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# Evaluation of Osteoprotgrin Level in Egyptian Beta Thalassemia Patients

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**Abstract:**

**Background**: Thalassemia is an inherited hematological disorder categorized by a decrease or absence of one or more of the globin chains synthesis. **Aim**: To investigate the diagnostic value of serum Osteoprotegerin in B-Thalassemia patients, and assay as an early biomarker for osteoporosis. **Patients and methods**: This case–control and comparative study was conducted on 80 subjects at hematology clinic Beni-Suef university hospital. It was held from It was held from June 2021 to June 2022. **Results**: There was a statistical significant high mean of Osteoprotegrin level with p-value <0.05 among thalassemia cases versus controls. Also significant lower mean in control group in comparison to both thalassemia subtypes (p-value <0.001), with no difference between the two thalassemia types (intermedia and major) (p-value 0.05). but it is higher in thalassemia major than thalassemia intermedia . Thalassemia cases show a positive correlation between age and hemoglobin levels, with a significant negative correlation between PLT and spine and femur scores. Ferritin levels are also negatively correlated with spine and femur scores p<0.05. **Conclusion**: Osteoporosis is a major issue in β-thalassemia patients, with osteoprotegrin (OPG) playing a crucial role in bone formation. Elevated OPG may compensate for osteoporosis in beta thalassemia patients, while reduced OPG may decrease osteoblast action. DEXA scans are the gold standard for early detection.

**Keywords: Osteoprotgrin level, Evaluation, Beta Thalassemia**

## 1. Introduction

Thalassemia is an inherited hematological disorder categorized by a decrease or absence of one or more of the globin chains synthesis. Beta-thalassemia is caused by one or more mutations in the beta-globin gene. The absence or reduced amount of beta-globin chains causes ineffective erythropoiesis which leads to anemia. People with thalassemia typically also require regular

chelation therapy to remove excess iron from their bodies. Different types of thalassemia exist, depending on the hemoglobin gene affected (alpha/beta) and severity, with beta-thalassemia major being the most common, but also one of the most severe types **(1)**.

Despite the improvement in survival rate in beta-thalassemia, many patients experience complications in several systems, including cardiopulmonary disorders, endocrine organ diseases, liver impairment,osteoporosis and thrombosis in different vascular beds **(2)**.

Osteoporosis is a frequent complication of beta thalassemia major with an etiology incompletely understood. Osteoporosis is resulted from too much breakdown in structure of bone, imbalance in bone formation, or an inadequate activity between the bone cells responsible for bone remodeling **(3)**.

The pathogenesis of osteoporosis in these patients is multifactorial. It includes endocrinological conditions, iron overload,bone marrow expansion, and genetic factors. Considering the pathogenesis of osteoporosis, osteoprotegerin (OPG) and receptor activator of nuclear factor kappa B ligand (RANKL) are essential mediators **(4)**.

The skeleton can be protected from excessive bone reabsorption by the secretion of OPG from osteoblasts and osteogenic stem cells and its binding to RANKL to inhibit the relations with RANK. Therefore, the RANKL/OPG ratio in bone marrow is thus an important determinant of bone mass in normal and disease states. OPG inhibits bone loss by acting as a decoy receptor to RANKL and preventing it from binding to RANK **(5)**.

This study aimed to investigate the diagnostic value of serum Osteoprotegerin in B-Thalassemia patients, and assay as an early biomarker for osteoporosis.

**Patients and methods**

This case–control and comparative study was conducted on 80 subjects at hematology clinic Beni-Suef university hospital. It was held from It was held from June 2021 to June 2022. patients group was further subdivided according to type of Beta thalassemia into 2 groups :Beta Thalassemia Major (20 patients) and Beta Thalassemia Intermedia (20 patients).

**Inclusion criteria:** 40 B thalassemia patients, B thalassemia patients with and without splenectomy, the ages of patients and healthy ranged from 13 to 35 years old, their diagnosis was based on Hb electrophoresis with HPLC and Patients on regular iron chelating agents.

**Exclusion criteria:** All α type of thalassemia and thalassemia-sickle anemia, Patients less than 13 and older than 35 years, postmenopausal females, endocrinal disorder affecting bone, Systemic disease affecting bone, Patients not on regular iron chelating agent, diabetic patients, patients with hypothyroidism, parathyroid dysfunction or liver disease, patients taking steroids, anticonvulsant therapies, vitamin D supplements, bisphosphonates excluded.

**Methods**

**All patients were subjected to the following:**

History taking, Clinical Examination, **investigations includes:** Laboratory investigations for patients and controls: (General Laboratory investigations) and **Specfic Laboratory investigations ) For patients :** Diagnosis with Thalassemia was confirmed by a pediatric hematologist based on having a hemoglobin level of 6–7 g/dl and maintaining this level without

regular blood transfusions for beta thalassemia intermedia and beta thalassemia major who required regular blood transfusions and by Hb electrophoresis and HPLC. (Data were collected from patients files).

**Radiological investigations for patients only**

The DEXA scan was used to measure bone mineral density (BMD) in the lumbar spine, left radius, and femur neck for all patients. The Z-scores were categorized into two subgroups of Beta Thalassemia: Normal (Z score > -1), Osteopenia (Z score between -1 and -2.5), and Osteoporosis (Z score < -2.5). The "T score" and "Z score" were calculated at these three sites after the DEXA scan. The T score is the number of standard deviations above or below the mean for a healthy 30-year-old adult, while the Z score is the preferred parameter in children.

**Ethical consideration:** Informed consent was taken from all the patients before the study and after ethical committee approval in Beni-Suef - Faculty of Medicine.

**Measurement of Osteoprotegrin**

**Sample collection and storage**

**Serum :** a serum separator tube was used and samples were allowed to clot for two hours at room temperature before centrifugetion for 20 minutes at approximately 1000 xg. The freshly prepared serum was Assayed immediately or stored samples in aliquid at-20°°C or-80°°C for later use. Repeated freeze/thaw cycles was avoided. **Reagent Preparation:** Before use, all kit components and samples were brought to room temperature, diluted the 25x wash buffer into 1x working concentration, and prepared a standard working solution. The standard was reconstituted with 1.0mL of Standard Diluent and kept at room temperature for 10 minutes. Seven tubes were prepared with 0.5mL Standard Diluent, and the diluted standard was used to produce a double dilution series. Biotinylated Antibody and Streptavidin-HRP were briefly spinned or centrifuged before use, and the TMB substrate was aspirated with sterilized tips.

**Assay Procedure**

A standard 1well blank was prepared by adding 100 uL of standard working solution or 100 µL of samples to 7 wells. The plates were covered with a plate sealer and incubated for 80 minutes at 37°C. After removing the liquid, aspirating the solution and washing with 200 uL of 1x Wash Solution, the plate was inverted and blotted against absorbent paper. Next, 100 µL of Biotinylated Antibody working solution was added, followed by 100 µL of Streptavidin-HRP working solution. The plate was then covered with a new plate sealer and incubated for 20 minutes at 37°C. The liquid turned blue with the addition of TMB Substrate Solution, and yellow with the addition of Stop reagent. The liquid was mixed by tapping the plate side and removed any water or fingerprints. The microplate reader was then run to conduct 450 nm measurements.

**Statistical Analysis**

Data collected and coded to facilitate data manipulation and entered into Microsoft Access and data analysis performed using the Statistical Package of Social Science (SPSS) software version 22 in windows 7 (SPSS Inc., Chicago, IL, USA). Simple descriptive analysis in the form of numbers and percentages of qualitative data, and arithmetic means as central tendency measurement, standard deviations as a measure of dispersion of quantitative parametric data. Tests

used were Independent samples t test, One-way ANOVA test, Chi square test and Bivariate Pearson correlation test. The P-value< 0.05 was considered as statistical significant

**Results**

**Table (1): Comparisons of sociodemographic characteristics (age and sex) in different study groups. (cases and healthy controls**)

| **Variables** | **Beta Thalassemia Cases** (N=40) | | **Healthy Control** (N=40) | | **P-value** | **Sig.** |
|---|---|---|---|---|---|---|
| **Age (years)** | | | | | | |
| Mean ± SD | 26.4±4.3 | | 25.03±3.6 | | 0.1 | NS |
| **Sex ; N(% )** | | | | | | |
| Female | 26 | 65% | 24 | 60% | 0.8 | NS |
| Male | 14 | 35% | 16 | 40% | | |
| Consanguinity **; N (**% **)** | +ve | 77.5% | | | NA | |
| | -ve | 22.5% | | | | |
| Family History**; N (**% **)** | +ve | 85% | | | NA | |
| | -ve | 15% | | | | |

NA: Not Applicable .

There was no statistical significant difference between cases and controls as regards age and sex distribution with p-value >0.05. (Table 1)

**Table (2): Comparisons of DEXA results in different Thalassemia types.**

| **Variables** | **Thalassemia intermedia** (N=20) | | **Thalassemia major** (N=20) | | **P-value** | **Sig.** |
|---|---|---|---|---|---|---|
| | **Mean** | **SD** | **Mean** | **SD** | | |
| **T-Score** | | | | | | |
| Radius | -1.4 | 1.9 | -4.3 | 1.4 | **<0.001** | **HS** |
| Spine | -0.92 | 1.4 | -2.6 | 1.3 | **<0.001** | **HS** |
| Femur | 0.09 | 1.2 | -1.1 | 1.2 | **0.002** | **HS** |
| **Z-score** | | | | | | |
| Radius | -1.4 | 1.9 | -4.3 | 1.4 | **<0.001** | **HS** |
| Spine | -0.88 | 1.3 | -2.5 | 1.1 | **<0.001** | **HS** |
| Femur | 0.15 | 0.94 | -1.1 | 0.93 | **<0.001** | **HS** |

There was a statistical significant lower mean of all DEXA (T and Z scores measures) among thalassemia major cases versus intermedia type with p-value <0.001.That mean that osteoporosis and bone affection more sever in beta thalassemia major. (Table 2)

**Table (3): Comparisons of BMD (Bone Mineral Density) in different Thalassemia types.**

| BMD (Bone Mineral Density) g/cm3 | Thalassemia intermedia (N=20) | | Thalassemia major (N=20) | | P-value | Sig. |
|---|---|---|---|---|---|---|
| | Mean | SD | Mean | SD | | |
| Forearm Radius | 0.77 | 0.16 | 0.52 | 0.12 | **<0.001** | **HS** |
| AP Spine | 1.1 | 0.16 | 0.88 | 0.17 | **0.001** | **HS** |
| LT Femur | 1.03 | 0.13 | 0.89 | 0.15 | **0.002** | **HS** |

There was a statistical significant lower mean of all BMD measures with p-value <0.001 among thalassemia major cases versus thalassemia intermedia,that mean that BMD was lower in thalassemia major cases. (Table 3)

**Table (4): Comparisons of osteoprotegrin level in different study groups.**

| Variables | Mean osteoprotegrin level | | P-value | Sig. |
|---|---|---|---|---|
| | Mean | SD | | |
| **Main groups** | | | | |
| Cases | 0.60 | 0.19 | **<0.001** | HS |
| Controls | 0.21 | 0.08 | | |
| **Subgroups** | | | | |
| Thalassemia intermedia | 0.58 | 0.21 | 0.5a | NS |
| Thalassemia major | 0.62 | 0.18 | **<0.001 b,c** | HS |
| Controls | 0.21 | 0.08 | | |

a: significance between thalassemia intermedia & major a:significance between thalassemia intermedia & control a:significance between thalassemia major & control

There was a statistical significant high mean of Osteoprotegrin level with p-value <0.05 among thalassemia cases versus controls. Also significant lower mean in control group in comparison to both thalassemia subtypes (p-value <0.001), with no difference between the two thalassemia types (intermedia and major) (p-value 0.05). but it is higher in thalassemia major than thalassemia intermedia . (Table 4)

**Table (5): Correlation between Osteoprotegrin level with age and laboratory investigations among Thalassemia cases.**

| Variables | Osteoprotegrin level | | |
|---|---|---|---|
| | R | P-value | Sig. |
| **Age (years)** | 0.08 | 0.5 | NS |
| **Hb** | -0.11 | 0.5 | NS |
| **TLC** | -0.13 | 0.4 | NS |
| **PLT** | -0.14 | 0.4 | NS |
| **S . iron** | -0.007 | 0.9 | NS |
| **S. ferritin** | -0.11 | 0.5 | NS |
| **TIBC** | -0.009 | 0.9 | NS |
| **TFS%** | -0.06 | 0.7 | NS |
| **TSH** | 0.11 | 0.5 | NS |
| **PTH** | -0.01 | 0.9 | NS |
| **Ca** | -0.03 | 0.8 | NS |

There was statistical no significant correlation with p-value >0.05 between Osteoprotegrin level and both age and all laboratory investigations among Thalassemia cases. (Table 5)

**Table (6): Correlation between Osteoprotegrin level and DEXA results (T and Z scores) and also with BMD levels among Thalassemia cases**

| Variables | Osteoprotegrin level | | |
|---|---|---|---|
| | R | P-value | Sig. |
| **DEXA T-score** | | | |
| Radius | -0.29 | 0.06 | NS |
| Spine | -0.17 | 0.3 | NS |
| Femur | -0.05 | 0.7 | NS |
| **DEXA Z-score** | | | |
| Radius | -0.30 | 0.06 | NS |
| Spine | -0.16 | 0.3 | NS |
| Femur | -0.07 | 0.7 | NS |
| **BMD** | | | |
| Forearm Radius | -0.29 | 0.07 | NS |
| AP Spine | -0.18 | 0.3 | NS |
| LT Femur | -0.007 | 0.9 | NS |

There was statistical no significant correlation between Osteoprotegrin level and DEXA results (T and Z scores) and also with BMD levels among Thalassemia cases P>0.05. (Table 6)

**Table (7): Correlation between DEXA scores and laboratory investigations among Thalassemia cases.**

| Variables | Age (yrs) | Hb | PLT | Ferritin | Transfertin saturation |
|---|---|---|---|---|---|
| | r(p-value ) | r(p-value ) | r(p-value ) | r(p-value ) | r(p-value ) |
| **DEXA T-score** | | | | | |
| Radius | **0.38(0.01*)** | **0.32(0.04*)** | -0.22(0.2) | -0.12(0.5) | -0.30(0.06) |
| Spine | 0.13(0.4) | 0.17(0.3) | **-0.47(0.002*)** | **-0.42(0.006*)** | **-0.48(0.002*)** |
| Femur | -0.004(0.9) | 0.27(0.08) | **-0.40(0.01*)** | -0.28(0.08) | **-0.40(0.01*)** |
| **DEXA Z-score** | | | | | |
| Radius | **0.38(0.01*)** | **0.32(0.04*)** | -0.22(0.2) | -0.12(0.4) | -0.30(0.06) |
| Spine | 0.16(0.3) | 0.13(0.4) | **-0.44(0.004*)** | **-0.38(0.01*)** | **-0.42(0.007*)** |
| Femur | 0.06(0.7) | 0.27(0.09) | **-0.39(0.01*)** | **-0.23(0.02*)** | **-0.39(0.01*)** |

Thalassemia cases show a positive correlation between age and hemoglobin levels, with a significant negative correlation between PLT and spine and femur scores. Ferritin levels are also negatively correlated with spine and femur scores $p<0.05$. (Table 6)

**Discussion**

The current study was a case control comparative study conducted on 40 patients having Beta Thalassemia (subdivided into two groups Beta Thalassemia Major = 20 and Beta Thalassemia Intermedia = 20) attending the Hematology Clinic-Beni Suef Univeristy Hospital-Egypt. and 40 subjects as healthy controls. To assess OPG level as abiomarker for osteoprosis . It was held from June 2021 to June 2022. Beta Thalassemic patients average age was 26.4±4.3 years old , and the average age of healthy controls was 25.03±3.6 years old with no statistical significant difference between cases and controls as regards age and sex distribution with p-value >0.05 . Female sex was predominant over male sex (26 females Vs. 14 males), parents consanguinity was detected in 77.5% (positive consanguinity) and other sibiling affection was reported in 85% of the studied BT patients, similar to our results, **Bejaoui & Guirat (6)** who documented that positive consanguinity was found in 244 among 324 (75.3%) of studied thalassemic patients .

As regards BMD by DEXA results (T-score) there was a statistical significant higher percentage of radius osteoporosis (90%) and osteopenia in spine (65%) among major thalassemia cases in comparison to thalasemia intermedia with radius osteoporosis (35%) and osteopenia in spine (30%) with no difference in femur T-score. Osteoporosis starts earlier in radius, so radius is affected earlier by osteoporosis.

As regards total DEXA diagnosis (T-score) there was a statistical significant higher percentage of osteoporosis (90%) and osteopenia (10%) among thalassemia major cases and percentage of osteoporosis (40%) and osteopenia (25%) among thalassemia intermedia cases ,so Osteoprosis is

more in BTM patients than BTI .There is (35%) of TI patients with normal DEXA results (T-score).No osteopenia or osteoporosis.

As regards BMD by DEXA results ( Z-score) there was a statistical significant higher percentage of radius osteoporosis (90%) and osteopenia in spine (70%) and femur (35%) among major thalassemia cases in comparison to thalasemia intermedia.

As regards total DEXA diagnosis (Z-score) there was a statistical significant higher percentage of osteoporosis (85%) and osteopenia (15%) among thalassemia major cases in agreement of **Samar Abbas Jaffri1 , et,al., (7).** who found that The DEXA profile showed that the BMD in the enrolled population (BTM) was collectively low. The mean T score of -3.17 ± 1.04 and the Z score of -3.06 ± 1.06. Of the total , 28 (77.7%) patients (most of them) were osteoporotic, while 8 (22.2%) had osteopenia.

Percentage of osteoporosis (35%) among thalassemia intermedia cases , osteopenia (35%) So Osteoprosis is more in BTM patients than BTI . and (30%) of TI patients with normal DEXA results (Z-score) .No osteopenia or osteoporosis.

Among thalassemia cases there was no statistical significant difference with p-value >0.05 in T-score DEXA results in different spelenctomy groups, but there was a statistical significant higher percentage of Osteoporosis diagnosis by Z-DEXA score among cases did spelenctomy, which may be due to increase severity and complications in cases who did spelenctomy,in agreement with our study **Khalid N.et,al.,. (8)**who detected association between splenectomy and low

bone mineral density and low Z-DEXA score, which may be due to the fact that ferritin was significantly elevated in patients with splenectomy and iron has direct toxic effects on osteoblasts.

In our study, there was a statistical significant lower mean of all BMD measures among thalassemia major cases versus intermedia , the lowest BMD is in forearm (Radius). with p-value <0.001. Generally there is decrease in BMD (Bone Mineral Density) among cases ( thalassemia major & thalassemia intermedia ) which is in agreement with **Adel Baghersalimi1,et,al. (9)** who found a high prevalence of BMD abnormality was noted in teenagers with TM **)** but he did not include patients with thalassemia intermedia in his study , in accordance with our study was **(7),** who found that the DEXA profile showed that the BMD in the BTM patients was collectively low.

When we measured OPG in cases there was a statistical significant high mean of Osteoprotegrin level with p-value <0.05 among males in Thalassemia cases than females as osteoporosis is more in females and males has elevated OPG which is protective against early osteoporosis ,in agreement of **Angelopoulos et al., (10)** OPG levels were also higher in males than females (2.73 ± 0.13 vs. 2.35 ± 0.14, respectively), but without statistical significance.

There was no statistical significant correlation between Osteoprotegrin level and all laboratory investigations (Hb,TLC, PLT , S. iron, S, ferritin ,TIBC,TFS% ,TSH , PTH and Ca ) among Thalassemia cases (thalassemia major & thalassemia intermedia) with p-value >0.05, in accordance with **Maryam Kadhim Al-Shemery,et,al. (11)** who found no significant correlation between osteoprotgrin and Hb level,iron &ferritin.

In our study there was a statistical significant high mean of Osteoprotegrin level among thalassemia cases both groups versus control with p-value <0.05 . Also significant lower mean in control group in comparison to both thalassemia subtypes (p-value <0.001), with no difference in OPG between the two thalassemia types (intermedia and major) (p-value 0.05). High OPG level may be explained as compensatory mechanism of Osteoprosis which occur in Thalassemic patient to increase osteoblast activity. In agreement with our study **Maryam Kadhim Al-Shemery,et,al. (11)** showed a significant increase of serum osteoprotgrin level in thalassemia patients than healthy controls, but he showed a significant increase of serum osteoprotgrin level in thalassemia intermedia male patients than thalassemia major male patients.

There was statistical significant positive correlation with p-value >0.05 between Osteoprotegrin level and number of affected joints (in DEXA scan) among Thalassemia cases, that means as OPG elevates ,the number of joints affected by osteopenia and osteoporosis increase.

There was statistical no significant correlation between Osteoprotegrin level and BMD levels among Thalassemia cases with p-value >0.05. Our data, in accordance with previous reports by **Szulc ,et ,al., (12)** who showed no correlation of OPG and BMD at any site of measurement. In agreement also with our results **Shahin Koohmanaee , et,al. (13)** who revealed that (BMD and Z – score) was not significantly associated with OPG level in all thalassemia cases. In agreement also with our study **Pietrapertosa , et al., (4)** who found that there was no significant relation between BMD and OPG in patients with osteopenia, but found that relation was significant in patients with osteoporosis and noted that with a unit increase in bone mineral density, the mean OPG decreased by 4.51 units. Inverse relation.

Among Thalassemia cases there was a statistical positive correlation with p-value <0.05 between each of age and hemoglobin level with Dexa (T and Z –scores) in radius, and a statistical significant negative correlation between PLT and both spine and femur (T and Z-DEXA scores).

In addition ferritin level was negatively correlated with spine ( T-DEXA score) and both spine and femur (T and Z –DEXA) scores. For transferin saturation there was a statistical significant negative correlation between it with both spine and femur ( T and Z DEXA )scores, against our study **Ali Reza Ansari-Moghada**,**et,al., (14)** who found no significant correlation between the hip and spine **Z-score** and the variables (age, hemoglobin, ferritin, calcium, phosphate, T3, T4, and TSH) with (P > 0.05).

## Conclusion

Osteoporosis is a significant issue in β-thalassemia patients, with osteoprotegrin (OPG) playing a crucial role in bone formation. Elevated OPG may compensate for osteoporosis and bone abnormalities in beta thalassemia patients, while reduced OPG may decrease osteoblast action. OPG may be used as a marker for osteoporosis and osteopenia in beta thalassemia patients, but there is no significant difference in OPG levels among Thalassemia cases. DEXA scans are currently the gold standard for early detection of osteopenia and osteoporosis.

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