# Correlation of β-Thalassemia Major Genotype and Bone Density in Beta-Thalassemic Patient.

by
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**Abstract**

**Background:** Certain genetic mutations in β-thalassemia result in total absence of β-globin chain production, which is signed as β° thalassemia. β+, on the other hand, denoted genetic alterations that allow for some β-globin synthesis. Patients require frequent iron-chelating agents to manage iron overload and blood transfusions on regular basis for anemia. Severe anemia beside extra iron accumulation have negative consequences such as retarded growth, liver, heart, and endocrine diseases, as well as negative impact on bone density.

**Aim of the work:** To record the correlation of β-thalassemia genotype and bone density that obtained by Dexa scan compared to the normal references.

**Methods:** This is a cross section study subjected to record the influence of β-thalassemia genotype on the incidence of low bone density scored Dexa scan with Z-scores results compared to the normal references in β thalassemia cases, it involved 50 cases that were selected by simple random method, registered & followed up at the Pediatric Hematology Unit of Zagazig University Hospital from may 2022 to April 2023.

**Results:** The most frequent bone consequence was low bone mineral density, while osteoprosis and osteopenia accounted in 34% and 28% of cases, respectively. Lower z-scores compared to standards accounted for 62% of cases while 38% of cases had normal values. The β°β° genotype had a significant lower z- scores compared to those with β°β+ and β+β+ genotypes.

**Conclusion:** The present study had proved that decreased bone density was common in β-thalassemia major cases with a clear correlation between its genotype and clinical disease progression as well as its severity.

**Keywords**
Z-score, Beta-globin ,Genotype ,Bone density

## INTRODUCTION

β-thalassemias are a group of recessively inherited hemoglobin disorders characterized by reduced synthesis of β-globin chain [1].

The degree of imbalance between α- and non-α-globin chains determines the clinical severity of β-thalassemia, which can range widely from asymptomatic to severe or even lethal forms [2].

More than 350 mutations that cause disease have been fully identified. The wide diversity side by side with phenotypic varieties of β-thalassemia alleles can lead to a multitude of distinct manifestations in the condition [3].

Thalassemia-causing mutations can impact any stage of the globin gene expression pathway. The most prevalent types result from mutations that cause abnormal mRNA precursor splicing or early mRNA translation termination. The resulting phenotype is a combination of the effects of B+, B++ thalassemia, which produces β-chain with a notable or mild decrease, and β0 thalassemia, which produces no B-globin gene [4].

Excessive iron overload as well as regular transfusions of blood can lead to a variety of medical issues, the most common ones being hepatic, cardiac, endocrine and bone problems [1].

In βTM cases, bone alterations are common and result from iron overload, iron-chelation therapy, nutritional deficiencies, sedentarism, and the hematological illness and its consequences. Osteoporosis sequelae, particularly long and vertebral bone fractures, are a major source of morbidity for these patients [1].

Osteopenia and osteoporosis are found in 40-50% of individuals with beta-thalassemia major; hence, osteoporosis can be regarded as a leading cause of co-morbidity in this population, which raises the risk of fracture considerably [6].

The collagen type Iα1 (COLIA 1) gene's polymorphism at the Sp1 location was discovered in about 30% of TM patients who were heterozygotes (Ss) and 4% who were homozygotes (SS) for the Sp1 polymorphism. Collagen type I is the main bone matrix protein. They claimed there were 2:1 more women than men. This indicates that male TM patients with the Sp1 mutation may experience severe hip and spine osteoporosis more commonly than those without this mutation [7].

Resorption was slightly elevated in beta TM patients. Baldini et al explain an interesting hypothesis that the chronic need for the production of blood cells may contribute to the etiology of osteoporosis by over stimulating the hematopoietic system, which in turn causes an increase in the number of osteoclasts and osteoblasts and accelerates bone turnover. However, it was speculated that elevated resorption may be the root of these patients' hypogonadism [8].

There is still much to learn about the pathogenesis of bone abnormalities in TM patients as it is still not clearly understood. These patients have an unbalanced bone mineral turnover with increased resorptive rates and suppressed osteoblast activity [9].

**Ethical Consideration:**

- Approval by the ethical committee of the Pediatric department at the Faculty of Medicine - Zagazig University .
- Participants in the study or their legal guardians gave their informed consent before enrollment in the study.
- All cases data was Confidentially ensured during all study steps.
- The recorded Cases and their legal guardians have the right to withdraw from the study at any time.
- There was no conflict of interest regarding the study or its publication.
- There is no financial support .

This study was authorized by an institutional review board and carried out in compliance with the Helsinki Declaration's ethical guidelines. Informed consent was provided by the study participants or their legal guardians.

**The calculation of sample size:**

The sample size is assumed that all cases met the inclusion and exclusion criteria will be then included. During the study period (12 months), 4-5 cases per month , 50 cases will be included as a comprehensive sample.

**Inclusion criteria:**

1- Children with the diagnosis of beta thalassemia major, based on conventional ,clinical and hematological criteria.

2- Both sexes was included.

3- Age range from 4 : 20 years.

**Exclusion criteria:** Any child with one or more of the following:

1. known bone and metabolic disease.
2. Hepatic and renal impairments.
3. Chronic hematological anemia.

**Study procedure:**

This study was a cross sectional study that was carried on fifty cases of Beta-thalassemia major that were selected by simple random method, who were recorded and followed up during the period from May 2022 to April 2023 at pediatric department of Zagazig University Hospital.

**All the studied cases were subjected to:**

**Complete history taking** including age, sex with special emphasis on the age at diagnosis, transfusion information (such as frequency and age at which transfusions started), and chelation data (such as type, compliance, and age of starting). , past surgical history and family history for cases of beta thalassemia major ).

**Physical examinations:**

**Including:** Anthropometric measurements such as height, weight, head circumference , and body mass index. Vital signs as pulse, respiratory rate, blood pressure and temperature.

**Systemic examination including**:

- Abdominal examination
- Cardiac examination
- Chest examination.
- Neurological examination.
- Assessment of pubertal status using the Tanner classification for both genders.

**laboratory investigations including**:

Complete blood count (Automated), serum ferritin (estimated by latex immune turbidimetric method), serum alkaline phosphatase (by spectrophotometer 5010 for chemical investigation), serum vitamin D (25 OH)(stat fax for Eliza test), calcium, phosphorus, Mg using Cornley AFT-C Electrolyte Analyzer. ESR (Westergren), CRP (Latex) and ASO level, fasting blood glucose, basal growth hormone, parathyroid hormone, thyroid-stimulating hormone (TSH), free T4 hormones such as testosterone (in boys), follicle-stimulating hormone (FSH), luteinizing hormone (LH), and estradiol (in girls) by immune-assay

**Imaging study:** including:

plain X-rays, and ultrasounds

DEXA scan with Z-scoring to assess bone mineral density. Echocardiography

**DNA sequencing** :

to determine and estimate the patient's genotype. using DNA sequencing techniques in Laboratory of Hemoglobinopathies (National health institute & Insurance department of Zagazig). **(University of Ulm, Ulm Germany)**

**STATISTICAL ANALYSIS**

In order to analyze the data , version 23.0 (SPSS Inc., Chicago, Illinois, USA). program was utilized. The data are presented as the mean ± standard deviation SD for quantitative variables, while numbers and percentages were used for qualitative variables. An independent Z-score and t-test were used to determine the difference in means for quantitative variables. Independent samples t-test of significance was used when comparing between two means & Mann Whitney U test: for two-group comparisons in nonparametric data. A one-way analysis of variance (ANOVA) when comparing between more than two means & Post Hoc test: Tukey's test was used for multiple comparisons between different variables. The Comparison between groups with qualitative data was done by using Chi-square test and Fisher’s exact test instead of Chi-square test only when the expected count in any cell less than 5.

## RESULTS

### Characteristics of cases:

The examined cases' ages ranged from 4 to 20 years old, with a mean age of 14.9±5.6 .There were (34 boys and 16 girls) involved in the study, with a level of serum ferritin and its mean was ranged 3589.5±1820 ng/ml. The average age at when blood transfusions began was 8.96±3.0, with sixty percent of cases transfusions were occurring every two weeks, while the remaining forty percent were 18% occurred every three weeks, and 22% every four weeks

**Table (1) Relationship between Z-score and each of demographic, transfusion, chelation characteristics and compliance:**

| | $X^2$ | P |
|---|---|---|
| Age | -0.76 | <0.001** |
| Age of start transfusion | -0.05 | >0.05 |
| Age of start chelation (years) | -0.11 | >0.05 |
| Compliance | 0.68 | <0.001** |

** Highly significant

Lower z-scores are more prevelant in patients with older age as well as those with poor compliance which were recorded with highly significant values.

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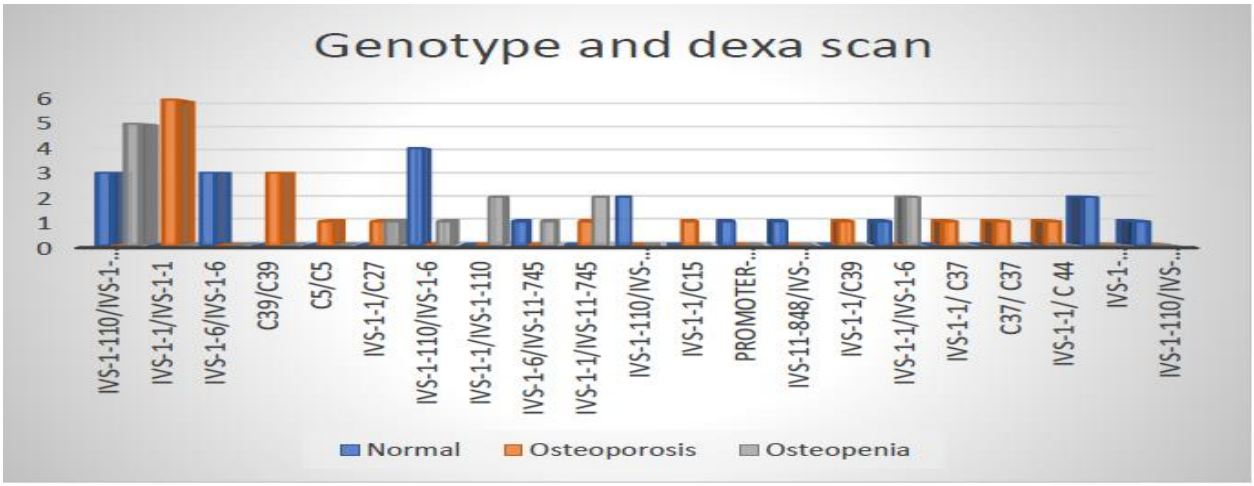
**Figure (1): Relationship between genotype and bone complications according to dexa scan :**



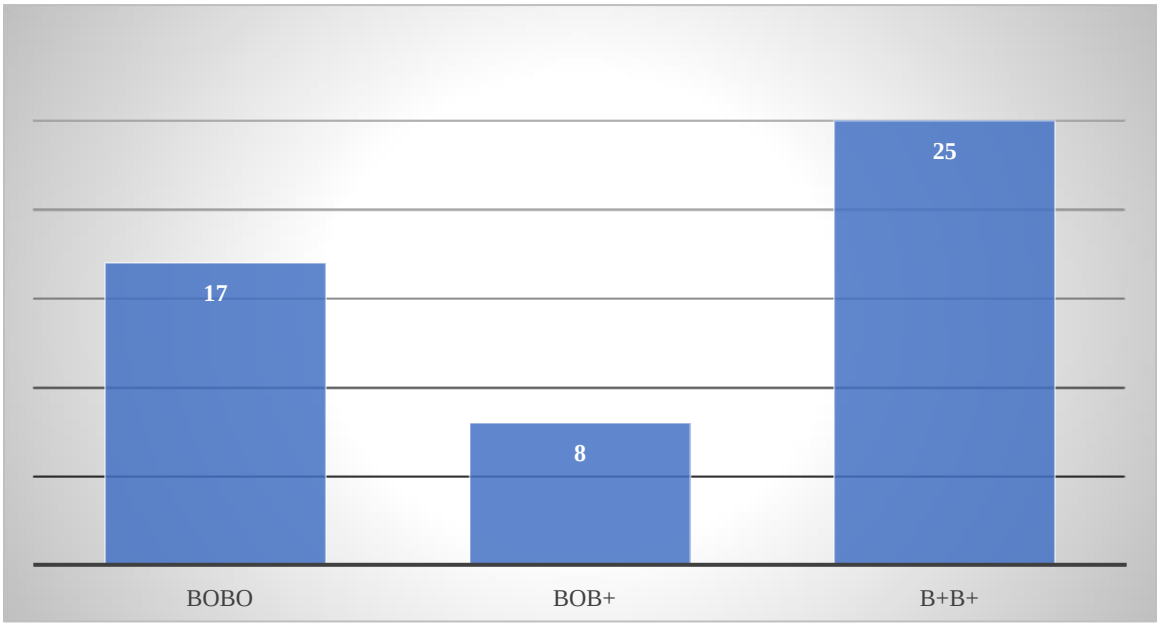

Patient with IVS-1-1/IVS-1-1 mutations had higher prevalence of osteoporosis followed by C39/C39 mutations, while IVS-1-110/IVS-1-110 mutations showed higher prevalence of osteopenia compared to other gene mutations.

**$\beta^0\beta^0$ genotype**

Cases that had βoβo genotype and associated with higher ferritin levels had more lower Z-score levels with higher prevalence of osteoporosis with as well as higher prevalence of other problems, including bone fracture compared to patients with β+β+ genotype. Besides, patients with βoβ+ genotype was shown to have more incidence to develop osteopenia compared to others.

**Table (2) & Figure (2) :** genotypes according to B globin gene production in patients.

| Genotype | No | % |
|---|---|---|
| $B^{+}B^{+}$ | 25 | 50.0 |
| $B^{o}B^{+}$ | 8 | 16.0 |
| $B^{o}B^{o}$ | 17 | 34.0 |




**$B^{+}B^{+}$** Had the higher prevalence in 50% 0f cases while **$B^{o}B^{o}$** was shown in 17% and in **$B^{o}B^{+}$** 8% of cases.

**Intervening sequence (IVS ) mutations**:

**Table (3):** Type and frequency of genotype in patients.

| Genotype | No | % |
|---|---|---|
| IVS-1-110/IVS-1-110 (homozygous) | 8 | 16.0 |
| IVS-1-1/IVS-1-1(homozygous) | 6 | 12.0 |
| IVS-1-6/IVS-1-6 (homozygous) | 3 | 6.0 |
| C39/C39 (homozygous) | 3 | 6.0 |
| C5/C5 (homozygous) | 1 | 2.0 |
| IVS-1-1/C27 ( compound heterozygous) | 2 | 4.0 |
| IVS-1-110/IVS-1-6 ( compound heterozygous) | 5 | 10 |
| IVS-1-1/IVS-1-110 ( compound heterozygous) | 2 | 4.0 |
| IVS-1-6/IVS-11-745 ( compound heterozygous) | 2 | 4.0 |
| IVS-1-1/IVS-11-745 ( compound heterozygous) | 3 | 6.0 |
| IVS-1-110/IVS-11-745 ( compound heterozygous) | 2 | 4.0 |
| IVS-1-1/C15 ( compound heterozygous) | 1 | 2.0 |
| Promoter-87/promoter-87(homozygous) | 1 | 2.0 |
| IVS-11-848/IVS-11-848(homozygous) | 1 | 2.0 |
| IVS-1-1/C39( compound heterozygous) | 1 | 2.0 |
| IVS-1-1/IVS-1-6( compound heterozygous) | 3 | 6.0 |
| IVS-1-1/ C37( compound heterozygous) | 1 | 2.0 |
| C37/ C37 (homozygous) | 1 | 2.0 |
| IVS-1-1/ C 44 ( compound heterozygous) | 1 | 2.0 |
| IVS-1-110/promoter-87( compound heterozygous) | 2 | 4.0 |
| IVS-1-110/IVS-11-848( compound heterozygous) | 1 | 2.0 |

There are three more prevalent mutations recorded ,while their prevalence were IVS-1-110, IVS-1-1 and IVS-1-6 (28%, 26%, 16% respectively). Besides, the three most prevalent genotypes were IVS-1-110/IVS-1-110, IVS-1-1/IVS-1-1 and IVS-1-110/IVS-1-6 then IVS1-6/IVS-1-6 (16%, 12%, 10% & 6% respectively) as in **Table (4)**.

**Bone complications in patients**:

↓ bone mineral density was a recorded in 62% of studied βTM participants, while osteoprosis and osteopenia recorded in (34% & 28%, respectively) ,while other skeletal problems were reported in 16% of cases.. Higher incidence of low bone density associated with lower z-score levels was significantly reported in cases with older age who began an early blood transfusion (less than nine months of age ), that underwent frequent transfusion (every 2–3 weeks), beganan early iron chelation (less than two years of age ) and had poor compliance to the therapy besides, they had high mean serum ferritin levels.

**Analytical Results:**

**Table (4): Relationship between Z-score and gender.**

| | **No** | **Z-score** X ± SD Range | **R P** |
|---|---|---|---|
| **Male** | 34 | -1.14±1.5 - 4.2 : 1.3 | 3.0 0.004* |
| **Female** | 16 | -2.37± 0.9 -3.4 :-0.3 | |

* Significant

This table (4) showed that more lower z-scores are signifacantly found in female patients compared to male group .

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**Table (5) Relationship between Genotypes based on β-globin gene production with bone complications z –score and serum ferritin level:**

| | n | $\beta^o\beta^o$ | | $\beta^o\beta^+$ | | $\beta^+\beta^+$ | | $X^2$ | P |
|---|---|---|---|---|---|---|---|---|---|
| | (50) | N=17 | % | N=8 | % | N=25 | % | | |
| **Bone complications** | 8 | 7 | 41.2 | 1 | 12.5 | 0 | 00.0 | 12.85 | 0.0016* |
| **Dexa scan** ( BMD) | | | | | | | | | |
| Normal | 19 | 0 | 0.00 | 1 | 12.5 | 18 | 72.0 | 52.3 | <0.001** |
| Osteoporosis | 17 | 16 | 94.1 | 1 | 6 | 0 | 7 | | |
| Osteopenia | 14 | 1 | 5.9 | 12.5 | 75.0 | 0.00 | 28.0 | | |
| **Z-score** | | | | | | | | | |
| Mean ±SD | | -3 ± 0.45 | | -1.67 ± 1.3 | | -0.47± 0.97 | | 41.4 | <0.001** |
| Range | | -4.2 : -2.1 | | -3.5 : 1.1 | | -2.4 : 1.3 | | | |
| **Serum Ferritin** | | | | | | | | | |
| Mean ±SD | | 6375.8 ± 3136 | | 4460± 2331 | | 1689± 1043 | | 19.2 | <0.001** |
| Range | | 1045: 10320 | | 769: 7560 | | 532 : 6500 | | | |

patients with $\beta^o\beta^o$ genotype had a significant high ferritin level, higher prevalence of osteoporosis with lower Z-scores as well as higher prevalence of other complications compared to patients with $\beta^+\beta^+$ genotype. Besides patient with $\beta^o\beta^+$ genotype showed higher prevalence of osteopenia compared to other types.

**Table (6):** Relationship between Genotypes based on β-globin gene production and other complications.

| | | $\beta^o\beta^o$ | | $\beta^o\beta^+$ | | $\beta^+\beta^+$ | | $X^2$ | P |
|---|---|---|---|---|---|---|---|---|---|
| | | **n** | **%** | **n** | **%** | **n** | **%** | | |
| **Growth Retardation** | | 16 | 94.1 | 6 | 75.0 | 15 | 60.0 | 6.1 | 0.04* |
| **Hypogonadism** | | 16 | 94.1 | 4 | 50.0 | 15 | 60.0 | 7.4 | 0.02* |
| **Hypothyrodism** | | 6 | 35.3 | 0 | 0.00 | 4 | 16.0 | 14.75 | <0.001** |
| **Hypoparathyrodism** | | 7 | 41.2 | 0 | 0.00 | 0 | 0.00 | 15.8 | <0.001** |
| **Diabetes Mellitus** | | 2 | 11.8 | 3 | 37.5 | 0 | 0.00 | 9.5 | 0.008* |
| **Cardiac complications** | | 3 | 17.6 | 0 | 0.00 | 0 | 0.00 | 6.2 | 0.04* |
| **Hepatitis** | | 1 | 5.9 | 1 | 12.5 | 1 | 4.0 | 0.78 | 0.67 |

* Significant ** highly significant

Table (6) shows that patients with $\beta^o\beta^o$ genotype had a significant higher prevalence of complications including growth retardation, hypogonadism, hypothyroidism, hyperparathyroidism, as well as cardiac complications compared to other studied patients having $\beta^o\beta^+$ and $\beta^+\beta^+$genotype.

**Table(7):** The Correlation of the mineral density of bone at the lumbar spine (LS), the distal radius (DR), and the neck of femur (NF) in cases.

| | | BMD LS | BMD DR | BMD NF |
|---|---|---|---|---|
| **Sex** | **r** | −0.078 | 0.013 | −0.155 |
| | P value | 0.680 | 0.956 | 0.404 |
| **Age** | **r** | −0.055 | −0.288 | −0.066 |
| | P value | 0.781 | 0.108 | 0.718 |
| **BMI** | **r** | 0.396 | 0.003 | 0.039 |
| | P value | 0.029 | 0.986 | 0.833 |
| **Chelation** | **r** | −0.064 | −0.209 | −0.065 |
| | P value | 0.725 | 0.255 | 0.729 |
| **S.Ferritin** | **r** | 0.203 | 0.041 | 0.180 |
| | P value | 0.273 | 0.815 | 0.327 |
| **Vit.D3 level** | **r** | 0.087 | 0.237 | 0.237 |
| | P value | 0.643 | 0.194 | 0.195 |
| **S.Calcium** | **r** | 0.118 | 0.065 | 0.391 |
| | P value | 0.528 | 0.735 | 0.026 |

According to table (7) there are lower BMD at the lumbar spine which is more affected in thalassemia cases compared to the other sites of BMD assessment. BMD at all the sites were negatively correlated to age although the difference was not statistically significant as shown in Table 8. No significant correlation was seen for BMI, serum ferritin, and

chelation with the BMD at any of the sites. No significant difference between BMD of male and female thalassemic cases.

**Table(8): Laboratory investigations in studied cases :**

| | **Mean ±SD** | **Range** | **P** |
|---|---|---|---|
| Serum ferritin level (ng/ml) | 3577.5±1826 | 836-8500 | **<0.001**** |
| Serum calcium level (mg/dl) | 8.46 ± 0.68 | 7.2 - 9.5 | **<0.001**** |
| Serum phosphorus level (mg/dl) | 3.87 ± 0.51 | 3.1–4.5 | **<0.001**** |
| Serum iron level ($\mu$g/dl) | 539.13 ± 129.67 | 323–862 | **<0.001**** |
| Serum alkaline phosphatase value (IU/L) | 397.45 ± 127.20 | 113–686 | **<0.001**** |
| vitamin D levels (pg/dl) | 16.71 ± 3.45 | 11.3–26 | **0.997** |
| Serum magnesium (mg/dl) | 1.67± 0.25 | 1.3-2.5 | **<0.001**** |
| Serum PTH (pg/mL) | 25.6 ± 6.7 | 9.1–41.2 | **<0.001*** |

**p≤0.001 is statistically highly significant

There is a significant decrease in serum calcium, magnesium, phosphorus and parathyroid hormone, and marked increase in iron , alkaline phosphatase and serum ferritin levels to be correlated with the clinical progress and decrease bone mineral density in the studied cases.

## DISCUSSION

Numerous studies have found that the most common cause of morbidity in patients with thalassemia major is osteopenia or osteoporosis, which affects 40–50% of those who are well treated [9].

Such complications are brought on by a lack of minerals like calcium, phosphorus and zinc, as well as vitamins like vitamin D, that can worsen bone health. Bone disease may be exacerbated by the presence of endocrinopathies including hypothyroidism, hypoparathyroidism, hypogonadism, and diabetes mellitus[10].

Patients with thalassemia are commonly to have osteoporosis and osteopenia as bone diseases. Numerous risk factors, including the severity of the condition, haemoglobin levels, iron toxicity, the onset of puberty, nutritional deficiencies, and hormonal imbalances, are associated with osteoporosis[12].

In the current study, almost two thirds of our patients had decreased BMD. Osteoporosis (34%) and osteopenia (28%) cases were discovered from the total number of thalassemic patients; these results matched and were compatible with other investigations conducted by Voskaridou E et al., Shawkat et al., and Cefalu CA[11,13&14].

We reported in this study that there is a positive correlation between the occurrence of osteoporosis and older patients, longer transfusion times, high ferritin levels and longer gaps between transfusions. Our results are matched with the study made by Hashemieh M et al [15].

In our study we found that the three most common mutations were IVS-1-110, IVS-1-1 and IVS-1-6 (28%, 26%, 16% respectively). Also we found the three most common genotypes were IVS-1-110/IVS-1-110 , IVS-1-1/IVS-1-1 and IVS-1-110/IVS-1-6 then IVS-1-6/IVS-1-6 (16%,12%,10% & 6% respectively).

Our findings are consistent with other earlier Egyptian investigations and studies, where in the study made by Al-Akhras et al 2016 has reported that The 3 most common mutations in their study were IVS-1-110, IVS-1-1 and IVS-1-6 (31.5, 23.5 and 20.5%,

respectively), while Hassan et al 2018 has reported that IVS 1–1, IVS 1–110 and IVS 1–6 were the commonest mutation in the studied cases (26.7%, 22.6% and 18.5% respectively), besides homozygous IVS 1–1, homozygous IVS 1–110 and homozygous IVS 1–6 were the commonest genotypes (19.17%, 15.06% and 10.95% respectively) [4&16].

In our study, we discovered that thalassemic cases with the genotypes IVS-1-110/IVS-1-110, IVS-I-6/IVS-1-6, IVS-11-745/IVS-11-745, and promoter 87/promoter87 were associated with $\beta^{+}\beta^{+}$ hematological phenotype where thalassemic cases with IVS-1-1/IVS-1-1, C39/C39, C5/C5 genotypes were associated with $\beta^{o}\beta^{o}$ hematological phenotypes.

Our findings are consistant with Gallanello and Origa 2010 in their comprehensive β thalassemia review that listed the common mutations of β thalassemia regarding the severity and ethnic distribution and they reported that IVS-1-110/IVS-1-110,IVS-1-1/IVS-1-1,IVS-I-6/IVS-1-6,C39/C39,IVS-11-745/IVS-11-745,C5/C5 and promoter 87/promoter87 genotypes were prevalent in the mediterranean region and also IVS-1-110/IVS-1-110, IVS-I-6/IVS-1-6, IVS-11-745/IVS-11-745, and promoter 87/promoter87 genotypes were associated with $\beta^{+}\beta^{+}$ hematological phenotypes where IVS-1-1/IVS-1-1, C39/C39, C5/C5 genotypes were associated with $\beta^{o}\beta^{o}$ hematological phenotype[17].

This study has reported that cases with the hematologic phenotypes β0β+ & β+β+ their age of transfusion beginning was delayed, also delayed in chelation therapy start, besides lower frequency of blood transfusions and they were significantly less in the prevalence of growth retardation, hypogonadism, hypothyroidism and hypoparathyrodism compared to cases with β0β0 genotype that had an earlier age of start transfusion and chelation, as well as more frequent transfusion rates. β+β+ was presented in our results as the most common genotype followed by β°β° then β°β+ (50%, 34.0% and 16% respectively). Incidence of bone complications in our study including low bone mineral density, lower Z-scores and other bone complications as pain and fracture had a significant correlations to genotype.

Similarly, studies that reported by Yaman et al that revealed βoβo hematologic phenotype was correlated significantly to the age of first transfusion and it was a well recognized disease severity indicator. Additionally ,in the study of Hassan et al showed that β+β+ was the most prevalent genotype followed by β°β° then β°β+ (49.3%, 37.0% and 13.7% respectively) [16&18].

## Conclusion

This study and collected data has emphasized that diminished mineral density of the bone associated with lower z-scores values as well as other bone complications were considered prevalent association in β-thalassemia major cases ,which clearly correlated to genotype beside clinical disease progression and also its severity. Genotype associated with the more decrease in β-globin production has the higher prevalence of lower values of z-scores and ↓bone mineral density. Serum ferritin levels and the existence of bone problems were found to be significantly correlated, which showed the critical role played by iron overload in the development of such bone problems.

## Recommendations

It is important to pay more attention to genotype determination and to perform it routinely at the time of initial diagnosis in order to identify the clinical phenotypes of these cases.

Regular follow-up are crucial for patients having IVS-1-1/IVS-1-1 genotype, $\beta^o\beta^o$ genotype as they are more likely to experience iron overload with its wide spectrum of complications and its effect on various organs like bone and endocrinal glands.

Importance of regular bone status assessment during routine follow up by laboratory investigations and bone imaging studies including Dexa scan specially for patients with high frequency rates of blood transfusion and poor compliance as well as during puberty.

Careful and regular monitoring of bone changes, pubertal development, growth, and other endocrinal problems of thalassemic cases to predict and assess such abnormalities and in order to initiate the more suitable as well as early appropriate treatment. Through such way, the future incidence of bone complications as well as endocrinal problems could be reduced.

Education and motivating strategies as well as the introduction of highly-tolerated chelation protocols should be utilized in order to combat the problem of compliance.

**Study Limitations**

- Most of history and data through patients and their records was subjected to the possibility of recall bias.
- Regular daily drug intake with proper dosing such as iron chelators are confined to the patients and their families and not under strict control nor monitoring of health care providers.
- Laboratory investigations and medical records were needed to be computerized and regularly recorded .
- Hospital based study and does not represent community data
- Relatively small sample size

## REFERENCES


**Abdo, A., Beshir, M., Hassan, T., Mohamed, A. (2024).** 'The Impact of Genotype on Bone Complications in Beta Thalassemia Major Patients.', *Zagazig University Medical Journal*, 30(3), pp. 935-945. doi: 10.21608/zumj. 189467.2731.

**Indrák K;Divoká M;Pospíšilová D;Čermák J;Beličková M;Horváthová M;Divoký V;** (no date) *[hemoglobinopathies], Vnitrni lekarstvi*. Available at: https://pubmed.ncbi.nlm.nih.gov/30193516.

**Finotti, A. & Gambari, R. (2014).** Recent trends for novel options in experimental biological therapy of β-thalassemia. Expert Opinion on Biological Therapy. 14, 1443–1454

**Al-Akhras A, Badr M, El-Safy U, Kohne E, Hassan T, Abdelrahman H et al. (2016).** Impact of genotype on endocrinal complications in β-thalassemia patients. Biomedical Reports, 4, 728-736. https://doi.org/10.3892/br.2016.646.

**Hassan TH, Salam MMA, Zakaria M, Shehab M, Sarhan DT, Zidan ESH, El Gerby KM. (2018)**. Impact of Genotype of Beta Globin Gene on Hepatic and Myocardial Iron Content in Egyptian Patients with Beta Thalassemia. Indian J Hematol Blood Transfus. 2019 Apr;35(2):284-291. doi: 10.1007/s12288-018-1034-x. Epub . PMID: 30988565; PMCID: PMC6439044.

**Gaudio A, Morabito N, Xourafa A, Currò M, Caccamo D, Ferlazzo N,**

**Macrì I, La Rosa MA, Meo A, Ientile R.( 2010)** Role of genetic pattern on bone mineral density in thalassemic patients. Clin Biochem. 2010 Jul;43(10-11):805-7. doi: 10.1016/j.clinbiochem.2010.04.070. Epub. PMID: 20444423.

**Saki N, Abroun S, Salari F, Rahim F, Shahjahani M, Javad MA. (2015 )** Molecular Aspects of Bone Resorption in β-Thalassemia Major. Cell J. 2015 Summer;17(2):193-200. doi: 10.22074/cellj.2016.3713. Epub PMID: 26199898; PMCID: PMC4503833.

**Baldini M, Forti S, Orsatti A, Marcon A, Ulivieri FM, Airaghi L, Zanaboni L, Cappellini MD. (2013)** Thalassemic osteopathy: a new marker of bone deposition. Blood Cells Mol Dis. 2014 Feb-Mar;52(2-3):91-4. doi: 10.1016/j.bcmd.2013.09.008. Epub. PMID: 24091145.

**Ali S, Mumtaz S, Shakir HA, Khan M, Tahir HM, Mumtaz S et al.( 2021)** Current status of beta-thalassemia and its treatment strategies. Mol Genet Genomic Med. (12):e1788. doi: 10.1002/mgg3.1788. Epub. PMID: 34738740; PMCID: PMC8683628.

**Fung EB. (2010)** Nutritional deficiencies in patients with thalassemia. Ann N Y Acad Sci. 1202:188-96. doi: 10.1111/j.1749-6632.2010.05578.x. PMID: 20712792.

**Shawkat, A. J., A. H. Jwaid, G. Marzouq Awad, and H. Adnan Fawzi. (2018)** "evaluation of osteopathy in patients with beta-thalassemia major using different iron chelation therapies". *Asian Journal of Pharmaceutical and Clinical Research*, vol. 11, no.. pp. 467-71, doi:10.22159/ajpcr.2018.v11i11.29079.

**Maggio A, Kattamis A, Felisi M, Reggiardo G, El-Beshlawy A, Bejaoui M et al. (2020)** Evaluation of the efficacy and safety of deferiprone compared with deferasirox in paediatric patients with transfusion-dependent haemoglobinopathies (DEEP-2): a multicentre, randomised, open-label, non-inferiority, phase 3 trial. Lancet Haematol.;7(6):e469-e478. doi: 10.1016/S2352-3026(20)30100-9. PMID: 32470438.

**Voskaridou E, Christoulas D, Xirakia C, Varvagiannis K, Boutsikas G, Bilalis A, Kastritis E, Papatheodorou**

**A, Terpos E. (2009)** Serum Dickkopf-1 is increased and correlates with reduced bone mineral density in patients with thalassemia-induced osteoporosis. Reduction post-zoledronic acid administration. Haematologica. 2009 May;94(5):725-8. doi: 10.3324/haema-tol.2008.000893. Erratum in: Haematologica. ;94(8):1182. PMID: 19407319; PMCID: PMC2675686.

**Cefalu CA. (2004):** Is bone mineral density predictive of fracture risk reduction? Curr Med Res Opin.;20(3):341-9. doi: 10.1185/030079903125003062. PMID: 15025843.

**Hashemieh M , Azarkeivan A , Radfar M , Saneifard H , Hosseini-Zijoud SM , Noghabaei G et al. (2014)** Prevalence of Osteoporosis among Thalassemia Patients from Zafar Thalassemia Clinic, Iran. JBC;6(3): 143-148.

**Hassan, T. et al. (2018)** Association between genotype and disease complications in Egyptian patients with beta thalassemia: A cross-sectional study, Scientific reports. Available at: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6286337.

**Galanello R, Origa R. Beta-thalassemia. Orphanet J Rare Dis. (2011)** ;5:11. doi: 10.1186/1750-1172-5-11. PMID: 20492708; PMCID: PMC2893117.

**Yaman A, Isik P, Yarall N, Kardemir S, Cetinkaya S, Bay A, et al.(2013)** Common complications in beta thalasemia patients. *Int J Hematol Oncol.* ;3:193–199. doi: 10.4999/uhod.12005.