Assessment of sexual maturation in patients with β-thalassemia major receiving iron chelation therapy in Assuit University Hospital

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Received 01 September 2021
Revised 28 November 2021
Accepted 18 December 2021
Published 14 September 2022

Journal of Current Medical Research and Practice
2022, 7:258–263

Background
As the survival of persons with thalassemia (TM) major has improved, extending into their third and fourth decades of life, growth, sexual development, and fertility have become crucial issues to address.

Aim and objectives
The aim of this work was to assess sexual maturation among patients with transfusion-dependent β-TM major on iron chelation therapy.

Patients and methods
This study was conducted at Assiut University Children's Hospital on previously diagnosed patients with transfusion-dependent TM undergoing chelation therapy.

Results
The study included 84 patients aged from 13 years in girls and 14 years in boys up to 18 years in both sexes. There were 38 (45.2%) females and 46 (54.8%) males. Overall, 59.6% of the cases had at least a Tanner stage 2 on the maturity rating scale appropriate to the age of onset of puberty (normal puberty), where 34 patients (40.5%) had no signs of puberty at the appropriate age of onset of puberty (delayed puberty).
Between individuals with normal puberty and those with delayed puberty, there was a significant difference in anthropometric measurements, clinical examination, chelation therapy compliance, and serum ferritin level.

Conclusion
Patients with transfusion-dependent TM major have a significant prevalence of delayed puberty, which is linked to a higher level of serum ferritin and poor adherence to chelation therapy. We advocate the use of modern treatment procedures, as well as transfusion optimization and chelation therapy, to reduce or eliminate such occurrences.

Keywords:
hypogonadism, iron chelators, thalassemia, transfusion

J Curr Med Res Pract 7:258–263

2357-0121

Introduction

Thalassemia (TM) is an autosomal recessive disorder in which the synthesis of one or more globin chains is hindered, affecting hemoglobin production (Hb).

TM produces anemia in varied degrees, ranging from minor anemia to a life-threatening illness. People of Mediterranean, Middle Eastern, African, and Southeast Asian ancestry are more likely to carry the TM genes [1].

However, recent human migrations have disseminated TM genes all throughout the world [2].

The homozygous state of β-TM causes severe anemia that needs blood transfusions on a regular basis. Patients with thalassemic disease can now expect to live into their fourth and fifth decades owing to the confluence of blood transfusions and chelation therapy [3].

Frequent blood transfusions, on the contrary, might result in iron overload, which can lead to hypogonadism, hyperglycemia, hypothyroidism, hypoparathyroidism, and other endocrine disorders. Several authors have observed a significant rate of endocrine problems in children, adolescents, and young adults with TM major in recent years [3].

The aim of this study was to assess sexual maturation and risk factors affecting it in patients with β-TM major on regular blood transfusions and receiving chelation therapy.

Patients and methods

Between October 2019 and October 2020, a cross-sectional study was carried out at the Hematology

Unit of Assuit University Children's Hospital in Assuit, Egypt. A total of 84 patients with homozygous β-TM aged 13–18 years (46 males and 38 girls) were evaluated. From childhood, homozygous β-TM was diagnosed using standard hematological criteria (peripheral blood examination and Hb electrophoresis of the children). The patients' or their parents' informed consent was acquired (in noncompetent patients). Their ages ranged from 13 to 18 years old. Age at diagnosis, first blood transfusion, start of iron chelation therapy, age at splenectomy, serum ferritin levels, type, dose, iron chelation therapy compliance, presence of nonendocrine TM complications (cardiac failure and liver disease), menstrual history, and family history of diabetes were all recorded. All children have received frequent packed red cell transfusions every 2–3 weeks since early childhood to keep their pretransfusion Hb levels over 9.5 g/dl. The study was approved by the ethics commitee of assuit university (Register No,17100466).

Procedures

The preceding year's mean serum ferritin readings were calculated. Radiographic assessment of the left wrist was used to determine bone age.

Nonendocrine complications

Symptomatic heart failure was identified during clinical examination. Left ventricular dysfunction was defined as having a resting left ventricular ejection fraction of less than 50%, as measured by echocardiography.

Hepatitis C infection was detected using elevated liver enzyme levels, the presence of hepatitis C virus antibodies, and PCR. Liver function tests, hepatitis B surface antigen, serum calcium, phosphorus, and blood sugar were all assessed for routine follow-up of TM complications.

Endocrine evaluation

Weight, height, and proportions between upper and lower body segments were all measured, as well as pubertal stage according to Tanner's pubic hair and testicular development classification system [4]. To make patient anthropometric data easier to be demonstrated, weight, height, and BMI measures were converted to *Z*-scores using Egyptian growth charts [5]. Hypogonadism was defined as the absence of breast growth in girls aged 13.5 years and testicular enlargement in boys aged 16 years or more. By Prader's orchidometer, testicular size 4 ml (long axis 2.5 cm) was designated stage I (prepubertal genitalia), whereas size 25 ml (5 cm in length) was termed adult genitalia. A delay in pubertal development of more over 2 SD beyond the mean for their sex was characterized as impaired puberty. Arrested puberty was defined as a lack of puberty progression for more than a year and no progression to Tanner stages 4–5 beyond the age of 16 years. Primary amenorrhea was present if the menarche had not appeared by the age of 16 years old [4].

Hormonal testing (Follicle-stimulating hormone [FSH], luteinizing hormone [LH], and testosterone) was not performed.

Results

The study included 84 patients aged from 13 years in girls and 14 years in boys up to 18 years in both sexes. They were 38 (45.2%) females and 46 (54.8%) males. Of the studied cases, 50 patients (59.6%) had at least a Tanner stage 2 of maturity rating scale appropriate to the age of onset of puberty. They were considered as having normal puberty. The other 34 patients (40.5%) had no signs of puberty at the appropriate age of onset of puberty, and they were considered as having delayed puberty (Fig. 1).

Table 1 shows a comparison between the demographic data of the studied cases.

Table 2 shows chelation therapy and investigations in patients with normal and delayed puberty.

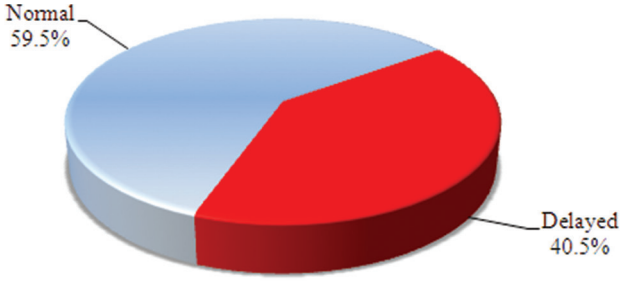
Table 3 shows the criteria of clinical examination and Tanner's classification in patients with normal and delayed puberty.

Table 4 shows the mean ± SD of ferritin levels in groups of different tanner stages.

Table 5 shows the mean ± SD of ferritin levels in groups of different maturation rate.

The ROC curve analysis of ferritin to predict sexual maturation is shown in Table 6 and Fig. 2.

Figure 1



Distribution of the studied patients with thalassemia according to sexual maturation.

Table 1 Comparison between the patients with normal puberty and those with delayed puberty according to demographic data

Demographic data	Total (*n*=84)	Sexual maturation [*n* (%)]		Test of significance	*P*
		Normal (*n*=50)	Delayed (*n*=34)		
Sex					
Male	46 (54.8)	31 (62.0)	15 (44.1)	χ^2=2.612	0.106
Female	38 (45.2)	19 (38.0)	19 (55.9)		
Age (years)					
Minimum–maximum	13.0-18.0	13.0-17.0	13.0–18.0	*U*=347.0*	<0.001*
Mean±SD	15.5±3.53	15.0±2.82	15.5±3.53		
Residence				0.2807	0.5962
Urban	40 (47.6)	25 (50)	15 (44.1)		
Rural	44 (52.4)	25 (50)	19 (55.8)		
Socioeconomic				3.799	0.1496
High	9 (10.7)	7 (14)	2 (0.5)		
Moderate	21 (25)	15 (30)	6 (17.5)		
Low	54 (64.3)	28 (56)	26 (82)		
Family history of thalassemia				2.052	0.1521
Negative	45 (53.3)	30 (60)	15 (45)		
Positive	39 (46.7)	20 (40)	19 (55)		
Family history of delayed puberty				11.58*	<0.001*
Negative	58 (55)	35 (70)	23 (67.7)		
Positive	26 (45)	15 (30)	11 (32.3)		

Table 2 Comparison between the patients with normal puberty and those with delayed puberty according to chelation therapy and the investigation

Chelation therapy	Total (*n*=84) [*n* (%)]	Sexual maturation [*n* (%)]		*P*
		Normal (*n*=50)	Delayed (*n*=34)	
Type				^{MC}P=0.167
Deferasirox	78 (92.9)	48 (96.0)	30 (88.2)	
Deferiprone	4 (4.8)	2 (4.0)	2 (5.9)	
Deferoxamine	2 (2.4)	0	2 (5.9)	
Dose (mg/kg)				0.280
Minimum–maximum	20.0-40.0	20.0-30.0	20.0-40.0	
Mean±SD	23.48±4.63	23.38±3.39	23.62±6.07	
Compliance				^{FE}P=0.027*
Regular	75 (89.3)	48 (96.0)	7 (20.6)	
Irregular	9 (10.7)	2 (4.0)	27 (79.4)	
Investigation				
Bone age on x-ray				
Minimum–maximum	9.0-17.0	10.0-17.0	9.0-17.0	0.001*
Mean±SD	12.48±1.97	13.26±1.83	11.94±1.89	
HB (g/dl)				<0.001*
Minimum–maximum	5.0–8.9	5.0–8.9	5.0–8.0	
Mean±SD	7.2±0.7	7.4±0.65	6.9±0.55	
Ferritin level				
Minimum–maximum	200-8000	500-5000	200-8000	0.022*
Mean±SD	2168 2±1404	1838.3±913.3	2653±1818.8	

FE, Fisher' exact text; MC, Monte–Carlo.

Multiple regression analysis was also performed for ferritin level, sexual Tanner stages, and age separately to predict ferritin level based on Tanner stage and age (Table 7 and Fig. 3).

Discussion

Failure of normal pubertal development and growth retardation are the most common endocrine adverse effects of iron overload. Iron chelation drugs have been shown to improve survival and slow or stop the progression of iron-induced heart disease [6].

Of the 50 patients studied, 50 (59.6%) had at least a Tanner stage 2 of the maturity rating scale appropriate for the age of puberty and were considered to have normal puberty. The other 34 patients (40.5%) had no signs of puberty at the appropriate age of puberty and were considered to have delayed puberty. According to Batubara *et al.* [7], who studied 72 patients with TM major aged 13–18 years old, delayed puberty

Table 3 Comparison between the patients with normal puberty and those with delayed puberty according to criteria of clinical examination and Tanner's classification

Clinical examination	Total (*n*=84) [*n* (%)]	Sexual maturation [*n* (%)]		*P*
		Normal (*n*=50)	Delayed (*n*=34)	
Tachycardia	11 (13.1)	6 (12.0)	5 (14.7)	^{FE}P=0.751
Murmurs	12 (14.2)	2 (4.0)	10 (29.4)	^{FE}P=0.512
Heart failure	7 (8.3)	2 (4.0)	5 (14.7)	^{FE}P=0.311
Cardiomegally	15 (17.85)	4 (8.0)	11 (32.3)	^{FE}P=0.452
Pulm HTN	12 (14.2)	5 (10.0)	7 (20.58)	^{FE}P=0.510
Hepatomegally	48 (57.0)	22 (44.0)	26 (76.4)	^{FE}P=0.303
Cirrhosis	28 (33.3)	9 (18.0)	19 (55.8)	^{FE}P=0.612
Spleenomegally	42 (50.0)	16 (38.0)	26 (62.0)	^{MC}P=0.002*
Mild	8 (9.5)	4 (9.5)	4 (9.5)	
Moderate	23 (29.7)	9 (21.4)	14 (33.3)	
Huge	11 (10.8)	3 (7.1)	8 (19.0)	
Splenectomy	42 (50.0)	26 (62.0)	16 (38.0)	<0.001*
Tanner's classification				
Age of first sign				<0.001*
Minimum–maximum	10.0–15.0	10.0–12.0	11.0–15.0	
Mean±SD	11.77±1.05	11.22±0.74	12.59±0.89	
Tanner stage on examination				
Stage 1	38 (45.2)	23 (46.0)	15 (44.1)	^{MC}P=0.011*
Stage 2	21 (25.0)	11 (22.0)	10 (29.4)	
Stage 3	16 (19.0)	7 (14.0)	9 (26.5)	
Stage 4	9 (10.8)	9 (18.0)	0	

HTN, hypertension.

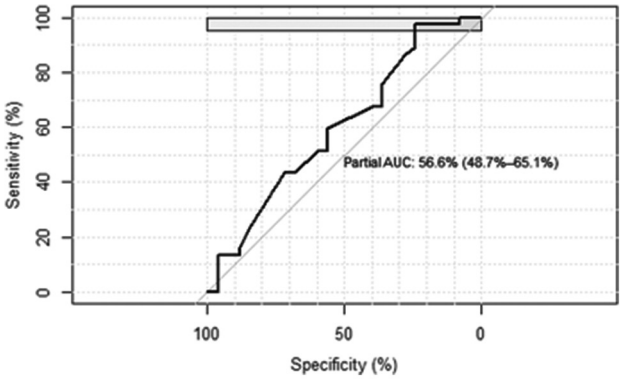
Table 4 The mean±SD of ferritin levels in groups of different Tanner stages

Sexual maturation rate	Ferritin level	
	Mean	SD
Stage 1	2339	1464
Stage 2	1735	1003
Stage 3	1797	537
Stage 4	1780	259

Table 5 The mean±SD of ferritin levels in groups of different maturation rates

Maturation rate	Ferritin level	
	Mean	SD.
Delayed	2384	1614.
Normal	1857	813

Figure 2



Receiver operating characteristic curve for ferritin level for the prediction of sexual maturation.

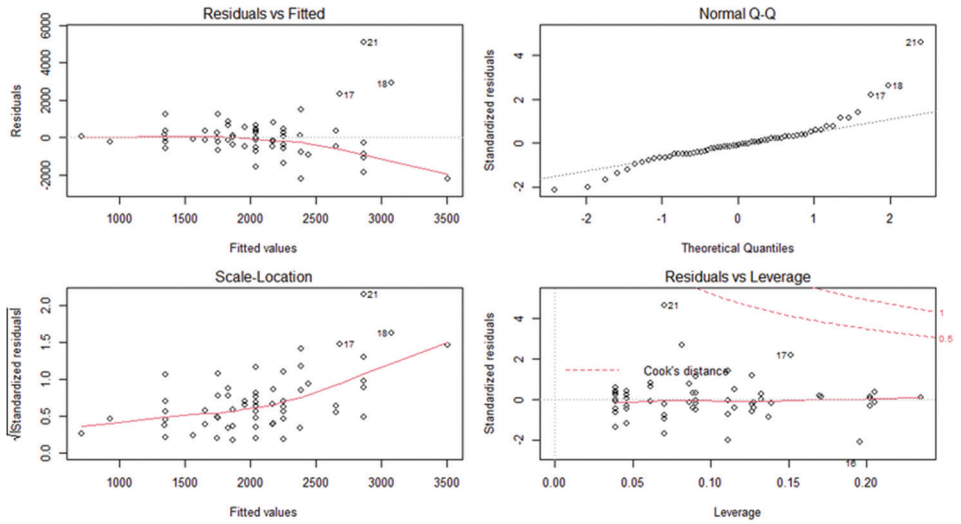
occurred in 40 patients (56%) consisting of 21 boys and 19 girls, whereas Wahidiyat *et al.*[8] found that 36 of 40 patients (90%) did not experience puberty, indicating that there has been an improvement in the management of patients with TM over the past 20 years. Furthermore, Abdelrazik and Ghanem[9] discovered that delayed puberty was evident in 80% of boys and 75% of girls above the age of 12 years old.

In a study of patients with TM, Moayeri and Oloomi[10] discovered that hypogonadism was common (69%). In individuals older than 14 years with impaired puberty, they discovered a low serum level of gonadotropins (FSH and LH), indicating that hypogonadotropic hypogonadism is to blamed.

Our findings were backed up by studies by Abdelrazik and Ghanem and Moayeri and Oloomi [9,10], which found that although the age of initiation of chelating therapy was similar in both groups, there was a substantial difference in the frequency and regularity of use.

In TM major, iron excess is the leading cause of delayed puberty. One of the approaches for detecting iron excess is transferrin saturation [11]. Iron overload was found in 36 patients (40%) with delayed puberty in a research by Batubara *et al.* [7]. Iron overload was found in 25 of 32 patients with normal puberty. It is possible that these individuals did not have iron overload when they developed secondary sex

Figure 3



Multivariate regression analysis for ferritin level, Tanner stages, and age.

Table 6 Sensitivity and specificity of ferritin levels to predict sexual maturation

Parameter	Area under the curve (%)	95% confidence interval	Selected cutoff value	Sensitivity at cutoff	Specificity at cutoff
Ferritin level	57	67.18	2850	97.29	24

Table 7 Association analysis between ferritin level and Tanner stages

Variable	Coefficient	SE of coefficient	*t* ratio	*P*
Constant	108.6	1882.7	0.058	0.9542
Tanner stage 2	−1117.2	428.1	−2.609	0.0116
Tanner stage 3	−1549.4	−624.0	−2.483	0.0161
Tanner stage 4	−1453.6	943.3	−1.541	0.1290
Age	212.4	141.7	−0.499	0.1395

characteristics, but our findings are corroborated by this study, which found that the delayed group had higher ferritin levels than the normal group (Table 2). The relationship of FSH, LH, and estrogen with chelation therapy duration was investigated by Sutay *et al.* [12], who found that there was no significant difference in FSH, LH, and estrogen levels with respect to chelation therapy duration. Those who had been on chelation therapy for more than 5 years had higher ferritin levels than those who had just been on it for a few months, which was statistically significant, with a *P* value of 0.044. This could be owing to the fact that those who received chelation for a longer period of time also received blood transfusions for a longer period of time. Dhouib *et al.*[13] also discovered that spontaneous puberty occurred in 16 cases (nine boys and seven girls) at an average age of 15 years (range 14–16 years) for boys and 13 years (range 11–15 years) for girls. Two indicators related to pubertal problems are transfusion demands before chelating therapy (*P* = 0.042) and myocardial iron measurement (*P* = 0.037). According to several studies, iron chelation therapy has a crucial role in gonadic function and growth in people with TM major, and most patients who begin treatment early in life and maintain good compliance may experience normal growth and sexual maturation [14].

In this study, the mean bone age on radiography in the normal group was 13.26 ± 1.83 years, with a range of 10.0–17.0, and in delayed group was 11.94 ± 1.89 years, with a range of 9.0–17.0 years. The etiopathogenesis of growth deficiency and delayed bone age is unknown; however, it is likely related to simultaneous processes such as chronic tissue hypoxia, siderosis, deferoxamine toxicity-induced bone dysplasia, zinc deficiency, hypothyroidism, hypogonadism, and growth hormone impairment. All of these factors interact in each subject and are influenced by transfusion regimen, age of initiation, and type of iron chelation. However, several factors, including the average Hb level before transfusion, nutritional status, and the presence of accompanying disease, could be responsible for this difference [14].

Hypogonadism is highly common in patients with TM. Several authors observed a high frequency of endocrine disorders. They proved that these anomalies were caused by an excess of iron in the body. This theory was validated by histology examinations of various endocrine glands [14].

Abdelrazik and Ghanem [9] found a substantial difference in mean blood ferritin levels between thalassemic patients with primary amenorrhea, irregular menses, and hypogonadism and those without endocrinopathies. These studies demonstrate the importance of iron overload in the development of

endocrine illnesses. On the contrary, several studies have revealed no correlation between ferritin levels and a range of other endocrinopathies [11]. It has been claimed that thalassemic patients with serum ferritin concentrations less than 2500 ng/ml have an excellent prognosis for survival. For a long time, iron excess has been thought to be the primary cause of endocrine problems in TM, and histology examinations of various endocrine glands back this up. Although there is evidence of free radical production and lipid peroxidation leading to mitochondrial lysosomal damage, the exact mechanism by which iron overload causes tissue damage is unknown. In iron-overloaded patients, the most prevalent results are delayed puberty and hypogonadism [15].

Transfusional hemosiderosis in the pituitary gonadotroph cell causes gonadotropin insufficiency, resulting in this endocrine disorder. The anterior pituitary gland is especially vulnerable to free radical-induced oxidative stress, and exposure to it, as measured by MRI, shows that even a small amount of iron deposition within the anterior pituitary gland can interfere with its function, and the signal intensity reduction in the anterior lobe of the pituitary gland was correlated with serum ferritin levels and the severity of pituitary dysfunction. On histologic inspection, the gonads show mild siderosis, with uncommon iron-containing macrophages in the ovaries and a reduced number of primordial follicles. The majority of iron in the testes is deposited in the seminiferous tubules and interstitial tissues, with only a minor amount in the Leydig cells, causing basement membrane breakdown [16].

In patients with β-TM, there is a link between the degree of organ damage and the degree of iron excess. This emphasizes the need of preventing iron overload by initiating early and consistent iron chelation therapy before damage to the pituitary gland and gonads occurs. It also emphasizes the importance of extensive chelation for β-TM normal puberty patients, particularly those who have just a partial response, to save the remaining working cells.

Conclusion

Patients with transfusion-dependent β-TM major have a significant prevalence of delayed puberty, which is linked to a higher level of serum ferritin and poor adherence to chelation therapy. We advocate the use of modern treatment procedures, as well as transfusion optimization and chelation therapy, to reduce or eliminate such occurrences.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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