Impact of genotype on endocrinal complications in β-thalassemia patients

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Abstract. In β-thalassemia, certain mutations cause a complete absence of β-globin chain synthesis, termed β^0-thalassemia, while others may allow certain β-globin production and are termed β^+- or β^{++}-thalassemia. The homozygous state results in severe anemia, which requires regular blood transfusion. By contrast, frequent blood transfusion can in turn lead to iron overload, which may result in several endocrinal complications. The present study aimed to investigate the impact of genotype on the development of endocrine complications in β-thalassemia patients. A cross-sectional study was conducted on 100 thalassemia patients >10 years. A data abstraction form was designed to capture the appropriate information from the individual medical records, including full clinical, laboratory, transfusion and chelation data. The genotype of the patients was identified by the DNA sequencing technique. Growth retardation and hypogonadism were the most prominent endocrinal complications (70 and 67%, respectively) followed by hypothyroidism, diabetes mellitus and hypoparathyrodism (8, 8 and 7%, respectively). The most common mutations identified were IVS-1-110, IVS-1-1 and IVS-1-6 (63, 47 and 41%, respectively). Patients with the $\beta^0\beta^0$ genotype had a significantly higher prevalence of growth retardation, hypogonadism, hypothyroidism and hypoparathyrodism compared to those with the $\beta^0\beta^+$ and $\beta^+\beta^+$ genotypes (P<0.001, P<0.001, P<0.001 and P=0.037, respectively). Patients with the homozygous IVS-11-745 mutation had a significantly higher prevalence of diabetes (P=0.001). The underlying genetic defect in thalassemia patients is a contributing factor for the development of endocrinal complications, as patients with the more severe defects have a greater rate of iron loading through higher red cell consumption.

Introduction

The β-thalassemias are a group of recessively inherited hemoglobin disorders characterized by reduced synthesis of β-globin chains (1). The severity of clinical manifestation and laboratory findings in thalassaemia largely depends on the type of underlying mutations of the β-globin gene, which are mostly point mutations or gene deletions (2). When no β-chains are produced (β^0) an excess amount of α-globin chains results in the destruction of the red cell precursors in the bone marrow. In cases where there is some production of β-globin chain ($\beta^+\beta^{++}$), the chain imbalance will be less, resulting in milder clinical phenotype (3). The homozygotes or compound heterozygote patients for β-thalassemia depend on frequent transfusions for life (4).

However, as a result of hypertransfusion therapy and increased longevity, iron tissue toxicity has become more common, and contributes significantly to morbidity in these patients (5). Despite intensive chelating therapy, growth retardation, hypogonadotrophic hypogonadism, diabetes mellitus, hypothyroidism and hypoparathyrodism represent the most common endocrinopathies in thalassemic patients (6). Data from a previous study suggests that the genotype, which determines the clinical severity of the disease, may also be a contributing factor in the development of such complications (7). The present study aimed to identify the association between genotype and endocrine complications in patients with β-thalassemia.

Materials and methods

General. A cross-sectional study was conducted on 100 thalassemic patients (54 males and 46 females) with a mean age of 14.2±1.37 years (range, 12-18 years), who were registered in and followed up at the Pediatric Hematology Unit of Zagazig University Hospital (Zagazig, Egypt) between July 2011 and June 2013. The data abstraction form was designed to capture the appropriate information, and the collected data included: Full clinical information including age, gender and age at

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Table I. Association between growth retardation and each of the demographic, transfusion, chelation characteristics, compliance and serum ferritin level.

| Characteristics | Patients, n | Growth retardation (n=100) | | χ^2 test | P-value |
		Negative (n=30), n (%)	Positive (n=70), n (%)		
Age, years				5.74	0.016[a]
≤14	66	25 (37.9)	41 (62.1)		
>14	34	5 (14.7)	29 (74.3)		
Gender				0.01	0.9
Male	54	16 (29.6)	38 (70.4)		
Female	46	14 (30.4)	32 (69.6)		
Age of start transfusion, months				11.98	<0.001[b]
≤9	68	13 (19.1)	55 (80.9)		
>9	32	17 (53.1)	15 (46.9)		
Frequency of transfusion, weeks				15.39	<0.001[b]
Every 2-3	43	4 (9.3)	39 (90.7)		
Every 4-5	57	26 (45.6)	31 (54.5)		
Age of start chelation, years				28.70	<0.001[b]
≤3	73	11 (15.1)	62 (84.9)		
>3	27	19 (70.4)	8 (29.6)		
Type of chelators				7.59	0.055
DFX	19	9 (47.4)	10 (52.6)		
DFP	40	11 (27.5)	29 (72.5)		
DFO	31	5 (16.1)	26 (83.9)		
DFO+DFP	10	5 (50.0)	5 (50.0)		
Compliance, %				9.90	0.0015[a]
<60	69	14 (20.3)	55 (79.7)		
≥60	31	16 (51.6)	15 (48.4)		
Mean serum ferritin level ± SD (range), ng/ml		2,227.8±796.1 (836-5,000)	4,155.9±1,841.3 (1,026-8,500)	5.50	<0.001[b]

[a]$P<0.05$; [b]$P<0.001$. DFX, deferasirox; DFP, deferiprone; DFO, desferrioxamine; SD, standard deviation.

diagnosis; transfusion data including age of start transfusion and frequency of transfusion; chelation data including age of start chelation, type of iron chelators and compliance; physical examination with special emphasis on assessment of anthropometric data and assessment of pubertal status in both genders according to the Tanner classification and girl's menstrual status; laboratory data including complete blood count, serum ferritin, fasting blood glucose, basal growth hormone, parathormone, thyroid-stimulating hormone (TSH), free T4, luteinizing hormone (LH), follicle-stimulating hormone (FSH), estradiol (in females) and testosterone (in males) and genotypes of patients were performed using DNA sequencing techniques in Laboratory of Hemoglobinopathies (University of Ulm, Ulm, Germany).

Treatment protocol. All the patients followed a standard treatment protocol and were transfused every 2-5 weeks to maintain the pretransfusion hemoglobin level >9 mg/dl. Patients received either desferrioxamine (DFO) subcutaneously or intravenously at a dose of 30-50 mg/kg/day alone or in combination with

deferiprone (DFP) at a dose of 50-75 mg/kg/day. Other patients received DFP alone or deferasirox (DFX) alone in a dose of 20-40 mg/kg/day with variable compliance.

Classification of patients according to genotype. Patients were divided into 3 groups according to their genotype based on the β-globin gene production. Group 1 consisted of 34 patients (34%) with mutations resulting in no β-globin chain synthesis ($\beta^0\beta^0$), group 2 included 6 patients (6%) with a mutation resulting in a small amount of β-globin chain synthesis ($\beta^0\beta^+$) and group 3 included 60 patients (60%) with a mutation resulting in a moderate amount of β-globin synthesis ($\beta^+\beta^+$).

Definitions. Short stature was defined as patient height >2 standard deviation below the mean for age, gender and ethnicity (6). Short stature was evaluated by assessment of patient height and plotted on Egyptian growth charts (source: Cairo University, Diabetic Endocrine and Metabolic Pediatric Unit and the National Research Center, Cairo, Egypt), and measurement of the basal growth hormone level.

Table II. Association between hypogonadism and each of the demographic, transfusion, chelation characteristics, compliance and serum ferritin level.

| Characteristics | Patients, n | Hypogonadism (n=100) | | χ^2 test | P-value |
		Negative (n=33), n (%)	Positive (n=67), n (%)		
Age, years				5.49	0.019[a]
≤14	66	27 (40.9)	39 (59.1)		
>14	34	6 (17.6)	28 (82.4)		
Gender				0.01	0.93
Male	54	18 (33.3)	36 (66.7)		
Female	46	15 (32.6)	31 (67.4)		
Age of start transfusion, months				14.81	<0.001[b]
≤9	68	14 (20.6)	54 (79.4)		
>9	32	19 (59.4)	13 (40.6)		
Frequency of transfusion, weeks				19.16	<0.001[b]
Every 2-3	43	4 (9.3)	39 (90.7)		
Every 4-5	57	29 (50.9)	28 (49.1)		
Age of start chelation, years				28.20	<0.001[b]
≤3	73	13 (17.8)	60 (82.2)		
>3	27	20 (74.1)	7 (25.9)		
Type of chelators				5.70	0.12
DFX	19	9 (47.4)	10 (52.6)		
DFP	40	13 (32.5)	27 (67.5)		
DFO	31	6 (19.4)	25 (80.6)		
DFO+DFP	10	5 (50.0)	5 (50.0)		
Compliance, %				9.69	0.0018[a]
<60	69	16 (23.2)	53 (76.8)		
≥60	31	17 (54.8)	14 (45.2)		
Mean serum ferritin level ± SD (range), ng/ml		2,207.2±755.9 (836-5,000)	4,252.4±1,824.1 (1,626-8,500)	6.12	<0.001[b]

[a]P<0.05; [b]P<0.001. DFX, deferasirox; DFP, deferiprone; DFO, desferrioxamine; SD, standard deviation.

Hypogonadotropic hypogonadism is LH and FSH levels <2 IU/l, with an estradiol concentration of <20 pg/ml in females or a testosterone concentration of <3 ng/ml in males (7). Hypogonadism was detected by the absence of breast development in females and absence of testicular enlargement in males (<4 ml), as measured by preorchidometer by the age of 16 years (8).

Primary hypothyroidism is a low serum thyroxine with an elevated serum TSH concentration (9). The criteria for diagnosis of hypoparathyroidism were low parathormone level, low total and ionized serum calcium, high serum phosphate, normal serum magnesium and alkaline phosphatase levels (10).

The criteria for diabetes mellitus was based on a family history of diabetes, and if the patient was on treatment with insulin and measurement of fasting blood glucose level according to American Diabetes Association, World Health Organization Criteria and National Diabetes Health Group 1979 (11).

Statistical analysis. Data were assessed, entered and analyzed using SPSS version 20 (IBM SPSS, Armonk, NY, USA). Data are expressed as the mean ± standard deviation for quantitative variables, number and percentage for qualitative variables. χ^2 test and t-test were used when appropriate to compare between different groups. P<0.05 and P<0.001 were considered to indicate statistically significant differences.

Statement of ethics. The present study was conducted in accordance with the ethical standards of the Helsinki Declaration of 1964 as revised in 2000, and was approved by the Institutional Review Board. Informed consent was obtained from the study participant or from their guardians.

Results

Patient characteristics. The mean age of the patients was 14.2±1.37 years with a range of 12-18 years. There were 54 males and 46 females, with a mean serum ferritin level of 3,577.5±1,826 ng/ml. In total, 68% of the patients started blood transfusion <9 months and 32% started blood transfusion >9 months. The mean age of start iron chelation was

Table III. Association between hypothyroidism and each of the demographic, transfusion, chelation characteristics, compliance and serum ferritin level.

| Characteristics | Patients, n | Hypothyroidism (n=100) | | χ^2 test | P-value |
		Negative (n=92), no. (%)	Positive (n=8), no. (%)		
Age, years				13.83	0.001[b]
≤14	66	66 (100.0)	0 (0.0)		
>14	34	26 (76.5)	8 (23.5)		
Gender				0.02	0.89
Male	54	50 (92.6)	4 (7.4)		
Female	46	42 (91.3)	4 (8.7)		
Age of start transfusion, months				4.05	0.04[a]
≤9	68	60 (88.2)	8 (11.8)		
>9	32	32 (100.0)	0 (0.0)		
Frequency of transfusion, weeks				9.14	0.002[a]
Every 2-3	43	35 (81.4)	8 (18.6)		
Every 4-5	57	57 (100.0)	0 (0.0)		
Age of start chelation, years				1.90	0.16
≤3	73	65 (89.0)	8 (11.0)		
>3	27	27 (100.0)	0 (0.0)		
Type of chelators				5.29	0.15
DFX	19	19 (100.0)	0 (0.0)		
DFP	40	34 (85.0)	6 (15.0)		
DFO	31	29 (93.5)	2 (6.5)		
DFO+DFP	10	10 (100.0)	0 (0.0)		
Compliance, %				3.87	0.0049[a]
<60	69	61 (88.4)	8 (11.6)		
≥60	31	31 (100.0)	0 (0.0)		
Mean serum ferritin level ± SD (range), ng/ml		3,247.2±1,482 (836-6,820)	7,376.2±839.2 (5,600-8,500)	7.70	<0.001[b]

[a]P<0.05; [b]P<0.001. DFX, deferasirox; DFP, deferiprone; DFO, desferrioxamine; SD, standard deviation.

2.78±1.1 years. A total of 40% of patients were receiving DFP, 31% were receiving DFO, 19% were receiving DFX and 10% was receiving combined DFO and DFP. The mean compliance was 55.52±16.2% with a range of 28-85%.

Growth retardation and hypogonadism were the most common endocrinal complications in the patients (70 and 67%, respectively) followed by hypothyroidism, diabetes mellitus and hypoparathyroidism (8, 8 and 7%, respectively).

IVS mutations. The most prominent mutations identified in the patients were IVS-1-110, IVS-1-1 and IVS-1-6 (63, 47 and 41%, respectively), followed by C39 (10.5%), IVS-11-745 (6%), promotor 87 (3%), C5 (2%), C15 (1%), IVS-1-5 (1%) and IVS-11-848 (1%).

Growth retardation in patients. Growth retardation was identified in 74.3% of patients >14 years old and 62.1% of patients <14 years old, and no significant difference was identified between males and females. A total of 78.5% of patients with growth retardation started earlier blood transfusion (<9 months), 55.7% received

frequent transfusion (every 2-3 weeks), 88.5% started iron chelation (<3 years) and 78.5% were poor compliant with high mean serum ferritin level (4,155.9±1,841.3 ng/ml) (Table I).

Hypogonadism in patients. Hypogonadism was identified in 82.4% of patients >14 years old and 59.1% of patients <14 years old with no significant difference between males and females. A total of 80.5% of patients with hypogonadism started earlier transfusion (<9 months), 58.2% of them received frequent transfusion (every 2-3 weeks). In total, 89.5% of patients with hypogonadism started iron chelation (<3 years) and 79.1% had a poor compliance with a high mean serum ferritin level (4,252.4±1,824.1 ng/ml) , as shown in Table II.

All the patients who developed hypothyroidism were >14 years old (4 males and 4 females) and no significant difference was identified between males and females. These patients all started earlier transfusion (<9 months), earlier iron chelation (<3 years) as well as frequent blood transfusion (every 2-3 weeks), and 11.6% had a poor compliant with

Table IV. Association between diabetes mellitus and each of the demographic, transfusion, chelation characteristics, compliance and serum ferritin level.

Characteristics	Patients, n	Diabetes mellitus (n=100)		χ^2 test	P-value
		Negative (n=92), n (%)	Positive (n=8), n (%)		
Age, years				1.92	0.16
≤14	66	63 (95.5)	3 (4.5)		
>14	34	29 (85.3)	5 (14.7)		
Gender				0.02	0.89
Male	54	50 (92.6)	4 (7.4)		
Female	46	42 (91.3)	4 (8.7)		
Age of start transfusion, months				4.05	0.04[a]
≤9	68	60 (88.2)	8 (11.8)		
>9	32	32 (100.0)	0 (0.0)		
Frequency of transfusion, weeks				2.35	0.12
Every 2-3	43	37 (86.0)	6 (14.0)		
Every 4-5	57	55 (96.5)	2 (3.5)		
Age of start chelation, years				1.90	0.16
≤3	73	65 (89.0)	8 (11.0)		
>3	27	27 (100.0)	0 (0.0)		
Type of chelators				5.29	0.15
DFX	19	19 (100.0)	0 (0.0)		
DFP	40	34 (85.0)	6 (15.0)		
DFO	31	29 (93.5)	2 (6.5)		
DFO+DFP	10	10 (100.0)	0 (0.0)		
Compliance, %				3.87	0.04[a]
<60	69	61 (88.4)	8 (11.6)		
≥60	31	31 (100.0)	0 (0.0)		
Mean serum ferritin level ± SD (range), ng/ml		3,336.1±1,627 (836-7,830)	6,353.2±786.8 (3,303-8,500)	4.99	<0.001[b]

[a]P<0.05; [b]P<0.001. DFX, deferasirox; DFP, deferiprone; DFO, desferrioxamine; SD, standard deviation.

a high mean serum ferritin level (7,376.2±839.2 ng/ml), as shown in Table III.

Diabetes mellitus in patients. Diabetes mellitus was identified in 8 patients and 62.5% of them were >14 years old with no significant difference identified between males and females. All the patients who developed diabetes mellitus started earlier transfusion (<9 months), earlier iron chelators (<3 years) and 75% of them received frequent transfusion (every 2-3 weeks) with a poor compliance and high mean serum ferritin level (6,353.2±786.8 ng/ml) (Table IV).

Hypoparathyroidism in patients. Hypoparathyroidism was observed in 7% of the patients and 71.4% of them were >14 years old, with no significant difference identified between males and females. A total of 85.7% of patients with hypoparathyrodism started earlier transfusion (<9 months) and earlier iron chelators (<3 years). There were 71.4% of patients with hypoparathyrodism who received frequent transfusion (every 2-3 weeks) and all

were poor compliant with a high mean serum ferritin level (5,952.6±3,022.6 ng/ml) (Table V).

$\beta^0\beta^0$ genotype. A total of 94.1% of patients with the $\beta^0\beta^0$ genotype started earlier transfusion (<9 months), 85.2% received frequent transfusion (every 2-3 weeks) and 91.1% started earlier iron chelators (<3 years). In addition, patients with the $\beta^0\beta^0$ genotype had a higher prevalence of growth retardation, hypogonadism, hypothyroidism and hypoparathyroidism (94.1, 91.1, 75 and 71%, respectively) (Table VI).

Discussion

Endocrine dysfunction is a frequent complication in thalassemic patients who are on regular transfusions. In a previous study, ≤66% of the patients had at least a single endocrine disorder and 40% have multiple endocrinopathies (9).

Iron overload has for a long time been considered as the major cause of endocrine abnormalities of β-thalassemia (12). Growth retardation is frequently profound in these children.

Table V. Association between hypoparathyroidism and each of the demographic, transfusion, chelation characteristics, compliance and serum ferritin level.

| Characteristics | Patients, n | Hypoparathyroidism (n=100) | | χ^2 test | P-value |
		Negative (n=93), n (%)	Positive (n=7), n (%)		
Age, years				4.65	0.03[a]
≤14	66	64 (97.0)	2 (3.0)		
>14	34	29 (85.3)	5 (14.7)		
Gender				0.05	0.82
Male	54	50 (92.6)	4 (7.4)		
Female	46	43 (93.5)	3 (6.5)		
Age of start transfusion, months				0.39	0.53
≤9	68	62 (91.2)	6 (8.8)		
>9	32	31 (96.9)	1 (3.1)		
Frequency of transfusion, weeks				1.39	0.23
Every 2-3	43	38 (88.4)	5 (11.6)		
Every 4-5	57	55 (96.5)	2 (3.5)		
Age of start chelation, years				0.12	0.73
≤3 years	73	67 (91.8)	6 (8.2)		
>3 years	27	26 (96.8)	1 (3.7)		
Type of chelators				3.08	0.37
DFX	19	19 (100.0)	0 (0.0)		
DFP	40	36 (90.0)	4 (10.0)		
DFO	31	28 (90.0)	3 (9.7)		
DFO+DFP	10	10 (100.0)	0 (0.0)		
Compliance, %				2.00	0.15
<60	69	62 (89.9)	7 (10.1)		
≥60	31	31 (100.0)	0 (0.0)		
Mean serum ferritin level ± SD (range), ng/ml		3,398.7±1,591.2 (836-7,830)	5,952.6±3,022.6 (1,026-8,500)	3.80	<0.001[b]

[a]P<0.05; [b]P<0.001. DFX, deferasirox; DFP, deferiprone; DFO, desferrioxamine; SD, standard deviation.

The present study showed that 70% of patients had evidence of growth retardation.

Similarly, Moayeri and Oloomi (13) reported that short stature was prevalent in 62% of patients. In addition, Mostafavi *et al* (14) reported a higher prevalence of growth retardation where 90.9% of patients were under the fifth percentile.

By contrast, other studies reported a lower prevalence of growth retardation compared to the present study, which ranged from 30 to 50% (6,12,15-17). Variability in the prevalence of growth retardation in different studies could be attributed to the age of the studied patients, regularity of blood transfusion, type and compliance to iron chelation therapy.

Hypogonadism is a well-recognized complication in thalassemic patients. In the present study, hypogonadism was a complication in 67% of patients and this was nearly consistent with multiple studies in which Moayeri and Oloomi (13) found hypogonadism in 69% of thalassemia major patients and Jensen *et al* (9) reported that 66% of patients had hypogonadism.

Other previous studies reported a higher prevalence of hypogonadism compared to the present study, which ranged from 70 to 100% (6,7,16,18). By contrast, other studies reported a lower prevalence of hypogonadism compared to the present study, which ranged from 12 to 54% (5,10,19-21).

Thyroid dysfunction is known to occur frequently in thalassemia major, but its prevalence and severity varies in different cohorts (22). In the present study hypothyroidism was present in 8% of patients. Similarly, Shamshirsaz *et al* (10) reported that the prevalence of hypothyroidism was 7.7%. Other studies have reported a higher prevalence of hypothyroidism, reaching 17-18% (23-25), while others had reported low prevalence from 0 to 9% (26-28).

Of note, even in the studies in which the prevalence of overt hypothyroidism as a complication of thalassemia major is relatively low, milder forms of thyroid dysfunction are much more common, although there are wide variations in different studies. These discrepancies can be attributed to differences in the age of patients and different treatment protocols, including differing transfusion rates and chelation therapies (29).

Table VI. Association between patient genotype and endocrinal complications.

Characteristics	Patients, n (n=100)	$\beta^0\beta^0$ (n=34), n (%)	$\beta^+\beta^0$ (n=6), n (%)	$\beta^+\beta^+$ (n=60), n (%)	χ^2 test	P-value
Gender					3.71	0.15
Male	54	14 (25.9)	3 (5.6)	37 (68.5)		
Female	46	20 (43.5)	3 (6.5)	23 (50.0)		
Age of start transfusion, months					18.60	<0.001[a]
≤9	68	32 (47.1)	5 (7.4)	31 (45.6)		
>9	32	2 (6.3)	1 (3.1)	29 (90.6)		
Frequency, weeks					79.20	<0.001[a]
Every 2	34	29 (85.3)	2 (5.9)	3 (8.8)		
Every 3	9	2 (22.2)	3 (33.3)	4 (44.4)		
Every 4	49	2 (4.1)	1 (2.0)	46 (93.9)		
Every 5	8	1 (12.5)	0 (0.0)	7 (87.5)		
Age of start chelation, years					13.05	<0.001[a]
≤3	73	31 (42.5)	6 (8.2)	36 (49.3)		
>3	27	3 (11.1)	0 (0.0)	24 (88.9)		
Growth retardation					16.35	<0.001[a]
Negative	30	2 (6.7)	1 (3.3)	27 (90.0)		
Positive	70	32 (45.7)	5 (7.1)	33 (47.1)		
Hypogonadism					16.09	<0.001[a]
Negative	33	3 (9.1)	1 (3.0)	29 (87.9)		
Positive	67	31 (46.3)	5 (7.5)	31 (46.3)		
Hypothyrodism					14.75	<0.001[a]
Negative	92	28 (30.4)	4 (4.3)	60 (65.2)		
Positive	8	6 (75.0)	2 (25.0)	0 (0.0)		
Hypoparathyroidism					6.58	0.037[b]
Negative	93	29 (31.2)	5 (5.4)	59 (63.4)		
Positive	7	5 (71.4)	1 (14.3)	1 (14.3)		
Diabetes mellitus					0.79	0.67
Negative	92	31 (33.7)	5 (5.4)	56 (60.9)		
Positive	8	3 (37.5)	1 (12.5)	4 (50.0)		

[a]P<0.001; [b]P<0.05.

Hypoparathyroidism in transfusion-dependent patients with β-thalassemia appears to be accompanied by other endocrinopathies. It is usually a late complication, and occurs after the age of 16 years (30). In the present study the prevalence of hypoparathyrodism was 7%. Similarly, Shamshirsaz et al (10) reported that the prevalence of hypoparathyroidism was 7.6%.

Other studies reported a higher prevalence of hypoparathyrodism compared to the present study. Jensen et al (9) and Gulati et al (31) reported that hypoparathyroidism was observed in 13% of patients. By contrast, Toumba et al (19) reported that the prevalence of hypoparathyrodism was 1.2%, which is low compared to the present study.

Diabetes mellitus is also a frequent complication later in life of thalassemic patients, mainly due to iron overload, chronic liver disease and genetic predisposition (19). In the present study the prevalence of diabetes mellitus was 8% in patients. In agreement with this study, Najafipour (6) reported that the prevalence of diabetes mellitus was 8.9%. Previous studies have reported a higher prevalence of diabetes mellitus in comparison to the present study, which ranged from 9 to 20% (9,19,32,33).

The 3 most common mutations in the present study were IVS-1-110, IVS-1-1 and IVS-1-6 (31.5, 23.5 and 20.5%, respectively). These results corresponded with numerous previous Egyptian studies in which the 3 most frequent mutations in Egyptian thalassemic patients in different parts of Egypt were IVS-1-110, IVS-1-1 and IVS-1-6 (34-39).

Furthermore, Huisman et al (40) found that the most common mutations in Mediterranean areas were IVS-1-110, IVS-1-6, IVS-1-1, promotor 87, IVS-11-745 and C39. Additionally, the most common mutations in Middle East areas were C8, C8/C9, IVS1-5, C39, C44 and IVS11-1.

In the present study growth retardation was significantly prevalent in the older age group (P=0.016) and there was a significant association between growth retardation and earlier age of

start transfusion, chelation and frequency of blood transfusion, poor compliance and higher mean serum ferritin level (P<0.001, P<0.001, P<0.001, P=0.0015 and P<0.001, respectively).

In agreement with this study, Kirti *et al* (17), Borgna-Pignatti *et al* (21) and Cario *et al* (41) reported that growth abnormalities were more prevalent in older and/or pubertal thalassemic patients.

In the present study hypogonadism was prevalent in the older age group (P=0.019) and there was a significant association between hypogonadism and earlier age of start transfusion, chelation and frequency of blood transfusion, poor compliance and higher mean serum ferritin level (P<0.001, P<0.001, P<0.001, P=0.0018 and P<0.001, respectively). Similarly, Shamshirsaz *et al* (10) reported a significant difference in the mean serum ferritin level between thalassemic patients with primary amenorrhea, irregular menses, hypogonadism and those without endocrinopathies. Furthermore, Borgna-Pignatti *et al* (21) reported that hypogonadism was detected less in patients whose serum ferritin was <2,500 ng/ml.

In the present study hypothyroidism was prevalent in the older age group (P<0.001) and there was a significant association between hypothyroidism and age of start and frequency of blood transfusion, as well as poor compliance and higher mean serum ferritin level (P=0.04, P=0.002, P=0.049 and P<0.001, respectively). Zervas *et al* (42) reported that hypothyroidism was prevalent among patients in second decade of life.

In the present study a significant association was observed between the prevalence of hypoparathyrodism and age of the patients as well as higher mean serum ferritin level (P=0.03 and P<0.001, respectively). Older age patients are more likely to have hypoparathyrodism. Jensen *et al* (9) reported a significant association between serum ferritin level and hypothyroidism, as well as hypoparathyrodism, suggesting a central role of iron overload in the development of these complications as serum ferritin level was >2,000 mg/l. This is also supported by the Italian Working Group who reported that the serum ferritin level was higher in patients with one or more endocrinopathies (8).

In the present study diabetes mellitus was more prevalent in patients who started blood transfusion at an earlier age and were poor compliant with a high serum ferritin level (P=0.04, P=0.04 and P<0.001, respectively). Similarly, Najafipour *et al* (43) reported that the age and transfusion periods are risk factors for developing diabetes and that the amount of transfusion is directly linked to the impaired fasting glucose level. Jensen *et al* (9) identified a significant association between the age of the patients and prevalence of diabetes; younger age is more likely to have a normal oral glucose tolerance test.

The present study found a significant association between the prevalence of endocrine complications and higher serum ferritin levels of >3,000 ng/dl (P<0.001). In agreement with this study, Toumba *et al* (19), Gamberini *et al* (44) and Low (45) reported that multiple endocrinopathies, including hypogonadism, hypothyroidism, hypoparathyrodism and diabetes mellitus, developed later in life and were associated with iron overload, which is one of the main risk factors for developing such complications in addition to poor compliance and early onset of transfusion therapy.

The present results showed that patients with the β⁰β⁰ genotype had an earlier age of start transfusion and

chelation, as well as more frequent transfusions (P<0.001, P<0.001 and P<0.001, respectively), while patients with the β⁰β⁺ and β⁺β⁺ genotypes had a delayed age of start transfusion, chelation therapy as well as less frequent blood transfusions and they had a significantly lower prevalence of growth retardation, hypogonadism, hypothyroidism and hypoparathyrodism.

Similarly, Chern *et al* (7) showed that the β⁰β⁰ genotype was significantly correlated with the age of first blood transfusion and it is an indicator of disease severity. The present results are also supported by Skordis *et al* (16) who demonstrated a significant association between the β⁰β⁰ genotype and frequency of blood transfusion where patients with the β⁰β⁰ genotype received more frequent blood transfusion.

The present study found that patients with the β⁰β⁰ genotype also had a significantly higher prevalence of growth retardation, hypogonadism, hypothyroidism and hypoparathyroidism (P<0.001, P<0.001, P<0.001 and P=0.037, respectively). This is in agreement with Yaman *et al* (46) who reported that the complication rates were significantly higher in thalassemia major patients with the β⁰β⁰ genotype compared to thalassemia intermedia patients with β⁺β⁺ (P<0.05). Similarly, Filosa *et al* (47) reported that all thalassemic patients with hypogonadism express the β⁰β⁰ genotype.

Chern *et al* (7) also showed that patients with the β⁰β⁰ genotype were significantly correlated with the development of hypogonadism (odds ratio=28.50, P=0.002). Similarly, Jensen *et al* (9) reported a significant association between the β⁰β⁰ genotype and development of hypogonadism. Also, Skordis *et al* (16) demonstrated the influence of β⁰β⁰ on the development of hypogonadism and associated it to the difference in the amount of blood transfusion and variability in free iron radicals.

The present study observed that patients with the IVS-11-745/IVS-11-745 genotype had a significantly higher prevalence of diabetes. Similarly, Khalifa *et al* (33) reported the association of the IVS-11-745/IVS-11-745 genotype and the prevalence of diabetes mellitus, in which 77.7% of patients with diabetes carry the IVS-11-745/IVS-11-745 genotype.

In conclusion, the present study revealed that endocrinal complications were more common in β-thalassemia patients with a clear association between genotype and clinical disease severity. A significant association was also identified between the serum ferritin levels and the presence of endocrine complications, emphasizing the important role of iron overload in the development of these complications.

Acknowledgements

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