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ORIGINAL ARTICLE

The Impact of Genotype on Bone Complications in Beta Thalassemia Major Patients

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Abstract

Background: Specific genetic mutations in β - thalassemia lead to complete lack of β - globin chain production, considered as β° thalassemia, others allow some synthesis of the β-globin and are known as β^+ thalassemia. Patients need regular blood transfusions to correct anemia and iron-chelating therapy, to control iron overload. Severe anemia, along with excess body iron, and chelation therapy, can result in complications, as bone abnormalities, growth retardation, liver, cardiac, and endocrine disorders.

Methods: Cross sectional study subjected to record the impact of genotype on occurrence of bone complications in β-thalassemia cases, while 50 thalassemic cases involved from July 2017 to June 2018. DNA sequencing allowed for the cases' genotype identification. Bone density is evaluated using a dexa scan, translated into a Z-score compared to an appropriate reference, as well as bone imaging and laboratory investigations, which all evaluated as biochemical variables.

Results: Low bone mineral density was the commonest bone complication, while osteoporosis and osteopenia represented 34% and 28% respectively, other bone problems presented in 16% of cases. Additionally, a positive correlation between occurrence of osteoporosis, older patients, longer transfusion times, high ferritin levels, and longer transfusion gaps. The three most common mutations discovered were IVS1-110, IVS1-1, and IVS1-6 (28, 26, and 16%, respectively). The β°β° genotype showed a significantly high incidence of complications and low bone density compared to those with $\beta^\circ\beta^+$ and $\beta^+\beta^+$ genotypes.

Conclusion: Bone complications are common association in β-thalassemia major cases with a clear correlation between genotype and clinical disease progression as well as its severity.

Key words**:** Beta-thalassemia major; Genotype; Bone density; Dexa scan.

INTRODUCTION

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

Patients need blood transfusions to correct anemia and iron-chelating therapy to control iron overload, Anemia, excess body iron, and iron-chelation therapy can result in splenomegaly, growth

retardation, liver and cardiac failure, bone abnormalities, and endocrine disorders [2].

Bone changes are frequent in βTM patients and occur as a consequence of the hematological disorder and its complications, as well as iron overload, iron-chelation therapy, nutritional deficits and sedentarism. The sequelae of osteoporosis, especially vertebral and long bone fractures represent a major cause of morbidity in these patients [3].

Osteopenia and osteoporosis are observed in 40–50% of beta-thalassemia major patients, and so osteoporosis can be considered a prominent cause of co-morbidity in this population, which significantly increases fracture risk [4].

Genetic factors have been shown to play a role in the pathogenesis of osteopenia/osteoporosis in βTM patients. For instance, a polymorphism G →T or TT in the regulatory region of COLIA1 at the recognition site for transcription factor Sp1 is associated with the presence of osteoporosis [5].

What is the impact of genotype in βTM patients on the occurrence of bone complications? Our study aimed to find an answer to that correlation.

METHODS

A cross sectional study was conducted on 50 thalassemic patients (34 males and 16 females) with a mean age of 14.9±5.6 (range 4-26 years) who were registered at and followed up at the Pediatric Hematology Unit of Zagazig University Hospital between July 2017 and June 2018.

The present study was conducted in accordance with the ethical standards of the Helsinki Declaration, and was approved by an institutional review board. Participants in the study or their legal guardians gave their informed consent.

The required information was captured using a data abstraction form, and the following information was gathered: including age, sex, age at diagnosis, information on transfusions, such as frequency and age at which transfusions first began, chelation data, such as its type, compliance and the age of starting; physical examination, with a focus on anthropometric data and assessment of pubertal status in both genders using the Tanner classification and girl's menstrual status; laboratory data including complete blood count, serum ferritin, serum alkaline phosphatase, serum calcium, serum phosphorus, vitamin D levels, fasting blood glucose, basal growth hormone, parathyroid hormone, thyroid-stimulating hormone (TSH), free T4, luteinizing hormone (LH), follicle-stimulating hormone (FSH), estradiol (in girls) and testosterone (in boys); imaging studies including (DEXA) scan to measure bone mineral density Echo-cardiography ,plain X-Ray and Ultrasonography according to bone complications and study need; genotypes of patients were done using DNA sequencing techniques.

Three groups of patients were created depending on their genotype and the production of the beta globin gene. 17 patients (34%) in Group 1 had mutations that prevented B-globin chain synthesis ($\beta^o\beta^o$), 8 patients (16%) in Group 2 had mutations that caused only a small amount of B-globin chain synthesis ($\beta^o\beta^+$), and 25 patients (50%) had mutations that caused moderate amounts of -globin synthesis ($\beta^+\beta^+$).

Statistical analysis: For data analysis, the SPSS program (version 20) was utilised. The data are presented as the mean ± standard deviation 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.

RESULTS

Patient characteristics

The mean age of studied cases was 14.9±5.6 years which ranged 4-26 years. There were 34 males and 16 females, with a mean serum ferritin level of 3577.5±1826 ng/ml. The mean age of starting blood transfusion was 8.96±3.0, while 60% transfused every 2 weeks, 18% every 3 weeks and 22% were transfused every 4 weeks. The mean age of start iron chelation was 2.79±1.0 years. A round 72% of cases were receiving DFX, 8% were receiving DFO, 6% were receiving DFP, 10% were receiving combined DFO+DFP, and 4% were receiving combined DFO+DFX. The mean compliance was 86.2±17.1% ranged between 50-100%.

Bone complications were prevalent in the studied patients, as low bone density was present in 62% of cases, other bone problems, including fracture were presented in 16% of cases. Endocrinal complications were shown as a common association ,while growth retardation and hypogonadism were the commonest in studied cases (74 and 70%, respectively) followed by hypothyroidism, hypo-parathyroidism and diabetes mellitus (20, 14 and 8%, respectively).

IVS mutations

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).

Bone complications in patients

Low bone mineral density was a common association in 62% of studied βTM participants, while osteoprosis and osteopenia were shown in (34% and 28%, respectively) and other bone problems were noticed in 16% of patients (Table 1). Higher incidence of low bone density associated with higher z-score levels was significantly identified in older patients who started earlier blood transfusion (<9 months), received frequent transfusion (every 2–3 weeks), started iron chelation (<2 years) and were poor compliant with high mean serum ferritin level (Table 2).

β0β0 genotype

$\beta^o\beta^o$ genotype with a high ferritin level had a higher prevalence of osteoporosis with higher z-score as well as a higher prevalence of other complications, including bone fracture compared to patients with B^+B^+ genotype, as shown in figure (1). Besides, patients with $\beta^o\beta^+$ genotype showed a higher prevalence of osteopenia compared to other types (Table 3).

Genotypes according to B globin gene production:

Patients with IVS-1-1, C39, C5, C27, C15, C37, C44 mutations were associated with B^oB^o genotypes while patients with IVS-1-110, IVS-1-6, IVS-11-745, IVS-11-848, promotor 87 were associated with B^+B^+ genotypes, as well as IVS-1-110, IVS-1-1and IVS-1-6 showed the highest frequency among the patients (Table 4).

Genotypes and other complications

Patient with $\beta^o\beta^o$ genotype had higher prevalence of complications regarding other genotypes including growth retardation that identified in 94.1% , hypogonadism represented 94.1% , hypothyroidism was identified in 35.3% , hypo-parathyrodism in 41.2% of cases , as well as hepatomegaly and cardiac complications compared to other studied patients having $\beta^o\beta^+$ and $\beta^+\beta^+$genotype (Table 5).

Table (1) :Prevalence of bone complications in patients

	NO	%
Bone problems including Fracture	8	16.0
Bone density according to dexa scan		
Normal (Z-score > -1)	19	38.0
Osteoporosis (Z-score -2.5 or lower SD below the mean for the age and sex)	17	34.0
Osteopenia (Z-score ranges from -1 to -2.5)	14	28.0

Table (2): 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**

*p<0.05 is statistically significant **p ≤0.001 is statistically highly significant

Table (3): Relationship between Genotypes based on β-globin gene production with bone complications z – score and serum ferritin level

	n (50)	$\beta^o\beta^o$ N=17	%	$\beta^o\beta^+$ N=8	%	$\beta^+\beta^+$ N=25	%	X^2	P
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									
X±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									
X±SD		6375.8 ± 3136		4460± 2331		1689± 1043		19.2	<0.001**
Range		1045: 10320		769: 7560		532 : 6500			

* Significant ** highly significant

Table (4): Genotypes according to B globin gene production in patients

Genotype	Genotypes based on B globin gene production					
	$\beta^o\beta^o$ n=(17)		$\beta^0\beta^+$ n=(8)		$\beta^+\beta^+$ n=(25)	
	n	%	n	%	n	%
IVS-1-110/IVS-1-110	0	0.0	0	0.00	8	32.0*
IVS-1-1/IVS-1-1	6	35.3*	0	0.00	0	0.00
IVS-1-6/IVS-1-6	0	0.0	0	0.00	3	12.0*
C39/C39	3	17.6*	0	0.00	0	0.00
C5/C5	1	5.9	0	0.00	0	0.00
IVS-1-1/C27	2	11.8	0	0.00	0	0.00
IVS-1-110/IVS-1-6	0	0.00	0	0.00	5	20.0*

IVS-1-1/IVS-1-110	0	0.00	2	25.0*	0	0.00
IVS-1-6/IVS-11-745	0	0.00	0	0.00	2	8.00
IVS-1-1/IVS-11-745	0	0.00	3	37.5*	0	0.00
IVS-1-110/IVS-11-745	0	0.00	0	0.00	2	8.00
IVS-1-1/C15	1	5.9	0	0.00	0	0.00
Promoter-87/promoter-87	0	0.00	0	0.00	1	4.00
IVS-11-848/IVS-11-848	0	0.00	0	0.00	1	4.00
IVS-1-1/C39	1	5.9	0	0.00	0	0.00
IVS-1-1/IVS-1-6	0	0.00	3	37.5*	0	0.00
IVS-1-1/ C37	1	5.9	0	0.00	0	0.00
C37/ C37	1	5.9	0	0.00	0	0.00
IVS-1-1/ C 44	1	5.9	0	0.00	0	0.00
IVS-1-110/promoter-87	0	0.00	0	0.00	2	8.00
IVS-1-110/IVS-11-848	0	0.00	0	0.00	1	4.00

Table (5): 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

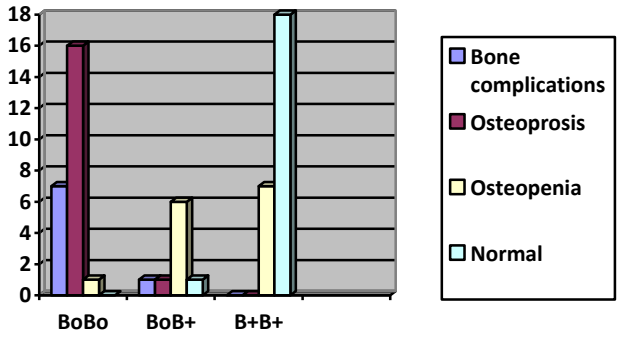

Figure (1): Illustrates relationship between Genotypes based on B globin gene production and bone complications

DISCUSSION

Bone disease is a frequent complication in thalassemic cases receiving repeated blood transfusions that emerged as a result of increased survival in thalassemia major patients. In this patient population, osteoporosis is brought on by a wide range of inherited and acquired factors [6].

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 [7].

Bone manifestations that are shown in cases of thalassemia usually occur as a result of expansion of bone marrow cavities, reduction in the volume of trabecular bone due to excess deposition of iron and extensive marrow erythropoiesis, which results in a decrease in bone tissue, and additionally, osteoporosis [8].

Such complications are brought on by a lack of minerals like calcium, phosphorus and zinc, as well as vitamins like vitamin D, which can worsen bone health. Bone disease may be exacerbated by the presence of endocrinopathies including hypothyroidism, hypoparathyroidism, hypogonadism and diabetes mellitus [9].

About two-thirds of our patients in the current study exhibited decreased BMD. From the total number of thalassemic patients, osteoporosis (34%) and osteopenia (28%) of cases were identified; these findings were consistent and matched with previous studies made by Voskaridou E et al& Shawkat et al and Cefalu CA [9,11&12].

Another study conducted by Lee SLK et al showed similar results while up to 55.84% of patients in Hong Kong , have low bone mineral density, whereas only 5.19% have osteoporosis/bone fragility state based on clinical fracture and DXA data [10].

We found in our 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 [13].

Current study showed that bone complications other than osteopenia or osteoprosis account for 16% of recorded cases, including bone pain, fracture and varying disabilities. Majority of cases had complained of bone pains while around 13% reported fractures cases. While poor quality of life in all domains (physical, emotional, schooling) in patients with TM have been reported worldwide .In our patients, we found a statistically significant correlation between age, serum ferritin and bone pain. High serum ferritin and increase prevalence of fracture demonstrated inadequate chelation insufficient to promote optimal bone health and hence increased risk of osteopenia, osteoporosis and fractures at an early age. The reported frequency of fractures in TM patients varies, from 20% in Italy according to Ruggiero L, De Sanctis V to 44% in Thailand as recorded by Sutipornpalangkul W et al [14, 15].

In our study, we found fractures in the upper and lower limbs in 13% of patients. The prevalence of fracture TDT in Hong Kong is comparable with the data of North American study conducted by Vogiatzi M et al, 15.58% vs. 12.1% (in North America) [10].

The older age of our patients associated with high ferritin levels, frequent blood transfusion rates and their poor compliance with iron chelation therapy, particularly desferrioxamine and deferiprone can be attributed to the higher prevalence of bone complications in our patients compared to other studies. Besides, new oral iron chelators were recently introduced and further studies should analyze their impact on patients.

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 Ahmed Al-Akhras et al 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 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). Also one study from

Hussin et al that listed the three most common mutations in Egyptian thalasemic patients in the Suez Canal Area were IVS-1-110, IVS-1-1 and IVS-1-6 [16-18].

Huisman et al. also discovered that the mutations IVS-1-110, IVS-1-6, IVS-1-1, promotor 87, IVS-11-745, and C39 were the most prevalent in Mediterranean regions. Additionally, C8, C8/C9, IVS1-5, C39, C44, and IVS11-1 were the variants that were most prevalent in the Middle East [19].

According to the genotyping data reported in the study by Willian R. Gomes et al, The CD39 mutation was the most prevalent among the subjects (44%) Next, IVS1-110 (16%) and IVS1-6 (36%) mutations were found [20].

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 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. These study data were in agreement with , Tamagnini et al 1983, Chehab et al 1987, Wong et al 1989, Yang et al 1989 and Diaz-Chico et al 1988 who reported that IVS-I-6/IVS-1-6 genotype was associated with mild $\beta^+\beta^+$ hematological phenotype, additionally IVS-1-110/IVS-1-110 genotype was associated with severe $\beta^+\beta^+$ hematological phenotype [21].

Results showed that patients with homozygous IVS-1-1/IVS1-1 genotype had early age of start transfusion and chelation as well as more frequent blood transfusion. And they had a significantly higher prevalence of growth retardation, hypogonadism, hypothyroidism and hypoparathyroidism. On the contrary, patients with IVS-1-110/IVS-1-110 and IVS-1-6/IVS-1-6 genotypes had a delayed age of starting transfusions, as well as less frequent blood transfusions and they had lower prevalence of growth retardation, hypogonadism, hypothyroidism and hypoparathyroidism.

Experienced experts have believed that overload of iron is the main factor contributing to the endocrine problems of β-thalassemia. These cases typically have severe growth retardation [22].

According to the current study, growth retardation was evident in 74% of the patients. In similar reports, Moayeri et al in their study reported that short stature was prevalent in 62% of patients. Furthermore, Mostafavi et al. showed an increased prevalence of growth retardation, with 90.9% of patients falling below the 5th percentile [23,24].

The prevalence of growth retardation in prior studies were in contrast to the current study, ranged from 30 to 50% [22,25,26-28]. The age of the patients who were studied, the frequency of blood transfusions, the kind of iron chelation therapy, and patient compliance are all factors that may affect the occurrence of growth retardation among studies.

Hypogonadism was a problem in 70% of the cases in our study, which is virtually in line with the findings of several other studies. Moayeri et al. Observed that 69% of patients with thalassemia major had hypogonadism. Also Chern et al has showed that hypogonadism was prevalenty in 72% [23,29]. The prevalence of hypogonadism in other previous research was higher than shown in the current study, as it ranged from 70 to 100%. [25,27,29,32]. In contrast to the current study, hypogonadism presented in other prior records a markedly lower prevalence that ranged from 12 to 54% [25,29,33].

Thyroid dysfunction is well recognized to be frequently shown in thalassemia major, but its prevalence and severity were variable in different cohorts .Through our study, we discovered that hypothyrodism was present in 20% of studied participants. These results were in close similarity

to some several other studies that reported a higher prevalence of hypothyrodism, reaching up to 17 – 18% as reported by Zervas A et al [35] while others reported low prevalence of 0 – 9% [31].

Through our study, hypoparathyrodism was prevalent in about 14% and it was usually presented as a late complication. In the current investigation, a significant correlation between the higher ferritin levels and the patients' ages with the prevalence of hypoparathyrodism was observed. Similarly, Gulati et al and Jensen et al reported that the prevalence of hypoparathyroidism was 13.5%&13% respectively. By contrast, Toumba et al recorded that the prevalence of hypoparathyroidism was as low as 1.2%, compared to current study.

In addition, diabetes mellitus is a common consequence for thalassemic individuals later in life, primarily because of iron overload, chronic liver disease, and genetic predisposition. Current study showed that the prevalence of diabetes mellitus was 10% . In agreement with our study, Najafipour reported that the prevalence of D.M. was 8.9%. Additionally, these results were in close similarity to the study by Ahmed Al-Akhras et al[16] that reported that the prevalence of diabetes was found in 8% of patients. Previous research showed a higher prevalence of diabetes mellitus compared to the current study, which ranged from 9 to 20%.[16,33,37] Through our study we recorded that hepatomegaly was prevalent in about 58% and was usually presented as a late complication, while the incidence of hepatitis was low, up to 3% [33].

Current study results showed that patients with the hematologic phenotypes $\beta^0\beta^+$ and $\beta^+\beta^+$ had delayed age of starting transfusion, chelation therapy, as well as less frequent blood transfusions and they had significantly lower prevalence of growth retardation, hypogonadism, hypothyroidism and hypoparathyrodism compared to cases with $\beta^0\beta^0$ genotype that had an earlier age of start transfusion and chelation, as well as more frequent transfusion rates. $\beta^+\beta^+$ was presented in our results as the most common genotype followed by $\beta^\circ\beta^\circ$ then $\beta^\circ\beta^+$ (50%, 34.0% and 16% respectively). Incidence of bone complications in our study including low bone mineral density, high Z-score and other bone complications as pain and fracture had a significant correlations to genotype (P value=0.001, 0.001 ,0.0016 respectively) that was correlated to a significant high serum ferritin levels (P value=0.001). Similarly, Yaman et al [38] reported $\beta^o\beta^o$ hematologic phenotype had s significant correlation with the age of first blood transfusion and it was a well recognized disease severity indicator. Additionally ,Hassan et al reported that $\beta^+\beta^+$ was the most common genotype followed by $\beta^\circ\beta^\circ$ then $\beta^\circ\beta^+$ (49.3%, 37.0% and 13.7% respectively) [17].

We had discovered that cases with $\beta^o\beta^o$ hematologic phenotype had a significantly high prevalence of low bone mineral density, growth retardation, hypogonadism, hypothyroidism, hepatomegaly and hypoparathyroidism . Similarly, there is an agreement with Yaman et al that showed in their reports that the rates of complications including low bone mineral density had a significant high prevelance in thalassemia major patients with $\beta^o\beta^o$ phenotype in a comparison to thalassemia intermedia patients with $\beta^+\beta^+$ ($P<0.05$). Similar to results found by Skordis et al who demonstrated the influence of $\beta^o\beta^o$ on development of hypogonadism and related it to difference in the amount of blood transfusion and variability in free iron radicals [27].

Conclusion

The present study has proved that bone complications were considered as common association in β-thalassemia major cases with a clear correlation between genotype and clinical disease progression as well as its severity. 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.

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