thalassemia reports

MDPI

Article

Association of Bone Disorder and Gene Polymorphism of PPAR-γ Pro12 Ala in Egyptian Children with β-Thalassemia

Ahmed M. Abdel Hamied [1], Heba Mostafa Ahmed [2], Dina H. Eldahshan [3], Dalia S. Morgan [2], Abdel Meged A. Abdel Meged [2], Marwa O. Elgendy [4,5,*], Mohamed S. Imam [6,7,*], Turki A. H. Alotaibi [8], Majed M. S. Alotaibi [9], Manal T. N. Alotaibi [10], Sarah S. S. Alshalan [10] and Sara O. Elgendy [3]

1 Department of Pediatric Medicine, The Egyptian Ministry of Health and Population, Beni-Suef 62513, Egypt; ahmadmada888@yahoo.com
2 Department of Pediatric Medicine, Faculty of Medicine, Beni-Suef University, Beni-Suef 62521, Egypt
3 Department of Clinical and Chemical Pathology, Faculty of Medicine, Beni-Suef University, Beni-Suef 62521, Egypt; drsaraosama88@med.bns.edu.eg (S.O.E.)
4 Department of Clinical Pharmacy, Faculty of Medicine, Beni-Suef University, Beni-Suef 62521, Egypt
5 Department of Clinical Pharmacy, Faculty of Pharmacy, Nahda University (NUB), Beni-Suef 62513, Egypt
6 Department of Pharmacy Practice, College of Pharmacy, Shaqra University, Shaqra 11961, Saudi Arabia
7 Department of Clinical Pharmacy, National Cancer Institute, Cairo University, Fom El Khalig Square, Kasr Al-Aini Street, Cairo 11796, Egypt
8 Boots Pharmacy, Riyadh 12272, Saudi Arabia; turki.alghuwiri@gmail.com
9 Alshafi Medical Company, Riyadh 14325, Saudi Arabia; majed-277@hotmail.com
10 College of Pharmacy, Shaqra University, Shaqra 11961, Saudi Arabia; s438480954@std.su.edu.sa (M.T.N.A.); s438480234@std.su.edu.sa (S.S.S.A.)
* Correspondence: marwa.elgendy@nub.edu.eg (M.O.E.); soliman@su.edu.sa (M.S.I.)

Abstract: β-thalassemia is a genetic disorder affecting chromosome 16, inherited from one or both parents. In spite of the improved treatment of the hematological disorder and its complications, β-thalassemic patients still exhibit an imbalance in bone mineral turnover, resulting in diminished bone mineral density (BMD), more evident in the lumbar spine. The purpose of this study was to investigate the association between genetic polymorphism of the PPAR-γ gene and the presence of osteopenia or osteoporosis in children with β-thalassemia. This case–control study was conducted on 50 children with β-thalassemia from the pediatric hematology unit of Beni-Suef University Hospital, including 50 healthy children as the control group. The age range was 8 to 18 years. Samples of patients and control subjects were analyzed for the presence of polymorphisms of the PPAR-γ gene and other blood labs. An assay of BMD measure using dual-energy X-ray absorptiometry (DXA) was performed to investigate osteopenia or osteoporosis. Statistical analysis was used to investigate the relationship between the risk of osteopenia or osteoporosis and the presence of PPAR-γ Pro12Ala gene polymorphism. Eighteen (eleven males and seven females) of fifty patients (representing 36% of the patients group) have osteopenia with low bone mineral density (Z-score is −1 or less than 1). There was no statistically significant difference between BMD measurements in males and females. By comparing the frequency of 12 Ala gene polymorphisms between the patient group and the control group, we found that no statistically significant difference was detected. The BMD values were not significantly different between the groups of PPAR-γ Pro12Ala gene polymorphism. In conclusion, decreased BMD levels are frequent in β-thalassemia patients. PPAR-γ Pro12Ala gene polymorphism is not common in Egyptian patients with β-thalassemia. No significant relationship was found between the PPAR-γ Pro12Ala gene polymorphism and low BMD levels or osteopenia in Egyptian β-thalassemia patients. However, further studies on a larger population of Egyptian patients are needed to confirm this finding.

Keywords: PPAR-γ gene; osteopenia; osteoporosis; β-thalassemia

Citation: Hamied, A.M.A.; Ahmed, H.M.; Eldahshan, D.H.; Morgan, D.S.; Meged, A.M.A.A.; Elgendy, M.O.; Imam, M.S.; Alotaibi, T.A.H.; Alotaibi, M.M.S.; Alotaibi, M.T.N.; et al. Association of Bone Disorder and Gene Polymorphism of PPAR-γ Pro12 Ala in Egyptian Children with β-Thalassemia. *Thalass. Rep.* **2023**, *13*, 230–240. https://doi.org/10.3390/thalassrep13040020

Academic Editor: Aurelio Maggio

Received: 2 July 2023
Revised: 23 September 2023
Accepted: 28 September 2023
Published: 30 September 2023

1. Introduction

β-thalassemia is a genetic disorder inherited from one or both parents. Individuals with thalassemia syndrome are most often African, Asian, Mediterranean, or Middle Eastern descent [1].

However, the life span of patients with thalassemia has improved both in duration and in quality in the recent decade in developed countries. Complications are still present and common, including heart failure, arrhythmias, and chronic liver hepatitis, which can evolve into cirrhosis and endocrine problems such as hypogonadism, hypothyroidism, diabetes, hypoparathyroidism, delayed growth, and osteoporosis [2,3].

Patients with β-thalassemia develop bone disease, which is a major cause of morbidity. Osteoporosis, rickets, scoliosis, nerve compression, spinal deformities, and fractures are among these bone disorders [4].

In β-thalassemic transfused patients, impaired calcium homeostasis is usual and primarily caused by iron overload. These patients have been found to have both impaired 25 OH vitamin D synthesis and/or hypoparathyroidism, both of which have a detrimental impact on bone metabolism [5].

Peroxisome proliferator-activated receptor-γ (PPARγ) is a subgroup of the PPAR family that controls lipid and glucose metabolism. Peroxisome proliferator-activated receptor-γ plays an important role in fat cell differentiation, insulin sensitivity, and glucose homeostasis in patients with type 2 diabetes [6].

On the other hand, the PPARγ gene is present on 3p25 in humans, and it contains nine exons [7].

Many single ribonucleotide polymorphisms (SNPs) have been shown in the mutations of the PPARγ gene in humans. One of these polymorphisms is Pro 12Ala (rs1801282), which has the replacement of proline amino acid with alanine amino acid at the codon 12 in exon B [8].

According to previous studies, PPARγ gene polymorphism plays an important role in osteogenesis [9–11]. Depending on this hypothesis that osteoblasts and adipocytes have a common mesenchymal precursor, recent studies have revealed that the activation or suppression of PPARγ promoted adipocyte differentiation, affecting the induction of osteoblast differentiation into osteoclasts or stimulating their apoptosis [12,13].

In this study, we aimed to evaluate osteopenia and osteoporosis in patients with β-thalassemia using DXA assay and measuring the BMD in these patients and study the prevalence of the PPAR-γ gene in children with β-thalassemia with regular blood transfusion. We also aimed to study the association between genetic polymorphisms of the PPAR-γ gene and its relation to BMD values, serum ferritin, calcium, phosphorus, and alkaline phosphatase enzyme.

2. Materials and Methods

2.1. The Study Design

This prospective observational study was conducted from June 2019 to February 2020. All the participants met the inclusion criteria. Participants' families provided written informed consent. The Research Ethical Committee of Beni-Suef University authorized the study protocol in accordance with the Declaration of Helsinki. The estimation of the minimum required sample size was carried out using G* power 3.1 software.

2.2. Inclusion Criteria

- Patients with β-thalassemia major ranging from 8 to 18 years old.
- Patients who were diagnosed at an early age based on Hemoglobin electrophoresis.
- Patients who were given blood transfusions.
- Children on iron chelation therapy, whether oral or subcutaneous.

2.3. Exclusion Criteria

The study excluded participants with endocrine disorders (such as thyroid disease), malabsorption, use of steroids and anticonvulsants, spinal radiological abnormalities (such as scoliosis), smokers, and HIV-positive individuals.

Patients diagnosed with β-thalassemia major attended the pediatric hematology outpatient clinic for follow-up at the Beni-Suef University Hospital.

One hundred subjects were included in the study. They were divided into two groups: Group 1 included patients diagnosed with β-thalassemia major (50 patients); Group 2 included healthy children as the control group (50 children). Group 2 was matched for age and sex to Group 1.

For all the subjects included in the study, routine hematological and radiological investigations were detected. Moreover, detection of the Pro12Ala polymorphism in the PPAR gamma gene (that is present on chromosome 3) was performed for all the subjects by polymerase chain reaction–restriction fragment length polymorphism PCR/RFLP.

(I) Routine hematological investigations:

Includes measurement of Hb, serum ferritin, serum calcium, serum phosphorus, and serum alkaline phosphatase enzyme. The samples of the patients were collected before their regular blood transfusion. All were determined by automated routine procedures.

(II) Radiological investigation:

Bone mineral density (BMD) was measured in all patients and control subjects by dual-energy X-ray absorptiometry (DEXA lunar DPX) at the lumbar spine (L2–L4) in A-P projection. BMD measurements were expressed in grams per centimeter squared and compared to BMD measurements of normal subjects of the same age and gender. If the Z-score is greater than −1, the BMD is considered normal; osteopenia is considered normal if the Z-score is between −1 and −2.5, and osteoporosis is considered normal if the Z-score is less than or equal to −2.5.

(III) Genotyping identification:

Detection of the Pro12Ala polymorphism in the PPAR gamma gene (that is present on chromosome 3) in the genomic DNA of patients and control subjects was performed by polymerase chain reaction–restriction fragment length polymorphism PCR/RFLP [14].

-A 295 base pair sequence of the PPARγ gene was amplified by PCR using oligonucleotide primers F: 5′-CTG ATG TCT TGA CTC ATG GG-3′ and R: 5′- GGA AGA CAA ACT ACA AGA GC-3′10.

Technique of PCR amplification:

Initial denaturation at 95 °C and each PCR was subjected to 35 cycles at 94 °C for 30 s, 53 °C for 30 s, and 72 °C for 30 s, followed by 7 min at 72 °C (18). Restriction of the PCR product with the HgaI enzyme generates fragments of 178/117 bp in rare homozygotes, 295/178/117 bp in heterozygotes, and 295 bp in common homozygotes. Samples were electrophoresed on 3.0% agarose.

(IV) Statistical studies to assess the obtained data:

All data were expressed as median and interquartile range (IQR) and count (percent). Statistical analysis was performed using statistical package for social sciences (SPSS) computer software (version 22), IBM V22.0 software, Armonk, NY, USA. Non-parametric tests were used to elucidate the significance between group means. A chi-square test was used to test association and significant differences in discrete variables. Differences were considered statistically significant at $p < 0.05$. The chi-square was used to test significant associations between different qualitative variables. Differences and associations were considered statistically significant at <0.05.

3. Results

3.1. The Demographic Data, Laboratory Data, and the Z-Score of BMD Levels of the Patient and Control Groups

A total of 100 subjects (56% of them are males) participated in the study. Regarding the demographic data of the patients and control groups, there were no significant differences among patient and control groups related to their age and gender, while the patients group had significantly lower weight, height, and BMI (body mass index) measures as compared to the control group. Regarding the laboratory data of the patients and control groups, we found that the patient group had significantly lower levels of hemoglobin and significantly higher levels of ferritin as compared to the control group. Additionally, no significant differences were found between patients and control groups related to the measurement of calcium, phosphorous, and alkaline phosphatase enzyme (ALP levels). The Z-score was significantly lower in the patient group than in the control group, with a *p*-value = 0.01. The BMD levels were significantly lower (osteopenia) in 36% of the patient group in comparison with the control group. There was no significant difference between the male and female patients. All these findings are shown in Table 1.

Table 1. The demographic data, laboratory data, and BMD measures of the patients and control groups.

	Patient (Median)	**Control (Median)**	***p*-Value**
Age (years)	11 (8–18)	11.5 (8–18)	0.85
Weight (kg)	23.5 (14–54)	35.5 (21–62)	0.01
Height (cm)	127 (106–168)	141 (119–173)	0.01
BMI (Kg/m^2)	14.95 (11.6–20)	17.65 (13.4–21)	0.003
Male n (%)	27 (54)	29 (58.0)	0.6
Female n (%)	23 (46)	21 (42)	
Hemoglobin (gm/dL)	8 (6–9.2)	1 (10–13)	**0.01** *
Ferritin (ng/mL)	1674 (474–7048)	50 (20–90)	**0.01** *
Calcium (mg/dL)	9 (7–9.8)	9.2 (8.5–9.8)	0.07
Phosphorous (mg/dL)	3.1 (2–3.8)	3 (2.4–4)	0.06
ALP (U/L)	135 (80–400)	130 (80–180)	0.69
Z-score	−0.7 (−1.5–1.2)	0.7 (0.6–1.2)	**0.01** *
Normal BMD measures	32 (64%)	50 (100%)	**0.01** *
Low BMD measures	18 (36%)	0 (0%)	

(*) means significance.

3.2. Frequency of PPAR-γ Gene Polymorphism of Patient Group and Control Group and Comparison between Them Regarding PPAR Allele Frequencies

There was no significant difference between the patients and control group regarding the different isotypes of the PPARγ genotype. There was no significant difference between the patients and controls regarding different alleles of the genotypes. These findings are shown in Table 2.

Table 2. Frequency of PPAR-γ gene polymorphism of patients group and control group.

Genotype	**Patients**	**Controls**	**OR (95% CI)**	***p*-Value**
C/C	45 (90%)	44 (88%)	1.00	0.95
C/G	5 (10%)	6 (12%)	1.06 (0.21–5.35)	
G/G	0 (0%)	0 (0%)	0	

3.3. Comparison between Males and Females Regarding PPARγ Gene Polymorphism

There was no significant difference between males and females of the patient group and control group regarding PPARγ gene polymorphisms, as shown in Table 3.

Table 3. Comparison between the two sex groups of patient and control group regarding PPARγ gene polymorphism.

	PPAR Genotypes		*p*-Value
Sex	**C/C**	**C/G**	
Male (No)	**51**	**6**	**0.88**
Female (No)	**38**	**5**	

3.4. Comparison between PPARγ Homozygous and PPARγ Heterozygous Gene Groups Regarding the BMD Levels and the Laboratory Data in Patient Group

There was no significant difference between patients of β-thalassemia with osteopenia (with low BMD levels) and patients with no osteopenia (with normal BMD levels) regarding PPARγ (C/C and C/G), as shown in Table 4.

Table 4. Comparison between PPARγ homozygous and PPARγ heterozygous gene groups regarding the BMD levels in patient group.

PPAR-γ Polymorphism		Low BMD Median (IOR)	Normal BMD Median (IOR)	*p*-Value
Homozygous (C/C)	Valid	**16 (35.6)**	**29 (64.4)**	**0.90**
Heterozygous (C/G)		**2 (40)**	**3 (60)**	

There was no significant difference in patients of β-thalassemia with PPARγ (C/C and C/G) gene polymorphisms regarding the laboratory data and Z-score, as shown in Table 5.

Table 5. Comparison between PPAR homozygous and PPAR heterozygous groups regarding the laboratory data and Z-score of BMD levels in patient group.

	Homozygous (CC) (n = 45)	Heterozygous (CG) (n = 5)	*p*-Value
Z-score	0.7 (−1.50–1.20)	0.7 (−1.50–90)	0.70
Hb (gm/dL)	8 (6.90–9.20)	8 (7.00–8.20)	0.91
Ferritin (ng/mL)	1635 (474–7048)	2220 (684–2420)	0.35
Calcium (mg/dL)	9 (7.00–9.80)	9 (8–9.2)	0.63
Phosphorous (mg/dL)	3.2 (2–3.8)	3 (2.9–3)	0.05
ALP (U/L)	135 (80–385)	130 (100–400)	0.85

4. Discussion

β-thalassemia is a genetic disorder inherited from one or both parents [1]. However, the life span of patients with thalassemia has improved in both duration and quality in the recent decade in developed countries. However, complications are still present and common [2,3]. Although conventional therapy has allowed children to grow normally during the first decade of life, growth retardation is still observed clearly in a significant ratio during adolescence [15]. In previous studies of the Iranian population with an age range of 20–45 years, 54.2% of thalassemic patients' weights and 65.7% of patients' heights dropped below the fifth percentile [16]. Additionally, 48% of Chinese β-thalassemic patients

with an age range of 9 months–17 years and short stature with heights below the third percentile and weights below the third percentile in 43.7% of cases [17]. Additionally, it was found that in Iraq, 79% of multi-transfused β-thalassemic patients aged 10–20 years old had short stature [18].

As shown in Table 1, according to our study, the weight, height, and BMI values of the patients of β-thalassemia were significantly lower in comparison with the corresponding values of subjects of the control group. Moreover, no statistical difference was found between patients of our study regarding the gender type, male or female, and that could be explained by the fact that β-thalassemia is an autosomal disease depending on mutations in chromosome 11, not depending on the sex chromosomes.

The lower measurements of weight, height, and BMI of the patients of the study may be explained by the chronicity of β-thalassemia and the many complications affecting the growth of the patients, including decreasing parathyroid hormone, thyroid hormones, growth hormone, sex hormone secretion and other unknown causes related to iron overload. Therefore, it was necessary to implement an aggressive iron chelating regimen to regularly monitor height to identify early signs of short stature in patients, screen β-thalassemic patients for hormonal deficiency, and treat hormone deficiency swiftly and effectively as needed [18].

In the context of hematological diseases, secondary iron overload is a frequent complication because iron builds up due to a variety of mechanisms, including chronic transfusion, increased gastrointestinal absorption, chronic hemolysis, and underlying genetic defects that cause increased gastrointestinal iron absorption. Since the body lacks a system to eliminate excess iron, it accumulates in the liver, endocrine system, and heart, with the latter two being less frequently impacted than in primary iron overload disorders like hemochromatosis [19].

Regarding the laboratory data of our study, the serum hemoglobin was significantly lower ($p = 0.01$), and the serum ferritin was significantly higher ($p = 0.01$) in the patient group when compared to the control group, which may be explained by the chronicity of β-thalassemia as hemolytic anemic disease and needing to the repeated blood transfusion since early childhood.

These patients are typically monitored with serum ferritin, an affordable and widely accessible method to monitor iron overload, in order to avoid the complications linked to iron overload [19].

A well-known clinical condition linked to thalassemia major called hypoparathyroidism appears to be related to hypocalcemia and hyperphosphatemia. The parathyroid gland accumulates iron from repeated blood transfusions, which interferes with its ability to function normally [20]. In patients with β-thalassemia, altered levels of serum calcium, phosphorus, and alkaline phosphatase enzyme are typical signs of bone impairment. Calcium is crucial for the mineralization of the skeleton. By raising the local concentration of inorganic phosphorus, alkaline phosphatase, which is in charge of phosphorus, encourages bone mineralization [21]. Therefore, we focused on studying the relationship between the measurement of BMD in the patients and the levels of serum calcium, phosphorus, and ALP enzyme to test the possibility of using them as monitoring markers of bone mineralization.

In our study, there were no significant differences between the patient group and control group regarding measurements of the serum calcium, phosphorus, and ALP enzyme. This may be explained by the increasing awareness of the importance of vitamin D and the need for calcium and vitamin D supplementation in β-thalassemic patients, especially children and adolescent patients.

In contrast to our findings, in a previous study [22] on 55 Egyptian β-thalassemia children aged (5–17 years), the patients of the study had significantly higher serum phosphorus and ALP levels, and serum calcium was significantly lower than the control group. The results in these studies are in agreement with Karim et al. [23], who showed that β-thalassemia patients had shown significantly low serum calcium levels in comparison with the controls. Also, Hagag et al. [24] found that serum calcium levels are significantly

lower in the patients group, and serum phosphorus and ALP levels are significantly higher. The same results by Saboor et al. [25] found that serum calcium level was low as compared with controls. All patients had a higher ALP level than the controls. Levels of inorganic phosphorus were also higher in patients than in controls. They explained their results by the presence of iron overload and hemosiderosis resulting in endocrinopathies, especially the parathyroid gland. Chelation therapy in addition to cirrhotic changes due to hemosiderosis may also play a significant role in hypocalcemia. Sultan et al. [26] found that hypocalcemia and hyperphosphatemia were seen in 66.6% and 19.4%, respectively, while 25 OH vitamin D deficiency was present in 72.2% of thalassemic patients.

In agreement with our results, Izzah et al. [27] found that vitamin D and phosphorus levels of patients do not differ significantly from healthy controls; however, they found that the mean calcium level was significantly lower in thalassemic patients. Also, Salama et al. [28] found that serum phosphorus level was significantly higher in their thalassemic patients than in the controls, but no alteration in calcium level in β-thalassemia major patients as compared with controls.

Chelators for iron are administered to avoid excessive iron overload. In the study, it was discovered that low serum calcium and high serum phosphorus levels may indicate parathyroid dysfunction. They also deduced from the study that parathyroid dysfunction worsens with age. As a result, it is recommended to evaluate serum calcium, phosphorus, and alkaline phosphatase enzyme levels, as well as administer regular calcium and vitamin D supplements [29].

Moreover, measurements of serum calcium, phosphorus, and ALP enzyme indicate the state of bone mineralization; therefore, further studies involving a larger number of β-thalassemia patients with older age are required to determine the reliability of these markers in screening or monitoring of osteopenia in β-thalassemia patients.

Moreover, further studies on the association between serum vitamin D and BMD and PPAR gene polymorphism are required.

Osteopenia or osteoporosis has been identified as a major cause of morbidity in patients with β-thalassemia major over the past ten years and has been reported in approximately 40–50% of well-treated patients [30,31]. The expansion of bone marrow cavities and the reduction in trabecular bone volume in β-thalassemic patients' bones are caused by increased marrow erythropoiesis and extensive iron deposition, which also results in decreased bone tissue and osteoporosis [32,33]. Chelation is a significant risk factor for osteoporosis in these patients as high-dose desferrioxamine therapy decreases collagen production, increases osteoblast programmed cell death, and decreases differentiation and proliferation of bone-forming cells. Additionally, chelation causes a deficiency in vitamins and minerals like zinc and vitamin D, which worsens the condition of the bones [34,35].

In our study, we reported that the Z score of BMD measurements was significantly lower in patients with β-thalassemia compared to members of the control group; eighteen (eleven males and seven females) of fifty patients (representing 36% of the patient group) have osteopenia with low bone mineral density where Z-score is -1 or less than -1. Also, we reported no statistically significant difference between BMD values in males and females of the patients. In a previous study on Iranian β-thalassemic patients, all patients were classified as having normal BMD levels with Z-score > -1 and osteopenic with Z-score < -1. Of the 156 subjects (mean age was 23.3 $\pm$ 8.2 years), 33 were normal, and 123 (78%) had osteopenia [36]. In a later study on 30 Egyptian β-thalassemic patients (with a mean age of 21.53 $\pm$ 5.44), all patients (100%) had osteopenia and low BMD values, and there was no statistically significant difference between BMD values in male and female patients [37]. In our study, a low number of osteopenic patients was found, and that may be explained by the younger age of patients in our study (the median age of 11 years) in comparison with the other studies with higher ages. Additionally, it was also interpreted by the increasing awareness of the importance of supplementation of vitamin D and calcium to the patients of β-thalassemia in the last decade.

It has been found that genetic factors affect the BMD levels in patients with β-thalassemia [38]. Peroxisome proliferator-activated receptor-γ (PPARγ) is a subgroup of the PPAR family that regulates glucose and lipid metabolism [39]. The PPARγ gene is located on 3p25 in humans and is composed of nine exons [8,40]. Several single nucleotide polymorphisms (SNPs) have been detected in the PPARγ gene in humans. One of these polymorphisms is Pro12Ala (rs1801282), which substitutes proline for alanine at codon 12 in exon B [8].

Recent research has suggested that PPARγ plays an important role in osteogenesis [9,41]. The PPARγ activity appears to be important in regulating bone metabolism through stimulation of osteoblast differentiation into osteoclasts [9,34].

As shown in the result of our study, there was no statistically significant difference in the frequency of PPAR-γ 12Ala polymorphism between patients and controls. In agreement with us, in the study by [42] that included 30 Egyptian thalassemic patients and 10 normal subjects as controls, there was no statistically significant difference between patients and controls regarding PPAR-γ 12Ala gene polymorphism.

Regarding allele frequencies, we reported that the G allele was observed in 5% of thalassemia groups and 6% of controls, with no significant difference in allele frequency between males and females. Similar results were reported by a previous study [42], which showed a G allele frequency of 6.67% among Egyptian thalassemia patients, while it was ∼10 and 11% in South Asians and Caucasians, respectively. The frequency of the PPARγ Pro12Ala CC genotype in normal subjects was significantly higher (p = 0.024) than in patients with osteopenia [16].

The lower frequency of 12Ala in Egyptian β-thalassemic patients compared to other populations can be interpreted by genomic manipulation, and the environment–gene interaction such as dietary conditions, lifestyle, and drug interaction can activate PPAR-γ and affect its metabolic response [17].

Our study showed no significant difference between PPARγ Pro12 Ala gene polymorphism groups regarding Z-score values or laboratory data, including levels of ferritin, calcium, phosphorus, and ALP enzyme when using the logistic regression tests. The same results were obtained by a previous study [42]. Conversely, in a previous study, patients with the Pro 12Ala genotype (CC) had a higher risk of osteopenia (low Z-score values) [16]. However, the latter study was performed on Iranian β-thalassemic patients, and the difference between that study and our study may be explained by the different genetic interactions and environments between Egyptian patients and Iranian patients.

5. Conclusions

Osteopenia or low BMD level (Z score is −1 or lower) is common in patients with β-thalassemia in comparison with the control group. Eighteen of fifty patients had low BMD measurements, representing 36% of the patient group.

Hemoglobin level was lower, while ferritin level was higher in the patients group in comparison with the control group. No significant difference was found between the patient and control group regarding the levels of calcium, phosphorus, and ALP enzyme. There were no association between low BMD levels and the laboratory data, including serum calcium, phosphorus, and ALP enzyme level in the β-thalassemic patients group. However, further studies are needed on a larger population of patients with β-thalassemia are needed to confirm the previous findings, especially with the possibility of depending on these markers in screening or diagnosis or screening of osteopenia in patients with β-thalassemia.

This study suggests that the Pro12 Ala gene polymorphism of the PPARγ gene was unrelated to low BMD levels and osteopenia in Egyptian children of β-thalassemia. However, larger-scale trials involving more patients are still needed for better assessment to evaluate or exclude the possibility of using the PPARγ gene polymorphism as a marker for osteopenia in patients with β-thalassemia. Further studies are still required to focus on the

relationship between the laboratory markers, including serum calcium, phosphorus, and ALP enzyme level, and the gene polymorphisms of osteopenia in β-thalassemic patients.

Author Contributions: Conceptualization, A.M.A.H.; methodology, formal analysis, investigation, resources, data curation, writing—original draft preparation, writing—review and editing, H.M.A., D.H.E., D.S.M., M.S.I., T.A.H.A., M.M.S.A., S.S.S.A., A.M.A.A.M., M.O.E. and M.T.N.A.; visualization, M.S.I.; supervision, S.O.E.; project administration, M.S.I. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki and approved by the Research Ethical Committee of Beni-Suef University.

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: Data are available upon request.

Acknowledgments: The authors would like to thank the Deanship of Scientific Research at Shaqra University for supporting this work.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Deo, M.G.; Pawar, P.V. Alpha thalassaemia in tribal communities of coastal Maharashtra, India. *Indian J. Med. Res.* **2014**, *140*, 231. [PubMed]
2. Zaki, A.; Elgendy, M.O.; Abdelrahman, M.A.; Ali, H.A.; Khalil, E.M.; Hassan, M.; Fahmy, A.M.; Gad, R.A.; Salem, H.F. The Efficacy of Using Different Antibiotics to Prevent Maternal Surgical Site Infections in COVID-19-Infected Cases. *Eur. Chem. Bull.* **2023**, *6*, 1342–1348.
3. Floris, F.; Comitini, F.; Leoni, G.; Moi, P.; Morittu, M.; Orecchia, V.; Perra, M.; Pilia, M.P.; Zappu, A.; Casini, M.R.; et al. Quality of life in Sardinian patients with transfusion-dependent Thalassemia: A cross-sectional study. *Qual. Life Res.* **2018**, *27*, 2533–2539. [CrossRef] [PubMed]
4. Das, D.S. Comparative Study to Assess the Relationship between Renal Calculi, Thyroid and Parathyroid Hormones and Urinary Infections at Tertiary Care Centre. Ph.D. Thesis, Sree Mookambika Institute of Medical Sciences, Kanyakumari, India, 2019.
5. Tharwat, R.J.; Balilah, S.; Habib, H.M.; Mahmoud, N.H.; Beek, F.S.; Almadani, F.K.; Elmaghraby, S.; Al-Loqmani, D.D.; Al-Mahdi, H. Ferritin and Vitamin D levels and its relation to bone diseases in thalassemic adults: A hospital-based retrospective cohort study. *J. Appl. Hematol.* **2019**, *10*, 15. [CrossRef]
6. Wagner, N.; Wagner, K.D. The role of PPARs in disease. *Cells* **2020**, *9*, 2367. [CrossRef] [PubMed]
7. Liu, F.; Rouault, C.; Clément, K.; Zhu, W.; Degrelle, S.A.; Charles, M.-A.; Heude, B.; Fournier, T. C1431T Variant of PPARγ Is Associated with Preeclampsia in Pregnant Women. *Life* **2021**, *11*, 1052. [CrossRef] [PubMed]
8. Minge, C.E.; Robker, R.L.; Norman, R.J. PPAR gamma: Coordinating metabolic and immune contributions to female fertility. *PPAR Res.* **2008**, *2008*, 243791. [CrossRef]
9. Elgendy, M.O.; Saeed, H.; Abou-Taleb, H.A. Assessment of educated people awareness level and sources about COVID-19. *IJCMR* **2023**, *1*, 19–27. [CrossRef]
10. Lecka-Czernik, B.; Moerman, E.J.; Grant, D.F.; Lehmann, J.M.; Manolagas, S.C.; Jilka, R.L. Divergent effects of selective peroxisome proliferator-activated receptor-γ2 ligands on adipocyte versus osteoblast differentiation. *Endocrinology* **2002**, *143*, 2376–2384. [CrossRef]
11. Li, Y.; Jin, D.; Xie, W.; Wen, L.; Chen, W.; Xu, J.; Ding, J.; Ren, D. PPAR-γ and Wnt regulate the differentiation of MSCs into adipocytes and osteoblasts respectively. *Curr. Stem Cell Res. Ther.* **2018**, *13*, 185–192. [CrossRef]
12. Park, J.S.; Kim, M.; Song, N.-J.; Kim, J.-H.; Seo, D.; Lee, J.-H.; Jung, S.M.; Lee, J.Y.; Lee, J.; Lee, Y.S.; et al. A reciprocal role of the Smad4-Taz axis in osteogenesis and adipogenesis of mesenchymal stem cells. *Stem Cells* **2019**, *37*, 368–381. [CrossRef] [PubMed]
13. Karadeniz, F.; Oh, J.H.; Jo, H.J.; Seo, Y.; Kong, C.-S. Myricetin 3-O-β-D-Galactopyranoside Exhibits Potential Anti-Osteoporotic Properties in Human Bone Marrow-Derived Mesenchymal Stromal Cells via Stimulation of Osteoblastogenesis and Suppression of Adipogenesis. *Cells* **2021**, *10*, 2690. [CrossRef] [PubMed]
14. Arat, A.; Yılmaz, Ü.; Yılmaz, N.; Fazlıoğulları, O.; Çelik, F.; Başaran, C.; Zeybek, Ü. Effects of Leptin, Resistin, and PPAR-Gama Gene Variants on Obese Patients with Acute Coronary Syndrome in the Turkish Population. JAREM. *J. Acad. Res. Med.* **2020**, *10*, 166. [CrossRef]

15. Hashemi, A.; Ghilian, R.; Golestan, M.; Akhavan, G.M.; Zare, Z.; Dehghani, M.A. The study of growth in thalassemic patients and its correlation with serum ferritin level. *Iran. J. Pediatr. Hematol. Oncol.* **2011**, *1*, 147–151.
16. Sahmani, M.; Gholami, A.; Azarkeivan, A.; Darabi, M.; Ahmadi, M.H.; Sabet, M.S.; Najafipour, R. Peroxisome proliferator-activated receptor-γ Pro12Ala polymorphism and risk of osteopenia in β-thalassemia major patients. *Hemoglobin* **2013**, *37*, 564–573. [CrossRef] [PubMed]
17. Contreras, A.V.; Torres, N.; Tovar, A.R. PPAR-α as a key nutritional and environmental sensor for metabolic adaptation. *Adv. Nutr.* **2013**, *4*, 439–452. [CrossRef] [PubMed]
18. Al-Salehe, Q.A.A.; Al-Awady, M.S.; Abbass, S.K. Growth retardation in β-thalassemia major. *Iraqi Postgrad. Med. J.* **2015**, *14*, 267–273.
19. Ali, M.; Yassin, M.A.; Aldeeb, M. Iron Overload in a Patient with Non-Transfusion-Dependent Hemoglobin H Disease and Borderline Serum Ferritin: Can We Rely on Serum Ferritin for Monitoring in This Group of Patients? *Case Rep. Oncol.* **2020**, *13*, 668–673. [CrossRef]
20. Mirhosseini, N.Z.; Shahar, S.; Ghayour-Mobarhan, M.; Banihashem, A.; Kamaruddin, N.A.; Hatef, M.R.; Esmaili, H.A. Bone-related complications of transfusion-dependent beta thalassemia among children and adolescents. *J. Bone Miner. Metab.* **2013**, *31*, 468–476. [CrossRef]
21. Saboor, M.; Qudsia, F.; Qamar, K.; Moinuddin, M. Levels of calcium, corrected calcium, alkaline phosphatase and inorganic phosphorus in patients' serum with β-thalassemia major on subcutaneous deferoxamine. *J. Hematol. Thromboembolic Dis.* **2014**, *2*, 130. [CrossRef]
22. Huang, Y.-L.; Liu, S.; Xia, T.; Hao, W.-G.; Liang, W.; Sun, X. Relationship between growth disorders and iron overload in children with beta-thalassemia major. *Chin. J. Contemp. Pediatr.* **2008**, *10*, 603–606.
23. Abdelmotaleb, G.S.; Behairy, O.G.; El Azim, K.E.A.; El-Hassib, D.M.A.; Hemeda, T.M. Assessment of serum vitamin D levels in Egyptian children with beta-thalassemia major. *Egypt. Pediatr. Assoc. Gaz.* **2021**, *69*, 20. [CrossRef]
24. Karim, M.F.; Ismail, M.; Hasan, A.M.; Shekhar, H.U. Hematological and biochemical status of beta-thalassemia major patients in Bangladesh: A comparative analysis. *Int. J. Hematol. Oncol. Stem Cell Res.* **2016**, *10*, 7–12. [PubMed]
25. Hagag, A.A.; El-Shanshory, M.R.; Abo El-Enein, A.M. Parathyroid function in children with beta-thalassemia and correlation with iron load. *Adv. Pediatr. Res.* **2015**, *2*, 98–105.
26. Sultan, S.; Irfan, S.M.; Ahmed, S.I. Biochemical markers of bone turnover in patients with β-thalassemia major: A single-center study from southern Pakistan. *Adv. Hematol.* **2016**, *2016*, 5437609. [CrossRef] [PubMed]
27. Izzah, A.Z.; Rofinda, Z.D.; Arbi, F. Vitamin D and parathyroid hormone levels and their relation to serum ferritin levels in children with thalassemia major: One-Center Study in Western Indonesia. *J. Adv. Med. Pharm. Sci.* **2017**, *15*, 1–5. [CrossRef]
28. Salama, O.S.; Al-Tonbary, Y.A.; Shahin, R.A.; Eldeen, O.A.S. Unbalanced bone turnover in children with beta-thalassemia. *Hematology* **2006**, *11*, 197–202. [CrossRef]
29. Uma Rani, R.; Ganesh, T. Study of serum calcium, phosphorus and alkaline phosphatase during fracture healing of femur in goats. *Indian Vet. J.* **2003**, *80*, 377–378.
30. Yıldız, M.; Canatan, D. Soft tissue density variations in thalassemia major: A possible pitfall in lumbar bone mineral density measurements by dual-energy X-ray absorptiometry. *Pediatr. Hematol. Oncol.* **2005**, *22*, 723–726. [CrossRef]
31. Fung, E.B. Nutritional deficiencies in patients with thalassemia. *Ann. N. Y. Acad. Sci.* **2010**, *1202*, 188–196. [CrossRef]
32. Ehlers, K.H.; Giardina, P.J.; Lesser, M.L.; Engle, M.A.; Hilgartner, M.W. Prolonged survival in patients with beta-thalassemia major treated with deferoxamine. *J. Pediatr.* **1991**, *118*, 540–545. [CrossRef] [PubMed]
33. Vogiatzi, M.G.; Macklin, E.A.; Fung, E.B.; Cheung, A.M.; Vichinsky, E.; Olivieri, N.; Kirby, M.; Kwiatkowski, J.L.; Cunningham, M.; A Holm, I.; et al. Bone disease in thalassemia: A frequent and still unresolved problem. *J. Bone Miner. Res.* **2009**, *24*, 543–557. [CrossRef] [PubMed]
34. Perrotta, S.; Cappellini, M.D.; Bertoldo, F.; Servedio, V.; Iolascon, G.; D'Agruma, L.; Gasparini, P.; Siciliani, M.C.; Iolascon, A. Osteoporosis in β-thalassaemia major patients: Analysis of the genetic background. *Br. J. Haematol.* **2000**, *111*, 461–466. [CrossRef] [PubMed]
35. Pennisi, P.; Pizzarelli, G.; Spina, M.; Riccobene, S.; Fiore, C.E. Quantitative ultrasound of bone and clodronate effects in thalassemia-induced osteoporosis. *J. Bone Miner. Metab.* **2003**, *21*, 402–408. [CrossRef] [PubMed]
36. Coakley, K.E. Translating Evidence to Practice: A Systematic and Clinical Analysis of Bone Health in Phenylketonuria. Ph.D. Thesis, Emory University, Atlanta, Georgia, 2015.
37. Coppola, G.; Fortunato, D.; Auricchio, G.; Mainolfi, C.; Operto, F.F.; Signoriello, G.; Pascotto, A.; Salvatore, M. Bone mineral density in children, adolescents, and young adults with epilepsy. *Epilepsia* **2009**, *50*, 2140–2146. [CrossRef]
38. Jensen, C. High incidence of osteoporosis in thalassemia major. *J. Pediatr. Endocrinol. Metab.* **1998**, *11*, 975–977. [PubMed]
39. Chinetti-Gbaguidi, G.; Fruchart, J.-C.; Staels, B. Role of the PPAR family of nuclear receptors in the regulation of metabolic and cardiovascular homeostasis: New approaches to therapy. *Curr. Opin. Pharmacol.* **2005**, *5*, 177–183. [CrossRef] [PubMed]
40. Fournier, T.; Handschuh, K.; Tsatsaris, V.; Evain-Brion, D. Involvement of PPARγ in human trophoblast invasion. *Placenta* **2007**, *28*, S76–S81. [CrossRef]

41. Mbalaviele, G.; Abu-Amer, Y.; Meng, A.; Jaiswal, R.; Beck, S.; Pittenger, M.F.; Thiede, M.A.; Marshak, D.R. Activation of peroxisome proliferator-activated receptor-γ pathway inhibits osteoclast differentiation. *J. Biol. Chem.* **2000**, *275*, 14388–14393. [CrossRef]
42. El-Dafrawy, M.; Hamed, N.A.M.; Ghallab, O.; Elneely, D.; Khalifa, M. Preliminary Report on the Frequency of Pro12Ala Polymorphism of the Peroxisome Proliferator-Activated Receptor-gamma Gene in Egyptian β-Thalassemia Major Patients. *Int. Blood Res. Rev.* **2016**, *6*, 1–7. [CrossRef]