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Original article

Estimation of Osteoprotgrin Level in β Thalassemia Children

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Abstract

Background: Beta-thalassemia major (□-TM) is a genetic disorder characterized by impaired hemoglobin synthesis, leading to hemolytic anemia and associated complications. This study aimed to assess the diagnostic value of serum osteoprotegerin (OPG) as an early biomarker for osteoporosis in B-TM children. **Patients and Methods:** A cross-sectional study was conducted at the Pediatric Department of Benha University Hospital from January 2022 to December 2022. The study included 64 children aged 2 to 18 years, divided into two groups: group 1 consisted of 40 children with β-thalassemia (cases group), and group 2 consisted of 24 age and sex-matched healthy children (control group). Various parameters including Hb, OPG, vitamin D3, and ferritin were measured using enzyme-linked immune sorbent assay. **Result:** The study also examined the relationship between these biomarkers, Body Mass Index (BMI), and splenic status. The results showed significantly decreased levels of Hb, and vitamin D3 in β-thalassemic patients compared to the healthy group ($P<0.05$). Serum ferritin levels, on the other hand, were significantly increased in β-thalassemic patients ($P<0.05$). ELISA assay of serum OPG demonstrated significantly higher levels in thalassemia patients than in healthy controls. Notably, OPG levels showed a positive correlation with thalassemia and a negative correlation with serum vitamin D3. **Conclusion:** this study suggests that OPG can serve as a diagnostic marker for B-thalassemia, with a negative association observed between OPG and vitamin D levels.

Keywords: Beta-thalassemia major (□-TM); serum Osteoprotegerin (OPG); osteoporosis; Vitamin D3.

Introduction

Beta-thalassemia major (β-TM) is a genetic disorder characterized by impaired synthesis of hemoglobin, resulting in hemolytic anemia and a variety of associated complications [1]. Osteoporosis, a condition characterized by reduced bone mass and heightened bone fragility, is a common complication of beta-thalassemia major (β-TM). This condition increases the risk of fractures and other skeletal issues [2]. Timely intervention and prevention of complications in beta-

thalassemia major (β-TM)necessitate early diagnosis of osteoporosis. Serum Osteoprotegerin (**OPG**) has been identified as a potential biomarker for the diagnosis of osteoporosis in **B-TM** patients [3].

Osteoprotegerin (**OPG**) is a secretory glycoprotein produced by osteoblasts that circulates in the body. **OPG** plays a significant role in various physiological processes, particularly in the genesis of osteoclasts [4]. Osteoprotegerin (**OPG**) functions as a decoy receptor for **RANKL** (receptor activator of nuclear factor kappa-B ligand), which is a cytokine responsible for promoting bone resorption by activating osteoclasts [5]. Osteoprotegerin (**OPG**) acts as a regulator of bone metabolism by binding to **RANKL**, thereby inhibiting osteoclastogenesis and reducing bone resorption. This mechanism highlights the significance of **OPG** as an important factor in maintaining bone homeostasis and suggests its potential as a biomarker for osteoporosis [6].

Several studies have investigated the diagnostic value of serum **OPG** in β-TM patients. For example, serum Osteoprotegerin (**OPG**) levels were compared between **76** thalassemia children and **50** healthy children. The results revealed a significant increase in serum **OPG** levels among thalassemia children compared to healthy children. Furthermore, the study found a significant correlation between serum **OPG** levels and bone mineral density (**BMD**), an indicator of bone mass, in thalassemia patients. Based on these findings, the study concluded that serum **OPG** could serve as a valuable biomarker for early detection of osteoporosis in thalassemia patients [7].

Similarly, serum Osteoprotegerin (**OPG**) levels were evaluated in 56 beta-thalassemia major (**B-TM**) patients. The results revealed a significant elevation in serum **OPG** levels among patients with osteoporosis compared to those without osteoporosis. Based on these findings, the study suggested that serum **OPG** could serve as a valuable marker for the early detection of osteoporosis in **B-TM** patients [8].

Furthermore, the relationship between serum Osteoprotegerin (**OPG**) levels and bone turnover markers was investigated in patients with beta-thalassemia major (β-TM). The results demonstrated that serum **OPG** levels were significantly elevated in **B-TM** patients compared to healthy controls. Furthermore, the study revealed a positive correlation between serum **OPG** levels and bone resorption markers, while a negative correlation was observed with bone formation markers. Based on these findings, the study suggested that serum **OPG** could serve as a valuable biomarker for monitoring bone turnover and predicting the risk of osteoporosis in β-TM patients [9].

In addition to its diagnostic value, serum **OPG** may also have prognostic implications in B-TM patients. For example, serum Osteoprotegerin (**OPG**) levels were evaluated in **30** beta-thalassemia major (β-TM) patients. The results revealed that elevated serum **OPG** levels were significantly associated with a higher risk of fractures and other skeletal complications. Based on these findings, the study suggested that serum **OPG** could serve as a valuable biomarker for predicting the risk of skeletal complications in β-TM patients [10].

Although there are promising findings, additional research is required to determine the sensitivity and specificity of serum Osteoprotegerin (**OPG**) as a biomarker for osteoporosis in beta-thalassemia major (**B-TM**) patients. Furthermore, further studies are necessary to establish appropriate cutoff values for serum **OPG** levels and to assess the effectiveness of combining serum **OPG** with other biomarkers for the diagnosis and monitoring of osteoporosis in **B-TM** patients [11].

The aim of this work was to investigate the diagnostic value of serum Osteoprotegerin in B-TM patients, and assay as an early biomarker for osteoporosis.

Patients and Methods

The study design of this research is a cross-sectional comparative study. The study group included **64** children, divided into two groups. Group **1**, or the cases group, consisted of **40** children with **β-thalassemia** who were attending the hematology clinic at the Pediatric Department of **Benha University Hospital**. Group **2**, or the control group, consisted of **24** age and sex-matched healthy children who were attending the outpatient clinic of the **same hospital** (the Pediatric Department of Benha University Hospital).

Inclusion criteria for the study were all children aged between **2 to 18** years old who were attending the hematology clinic at the Pediatric Department of **Benha University Hospital** with β-thalassemia. The diagnosis of β-thalassemia was confirmed based on personal history, clinical symptoms, physical exam, and Hb electrophoresis.

Exclusion criteria for the study included all α-type thalassemia and thalassemia-sickle anemia, patients less than **2** years or older than **18** years, any unconfirmed blood transfusion-dependent children, and other diseases such as chronic liver disease (**CLD**), chronic kidney disease (**CKD**), and cardiovascular disease (**CVD**).

This study was conducted with due consideration for ethical principles and guidelines. Prior to the commencement of the study, the research design was reviewed and approved by the local ethics committee at the **Faculty of Medicine Benha University** from **January 2022 to December 2022**. Throughout the study, confidentiality and personal privacy of the participants were strictly observed and maintained at all levels of data collection and analysis. The guardians of the children were informed of their right to withdraw from the study at any time without any consequences. It is important to note that the data collected in this study was solely for the purpose of this research and will not be utilized for any other purpose.

Research ethics committee :{M.S. 22.10.2021}

Methodology

This study employed a comprehensive methodology to evaluate cases and controls. All cases underwent a thorough assessment, including complete history taking, clinical examination, and laboratory analysis. History taking gathered information on demographics, disease history, transfusion details, drug history, and splenectomy. Clinical examination involved a full examination of children, including general and local examinations. Laboratory analysis

encompassed routine investigations along with serum ferritin, vitamin D3, and human osteoprotegerin levels. The gathered data were analyzed to assess the diagnostic value of serum osteoprotegerin in beta-thalassemia patients. The methodology ensured a comprehensive evaluation, leading to reliable and robust results.

Blood samples collection

The blood samples were collected using sterile syringes, with a volume of 5 milliliters. Each sample was divided into two labeled tubes. The first group of tubes contained EDTA as an anti-coagulant to prevent blood clotting for physiological studies. The second group of tubes, without anti-coagulant, served as serum separation tube for subsequent biochemical and biomarker analysis. The samples were left at room temperature for 10 minutes to allow clotting, followed by centrifugation at 6000 rpm for 10 minutes. The resulting serum was separated and stored at -80 ºC until the laboratory analysis was performed for the study.

BMI (Body Mass Index)

The weight and height were measured using an electronic balance and height device, respectively. The BMI (Body Mass Index) was calculated using the formula: BMI = Weight [kg] / Height [m2].

Assessment of Serum vitamin D3 level.

Serum vitamin D3 levels were assessed using the Human (VD3) ELISA Kit from Sun Red Biotechnology, employing a double-antibody sandwich ELISA method (Kit number: 201-12-1547).

Calculation of the results:

The standard curve was plotted using the standard density as horizontal and OD value as vertical, and the sample density was determined by finding the corresponding density from the sample OD value using the sample curve or calculating the regression equation using the standard curve and the sample OD value.

Assessment of Serum Human (OPG) level.

In this study, the Human Osteoprotegerin (OPG) ELISA Kit (Sun Red Biotechnology) was used to quantitatively assess Human Osteoprotegerin (OPG) levels in samples via a double-antibody sandwich enzyme-linked immunosorbent assay (ELISA) approach.

Calculation of the results:

A standard curve was created on graph paper using standard density as horizontal and optical density (OD) value as vertical. Sample density was determined by finding the corresponding density on the sample curve or calculating with the straight line regression equation using the standard density and OD value, and the sample OD value.

Statistical analysis

The study data were analyzed using IBM SPSS (version 27) from Chicago, USA, with descriptive and analytical statistics.

tests, Mann-Whitney U tests, and Pearson's/Spearman's correlations to test associations and correlations between variables. The "r" values represented the strength of the correlation.

Univariate and multivariate logistic regression analyses identified risk predictors for binary categorical outcomes. The significance level for all tests was set at 0.05, and P-values determined the level at which the null hypothesis was rejected. P-values < 0.05 were significant, P-values ≥ 0.05 were non-significant, and P-values < 0.01 were highly significant.

Results

In table (1) The mean age of the cases group was 7.80 ± 1.50 years and there were 32.5% males and 67.5% females in the cases group with no significant difference as compared with the mean age of control group 7.2 ± 1.1 years and there were 41.7% males and 58.3% females, also there was no statistically significant difference in the mean BMI between the cases with β-thalassemia and the control group p-value > 0.05.

In the table (2) hemoglobin level was statistically significantly lower in the cases group as compared with the control (9.27 ± 0.82 g/dL and 11.77 ± 0.90 g/dL respectively). On the other hand, serum levels of ALT (42.03 ± 43.52 IU/L and 22.21 ± 7.23 IU/L respectively) and AST (40.13 ± 18.21 IU/L and 23.88 ± 7.50 IU/L respectively) were statistically Descriptive statistics included frequency tables for qualitative data and central indices with dispersion for quantitative variables. Analytical statistics involved Chi-square tests, independent sample t-significantly higher in the cases group as compared to the controls p-value < 0.05.

In table (3) the serum level of vitamin D showed highly statistically significant decreases in the cases group as compared with the healthy controls (mean level of 13.25 ng/ml vs 27.6 ng/ml respectively) (p<0.001).

In table (4) the serum level of osteoprotegerin showed highly statistically significant increase in the cases group as compared with the healthy controls (median level of 140.3 ng/l vs 58 ng/l respectively) (p<0.001).

In table (5) the results of correlation and linear regretion between OPG level and another paramters are indicated. there was non-statistically significant negative correlation between osteoprotgerin level and BMI.

Hb: As shown in figure (3), there was a statistically significant negative correlation between OPG and Hb

As shown in figure (4) there was a statistically significant positive correlation between osteoprotgerin level with SGPT.

As shown in figure (5) there was a statistically significant positive correlation between osteoprotgerin level with Serum Ferritin.

As shown in figure (6) there was a statistically significant negative correlation between osteoprotgerin level and Vit D3 level.

Table (1): Comparison of the study groups regarding demographic data

Characteristics		Group 1 (n=40)		Group 2 (n=24)		Test of sig.	p-value
Age (yrs) (mean ± SD)		7.80	±1.50	7.2	±1.1	1.7	0.09
Sex No. (%)	**Female**	27	67.5%	14	58.3%	0.5	0.5
	Male	13	32.5%	10	41.7%		
BMI (mean ± SD)		20.03	±3.66	19.77	±2.65	0.3	0.8

Table (2): Comparison of the study groups regarding laboratory findings

	Group 1 (n=40)		Group 2 (n=24)		T test	p-value
	Mean	SD	Mean	SD		
Hb (g/dL)	9.27	0.82	11.77	0.90	11.3	<0.001*
SGPT (IU/L)	42.03	43.52	22.21	7.23	2.8	0.007*
SGOT (IU/L)	40.13	18.21	23.88	7.50	4.9	<0.001*
S. Urea (mg/dL)	24.53	5.69	22.46	4.23	1.5	0.1
S. Creatinine (mg/dL)	0.56	0.18	0.52	0.12	0.9	0.3

Table (3): Comparison of the study groups regarding S. Vitamin D3

	Group 1 (n=40)		Group 2 (n=24)		T test	p-value
	Mean	SD	Mean	SD		
S. Vitamin D3 ng/ml	13.25	3.01	27.60	3.60	17.4	<0.001*

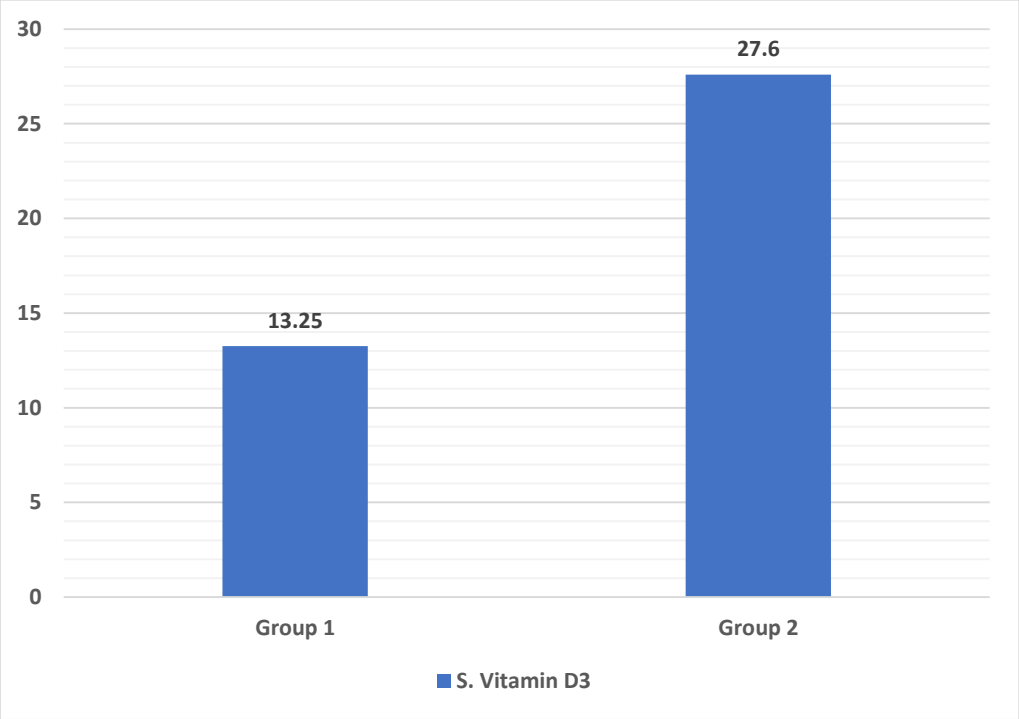

Figure (1): Study Groups regarding Vitamin D3

Table (4): Comparison of the study groups regarding S.OPG

	Group 1 (n=40)		**Group 2 (n=24)**		Mann-Whitney U	p-value
	Median	Range	Median	Range		
S.OPG ng/L	140.3	63.1-524	58.00	33.2-90.2	6.5	<0.001*

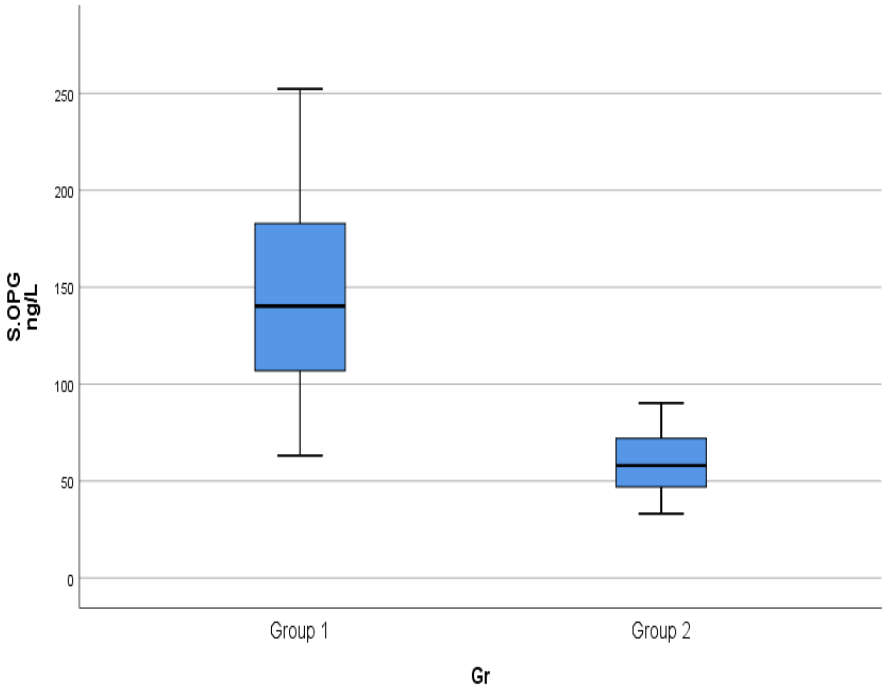

Figure (2): Study Groups regarding S.OPG

Table (5): Correlation between S.OPG (ng/L) and different variables

	r	p-value
Age	0.186	0.140
BMI	-0.107	0.402
Hb	-0.394	0.001*
SGPT	0.486	<0.001*
SGOT	0.411	0.001*
S. Urea	0.296	0.017*
S. Creatinine	0.068	0.595
S. Ferritin	0.275	0.028*
S. Vitamin D3	-0.415	0.001*
Duration of illness	-0.132	0.417

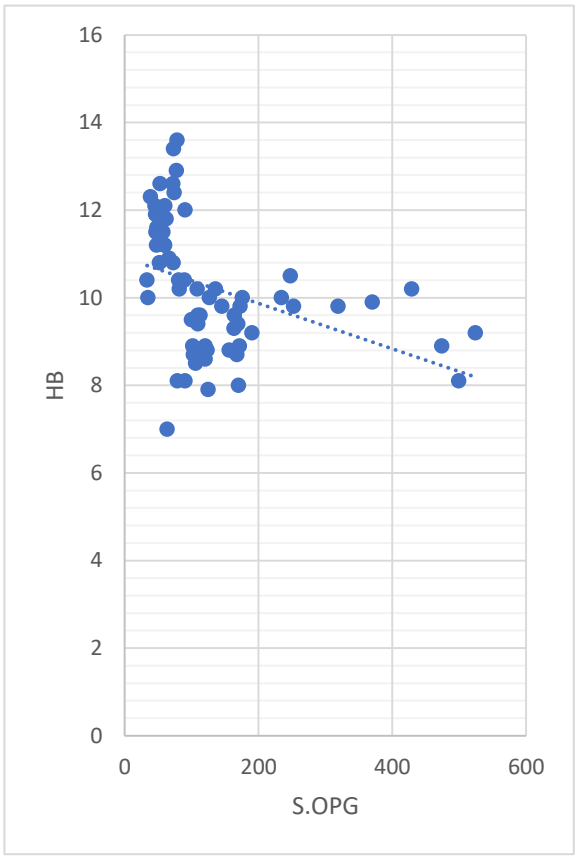

Fig. (3): correlation between S.OPG and HB

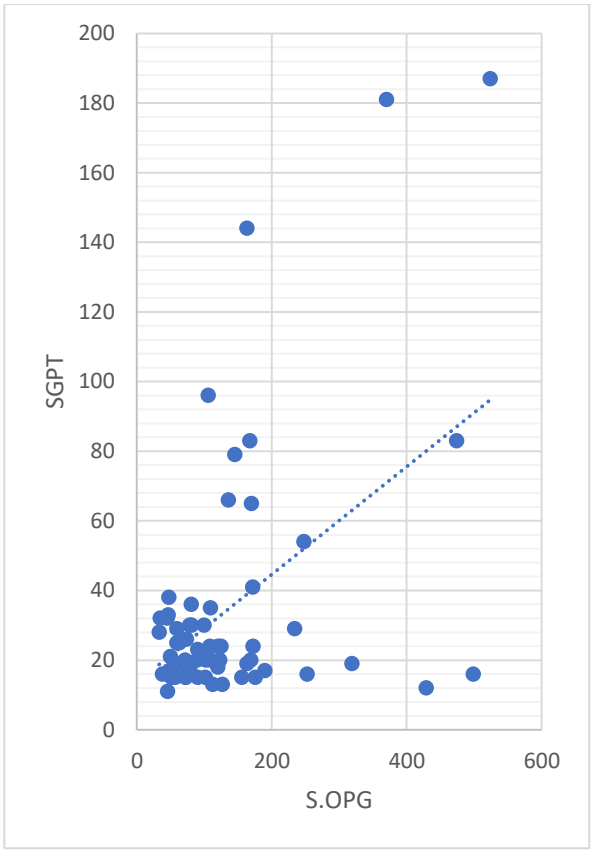

Fig.(4): correlation between S.OPG and SGPT

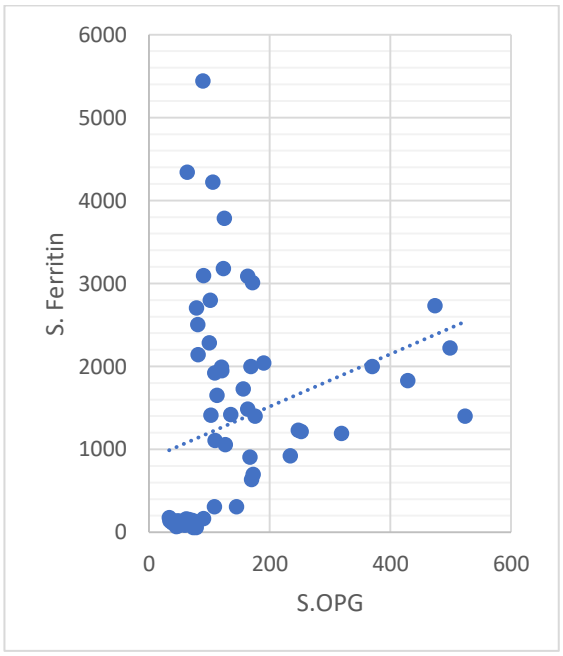

Fig.(5): correlation between S.OPG and S. Ferritin

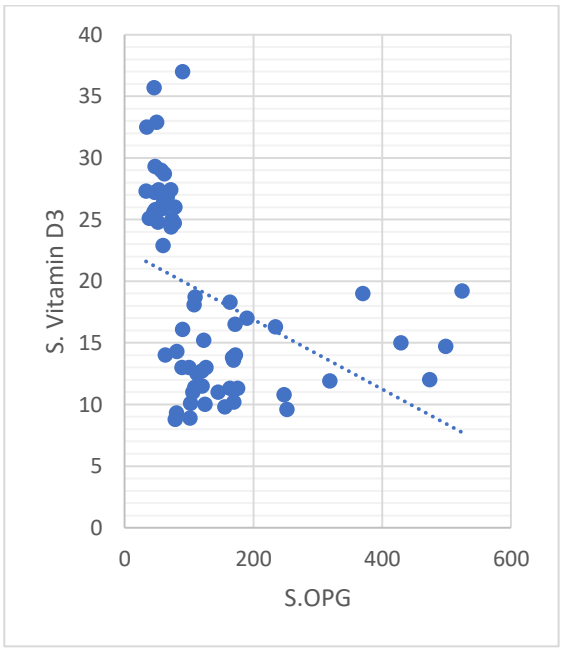

Fig.(6): correlation between S.OPG and S. Vitamin D3

Table (6): Univariate and Multivariate regression analyses of various variables for prediction of S.OPG.

	Univariate analysis		Multivariate analysis	
	β	p-value	β	p-value
Age	4.2	0.1		
Sex	31.6	0.3		
BMI	-3.6	0.4		
Hb	-29.9	0.001*	-9.4	0.5
SGPT	1.5	<0.001*	1.5	0.003*
SGOT	2.7	<0.001*	-0.6	0.6
S. Urea	3.2	0.02*	0.7	0.6
S. Creatinine	46.2	0.6		
S. Ferritin	0.03	0.03*	-0.005	0.7
S. Vitamin D3	-6.1	<0.001*	-4.1	0.2
Duration of illness	-3.5	0.4		
Frequency of blood transfusion	-0.4	0.9		

Discussion

Beta thalassemia major (**β-TM**) is a genetic disorder characterized by chronic hemolysis and ineffective erythropoiesis, which ultimately leads to severe anemia. This condition is well-established as a cause of skeletal morbidity and an increased risk of bone fractures among thalassemic patients [12].

The pathogenesis of β-TM-induced skeletal morbidity is multifactorial, involving bone marrow expansion, endocrine dysfunction, and iron overload as the major contributing factors. However, the precise mechanisms through which these factors lead to bone loss in beta thalassemia major are still not fully understood [13]. According to recent studies it has been suggested that Osteoprotegerin (**OPG**) and the receptor activator of nuclear factor-kappa B (**RANK**)/receptor activator of nuclear factor-kappa B ligand (**RANKL**) cytokines play a critical role in the regulation of bone resorption.

The **RANK/RANKL/OPG** pathway is recognized as the primary mediator of osteoclast proliferation and activation [14] [15].

The current study aimed to investigate the diagnostic value of **serum Osteoprotegerin** as a potential early biomarker for osteoporosis in patients with **β-TM**. The study was conducted as a cross-sectional case-control study over a **one-year** period at the **Pediatric Department of Benha University Hospitals**. The study included **64** children divided into **two** groups: the Cases Group (**40** children with β-thalassemia) and the Control Group (**24** age and sex-matched healthy children attending the same hospital's outpatient clinic). The cases group underwent history taking (including demographic data and history of present illness) and clinical examination. Laboratory investigations were also carried out, including complete blood count, liver enzymes, kidney function tests, serum ferritin level, serum vitamin D3 level, and estimation of human osteoprotgrin serum levels.

The study results showed no statistically significant differences between the studied

groups in age and sex, this agreed with the results of a previous study [16]. The study results showed no statistically significant differences between the studied groups in BMI, this agreed with the results of proved before[17]. However, the cases group had a statistically significantly lower hemoglobin level and higher levels of alanine transaminase and aspartate aminotransferase, in agreement with the recent Egyptian studies [18] [19]. There were no statistically significant differences between the groups in serum urea and creatinine levels reported [17].

The cases group also had statistically significantly lower levels of serum vitamin D-3. This came in agreement with the results of the recent Egyptian study 2022 [18] [19]. This could be due to hypoparathyroidism due to iron deposition in the parathyroid gland evidenced by elevated bone alkaline phosphatase in these patients [20]. Other factors also play a role including deficient calcium intake, IGF-I deficiency, delayed puberty and hypogonadism and decreased synthesis of 25-OH-D, due to hepatic siderosis [21]

Furthermore, the osteoprotgrin serum level was statistically significantly higher in the cases group, this agreed with [18]. This could be explained based on the results of [22] who showed that a correlation between high level of OPG and diastolic dysfunction and left ventricular heart disease [LVH] in thalassemia major due to induction of matrix metalo-proteinase 9 [MMP9] also by pathway of implicated iron mediated heart disease by increasing reactive oxygen species [ROS] and receptor activator of nuclear Kappa-B ligand also the RANKL/Osteoprotegrin axis mediated inflammation, They linked the increased OPG based on the inflammation and oxidative stress theory.

The study also found a statistically significant positive correlation between osteoprotgrin and Alanine transaminase, aspartate aminotransferase, urea, and ferritin [16] [18].

The study also found a statistically significant negative correlation between osteoprotgrin and hemoglobin and vitamin D-3 [23].

Univariate and multivariate linear regression analyses showed that hemoglobin, Alanine transaminase, aspartate aminotransferase, Urea, Ferritin, and Vitamin D3 were significant predictors for osteoprotgrin, while only Alanine transaminase was a significant predictor of osteoprotgrin in multivariate analysis.

Conclusion

Our study results indicate that serum osteoprotgrin may serve as a sensitive marker for diagnosing Beta thalassemia, and that Beta thalassemia is associated with lower levels of vitamin D-3, which may increase the risk of osteoporosis in affected individuals. As recommendations for future research, we suggest conducting larger studies with longer follow-up durations to further evaluate these observations. Additionally, further research should be conducted to investigate osteoprotgrin levels in other forms of thalassemia and hematological diseases.

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