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Original Article

Glucose Homeostasis and Assessment of β-Cell Function by 3-hour Oral Glucose Tolerance (OGTT) in Patients with β-Thalassemia Major with Serum Ferritin below 1,000 ng/dL: Results from a Single ICET-A Centre

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Abstract. ***Aims*: The primary aim of this study was to evaluate retrospectively the glucose homeostasis and surrogate indices of insulin sensitivity and resistance, during a 3-hour oral glucose tolerance test (OGTT), in β- thalassemia major patients (β-TM) with serum ferritin (SF) below 1,000 ng/mL.**

***Patients and methods:* The retrospective cohort study evaluated the medical records of 24 β-TM patients from 2010 to 2022. At the year of study the mean age of patients was 31.0 ± 4.1 (20-37.11) years; 13 (54.1%) were females. The most commonly used iron chelator was deferoxamine (DFO: 75%), followed by deferiprone (DFP:12.5%) and deferasirox (DFX: 12.5%). Insulin sensitivity and resistance indices were derived from OGTT. A liver iron concentration (LIC) < 3 mg/g d.w. and a global heart T2* value > 20 ms were considered as conservative cut-off values for insignificant iron overload (IOL).**

***Results:* The mean SF levels in the whole study cohort population at the age of evaluation was 549.6 ± 232.3 ng/mL. Based on the SF levels, two groups were identified: Group A (N = 14) < 500 ng/mL and Group B (N=10) 500-1,000 ng/mL. Normal glucose tolerance (NGT) during OGTT was observed in 4 patients of Group A (28.5 %) and in 5 patients of Group B (50%) (P: 0.29). The remaining 15/24 patients (62.5%) had glucose dysregulation (GD). The mean age at starting iron chelation therapy (ICT) and the mean SF peak in Group A versus Group B were significantly higher in group A. The GD was associated with significantly attenuated IGI (first phase of insulin response) and impaired oral disposition index (oDI). Hypogonadotropic hypogonadism (HH) was the most common associated endocrine complication in both groups of patients.**

***Conclusions:* This study showed that efficient iron chelation monotherapy in patients with β-TM and SF < 1,000 ng/ml did not entirely prevent glucose metabolism disorders, abnormalities of insulin secretion and sensitivity, and development of acquired hypogonadism.**

Keywords: β-thalassemia major; Iron overload; Oral glucose tolerance test; Glucose tolerance abnormalities; Insulin

sensitivity; Insulin resistance.

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Introduction. The basic defect in β-thalassaemia is the reduced or absent production of β globin chains leading to a relative excess of α chains. The excess of α globin chains in the form of inclusion bodies accumulates and precipitates in the erythroid precursors causing oxidative membrane damage and ineffective erythropoiesis.[1] Based on the severity of the clinical phenotype, β-thalassemia has been classified into three main types: transfusion-dependent thalassaemia (TDT) or β-thalassemia major (β-TM), non-transfusion- dependent thalassaemia (NTDT) or thalassemia intermedia (TI), and thalassemia minor (heterozygote, silent carrier state).[2]

The recommended treatment for β-TM patients is lifelong, regular packed red blood cell transfusions (Transfusion Dependent Thalassemia-TDT).

Transfusions lead to excess iron input and systemic iron overload accumulating in the heart, liver, spleen, endocrine glands, and other tissues; if iron overload is not treated, it can lead to a wide array of complications. As a rule, the liver has the highest iron overload, followed by the pancreas and the heart.[4]

Iron chelation therapy (ICT) is essential to prevent and/or reduce complications of iron overload (IOL) and to decrease morbidity and mortality in the TDT population. An appropriate dose of ICT should be given to be efficient, and that requires a deep commitment to the chelation protocol prepared for the individual patient (personalized treatment). Moreover, adherence to treatment is very critical in these patients.[5]

Three iron chelators are currently available for the treatment of IOL: deferoxamine (DFO) as a subcutaneous or intravenous infusion; oral deferiprone (DFP) in tablet or solution form; and oral deferasirox (DFX), as a dispersible tablet and more recently, film-coated tablet. Studies have shown differences in chelators' efficacy in controlling IOL. DFP is superior in controlling or reducing myocardial iron load, while DFO is more effective in controlling or reducing hepatic iron load.[6]

Older patients with β-TM were initially treated with a single iron chelating agent (DFO). ICT's effectiveness was followed by serial iron quantification of serum ferritin (SF) and transferrin saturation. Later, additional methods for monitoring ICT were developed, namely magnetic resonance imaging (MRI) for assessment of liver iron concentration (LIC) and T2* for cardiac iron load for monitoring chelator efficacy or stratifying end-organ risk.[4] Each method has pros and cons to quantifying and monitoring iron burden. When intensification of chelation is required for patients with a very high iron burden, combination therapy is used. A long experience with combination therapy concerns DFO and DFP chelators.

SF levels >1,000 ng/mL are indicators of iron overload and are associated with iron-mediated tissue damage. A recent report has documented the occurrence of diabetes [based on the American Diabetes Association (ADA) criteria],[8] in three groups of β-TM patients with different levels of SF.

Based on their mean SF levels, they were divided into three groups: <500 ng/mL (N = 32 patients), between 500 and 1,000 ng/mL (N = 43 patients), and between 1,000 and 2,500 ng/mL (N = 28 patients). Diabetes mellitus was detected in 15.6%, 7% and 10.7%, respectively (P: 0.487).[7]

No information was reported about other abnormalities of glucose homeostasis. MRI pancreatic IOL was comparable among the three groups. No difference was found in the frequency of hypogonadism, hypothyroidism, hypoparathyroidism, osteopenia/ osteoporosis, arrhythmias, and hepatic cirrhosis among the 3 groups.[7] Chelation therapy was started at the mean age of 7.3 ± 5.1 years; 5.8± 4.3 years; and 6.2 ± 6.7 years, respectively. The difference was not statistically significant between groups of patients (P: 0.25). No signs of hematological/renal toxicity were documented in any of these patients. [7]

The primary aim of this study was to evaluate in depth the variations of glucose homeostasis and surrogates indices of insulin sensitivity and resistance, during a 3-hour OGTT, in adult β-TM patients with SF below 1,000 ng/mL.

Patients and Methods.

Study population and design. All β-TM patients consecutively referred for consultation or second opinion, basically for endocrinological and metabolic problems, to a single Italian centre (Pediatric and Adolescent Outpatient Clinic, Private Accredited Quisisana Hospital, Ferrara, Italy) from October 2010 to July 2022, were reviewed. Patients who had SF levels below 1,000

ng/mL at the time of referral (assessed as the mean of values collected during the last 12 months prior to referral) were selected for the study. All patients were of Italian ethnic origin. This study was conducted in the context of an ongoing observational study of the natural history of GD in patients with β-TM, promoted by the International Network of Clinicians for Endocrinopathies in Thalassemia and Adolescence Medicine (ICET- A).

Eligibility Criteria. The inclusion criteria for the study were patients with β-TM: (a) receiving routine blood transfusion (TDT) and chelation treatment; (b) over the age of 18 years with SF levels < 1,000 ng/mL on the year of referral; (c) quantification of liver and myocardial iron deposition assessed within the previous 8 months by MRI, before referral. Exclusion criteria were β-TM patients with: (a) diabetes, either insulin dependent or on antidiabetic agents; (b) mean SF values > 1,000 ng/mL, in the previous 12 months before performing OGTT; (c) major chronic illness other than β-TM; (e) non-transfusion dependent β-thalassemia; (d) bone-marrow transplanted and (e) systemic glucocorticoid treatment within the previous 4 weeks before OGTT.

The medical records of 24 β-TM patients with the inclusion criteria were retrieved. The following data were collected: age, gender, ethnicity, anthropometry, pubertal status, age at first transfusion, interval between transfusions, age at starting chelation, peak of SF (defined as the highest level registered from the beginning of chelation therapy to the last observation), age at splenectomy, and associated endocrine complications. β -TM patients were diagnosed on the basis of clinical and laboratory data and the related phenotypes.[1,2]

Anthropometry and assessment of associated endocrine complications. Height and weight were measured according to international recommendations.[9,10] Short stature was defined as height 2 SD below the mean height for age and sex. Body Mass Index (BMI) was calculated as weight in kilograms divided by the square of height in meters. BMI was evaluated based on the World Health Organization (WHO) recommendations: underweight (<18.5 kg/m^2); normal range (18.5–24.9 kg/m^2); overweight (25.0– 29.9 kg/m^2); obese (≥ 30 kg/m^2).[11] Associated endocrine complications were assessed and defined according to the I-CET position statement published in 2013.[10]

Serum IGF-1 concentration was determined by chemiluminescent immunoassay (Siemens Healthcare Medical Diagnostics, Bad Nauheim, Germany). The assays were performed according to the manufacturer's recommendations.

OGTT: Method and definitions. OGTT was performed after an overnight fast of 10 hours. Blood was sampled through a peripheral intravenous catheter at times 0, 30, 60, 90,120, and 180 minutes after 1.75 g/kg (maximum 75 g) of glucose was administered as a 20% oral solution. Informed consent was obtained before study procedures were performed. Plasma glucose and insulin levels were determined, and indices of β-cell function, insulin sensitivity, and insulin secretion were calculated. Based on plasma glucose results on OGTT, each patient's glycemic status was classified according to the American Diabetes Association (ADA) criteria.[8]

In addition to the traditional criteria for glucose level during OGTT, we also used other criteria for glucose dysregulation (GD), based on current published definitions: (a) the time of glucose considered as "late response" when the glucose peak was > 30 min during OGTT; (b) PG levels ≥155 mg/dL at 1-h; (c) the presence of indeterminate glucose tolerance (INDET), defined as a normal fasting PG and normal 2- h post-challenge glucose with any intermediate OGTT plasma glucose level ≥ 200 mg/dL);[12] (d) the glucose curve shapes during OGTT,[13] and (e) the presence of hypoglycemia ≤ 55 mg/dL at any time during the OGTT with or without symptoms of hypoglycemia.[14]

The glucose curve shape was classified as 'monophasic' when PG increased after an oral glucose load between 30 and 90 min until a peak was reached, followed by a subsequent decline of ≥ 4.5 mg/dL. A biphasic curve was defined as a rise of blood glucose to a peak followed by a fall (as in the monophasic curve), then followed by a second rise of ≥ 4.5 mg/dL.[13]

The control group consisted of the data reported in a previous study in eleven healthy adult volunteers **(**mean age: 23.8 ± 3.2 years) who were not carriers for β-thalassemia, overweight or obese.[15]

PG was measured using an automated glucose oxidase reaction (Glucose Analyser, Ames). Plasma was separated within 60 min for storage at − 60°C until assay. Plasma insulin was determined by a commercial chemiluminescence solid phase immunometric assay (Immulite and Immulite 2000, Diagnostic Products, Corp, Los Angeles, CA). All insulin samples were tested in duplicate, and the values were expressed in μU/ml.

Surrogate β-cell function indices related to OGTT. Different indirect indices were also applied to evaluate insulin resistance and sensitivity.

These were:

(a) Early-phase insulin secretion (IGI) calculated as the ratio between the incremental plasma insulin and glucose concentrations during the first 30 min of the OGTT (ΔI 0–30/ΔG 0–30);[16]

(b) Insulin sensitivity estimated using the Matsuda index (MI 0-120), a well-established measure of whole-body insulin sensitivity for both hepatic and peripheral tissues that has been validated against the euglycemic-

hyperinsulinemic clamp;[17]

(c) As a secondary measure of insulin sensitivity (largely hepatic), the reciprocal of the homeostasis model assessment of insulin resistance (HOMA-IR) was assessed;[18]

(d) Finally, the oral disposition index (oDI) was calculated as the product of the IGI and the MI 0-120. This index reflects the relationship between β-cell function (first-phase insulin secretion) and peripheral insulin sensitivity (hepatic and peripheral tissues). The oDI provides an evaluation of pancreatic β-cell function adjusted for insulin sensitivity and is predictive for deterioration of glucose dysregulation.[19]

Iron Overload Assessment. Iron overload was evaluated by direct and indirect methods namely by SF and Magnetic Resonance Imaging (MRI) T2* of liver and heart.

SF was measured by chemiluminescence immunoassays (Beckman Access Dxl). The normal reference range values are 30-350 ng/mL in males and 15-150 ng/mL in females. Generally, a cut-off value of SF concentration higher than 800 ng/mL indicates significant IOL.[20]

Reported global myocardial and liver IOL data, assessed by MRI and registered not more than 8 months before OGTT, were collected. The values were expressed in mg/g dry weight (d.w.) for LIC, classified into normal (LIC < 3), mild (LIC > 3 and < 7), moderate (LIC > 7 and < 14) and severe overload (LIC > 14).[21] A global heart T2* value < 20 ms was considered as a conservative cut-off for significant myocardial iron overload.[22]

Biochemical assays and hepatitis C seropositivity. Blood samples were taken in the morning, one or two weeks after the blood transfusions.

Biochemical parameters (liver enzymes, serum creatinine and plasma glucose) were assessed in the same laboratory using commercially available kits. Based on the registered presence of HCV antibodies (anti-HCV) and HCV ribonucleic acid (HCV RNA), patients were categorized in three groups: negative patients, patients who eradicated the virus spontaneously or after treatment with antiviral therapy, and patients with chronic HCV infection (anti-HCV and HCV-RNA positive).

Statistical analysis. All numeric variables were expressed as mean ± standard deviation (SD). Comparison of variables in the two groups of patients was made using an independent sample *t*-test and variables with non-normal distribution were compared using non-parametric Wilcoxon's signed rank test.

Pearson's correlation tests (2-tailed) were used to study correlations between variables with parametric and non-parametric distributions. For the statistical analysis, a software program was used and validated, according to Alder and Roesser.[23] A P value < 0.05 was considered statistically significant.

Ethics. All procedures were in accordance with the 1964 Helsinki declaration and its later amendments.

According to the Italian regulations, ethics approval by the local Ethics Committee was not required for the following reasons: no identifiable private information was collected; patients underwent only routine diagnostic and therapeutic procedures according to current guidelines[24] and an anonymized dataset was analyzed. Furthermore, informed consent was obtained from all patients after a detailed explanation of the nature and purpose of the study and the likely risks and benefits associated with study participation.

Results

a. Patients characteristics and iron overload. Out of the 94 β-TM patients followed at a single Centre, 24 (25.5 %) fulfilled the criteria and were included in the study. The mean age of patients at the time of the study was 31.0 ± 4.1 years (range: 20-37.11 years); 13 (54.1%) were females. The chelator most often used for the longest period was DFO (75%) followed by DFP or DFX (12.5%), respectively.

The clinical, demographic, and laboratory data of recruited β -TM patients are summarized in **Table 1**.

The mean SF level in the whole study population was 549.6 ± 232.3 ng/mL. Two groups of β-TM patients were identified based on the SF levels: <500 ng/mL (Group A; N = 14) and between 500 and 1,000 ng/mL (Group B; N = 10). No significant differences were found between the two groups regarding mean age, BMI, IGF-1, ALT and γ-GT variables (**Table 1**). 13/24 patients (54.2%) had chronic hepatitis C; 4/13 did not previously respond to antiviral therapy, 5 refused antiviral therapy, and 4 were not treated because of normal serum ALT levels.

One female patient of Group A was overweight, and two females of Group B were obese. In addition, MRI global myocardial T2* (ms) was significantly lower in group B (i.e., more iron in the myocardium).

The mean age at starting ICT and the mean SF peak of Group A versus Group B differed significantly between the two groups (**Table 1**).

b. OGTT. Normal glucose tolerance (NGT) during OGTT was observed in 9 of 24 (37.5%) patients, in 4 of Group A (28.5 %), and 5 of Group B (50%) (P: 0.29).

A late glucose peak (at 60' or 90' minutes) was observed in 12 patients of Group A (85.7%) and in 7 patients (70%) of Group B. The OGTT in the remaining patients of Group A showed: isolated IFG in 1 patient, IGT in 5 patients, IFG+IGT in 1 patient, and 1-h PG > 155 mg/dL in 3 patients; Group B showed: IGT in 3 patients and 1-h PG > 155 mg/dL in 2 patients. No patient

Table 1. Clinical, laboratory, and iron chelation treatment data in two groups of 24 β-thalassemia major (β-TM) patients, above the age of 18 years at the time of study with serum ferritin < 500 ng/mL (Group A) and 500 to 1,000 ng/mL (Group B). The values are expressed as mean ± SD.

Variables	(Group A) β-TM patients with SF < below 500 ng/mL	(Group B) β-TM patients with SF between 500 to 1,000 ng/mL	P value
Number of patients	14	10	-
Age (yr)	31.1 ± 3.9	30.9 ± 4.4	NS
Sex (M/F)	8/6	3/7	-
Body Mass Index (kg/m2)	22.2 ± 1.9	21.8 ± 2.2	NS
Splenectomy (n and %)	9 (64.2 %)	6 (60 %)	NS
Mean age at first blood transfusion (months)	8.3 ± 4.8	8.4 ± 4.1	NS
Mean pre-transfusional Hb level (g/dL) at the year of study	8.93 ± 0.48	8.98 ± 0.39	NS
Age at starting iron chelation therapy (months)	38.11 ± 4.7	31.6 ± 5.4	0.0047
Iron chelation therapy at OGTT: **Desferrioxamine (DFO) (n)** **Deferiprone (DFP) (n)** **Deferasirox (DFX) (n)**	- 10 2 2	- 8 1 1	- - - -
Peak of serum ferritin (ng/mL)	5033.0 ± 1380.4	3853.6 ± 1255.1	0.043
Mean SF ng/mL in the last 12 months **Range**	374.7 ± 66.2 229-480	794.6 ± 142.0 548-981	< 0.0001
Family history of diabetes: **None** **Yes (n. and %)**	 13 1 (7.1%)	 6 4 (40%)	 - 0.055
ALT (IU/L; normal values: < 33)	33.3 ± 15.9	32.8 ± 20.0	NS
γ-GT (IU/L; normal values: < 30)	23.0 ± 15.5	15.7 ± 3.5	NS
IGF-1 (ng/mL)	73.8 ± 45.2	85.7 ± 39.3	NS
MRI LIC (mg/g d.w.) **LIC: > 3 and < 4.5 (n. and %)**	1.5 ± 0.48 0	2.1 ± 1.20 2 (20%)	NS -
MRI global myocardial T2* (ms) **Global heart T2* < 20 ms, N (%)**	34.7 ± 9.1 0	26.3 ± 4.4 2 (20%)	0.013 -
Growth and endocrine complications: **1. Short stature (≤ 3rd centile) (n and %)** **IGF-1 < 2.5th percentile (n)** **2. HH (n and %)** **3. Acquired secondary HH (n and %)** **4. Central HT (n)** **5. Hypoparathyroidism (n)** **6. Hypocortisolism (n)**	- 7 (50 %) 6/7 4/14 (28.5%) 7/14 (50%) 1/14 0 0	- 4 (40 %) 3/4 4/10 (40%) 3/10 (21.4%) 0 0 0	- NS - NS NS - - -

Legend = **N:** number; **SF:** serum ferritin, **ALT:** alanine aminotransferase **γ-GT:**gamma-glutamyl transferase; **MRI:** magnetic resonance imaging; **LIC**: liver iron concentration; **HH:** hypogonadotropic hypogonadism; **Central HT**: Central hypothyroidism; **NS:** not significant.

had fasting PG or OGTT values consistent with diabetes or INDET.

A 'monophasic' glucose curve shape during OGTT was observed in 11 patients of Group A (78.5 %), and 6 patients of Group B (60%) (P: 0.33) and a 'biphasic' glucose curve shape was observed in all the remaining patients but one in whom the glucose curve shape could not be classified.

Two male patients (one from each group of patients) showed a PG level, at 3-h during OGTT, compatible with the diagnosis of biochemical hypoglycemia[14] (52 mg/dL and 51 mg/dL, respectively).

In two patients of group B (1 male and 1 female on treatment with DFO and DFX, respectively), a LIC of 3.5 and 4.5 mg/g d.w. was reported 6 months before the OGTT and, in the same group B, a MRI myocardial T2* < 20 ms was reported in 1 male and 1 female patient (18,8 and 18.5 ms, respectively) 8 months before OGTT; both were on treatment with DFO). The OGTT in the 4 patients showed an NGT in 2 and an IGT and 1-h PG > 155 mg/dL in the remaining two patients.

A detailed report of PG and insulin levels and the surrogate β-cell function indices of the two groups of patients compared to controls are shown in **table 2**.

c. Correlations. Pearson's test revealed no statistically significant correlation between SF and LIC, myocardial T2* and LIC, age, and myocardial T2*, PG at baseline and at 2-h during OGTT, and myocardial T2*, IGI and oDI versus myocardial T2*. A weak but not significant inverse correlation was found between SF and myocardial T2* (r: - 0.381; P: 0.066) and a positive correlation between myocardial T2* and LIC (r: 0.4161; P: 0.043). A similar finding was reported by Dissanayake et al. in patients with transfusion dependent β-thalassaemia.[25]

d. Growth and associated endocrine complications.

Table 2. Characteristics of plasma glucose and insulin levels during OGTT in β-TM patients with serum ferritin (SF) < below 500 ng/mL and with SF between 500 to 1,000 ng/mL versus normal controls.

Variables	- Group A - 14 β-TM patients with SF < below 500 ng/mL	- Group B - 10 β-TM patients with SF between 500 to 1,000 ng/mL	Controls 11 normal subjects
Fasting glucose (mg/dL)	93.7 ± 11.6 **(*)**	90.5 ± 4.6	84.6 ± 7.2
1-h glucose (mg/dL)	157.7 ± 43.9 **(**)**	149.5 ± 30.8	95.4 ± 18.0
2-h glucose (mg/dL)	133.2 ± 34.5 **(***)**	137.2 ± 23.0	84.6 ± 18.0
3-h glucose (mg/dL)	100.4 ± 29.9	107.5 ± 27.4	77.4 ± 14.4
Fasting insulin (µU/mL)	5.2 ± 2.1	5.8 4 ± 3	7.0 ± 3.0
1- h insulin (µU/mL)	43.4 ± 28.6	45.2 ± 27.0 **(*)**	37.0 ± 17.6
2 -h insulin (µU/mL)	35.0 ± 26.7	41.4 ± 31.1	24.1 ± 12.2
3- h insulin (µU/mL)	17.2 ± 7.2 **(*)**	27.8 ± 2.8 **(***) (§)**	10.7 ± 7.7
Insulinogenic Index (IGI)	0.61 ± 0.33 **(*)**	0.64 ± 0.36 **(*)**	1.54 ± 0.99
HOMA-IR	1.20 ± 0.54	1.25 ± 0.90	1.16 ± 0.76
Matsuda IR Index (0-120)	7.72 ± 3.0	7.84 ± 3.29	8.71 ± 2.85
Oral Disposition Index (ODI)	4.60 ± 3.05 **(***)**	4.66 ± 2.35 **(***)**	12.11 ± 6.55

Legend: Group A and B vs. Controls = (*): < 0.05; (**): < 0.01; (***): < 0.001. Group A vs Group B = (§): < 0.001.

Short stature (< 3rd centile) was observed in 11/24 (45.8%) of patients; 7 in Group A (3 males and 4 females; 50%) and 4 in Group B (2 males and 2 females; 40%). The mean IGF-1 levels in the two groups were: 59.6 ± 28.5 and 81.4 ± 32.5 ng/mL (P: 0.27), respectively. Only seven patients (3 of Group A and 4 of Group B) had IGF-1 above the 2.5th percentile of the normal values for the Italian population.[26]

Associated hypogonadotropic hypogonadism (HH) was the most common endocrine complication in both groups. Interestingly, after a full pubertal development, an acquired HH was reported in 5 females and 2 males of Group A (35.7%) and 3 females of Group B (30 %). All but four were on hormone replacement therapy with sex steroids.

e. Survey during chelation therapy. No previous serious adverse events were reported in patients with a SF level < 500 ng/mL. An adjustment of iron chelating dose of monotherapy with DFO was done in one male and with DFP in one female patient (SF: 229 and 303 ng/mL, respectively), and a temporarily interruption in one male patient on monotherapy with DFO (SF:180 ng/mL).

Discussion. The recommended treatment for β-TM is lifelong regular blood transfusions (TDT) supplemented by iron chelation at the earliest time. IOL is inevitable as humans lack mechanisms to excrete the excess iron input accumulated via transfusions. Iron accumulation is toxic to many tissues, such as the liver, heart, pancreas and endocrine glands, causing dysfunction of organs and multiple complications. A significant association between persistently elevated SF levels and endocrine glands dysfunction has been documented; in particular, a 14-fold increased risk of diabetes has been reported in β-TM patient populations with a median age of 20.7 years.[27]

ICT aims to balance the increased rate of iron input of blood transfusions by increasing iron excretion in urine and /or stools with effective chelating drugs. This requires a profound commitment from patients and parents to iron chelation protocols and recommendations to monitor efficiency and unwanted side effects for the individual patient.

SF levels >1,000 ng/mL are indicators of iron overload and are more frequently associated with iron-mediated tissue damage. Although SF is considered a reliable surrogate marker for total body iron load in β-TM patients, its elevation may also reflect other conditions not strictly linked to iron metabolisms, such as inflammation, liver damage, and steatohepatitis.[27]

The average SF levels in healthy individuals range from 12 to 300 ng/mL in men and from 12 to 150 ng/mL in women.[27] Increased SF concentrations (> 300 ng/mL for men and > 200 ng/mL for women) in non-pathologic conditions, reflecting subclinical IOL, have been reported to be associated with insulin resistance (IR) and an increased risk of type 2 diabetes.[28,29]

It has been shown that "normalizing" iron stores in β-TM patients, in the absence of chelator-mediated toxicity, prevents new morbidities and reverses many complications, such as cardiac failure, hypothyroidism, hypogonadism, and GD.[30] In general, chelation therapy's unwanted effects are more likely at high chelator doses and low levels of iron overload (SF levels < 500 ng/mL).[31]

The present retrospective study was conducted to investigate glucose homeostasis in depth during OGTT in a selected group of β-TM patients with SF <1,000 ng/mL, classified in two groups: SF <500 ng/dL Group A:N = 14) and SF between 500–1,000 ng/mL (Group B:N = 10). We used a 3-h OGTT and insulin level estimation to assess glucose tolerance status and β-pancreatic islet-cell hormone secretory response simultaneously. No patient had fasting glucose or OGTT levels consistent with the diagnosis of diabetes,

according to the ADA criteria. However, a GD was disclosed in 15/24 (62.5 %) patients; 10 of Group A (71,6%) and 5 of group B (50%) (P: 0.29).

Interestingly, but not easily explained, the mean age in months at starting iron chelation therapy and the mean peak of SF were statistically higher in Group A compared to Group B. Unfortunately, the data on pancreatic IOL, assessed by MRI, were unavailable in our patients. In Spasiano et al. report,[32] no patient with an SF < 500 ng/mL had significant myocardial IOL, but pancreatic IOL was present in all but one patient. No significant liver and myocardial IOL, assessed by MRI, was also detected in our 14 patients of Group A with SF < 500 ng/mL. In Group B, 2/10 patients had mildly increased LIC, and heart iron overload. It is also of interest that in Spasiano's paper,[32] patients with SF <500 ng/mL had a higher prevalence of diabetes and started chelation at the most advanced age.

Speculating on the findings of our study, early deposition of iron in the first years of life may have induced permanent organ damage and/or the available iron chelating agents used to treat IOL were not effective enough to remove iron deposition from the pancreas; a more aggressive ICT would have been necessary to remove pancreatic iron deposits in patients with severe IOL (assessed in our patients by SF peak).

The data of Farmaki et al.[32] support the latter hypothesis. After 5–7 years of intensive combined ICT with DFO and DFP (SF at baseline: 3421.6 ± 882.0 ng/mL), the proportion of β-TM patients with glucose metabolism abnormalities declined from 78% to 34%. A significant increase in insulin secretion and sensitivity was also observed. At the end of the study, the mean age for their patients was 32.9 ± 9.8 years and the mean SF was 87 ± 25 ng/mL. Moreover, in 18 β-TM patients requiring L-thyroxine for hypothyroidism, 10 patients discontinued treatment, and 4 reduced L-thyroxine dose. In 14 hypogonadal males on testosterone therapy, 7 stopped treatment. Of the 19 females, who were hypogonadal on DFO monotherapy, six were able to conceive after combination therapy.

These results have not been replicated in other studies. Although these are very interesting, it should be kept in mind that, l there is marked intra-individual variability in reproducibility of the OGTT results in patients with TM as with other diseases.[33] In addition, the lack of long-term follow-up data urges further caution in interpretation of these results.

Although iron deposition in the pancreatic parenchyma seems to play a major role in the onset of diabetes, no link has been shown between the development of the disease and SF level in long-term chelated patients. This indicates that additional factors may also be involved in the development of GD. Chronic hepatitis C, common in older β-TM patients, may also play a role in the pathogenesis of abnormal glucose tolerance.[34] Moreover, we can also speculate that zinc (Zn) deficiency, encountered frequently in β-TM patients, might lead to an exacerbation of the inability of the pancreas to secrete sufficient amounts of insulin in response to glucose stimulation.[34] Zn is a potent physiological regulator of insulin signal transduction through its inhibitory effect on protein tyrosine phosphatase 1b, the key phosphatase that dephosphorylates the insulin receptor.[35]

Although the gold standard for assessing insulin secretion and sensitivity is the hyperinsulinemic-euglycemic clamp,[36] the OGTT provides information about insulin resistance and secretion assessment both directly and indirectly through surrogate indices, such as the HOMA-IR, the Matsuda index, and the oDI .

In our patients, GD was associated with a significantly attenuated IGI (first phase of insulin response), which is considered the earliest sign of glucose intolerance and impaired oral disposition index (oDI). The latter index reflects the relationship between β-cell function (first-phase insulin secretion) and peripheral insulin sensitivity (hepatic and peripheral tissue sensitivity to insulin). These indices may provide an evaluation of pancreatic β-cell function adjusted for insulin sensitivity that may represent a predictive value for glucose deterioration.[19]

The additional novel parameters were used to evaluate insulin secretion and resistance. Such indices included time to peak glucose, shape of the glucose curve, and 1h-PG ≥ 155mg/dL.

In individuals with and without diabetes[37] or IFG,[38] delayed nadir during post-load glucose level indicates a higher insulin resistance combined with a reduction in insulin secretion, while those individuals with longer time to peak glucose have a greater likelihood of diabetes; subjects with a monophasic response curve tend to have impaired insulin sensitivity and β-cell function, and have higher risk for the development of type 2 diabetes compared to those with a biphasic response curve;[39,40] in addition, 1h-PG ≥ 155mg/dL is predictive for detecting progression to type 2 diabetes.[41]

However, to improve evidence in this area, more studies are needed in β-TM patients to provide data on long-term follow-up and for evaluating risk of diabetes in the future.

The mechanisms underlying biochemical hypoglycemia remain undefined, potentially being a manifestation of islet dysfunction and an inadequate counter-regulatory response.

We are aware of the limitations of our study, particularly that of small sample size. However, our findings can generate hypotheses, warrant larger studies, and encourage further research. Our next step is to evaluate the progression of glucose abnormalities longitudinally in this group of TDT patients, representing the majority of adult TDT patients, and to

address the long-term effects of ICT.

Conclusions. Our study showed that effective iron chelation monotherapy in patients with β-TM with SF < 1,000 ng/ml and even < 500 ng/mL did not entirely prevent glucose metabolism disorders and the development of acquired hypogonadism. Certain tissues are more susceptible to excess iron loading (liver>pituitary>pancreas>heart), and iron toxicity and iron toxicity may have already started in early childhood in patients receiving suboptimal ICT.[30,34]

Serum ferritin < 1,000 ng/ml is traditionally regarded as a marker of no significant iron overload. However, the majority of β-TM patients with this level have some form of GD and a large proportion have hypogonadotropic hypogonadism. Detailed analysis of OGTT results suggests reduced insulin secretory capacity as the main cause of GD after the second decade of life. Further studies are warranted to explore the potential predictive role of the shape of glucose curve / the time of glucose peak / the development of late hypoglycemia in glucose regulation and whether performing OGTT with longer duration and samples at more time points would add valuable clinical information. Extending the duration of OGTT beyond the standard clinical 2-h OGTT may be useful to evaluate subjects with delayed hypoglycemia. Prompt intervention at early stages is essential to avert progression to prediabetes and diabetes in high-risk individuals.[42]

Adequate assessment and monitoring of IOL, based on tailored ICT, is crucial for preventing these complications. Identifying the earliest moment in which to intervene to avert the progression to prediabetes and diabetes in high-risk individuals is a substantial challenge. The present recommendation to interrupt chelation therapy when the SF level is below 1,000 ng/mL[43] should be revised. Recent studies recommend a SF near to "normality". To avoid over-chelation, IOL should be appropriately managed and closely monitored, ideally in specialized thalassemia centers.

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