study are available on request from the corresponding author [N.T.]. The data are not publicly available due to "them containing information that could compromise research participant privacy/consent"

Compliance with ethical standards

Conflict of Interest Disclosures:

The authors report no conflicts of interest.

Ethics approval and consent:

The research protocol was approved by the Ethics Review Board of Human Research of the Faculty of Medicine Khon Kaen University Thailand (HE641608) and performed following the Declaration of Helsinki. All participants gave consent to participate and publish the data.

References

1. Toumba, M. & Skordis, N. Osteoporosis syndrome in thalassaemia major: an overview. *Journal of Osteoporosis* **2010**,(2010).
2. Terpos, E.& Voskaridou, E.Treatment options for thalassemia patients with osteoporosis.*Annals of the New York Academy of Sciences* **1202**, 237–243(2010).
3. Baldini, M.*et al.* Endocrine and bone disease in appropriately treated adult patients with beta-thalassemia major.*Annals of Hematology* **89**, 1207–1213(2010).
4. Fung, E.B.*et al.* Relationship between Chronic Transfusion Therapy and Body Composition in Subjects with Thalassemia.*J Pediatr* **157**, 641-647.e2(2010).
5. Fung, E.B.*et al.* Characterization of low bone mass in young patients with thalassemia by DXA, pQCT and markers of bone turnover.*Bone* **48**, 1305–1312(2011).
6. Musallam, K.M.*et al.* Levels of growth differentiation factor-15 are high and correlate with clinical severity in transfusion-independent patients with β thalassemia intermedia.*Blood Cells Mol Dis* **47**, 232–234(2011).
7. Rivella, S.The role of ineffective erythropoiesis in non-transfusion-dependent thalassemia.*Blood Rev.* **26 Suppl 1**, S12-15(2012).
8. Vichinsky, E.P.The morbidity of bone disease in thalassemia.*Ann N Y Acad Sci* **850**, 344–348(1998).
9. Tanno, T.*et al.* High levels of GDF15 in thalassemia suppress expression of the iron regulatory protein hepcidin.*Nat.Med.* **13**, 1096–1101(2007).
10. Porter, J.B.*et al.* Iron overload across the spectrum of non-transfusion-dependent thalassaemias:role of erythropoiesis, splenectomy and transfusions.*Br J Haematol* **176**, 288–299(2017).
11. Hinoi, E.*et al.* Positive regulation of osteoclastic differentiation by growth differentiation factor 15 upregulated in osteocytic cells under hypoxia.*J Bone Miner Res* **27**, 938–949(2012).
12. Westhrin, M.*et al.* Growth differentiation factor 15(GDF15)promotes osteoclast differentiation and inhibits osteoblast differentiation and high serum GDF15 levels are associated with multiple myeloma bone disease.*Haematologica* **100**, e511-514(2015).
13. Wakchoure, S.*et al.* Expression of macrophage inhibitory cytokine-1 in prostate cancer bone metastases induces osteoclast activation and weight loss.*Prostate* **69**, 652–661(2009).
14. Xu, Z.*et al.* The regulation of iron metabolism by hepcidin contributes to unloading-induced bone loss.*Bone* **94**, 152–161(2017).
15. Li, W.*et al.* Relationship between GDF15 level and bone metabolism in postmenopausal Chinese women.*Gynecol Endocrinol* **36**, 714–717(2020).

16. Gardenghi, S.*et al.* Hepcidin and Hfe in iron overload in beta-thalassemia.*Ann.N.Y.Acad.Sci.* **1202**, 221–225(2010).
17. Shamshirsaz, A.A.*et al.* Metabolic and endocrinologic complications in beta-thalassemia major:a multicenter study in Tehran.*BMC Endocr Disord* **3**, 4(2003).
18. Jensen, C.E.*et al.* High prevalence of low bone mass in thalassaemia major.*Br.J.Haematol.* **103**, 911–915(1998).
19. Vogiatzi, M.G.*et al.* Low bone mineral density in adolescents with beta-thalassemia.*Ann.N.Y.Acad.Sci.* **1054**, 462–466(2005).
20. Ruckle, J.*et al.* Single-dose, randomized, double-blind, placebo-controlled study of ACE-011(ActRIIA-IgG1)in postmenopausal women.*J Bone Miner Res* **24**, 744–752(2009).
21. Sherman, M.L.*et al.* Multiple-dose, safety, pharmacokinetic, and pharmacodynamic study of sotatercept(ActRIIA-IgG1), a novel erythropoietic agent, in healthy postmenopausal women.*J Clin Pharmacol* **53**, 1121–1130(2013).
22. Cappellini, M.D.*et al.* A Phase 3 Trial of Luspatercept in Patients with Transfusion-Dependent β-Thalassemia.*N Engl J Med* **382**, 1219–1231(2020).
23. De Sanctis, V., Eleftheriou, A.& Malaventura, C.Prevalence of endocrine complications and short stature in patients with thalassaemia major:a multicenter study by the Thalassaemia International Federation(TIF).*Pediatr Endocrinol Rev* **2 Suppl 2**, 249–255(2004).
24. Anapliotou, M.L.*et al.* The contribution of hypogonadism to the development of osteoporosis in thalassaemia major:new therapeutic approaches.*Clin.Endocrinol. (Oxf)* **42**, 279–287(1995).
25. Fung, E.B.*et al.* Fracture prevalence and relationship to endocrinopathy in iron overloaded patients with sickle cell disease and thalassemia.*Bone* **43**, 162–168 (2008).

Figures

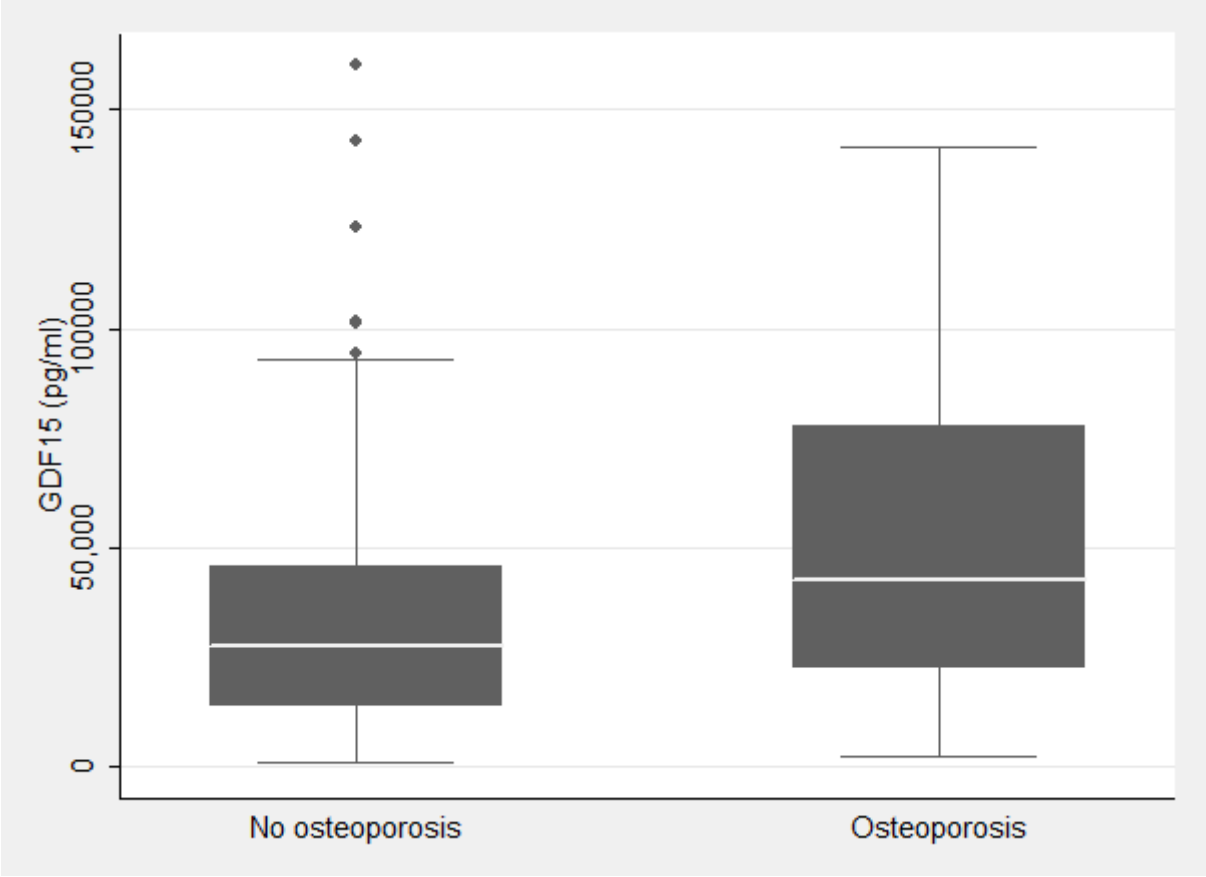

Figure 1

Graph box of the GDF15 levels and osteoporosis in patients with thalassemia.

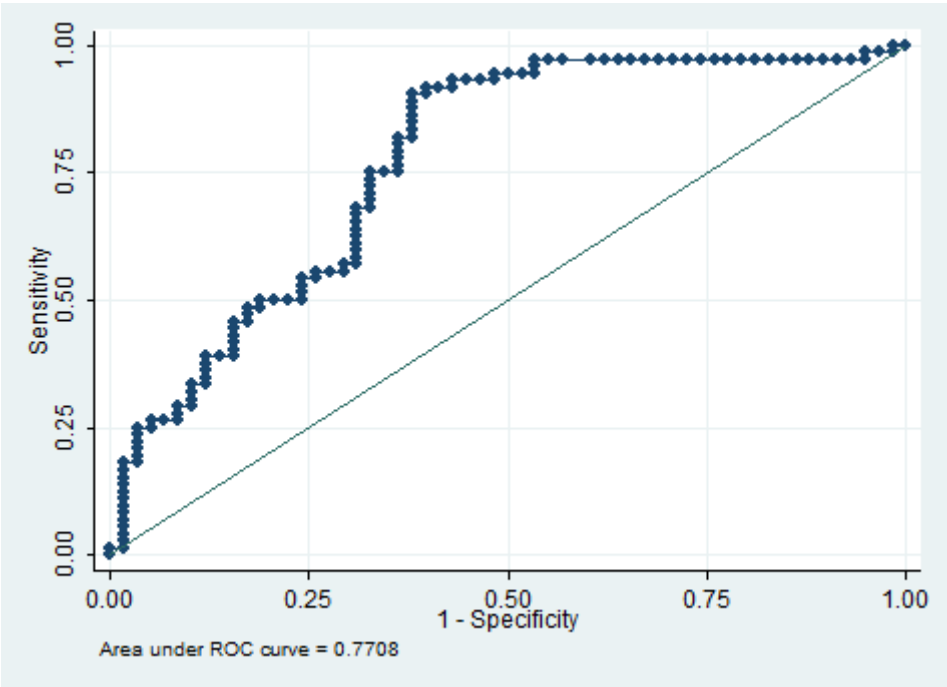

Figure 2

Receiver-operating characteristics (ROC) curve of the GDF15 levels and osteoporosis.