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Original Article

Final Height and Endocrine Complications in Patients with β-Thalassemia Intermedia: Our Experience in Non-Transfused Versus Infrequently Transfused Patients and Correlations with Liver Iron Content

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Abstract. ***Background:*** **β-thalassemia intermedia (TI) spans a wide spectrum of severity and carries higher morbidity than previously recognized, including extramedullary hematopoiesis, leg ulcers, gallstones, thrombosis, secondary heart failure, pulmonary hypertension, skeletal deformity, growth retardation and endocrine abnormalities, such as diabetes mellitus, hypothyroidism, osteoporosis, and hypogonadism.**
Objectives: **To evaluate the final height and the endocrine complications encountered in young adult patients with TI followed at Hematology Section, Doha (Qatar) in relation to liver iron content in non-transfused versus infrequently transfused TI patients.**
Patients and Methods: **This retrospective cohort study was performed on 28 young adults with TI who were randomly selected from the Hematology Clinic of the Hematology Section, National Centre for Cancer Care and Research, Hamad Medical Corporation of Doha (Qatar).**
Eligibility criteria for this retrospective analysis included TI patients diagnosed by complete blood count, hemoglobin electrophoresis and young adult age (≥ 18 years).
Group 1 included nine patients who did not receive any blood transfusion, and Group 2 included 19 patients who infrequently received blood transfusions.
Data recorded from charts included demographic characteristics (gender, date of birth, ethnicity), disease and treatment characteristics (e.g., transfusion frequency, history of chelation therapy, and splenectomy), auxological and pubertal data [growth percentiles and pubertal stages, and body mass index (BMI)], laboratory data and target organ complications (including endocrinopathies and liver disease). Iron overload was assessed by direct (liver iron content; LIC) and indirect methods (SF), and bone mass index (BMA) by dual-energy X-ray absorptiometry (DXA).
Results: **Short stature [Final Height (Ht) SDS < -2] occurred in 25% of patients with no difference between the two groups of patients. Insulin growth factor 1 (IGF-1) SDS was low in 35.7 % of patients with no statistical difference among the two groups. Impaired fasting blood glucose occurred in 17.8% of patients, diabetes mellitus in 25% and hypogonadotropic hypogonadism in 10.7% of them. Morning cortisol was low in one patient. No thyroid or hypo-parathyroid abnormalities were detected in any patient. Liver iron content (LIC) > 15 mg/g dry weight and SF > 2,000 ng/mL were detected in 75% of the patients. The values resulted significantly higher in the transfused group (Group 2). High liver enzyme level (ALT) was detected in 42.8 % of patients, and the values were significantly higher in the transfused group (Group 2). Total and fetal Hb was significantly higher in group 1 versus group 2. Osteopenia was diagnosed in 14.3% of patients. Females had significantly better final height SDS, higher IGF-1 SDS, lower LIC and fasting blood glucose level compared to males. Significant correlations were**

found between Ht-SDS and IGF-1 SDS; LIC and SF, level; ALT and LIC, SF levels. Total and fetal Hb did not correlate significantly with Ht-SDS or IGF-1 level.
Conclusions: **A significant number of TI patients have high LIC, short stature and endocrine disorders. Patients who require occasional transfusions have more liver iron overload and higher hepatic dysfunction. Females appear to attain a better final adult height and have higher IGF1-SDS versus males. Our data emphasize the need for long term surveillance for identification of organ-specific risk factors and early disease manifestations. We also recommend close monitoring of endocrine and other complications, according to the international guidelines.**

Keywords: Thalassemia intermedia; Gender; Growth; Endocrine complications; IGF-1; Iron overload; Liver iron concentration; Serum ferritin.

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Introduction. According to the WHO, the carrier rate of β -thalassemia is around 1.5% of the world population. β-thalassemia intermedia (TI) defines a group of patients whose anemia is not so severe to necessitate regular transfusions but are at significant risk of iron accumulation from underlying disease-related mechanisms distinct from transfusional iron overload. TI is characterized by significant genetic and clinical heterogeneity.[1,2]

Three mechanisms are responsible for having a milder phenotype of thalassemia. First, is the inheritance of a mild or silent beta-chain mutation. The second is the coinheritance of *α*-thalassemia. The third is the hereditary persistence of HbF, *δβ*-thalassemia, and G*γ* XMN1 polymorphism.[3] Due to imbalances in α- and β-globin chains, globin tetramers are unstable resulting in precipitation and degradation. This process releases free iron and leads to the formation of reactive oxygen species (ROS), causing membrane damage and premature cell death in bone marrow (ineffective erythropoiesis) or peripheral circulation.[3-5]

Clinically, they present in later childhood or even in early adulthood with a mild to moderate anemia, with a hemoglobin (Hb) level ranging between 7 and 10 g/dL, that only requires occasional or short-course of regular transfusions in certain clinical settings (e.g., in the course of infections or pregnancy).[1,2] More severely affected patients present younger, between 2 and 6 years of age, and require transfusions for normal and sustained growth.

The triad of chronic anemia, ineffective erythropoiesis, and iron overload characterizes TI and eventually culminate in the clinical sequelae that we see in these patients, including extramedullary hematopoiesis, leg ulcers, gallstones, thrombosis, skeletal deformities, osteoporosis, heart failure, pulmonary hypertension, and endocrine complications (such as diabetes mellitus, hypothyroidism, and hypogonadism).[4-8] The complications are associated with the severity of anemia and iron overload, splenectomy, older age, the rate of ineffective erythropoiesis, and low fetal Hb levels.[8,9]

Conventional treatment consists of transfusions, iron chelation, splenectomy, Hb F-inducing agents, supportive therapies, and psychological support. However, management of these patients is particularly challenging for clinicians because they can present with a wide variety of complications which can vary in severity. Therefore, the treatment of TI must be individualized and tailored to the patient's clinical scenario.

Qatar is a peninsula bordering the Arabian Gulf and Saudi Arabia. The country's population has been roughly split with 20% native Qatari, mostly tribal, and 25% other Arabs from Egypt, Syria, Iraq, Lebanon, Yemen, Palestine, and Jordan. The rest of the population (55%) consists of expatriate workers from the East and the West.[10]

The frequency of β- thalassemia heterozygotes is estimated to be 2-3%. Al-Obaidli et al.[11] analyzed the molecular basis of β-thalassemia in Qatar. They found the most common mutant alleles were IVS-1-5 (G>C) and codon 8/9 (+G), representing 35.4% and 26.1% of the total, respectively. Most of these two mutations are homozygous, probably because of the high rate of consanguinity. Recently, a novel β-thalassemia deletional variant allele in an ethnic Qatari patient was reported.[11] The deletion spans exon 1, the entire intron 1 and the first two bases of exon 2 causing a frameshift and the premature appearance of a stop codon. The presence of this novel deletional allele in a compound heterozygous state with a non-deletional allele is

alarming in a diagnostic setting, especially in the absence of family studies. The frequencies of three other common mutant alleles, IVS-2-1 (G>A), 25bp deletion and IVS-1-110 (G>A) are higher in surrounding locations, such as Southern Iran, Kuwait, eastern Saudi Arabia, and Bahrain.[12]

We report for the first time the endocrine complications encountered in young adult patients with TI followed at Hematology Section, Doha (Qatar) and we shed further light on their final height and hormonal changes in relation to liver iron content in non-transfused versus infrequently transfused TI patients.

Patients and Methods. Patients with β-thalassemia and sickle cell disease are treated comprehensively in Hamad Medical Corporation (HMC) where the Pediatric Hematology Department takes care of these patients up to the age of 14 years. After the age of 14 years, patients are treated in the Hematology Sections of the National Centre for Cancer Care and Research. The center is following the Thalassemia International Federation (TIF) guidelines for treatment of patients with Hemoglobinopathies. In addition, our health care system in Qatar provides standard high-quality care for these patients. The center is accredited by a joint International Commission, and the laboratories are accredited by American College of Pathologist.

This retrospective cohort study was performed on twenty-eight young adults (20 males and 8 females) with TI who were randomly selected from the Hematology Clinic of the National Centre for Cancer Care and Research (NCCCR) of Doha (Qatar). Two patients had splenectomy and one developed extramedullary mass with spinal compression. None was hepatitis B or C positive.

Eligibility criteria for this retrospective analysis included TI patients diagnosed by complete blood count, hemoglobin electrophoresis and young adult age (≥ 18 years).

Data recorded from medical charts included the demographic characteristics (gender, date of birth, ethnicity), disease and treatment characteristics (e.g., transfusion frequency, history of chelation therapy, and splenectomy), auxological and pubertal data [growth percentiles and pubertal stages, and body mass index (BMI)]. Laboratory data included the Hb level, mean corpuscular volume (MCV), indirect bilirubin, and target organ complications (including endocrinopathies and liver disease).

Short stature was defined as patient height < 2 standard deviations below the mean for age, gender, and ethnicity. Short stature was evaluated by assessment of patient height and plotted on international (WHO) adjusted growth charts.[11] Body mass index (BMI) was calculated with the following formula: weight in Kg/ height in m^2.[13]

Hypogonadism was suspected by the absence of breast development or secondary amenorrhea and by the absence or arrest of testicular enlargement, measured with Prader's orchidometer.[14] Male and female patients with suspected hypogonadism were evaluated for pituitary-gonadal axis integrity.[3,15] Low blood testosterone levels (in males) or 17βestradiol (in females) associated with low pituitary gonadotropin levels (LH and FSH) indicated a diagnosis of hypogonadotropic hypogonadism (HH). Hyperprolactinemia was defined as a basal level higher than the locally derived normal assay reference range.

Primary hypothyroidism was diagnosed in the presence of low serum free thyroxine (FT4) and elevated serum thyroid stimulating hormone (TSH) concentration[10] and central hypothyroidism was diagnosed when FT4 was low, and TSH was low or inappropriately normal for the FT4 level.[13,15] The criteria for the diagnosis of hypoparathyroidism were: low parathormone (PTH) level with low total and ionized serum calcium, high serum phosphate, normal serum magnesium, and alkaline phosphatase levels.[10]

The criteria for diabetes mellitus were based according to the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus (ADA), 1998 World Health Organization (WHO) consultation criteria, and 1985 WHO criteria. [13] Impaired fasting blood glucose (FBG) was diagnosed in the presence of fasting glucose ≥ 5.6 mmol/L but < 7.0 mmol/L and diabetes mellitus (DM) in the presence of fasting glucose ≥ 7.0 mmol/L or two-hour postprandial glucose ≥ 11.1 mmol/L.[16]

Hypoadrenalism was suspected in the presence of basal morning cortisol level < 3.5 μg/dl (98 nmol/liter) or less.[17]

Other laboratory investigations included: alanine transferase (ALT), aspartate transferase (AST), and insulin-like growth factor (IGF-1).

Plasma total IGF-1 was measured by a chemiluminescent immunometric assay (CLIA) method (Nichols Institute Diagnostics, San Juan, CA). For ethical reasons, we used as a reference for normal the IGF-1 values determined in a large population of healthy subjects, reported in the literature [18] using the same automated chemiluminescence immunoassay system (CLIA). A serum IGF-1 level < -2 SDS was considered a deficiency. Other hormonal parameters were determined using commercially available automated immunoassays.

Patients were divided into two groups based on their transfusional history: Group 1 included 19 patients (7 females and 12 males) who did not receive any blood transfusion and group 2 included 9 patients (2 females and 7 males) who infrequently received blood transfusion; no more than five transfusions in the last 3 years). None had received HbF induction therapy.

Iron overload was assessed by indirect (SF) and direct methods (liver iron content; LIC).

Electrochemiluminescence immunoassays measured SF level. The manufacturer's normal reference range values for SF were 30–350 ng/L, in males, and 15–150 ng/mL in females. Iron status was classified as mild (serum ferritin < 1,000 ng/mL), moderate (serum ferritin >1,000 ng/mL and < 2,000 ng/mL) or severe (serum ferritin >2,000 ng/mL).[19]

Liver iron content (LIC) was measured by FerriScan ® and values were expressed in mg Fe/g weight.4 Four classes of LIC have been reported in thalassemic patients: Class 1 = normal LIC < 3 mg Fe/g dry liver, Class 2 = mild overload LIC 3–7 mg Fe/g dry weight, Class 3 = moderate LIC overload 7–15 mg Fe/g dry weight, and Class 4 = severe LIC overload ≥15 mg Fe/g dry weight.[19-21]

Patients with serum ferritin > 2,000 ng/mL were treated with subcutaneous daily desferrioxamine during childhood. All patients were shifted, 7 years ago, to oral chelation therapy with deferasirox (20 mg/Kg/ body/d), with dose escalations up to 30 mg/kg/d. In the last year, deferasirox (DFX) was shifted to the new tablet DFX formulation (Jadenu® :14 mg/kg/d with dose escalations up to 21 mg /kg/d).

The assessment of bone mineral density (BMD) was was measured by dual-energy

X-ray absorptiometry (DXA). BMD of the lumbar spine (L1–L4) and right femoral neck were measured. The BMD values were expressed as mean values (g/cm^2). Z-score, which is the number of SD above or below the average of age- and sex-matched control subjects' BMD, was calculated.[17] Based on the World Health Organisation definition, a Z-score of less than –2.5 SDS below the mean in relation to the patient's age was defined as osteoporosis, and between –1.0 and –2.5 SDS as osteopenia.[22]

Ethical approval for the study was obtained by the IRB of the Medical Research Center at Hamad Medical Corporation. All procedures were carried out with the adequate understanding and consent of patients.

The Kolmogorov-Smirnov (K-S) test and PP plot were used to test for normality of the data. Quantitative data between the two independent groups were analyzed using unpaired 't' test or Mann Whitney U test as appropriate. The relationship between two quantitative variables was examined using Pearson's correlation coefficients. We used. All p values presented were two-tailed, and p values <0.05 was considered as statistically significant. All statistical analyses were done using Excel statistical packages 2010 software.

Results. Patients' characteristics and biochemical parameters in 28 young adult patients with β-thalassemia intermedia are reported in **table 1**.

A short final stature (Ht-SDS < -2) occurred in 25% of patients and in 35.7 %. The IGF-1

SDS level was low, with no statistical difference among the two groups. FBG occurred in 17.8% of patients, DM in 25% and acquired hypogonadotrophic hypogonadism in 10.7% of them (2 patients with hypogonadotropic secondary amenorrhea and one male patient with acquired HH). The two females had secondary amenorrhea after a complete spontaneous pubertal development. Morning basal cortisol was low in one patient in the non-transfusion group. No thyroid or parathyroid abnormality was detected in any patient (**Table 2**).

Increased ALT levels (> 40 U/L) were detected in 42.8 % of patients. The levels resulted significantly higher in the transfused group (73.3±34.3 vs. 42.2±32.1 in NTD of Group 1). Total Hb and fetal Hb were significantly higher in Group 1 (8.2 ± 2.8 g/dL and 2.8 ± 1.6 %, respectively) versus Group 2 (7.0 ± 0.4 g/dl and 1.2 ± 0.42 g/dL, respectively) (**Tables 1** and **2**).

SF was > 2,000 ng/mL in 12/19 (63%) and LIC > 15 mg/g liver dry weight in 12/19 (63.1%) of patients who did not receive blood transfusion (Group 1) and in 100 % of patients who received occasional blood transfusions (Group 2). Liver iron content > 15 mg/g dry weight and serum ferritin level > 2, 000 ng/mL were detected in 75% of the patients. Four patients with SF concentration < 1,000 ng/mL had a high LIC >15 mg/g dry weight. Although the mean SF level in males was comparable to the mean SF level in females, LIC of females was significantly lower.

Comparison of males versus females with TI showed that females had significantly better adult final Ht-SDS (- 0.45 ± 1.06 vs. -1.80 ± 0.88: p: 0.0001), higher IGF-1 SDS (-1.09 ± 1.73 vs. -1.80 ± 1.71;p: 0.01), lower LIC (24.4± 8.5 vs.32.3 ± 12.5 mg Fe/g

Table 1. Patients' characteristics and biochemical parameters in 28 patients with β-thalassemia intermedia

Groups 1 and 2	Age (years)	Fetal Hb (%)	Hb (g/dL)	Height-SDS	SF (ng/mL)	LIC (mg Fe/g dry weight)	AST (U/L)	ALT (U/L)	ALP (U/L)
NTD (19)	24.7±7.8	2.9±1.6*	8.2±0.8	1.5±1.2	2,874±732	23.5±9.5	33.7±14.7	42.2±32.1	133.1±92.3
OTD (9)	24.3± 7.2	1.2±0.4	7.0±0.3	1.6±1.0	7,518±618*	40.5±10.7 *	48.2±15.1	73.3±34.3*	118.3±46.6

Legend= Groups: NTD: TI patients not receiving blood transfusions; **OTD:** TI patients infrequently receiving blood transfusions; **SF:** serum ferritin; **LIC:** liver iron concentration; **ALT**: alanine transferase; **AST**: aspartate transferase (**AST**); **ALP**: alkaline phosphatase; (***p:** < 0.05,non-paired t test).

Table 2. Final adult height (Ht-SDS), endocrine complications, biochemical parameters and bone densitometry in 28 patients withβ-thalassemia intermedia.

Variables and endocrine complications	Total N = 28	Group 1 N = 19	Group 2 N = 9	P value (Group 1 vs. Group 2)
Ht-SDS < -2	7/28	4/19	3/9	0.45
IGF-1 SDS < -2	10/28	5/19	5/9	0.13
Hypoparathyroidism	0/28	0/19	0/9	NA
Hypothyroidism	0/28	0/19	0/9	NA
Low basal cortisol	1/28	1/19	0/9	0.48
Impaired FBG (mmol/L)	5/28 --	2/19 5.5 ± 1.2	3/9 7.5± 2.3	0.14 <0.05
Diabetes mellitus	7/28	3/19	4/9	0.1
Hypogonadism	3/28	1/19	2/9	0.17
Serum ferritin > 2,000 ng/mL	21/28	12/19	9/9	0.035
High ALT (> 40 U/L)	12/28	5/19	7/9	0.01
LIC > 15 mg Fe/g dry weight	21/28	12/19	9/9	0.035
Hb < 8 g/dL	16/28	7/19	9/9	0.001
Osteopenia (DXA scan)	4/28	3/19	1/9	0.74

Legend: NTD: TI patients not receiving blood transfusions; **OTD:** TI patients infrequently receiving blood transfusions; **Ht:** standing height; **NA:** not applicable; **IGF-1**: insulin growth factor 1; **FBG:** fasting blood glucose; **ALT**: alanine transferase; **LIC:** liver iron concentration: **Hb**: hemoglobin level; **DXA:** dual-energy X-ray absorptiometry (p value for t-test between two percents).

Table 3. Correlations (r values) between anthropometric and biochemical variables in patients with β-thalassemia intermedia.

	Ht-SDS	BMI	AST	ALT	FBG	LIC	Serum ferritin	FT4	IGF-1 SDS
Ht-SDS	1.00								
BMI	0.03	1.00							
AST	0.01	0.26	1.00						
ALT	-0.10	0.17	0.72	1.00					
FBG	-0.01	0.04	0.20	0.20	1.00				
LIC	0.11	-0.21	0.52	0.43	0.15	1.00			
Serum ferritin	0.00	-0.06	0.36*	0.35*	0.21	0.47*	1.00		
FT4	0.05	0.08	-0.06	0.06	0.10	-0.30	-0.18	1.00	
IGF-1 SDS	0.65**	0.35*	-0.04	0.17	-0.15	-0.19	-0.03	0.42	1.00

Legend: Ht: standing height; **BMD:** body mass index; **AST**: aspartate transferase (**AST**); **FBG:** fasting blood glucose; **LIC:** liver iron concentration; **FT4:** free thyroxine; **IGF-1**: insulin growth factor 1; ***p < 0.01, **p<0.001.** Strength of correlation (r) – 0.70 = A strong negative linear relationship, – 0.50 = A moderate negative relationship, – 0.30 = A weak negative linear relationship, 0 = No linear relationship, + 0.30 = A weak positive linear relationship, + 0.50 = A moderate positive relationship and + 0.70 = A strong positive linear relationship)

dry weight; p: 0.03) , ALP (85.5 ± 20.9 vs. 144.6 ± 84.5 U/L; p:0.04 , Normal values: 40-129 U/L) and FBG (5.7 ± 2.2 vs. 7.1 ± 4.4 mmol/L; p: 0.04) compared to males.

ALT was not significantly lower in females versus males (43.1± 16.7 vs.55.1 ± 38.7 U/L; p: 0.6. Normal values: < 40 U/L).

LIC was correlated significantly with serum ferritin level (**Figure 1**). Ht-SDS was correlated significantly with IGF-1 SDS (**Figure 2**) and BMI. ALT concentrations were correlated significantly with LIC and SF level. Total Hb and fetal Hb did not correlate significantly with Ht-SDS or IGF-1 level. Osteopenia was diagnosed by DXA scan in 14.3% of patients (**Table 2**).

Discussion. β-thalassaemia intermedia (TI) is an inherited genetic disorder that affects haemoglobin chain synthesis, leading to ineffective erythropoiesis and anemia. Despite absent or infrequent blood transfusions, patients with TI are at risk of iron overload mainly because of increased iron uptake from the gastrointestinal tract, because of ineffective erythropoiesis, accompanied by anemia and hypoxia.[20]

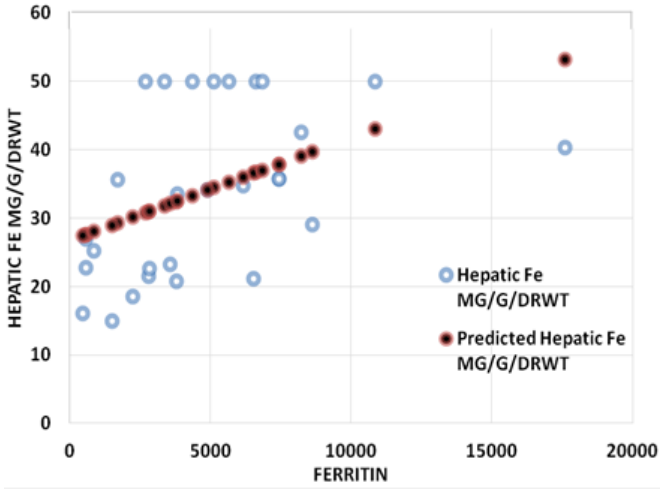

Figure 1. Correlation between liver iron concentration (LIC) and serum ferritin (r = 0.47; P: 0.01).

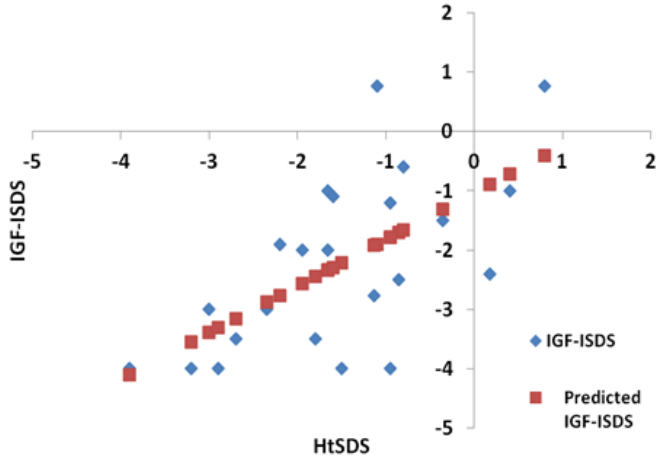

Figure 2. Correlation between standing height - SDS (Ht-SDS) and insulin growth factor- 1 SDS (IGF1SDS) (r = 0.65; P: < 0.001).

In these patients, iron absorption is 3–10 times normal, which is 17–89 % compared to 14.8 ± 9.5 % in normal subjects and increases after splenectomy. [23-25] This iron burden promotes oxidative damage in a variety of organs, inducing a variety of endocrine and end-organ dysfunction.

When comparing our patient's data with data published by other groups from Iran, Lebanon, and Italy, we clinically noticed that minor β- thalassemia and mild phenotype of the disease is more common in Qatar compared to others gulf countries.[10.12]

Our patients with and without occasional transfusion had markedly high SF level and LIC. A significant correlation between SF and LIC was observed in our patients as reported in another study. [26] Four patients in spite of an SF concentration not particularly elevated (< 1,000 ng/mL) had a high LIC > 15 mg/g dry weight, thus remaining at risk of being denied iron chelation therapy although they need it, and exposing patients to morbidity and mortality risks associated to iron overload. Vascular, endocrine, and bone morbidity in TI are significantly associated with SF > 800 ng/mL and LIC > 6-7 mg Fe/g dry weight.[27-30]

The main aim of our retrospective study was to evaluate the final height and the endocrine complications in relation to their iron status and degree of anemia and to compare LIC and endocrine status in patients on occasional transfusion (Group 2) versus those who did not receive a blood transfusion (Group 1). Twenty-five percent of patients had short stature (Ht-SDS < -2), and 35.7% had low IGF-I (<-2SD), with no statistical difference among the two groups. Females attained a better final height and showed a higher IGF1- SDS versus males. Impaired fasting blood glucose occurred in 17.8%, DM in 25% and hypogonadotropic hypogonadism in 10.7% of them. Morning cortisol was low in one patient. No thyroid or parathyroid abnormalities were detected in our study.

Karamifar et al.[31] investigated 93 patients with TI (40 males, 53 females), aged 11-40 years (mean 19.4 yr). Short stature (height < 3rd percentile) was documented in 47% and growth hormone deficiency in 31% of their patients. A lower incidence of final short stature (7%) was reported by De Sanctis et al.[6] in 50 patients (21 males, 29 females) with TI (aged 15-46 years; mean age 28.7 yr), with raised SF levels (mean 1,540 ng/mL). Mean hemoglobin concentration was 8.1 g/dL. Half of them had had more than 50 transfusions in their life and had received irregular intramuscular or subcutaneous chelation therapy with desferrioxamine.

The difference in the prevalence of short stature may be due to several factors: gender, low fetal hemoglobin level,[32] severity of anemia,[30-33] treatment of iron overload and compliance to medical care.[4,6,8]

The Ht-SDS of our patients was correlated significantly with IGF-1 level, suggesting that IGF-1 deficiency may play a key role in growth retardation of these patients. The Ht-SDS was not correlated with SF, LIC, Hb F or total Hb levels. However, the lower LIC levels found in our female patients can explain their higher IGF1-SDS and better final adult Ht-SDS as well as lower ALT concentrations compared to males. The lower LIC levels found in our female patients can explain their higher IGF1-SDS and better final adult Ht-SDS as well as lower ALT concentrations compared to males.

A low Hb has been associated with low IGF-1 levels and slow growth in many disorders including iron deficiency anemia and thalassemia major.[33-36] Correction of the anemia was associated with increasing IGF-1 secretion and acceleration of growth in these disorders.[31]

Thus, the adverse effects of iron overload, the severity of anemia and a defective secretion of growth hormone GH-IGF-1 axis could explain the short stature and the reduced synthesis low IGF-1 in patients with TI. Additional factors include hepatic impairment, other associated endocrine complications, and zinc deficiency.[36,37]

Our patients had a high prevalence of impaired FBG (17.8%) and DM (25%).

Patients on occasional blood transfusion who had higher iron overload (SF and LIC) had a higher FBG compared to the non-transfused group.

In a study by Flatau et al.[38] abnormal glucose tolerance was detected in two out of 4 patients. In these two patients, the SF levels were more than 20 times the average value. Abnormal glucose tolerance test (OGTT) was found in 24% of patients in De Sanctis et al. study.[6] These patients had a higher serum SF level and a lower Hb level than those with normal OGTT. A diagnosis of DM was reported by Karimi et al. [39] in 11% of 721 TI patients from twelve comprehensive thalassemia centers.

Although our patient did not have any thyroid dysfunction or calcium homeostasis abnormality, primary hypothyroidism was detected in 5.7% of patients reported by De Sanctis et al. [6] Compensated hypothyroidism and primary hypothyroidism was documented respectively in 19% and 2% of patients reported by Karamifar et al.,[31] and central hypo-thyroidism in 8.3% of 721 TI patients.[39]

Thus, different studies have revealed that several changes in thyroid hormone concentrations may occur with a broad spectrum of severity from compensated to overt hypothyroidism. Central hypothyroidism, considered an uncommon clinical entity in the past, is an emerging endocrine complication due to inadequate stimulation of a normal thyroid gland by TSH (low FT4 and a low or "inappropriately normal" TSH).

HH occurred in 10.7 % of our patients. Prevalence of hypogonadism ranging from 4.2 % to 16 % has been reported by different authors.[6,31,39,40] The detailed description of this complication, in the current literature, is quite scanty. De Sanctis et al.[6] found a delayed puberty in 36% of patients, primary amenorrhea in 4% and secondary amenorrhea in 4 out of 50 TI patients (8%). Two adult males (aged 19 and 36 years) had HH (4%).[6] A poor response to Gn-RH stimulation test, found in three females and in both males tested, suggested that pituitary dysfunction was wholly or partially responsible for hypogonadism. The gonadal function was normal in all patients studied. The appearance of endocrine complications was not necessarily related to the degree of iron overload as evaluated by SF.[6]

However, lack of concordance of SF concentrations and different endocrine dysfunctions can be explained by the fact that serum ferritin levels increase linearly with the transfusion load up to 100 units of transfused blood, but after that there is no simple relation-ship.[31,39,40] Moreover, there is no direct linear relationship between the amount of iron accumulated and organ dysfunctions.[41] Furthermore, it is possible that endocrine glands are more sensitive to iron toxicity compared to other organs and that even small amounts of it accumulated in the first few years of life produce damage that cannot be reversed.

Cut-offs of < 300 ng/mL for the absence of iron overload and > 800 ng/mL for the presence of clinically significant iron overload in TI have been suggested.[38.] However, recent re-evaluation found that a considerable number of patients with SF levels between 300 and 800 ng/mL to have iron overload requiring management.[42,43] Iron chelation therapy and hydroxyurea therapy have been associated with a lower frequency of endocrine complications. [9] One postulated mechanism of action of hemoglobin F inducers is based on reducing the imbalance between α-globin chains and non-α chains.

DFX, an oral chelator with a once-daily dosage, was developed to provide day-long chelation coverage with a suitable safety and efficacy profile. A recent study, called THALASSA (Assessment of Exjade ® in Non-Transfusion-Dependent Thalassemia), on 166 patients with TI (>10 years of age with a LIC ≥ 5 mg Fe/g dry weight, and SF level of at least 300 ng/mL) showed the efficacy of DFX at starting doses of 5 and 10 mg/kg/d, with dose escalations up to 20 mg/kg/d in patients with high levels of iron overload.[44]

However, despite the availability of long term chelation therapy including oral agents for 7 years, in our patients iron overload remains a serious problem because of poor compliance to treatment. The uncomfortable side effects of therapy can have a negative impact on daily activities and well-being, which may affect adherence.[45] A new tablet DFX formulation (Jadenu®) has been recently developed in an attempt to overcome tolerability issues and to improve the patients' compliance.[46]

These observations suggest that doctors taking care of patients with thalassemias should be aware of the importance of monitoring iron load and should implement strategies for improving compliance with the treatment.

Bone abnormalities in TI are quite frequent and range from a decrease in the bone mineral density (BMD) and consequent osteoporosis to spinal cord compression and increased risk of fractures. [43] Osteopenia occurred in 14.3 % of our young adults with TI. Other authors have reported a prevalence of bone disease in 53/70 (76%) patients, osteoporosis in 26/53 (49%), and osteopenia in 27/53 (51%). [43] Relatively lower prevalence of osteopenia in our patients may be due to their young age, with high activity, regular intake of vitamin D, calcium and dairy products.

Most of our patients with TI had decreased levels of IGF-1 that usually plays an essential role in the bone remodeling cycle that stimulates osteoclasts and the differentiation and proliferation of osteoblasts. An increased level of the receptor activator of nuclear factor KB ligand (RANKL) leads to decreased bone

thickness followed by bony deformities, osteopenia, and ultimately fractures.[40] However, several other factors including bone marrow expansion, ineffective erythropoiesis, vitamin D deficiency, genetic factors, and reduced physical activity may contribute to lower bone mineral accretion in these patients.[44]

The most recent guidelines by the Thalassemia International Federation (TIF) recommend that all patients >10 years of age be screened by yearly assessment of lumbar spine, femoral neck, and distal ulna BMD by DXA.[47,48]

Conclusions. Although many TI patients do not present serious health problem and they generally reach adult age without or with a little requirement of blood transfusion, growth impairment, and endocrine disturbances have been reported with variable prevalence among these patients. The degree of iron overload and the effect of anemia and variation in iron chelation therapy prescription could explain the variation in the prevalence of endocrine complications reported in the literature.

In TI the pattern of iron overload is preferentially hepatic, and it develops gradually throughout life, manifesting as early as ten years of age. Our patients who received occasional transfusions (Group 2) had higher ALT and LIC versus those who did not receive transfusions (Group 1). LIC correlated significantly with ALT level stating that liver impairment increase with the iron overload.

Spot ferritin measurements may underestimate the burden of iron overload and subsequently delay the iron chelation therapy but its monthly measurement could have some value in countries were LIC assessment is not available. LIC is the most reliable and noninvasive gold standard method to assess iron overload.

Finally, our data also emphasize the need for long term surveillance for identification of organ-specific risk factors, early diagnosis, and treatment of growth and endocrine complications. We also recommend close monitoring of endocrine and other complications, according to the international guidelines.

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