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Original article

Effects of iron-chelation therapy intensification on glucose homeostasis during 3-h oral glucose tolerance test (OGTT) in transfusion-dependent β-thalassemia patients (β-TDT)

Vincenzo De Sanctis[1], Shahina Daar[2], Ashraf Soliman[3], Saveria Campisi[4], Ploutarchos Tzoulis[5]

[1] Coordinator of ICET-A Network (International Network of Clinicians for Endocrinopathies in Thalassemia and Adolescent Medicine) and Pediatric and Adolescent Outpatient Clinic, Quisisana Hospital, Ferrara, Italy; [2] Department of Hematology, College of Medicine and Health Sciences, Sultan Qaboos University, Muscat, Sultanate of Oman; [3] Department of Pediatrics, Division of Endocrinology, Hamad General Hospital, Doha, Qatar; [4] UOSD Thalassemia, Umberto I° Hospital, Siracusa, Italy; [5] Department of Diabetes and Endocrinology, Whittington Hospital, University College London, London, UK

Abstract. *Background:* Iron overload (IOL) due to chronic transfusion therapy in β-thalassemia major (β-TDT) patients leads to multi-organ damage, including glucose dysregulation (GD). The effectiveness of intensified iron chelation therapy (ICT) on glucose homeostasis and its ability to reverse iron-induced endocrinopathies is not fully understood. *Objectives:* To assess the effects of intensified ICT on glucose homeostasis, insulin secretion, and sensitivity in adolescent and very young adult β-TDT patients with moderate to severe IOL. *Methods:* This retrospective study evaluated 19 β-TDT patients who underwent intensified ICT with the aim of reducing serum ferritin (SF) to 500-1,000 ng/mL. Over a median follow-up of 4.2 years, parameters including oral glucose tolerance test (OGTT), insulin response, and serum ferritin levels were analyzed. *Results:* Despite a marked reduction in SF and IOL, the prevalence of GD remained unchanged (P = 0.75). Some patients showed improvement in glucose tolerance, whereas other developed new endocrine disorders, such as hypogonadotropic hypogonadism and secondary amenorrhea. Improvements were seen in insulin sensitivity, but not in pancreatic β-cell function. *Conclusion:* Intensified ICT in β-TDT patients, albeit effectively reducing iron burden, did not uniformly reverse established glucose homeostasis disorders. While some endocrine functions improved, others deteriorated or developed a new complication, suggesting that more aggressive or prolonged ICT may be necessary. Long-term studies are required to better understand the impact of ICT on endocrine organ function. (www.actabiomedica.it)

Key words: transfusion-dependent thalassemia, iron overload, intensification chelation therapy, ogtt, follow-up

Introduction

Advances in understanding the pathophysiology of complications classically associated with transfusion-dependent β-thalassemia (β-TDT), also known as β- thalassemia major (β-TM), have contributed to the optimization of transfusion programs and the timely implementation of iron chelation therapy (ICT). The overall goal of ICT is to limit iron loading from ongoing transfusions and to safely remove excess iron (1-3).

Various indices and methods are used to assess iron overload (IOL) in patients, each with its advantages and limitations. Recently, the quantitative

measurement of iron via magnetic resonance imaging (MRI) has been employed to assess iron levels in the liver, heart, pancreas, and pituitary gland. However, these techniques have not yet become widespread for clinical use in many countries. Liver iron concentration (LIC) values exceeding 7 mg/g dry weight (d.w.) indicate an increased risk of complications associated with IOL. Furthermore, values above 15 mg/g d.w. are predictive of advanced liver fibrosis and a heightened risk of cardiac disease.

In the clinical setting, serum ferritin (SF) is the most widely used non-invasive, indirect parameter for assessing body iron stores globally. Its advantages include ease of access, low cost, and the utility of serial measurements for monitoring iron chelation therapy (ICT). However, SF reliability diminishes in the presence of concomitant illnesses such as malignancies, infectious diseases, hepatitis, and critical and inflammatory conditions. SF levels significantly correlate with liver iron concentration (LIC) but it is not effective for assessing cardiac iron (1-3). For the management of patients with β-TDT, targeting SF levels between 500 and 1,000 ng/mL is recommended, as this range is associated with improved survival rates and enhanced heart and liver functions (1,2).

Currently, three chelators are licensed in most countries: desferioxamine (DFO), deferiprone (DFP) and deferasirox (DFX) (1,2). The choice regarding the optimal agent depends on the cost, severity of iron burden, and patients' comorbidities. Trends in SF, liver iron concentration (LIC) and cardiac iron burden assessed by MRI, along with ongoing transfusional iron intake, are usually used to adjust the dosing and type of chelator (1). However, adherence to ICT can be critical as IOL is often asymptomatic and many patients do not perceive direct benefits from ICT. Additional factors for poor adherence to treatment are represented by the cost of medication, suboptimal patient education, limitation in availability and/or psychosocial factors (4).

The average SF levels in healthy individuals range from 80 to 180 ng/mL in men and from 60 to 120 ng/mL in women (5). Levels of SF, especially those exceeding 2,500 ng/mL, are linked with iron-related organ failure, notably cardiac siderosis and endocrine complications (6). A univariate analysis of β-TDT patients in the UK showed a higher risk of diabetes mellitus (DM) in those with persistently elevated SF levels (an average 10-year SF level >1,500 ng/mL) compared to those with a lower average SF level (7). Furthermore, in a study of 165 adult β-TDT patients followed in Italy (mean age 39.9 ± 8.3 years, 43% males), a SF level >1,300 ng/mL was predictive for the development of endocrinopathies (P = 0.025) (8).

Therefore, intensifying the chelation regimen, whether through monotherapy or combined ICT, is recommended in patients with persistently high SF levels and/or associated comorbidities in order to minimize the free labile iron-induced organ damage (9,10). To personalize ICT, ten different combinations of iron chelators are employed in clinical practice for those with persistent IOL and/or organ damage. These combinations include DFO + DFP, DFO + DFX, and DFP + DFX (9). Among these, the combination of DFO and DFP has the longest clinical experience (10).

Although intensification ICT is well established to reverse hepatic or cardiac dysfunction, the effects on endocrinopathies in patients with β-TDT remain unpredictable (11-14).

Therefore, the primary aim of this retrospective study, designed and supported by the International Network of Clinicians for Endocrinopathies in Thalassemia and Adolescence Medicine (ICET-A), was to examine the evolution of oral glucose tolerance tests (OGTT) in adolescents and very young adult β-TDT patients with moderate to severe iron overload (IOL), following the intensification of their ICT regimen. As a secondary endpoint, the study aimed to evaluate markers of insulin secretion and sensitivity/resistance during a 3-hour OGTT, from baseline up to a target serum ferritin (SF) level of 500-1,000 ng/mL, in line with current guidelines (1).

Patients and Methods

Studied patient population, assessment of iron overload and setting at baseline

This retrospective study included β-TDT patients with moderate-severe IOL and inadequate adherence to ICT referred for endocrine and metabolic

assessment, between October 2016 and January 2024, to a single Italian outpatient clinic.

β-TDT was diagnosed by the referring Centers through hemoglobin HPLC and/or molecularly characterized genotype. The exclusion criteria were: (a) the presence of hemoglobinopathies other than β-TDT; (b) patients who had undergone bone marrow transplantation; (c) β-TDT patients taking medications that affect glucose metabolism; (d) individuals with chronic illnesses in addition to β-TDT; and (e) patients lacking clinical and OGTT data.

Iron overload was evaluated by the referring Centers through serial serum ferritin (SF) measurements at regular intervals using an automated immunoassay system, alongside hepatic and cardiac MRI T2*. The severity of IOL was arbitrarily classified based on mean SF levels: mild (500 -1,000 ng/mL), moderate (>1,000 ng/mL to < 2,000 ng/mL), or severe (>2,000 ng/mL). Liver iron concentration (LIC) assessed by MRI T2* < 1.5 mg/g dry weight (d.w.) was considered iron-free, from 1.5 to 7.0 as mild, from 7.0 to 14.0 as moderate, and over 14.0 as severe. For global heart iron concentration, MRI T2* >22 msec was considered iron-free, from 14 to 22 msec as mild, from 8 to 14 msec as moderate, and < 8 msec was categorized as heavy cardiac iron load (15).

Data collection and study procedures

Data collected included demographics such as age, gender, anthropometric measures (standing height, weight and body mass index), family history of diabetes in siblings or parents, history of splenectomy, age at starting of blood transfusion treatment, mean annual pre-transfusion hemoglobin (Hb), type of chelation therapy, biochemical values of liver enzymes, serum creatinine, assessment of IOL (SF, LIC and global heart T2*) and evaluation of endocrine profile. The ICET-A criteria previously described were used to diagnose associated endocrine complications (16).

Height and weight were measured using standard techniques. Body mass index (BMI) was calculated as body weight divided by the square of height and categorized as underweight (<20 kg m^2), normal weight (20–24.9 kg m^2), overweight (25–29.9 kg m^2) and obesity (≥ 30 kg m^2).

OGTT and classification of glucose abnormalities

A standard OGTT (max 75 g of glucose in 250–300 mL water) was performed in the morning, after an overnight fast, in subjects clinically stable and without a history of acute infection in the previous 3 weeks. Venous blood samples were collected at 0, 30, 60, 90,120 and 180 minutes after glucose load, for plasma glucose (PG) and insulin assay. PG was measured using glucose oxidase method and insulin was measured using the Immulite immunoassay (Diagnostic Products Corporation, Los Angeles, CA, USA). The analytical sensitivity was 2 μIU/mL.

Impaired fasting glucose (IFG) and impaired glucose tolerance (IGT) were defined according to the criteria of the American Diabetes Association (ADA) (17). Early glucose intolerance (EGI) was defined by the combination of 1-hour post-glucose (1-h PG) level ≥155 mg/dL and 2-h PG level <140 mg/dL (18).

Selection of insulin secretion and insulin sensitivity/resistance indices.

Pancreatic β-cell function was estimated by the insulinogenic index (IGI), calculated as the ratio of the early phase increment in the plasma insulin level to that in the PG level during the first 30 min (IGI 0-30: ΔIns 30–0/Δgluc30–0), after the ingestion of glucose (19,20). An IGI value <0.4 was considered to be an index of defective acute insulin response.

The updated index of the homeostasis model assessment of insulin secretion (HOMA2-% β), insulin sensitivity (HOMA2-%S) and the reciprocal index of HOMA2-IR values were calculated from fasting PG and insulin measures using the Oxford Diabetes Trials Unit calculator (21,22). A HOMA2-% β value < 40 is considered as an index of decreased pancreatic β cell function and HOMA2-IR ≥1.2 is considered as an index of IR (22). Moreover, the dynamic insulin sensitivity index, proposed originally by Matsuda and DeFronzo (oMI 0-120) (23) and the oral disposition index (oDI), as the product of the oMI 0-120 and IGI were also calculated (24). oDI index provides a measure of β-cell function adjusted for insulin sensitivity and has been shown to be predictive of development of diabetes in the general population and patients with

thalassemia (25). oMI 0-120 index ≤ 2.5 indicates the presence of IR (26).

Statistical analysis

Qualitative variables are presented as mean ± standard deviation (SD), median, and percentages. Data were assessed for normal distribution using the Shapiro–Wilk test. Non-normally distributed data were analyzed using the Wilcoxon-Mann-Whitney test for two independent samples and the Kruskal–Wallis test for multiple independent samples with non-normal distributions. Statistical correlation between two variables was determined using Pearson's correlation for normally distributed data and Spearman's correlation for non-normally distributed data. A software program was employed for statistical analysis, validated according to Alder and Roesser (27). The chi-square ($\chi 2$) test was applied to compare frequencies of qualitative variables across different groups. Two-tailed P-values < 0.05 were considered statistically significant.

Ethics

All procedures were in accordance with the 1964 Helsinki declaration and its later amendments (www.wma.net). 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 (1,2,8,15), 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 at first consultation

The demographic, anthropometric, clinical data and associated endocrine complications of enrolled patients, at first consultation, are summarized in Table 1. Patients with β-TDT were on regular packed red blood cell (RBCs) transfusion, every 2-3 weeks, to keep pre-transfusional Hb level > 9 g/dL. Eleven out of 19 β-TDT patients (57.8%) were on treatment with DFO and the remaining 8 patients on monotherapy with oral chelators. The most documented associated complications related to IOL were short stature (21%) and hypogonadotropic hypogonadism (31,5%) (Table 1).

B. Glucose homeostasis, insulin secretion and resistance/ sensitivity during OGTT at first consultation

A normal OGTT test (NGT) was reported in 8/19 β-TDT patients (42.1 %). The remaining 11 patients had abnormal OGTT values, including 6/19 (31.5%) with isolated impaired fasting glucose (i-IFG), 2/19 (10.5%) with isolated early glucose intolerance (EGI:1-h OGTT ≥ 155 mg/dL), 1/19 isolated glucose tolerance (i-IGT), and 2/19 (10.5%) with combined IFG and impaired glucose tolerance (IFG + IGT).

Interestingly, a negative correlation was observed between global heart MRI T2* and 1-h PG after OGTT load [Spearman's analysis: r_s = -0.49517, P (2-tailed) = 0.031].

The insulin response during OGTT was compared to 11 healthy adult volunteers with BMI < 25 Kg/m^2 who were not heterozygotes for β-thalassemia, matched for age (23.8 ± 3.2 years) and gender (5 males and 6 females) (28). No statistically significant differences were found between the two groups (Ins t_0: P = 0.12; Ins t_{30}': P = 0.31; Ins t_{60}': P = 0.24; Ins t_{120}': P = 0.20; Ins t_{180}' P = 0.12). However, the assessment of glucose homeostasis during OGTT, compared to 11 healthy controls, was associated with a significant deterioration of insulin sensitivity/resistance = HOMA 2-IR: 1.12 ± 0.30 vs. 0.76 ± 0.24, P = 0.0021; HOMA 2-%S: 94.5 ± 24.0 vs. 144.7 ± 49.8, P= 0.0008; Matsuda index: 5.35 ± 1.61 vs. 8.71 ± 2.85, P = 0.0003, and oDI: 5.15 ± 2.97 vs. 12.11 ± 6.55, P = 0.0004.

Two patients presented an IGI value <0.4. No statistical differences were found between β cell function (HOMA 2-%β) and early insulin response (IGI), after OGTT load. HOMA 2-%β: 93.9 ± 24.0 vs. 99.2± 38.5, P= 0.64 and IGI:1.0 ± 0.60 vs. 1.54 ± 0.99, P = 0.0721.

Table 1. Summary of clinical and laboratory characteristics.

Variable	Results
Mean Age (yrs)	18.5 ± 2.9
Gender distribution	Males: 9 ; Females: 10
Positive family history for diabetes type 2	5 out of 19 (26.3%)
History of splenectomy	9 out of 19 (47.4%)
Mean Body Mass Index (BMI: Kg/m²)	20.3 ± 1.3
BMI: 18.5 - 25 kg/m²	19 out of 19 (100%)
Mean serum ferritin (SF: ng/mL)	2,670.6 ± 778.2 (Range: 1,865-4,532)
SF ˃ 2,500 ng/mL	8 out of 19 (42.1%)
Mean SF (ng/mL) in year preceding first consultation	2,193.0 ± 638.2
Mean alanine aminotransferase (ALT)	42.4 ± 19.6 (Range: 14-79)
ALT ˃ 40 IU/L	7 out of 18 (38.8%)
LIC (mg Fe/g dry weight)	7.8 ± 6.9 (Range:1.3-24.6)
Global heart MRI T2* (msec)	23.9 ± 9.0 (Range: 12.6-43)
Iron chelation therapy	- DFO monotherapy: 11 out of 19 (57.8%)
	- DFP monotherapy: 7 out of 19 (36.8%)
	- DFX monotherapy: 1 out of 19 (5.2%)
Short stature and endocrine complications	- Short stature (≤ 3rd centile): 4 out of 19. In 1/4 patients, the IGF-1 was very low: 27.3 ng/mL
Insulin growth factor 1 (IGF-1)	- 91.1 ± 41.2 ng/mL
Delayed puberty	- 2 out of 19 (10.5 %)
Hypogonadotropic hypogonadism	- 6 out of 19 (31.5 %)
Secondary amenorrhea	- 3 out of 19 (15.7 %)
Primary hypothyroidism	- 3 out of 19 (15. 7 %)
Hypoparathyroidism	- 1 out of 19 (5.2 %)
Hypocortisolism	- 0 out of 19 (0 %)

C. Iron overload and chelation therapy

Eight out of 19 β-TDT patients (42.1%) had SF levels exceeding 2,500 ng/mL.

Three out of 19 (15.7%) exhibited moderate LIC (7 to 14 mg/g dry weight (d.w.), and 4 out of 19 (21.0%) had severe LIC (>14.0 mg/g d.w.), while in the remaining 12 patients, the mean LIC was 3.4 ± 1.37 mg/g d.w. (A LIC value < 1.5 mg/g d.w. was considered iron-free). Six out of 19 β-TDT patients (31.5%) presented with a global heart T2* from 8 to 14 msec, and the remaining 13 patients had a mean cardiac T2* value of 28.5 ± 7.0 msec.

A significant direct correlation was observed between LIC and SF [Spearman's analysis: r_s = 0.45654, P (2-tailed) = 0.049].

The majority of patients,11/19 (57.8%), were on monotherapy with DFO (40-45 mg/kg/day s.c. over 8–10 hours, 6-7 days/week) and the remaining 5 patients on monotherapy treatment with DFP (75 mg/kg/day in 3 divided doses daily), and 3 patients were on monotherapy treatment with DFX (20- 25 mg/kg/day). The reported inadequate adherence to ICT was mainly due to the patients' younger age, psychosocial factors and lack of perception of immediate ICT benefit.

Based on the severity of iron burden assessed by SF, LIC, and global heart MRI T2* or both, glucose abnormalities and associated endocrine complications, the patients were discharged from the Outpatient Clinic with the recommendation to use as first step intensive combined ICT with DFO (at doses of 40 mg/kg/day s.c. over 10-12 hours, 2–5 infusions per week, depending on SF levels) plus DFP (75 mg/kg/day in 3 divided doses). Referring Centers were also invited to tailor the ICT based on the individual patient's response, to monitor the reduction of iron burden, and to decide the switch to monotherapy with oral chelators according to the available TIF guidelines for the management of TDT patients (1) and based on clinicians' experience with the patients.

D. Patients' characteristics, variations of metabolic profile, insulin secretion and/or sensitivity markers at last consultation

The demographic, clinical data, biochemical and diagnostic parameters at last consultation are summarized in Table 2.

After 18-24 months of combined ICT therapy with DFO+DFP, a sustained reduction of SF level was observed [1,575.9 ± 416.3 vs. 2,670.6 ± 778.2

Table 2. Clinical and laboratory characteristics at first and last consultation.

Variables	At first consultation	At last consultation	P value
Number of β-TDT patients	19	19	=
Age (yr), median	18.5 ± 2.9 (17.10)	23.2 ± 3.0 (23.1)	**0.0001**
Males/Females	9/10	9/10	=
Body Mass Index (BMI:18.5 -25 kg/ m^2) (n.)	19/19	17/19	0.15
Positive family history (n.)	5/19	5/19	=
History of splenectomy (n.)	9/19	9/19	=
Mean pre-transfusional Hb level (g/dL)	9.3 ± 0.3	9.1 ± 0.2	**0.020**
Mean serum ferritin (SF: ng/mL), range	2,670.6 ± 778.2 (1,865 - 4,532)	623.0 ± 227.1 (182 - 948)	**0.0001**
Mean ALT (normal values: < 40 IU/L), range	39.7 ± 16.4 (12-71)	33.0 ± 20.0 (13-57)	0.26 =
Iron chelation therapy (*) **Desferrioxamine (DFO) monotherapy (n.)** **Deferasirox (DFX) monotherapy (n.)** **Deferiprone (DFP) monotherapy (n.)**	= 11/19 (57.8%) 1/19 (5.2%) 7/19 (36.8%)	= 0/19 (0%) 13/19 (68.4%) 6/19 (31.5 %)	= **0.0001** **0.024** 0.73
Mean LIC (mg Fe/g dry weight), range	7.8 ± 6.9 (1.3-24.6)	2.2 ± 1.0 (1.0- 4.6)	**0.0013**
Mean Global heart T2* (msec), range **Heart T2* from 8 to 14 msec (n. and %)**	23.9 ± 9.0 6 (31.5 %)	28.3 ± 8.3 1 (5.2%)	0.12 **0.038**
Insulinogenic Index (IGI)	1.0 ± 0.60	0.82 ± 0.40	0.17
HOMA 2-% β	93.9 ± 24.0	79.9 ± 29.1	**0.048**
HOMA 2-IR	1.12 ± 0.30	0.95 ± 0.40	0.089
MATSUDA Index (MI 0-120)	5.35 ± 1.61	6.45 ± 2.35	0.098
HOMA 2-%S	94.5 ± 24.0	124.5 ± 55.4	**0.022**
Oral disposition Index (oDI)	5.15 ± 2.97	4.98 ± 2.10	0.71

Legend = (*) DFX dosage: dispersible tablets 25-35 mg/kg/day, once daily dose; DFX -FCT (film-coated tablets): 15-22 mg/kg/day, once daily; DFP dosage: 75 mg/kg/day in 3 divided doses.

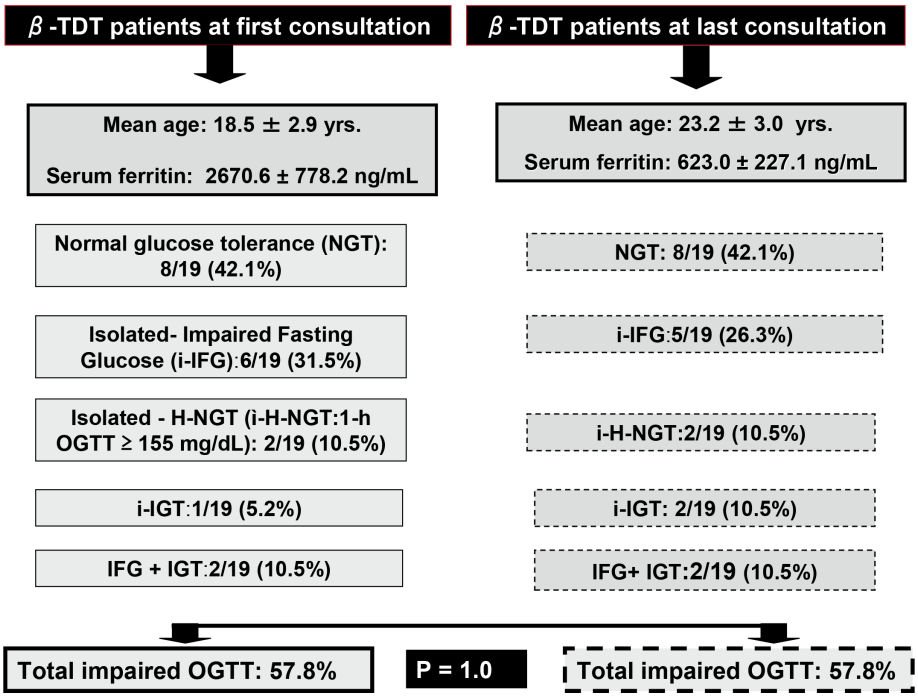

Figure 1. Glucose homeostasis in 19 transfusion-dependent β-thalassemia (β-TDT) patients with moderate - severe iron overload

(at baseline); P = < 0.0001]. After discontinuation of 2 years combined ICT, all patients were treated with oral monotherapy with DFX or DFP. During monotherapy treatment, a further reduction of SF level was documented. One year before last consultation, the mean SF level was 943.2 ± 363.6 ng/mL.

At last consultation, the mean SF level was 623.0 ± 227.1 ng/mL (range:182 - 948; median: 666 ng/mL), LIC was consistently lower compared to the baseline (P = 0.0013) and the number of patients with low global heart T2* (from 8 to 14 msec) declined from 6 to 1 (P = 0.038). Moreover, during the follow-up, primary hypothyroidism resolved in 3 patients. However, 2 males developed hypogonadotropic hypogonadism and 2 females developed secondary amenorrhea.

In general, ICT was well tolerated. No patients treated with a combination of deferiprone (DFP) and deferasirox (DFX) developed leukopenia or agranulocytosis. Among DFP-treated patients, mild hypercalciuria was reported in one patient. Among patients receiving DFX, a transient increase in serum creatinine (≥ 30%) and hypercalciuria were reported in 3 out of 13 patients (23%).

Glucose homeostasis at first consultation and after 4.2 -years (median) of follow-up is summarized in Figure 1. According to ADA criteria, 8/19 patients (42.1%) were classified as having a normal glucose tolerance test (NGT) and the remaining 11 patients presented various phenotypes of prediabetes or early glucose intolerance (EGI).

Interestingly, 3 out of 6 patients with isolated impaired fasting glucose (i-IFG) reverted to normal glucose tolerance (NGT), and 1 patient with impaired fasting glucose plus impaired glucose tolerance (IFG+IGT) transitioned to i-IFG. Nevertheless, the percentage of glucose dysregulation (GD) during the oral glucose tolerance test (OGTT) did not change significantly compared to baseline (P = 0.75). Furthermore, a lower insulin response was documented during OGTT at 30 minutes (Ins T_{30}': 56.7 ± 28.4 vs. 39.5 ± 19.2, *P* = 0.0065) compared to baseline. In addition, at last consultation, a reduction of mean HOMA 2-%β value (P= 0.048) and an increase of HOMA 2-%S value (P= 0.022) was documented.

E. Correlations

A significant improvement of SF was observed at last evaluation compared to SF baseline (r: 0.4256, P = 0.0077), PG at $t_{30'}$ vs. Ins at $t_{30'}$ (r: 0.4804, P = 0.037), and PG at $t_{120'}$ vs. Ins at $t_{120'}$ (r: 0.5865, P = 0.0083).

Other correlations, including all collected variables, were not statistically significant. Nevertheless, a tendency for a negative correlation was found between LIC values and. HOMA 2-% β [Spearman's analysis: r_s : - 0.44747, P (2-tailed) = 0.054].

Discussion

Most guidelines for the management of thalassemia now rely on non-invasive detection through MRI imaging of preclinical iron deposition in most organs, especially the liver and heart. It has also been considered a key tool for the assessment of IOL in other organs, such as pituitary, pancreas, adrenals, spleen, bone marrow, and kidney (28,29).

A number of studies have reported a marked hypointense pancreatic MRI signal in 75-100% of TDT patients (30). Pancreatic iron loading starts in early childhood in patients receiving suboptimal ICT (31), is more evident in patients with abnormal glucose tolerance (IGT and DM) and accelerates after splenectomy (32). Therefore, early detection of GD plays an important role and is an area of considerable interest for patients with thalassemia.

Farmaki et al. (13,14) have shown that "normalizing" iron stores in β-TDT patients, in the absence of chelator-mediated toxicity, prevents new morbidities and reverses many complications, such as cardiac failure, hypothyroidism, hypogonadism and GD, but the response remains unpredictable.

The results of the current study confirm the high prevalence of glucose dysregulation (GD) (52.6%) in β-thalassemia major (β-TDT) patients with inadequate adherence to iron chelation therapy (ICT) and moderate-to-severe iron overload (IOL). However, despite a sustained reduction in serum ferritin (SF) levels, liver iron concentration (LIC) values, and improvement in cardiac dysfunction, as assessed by MRI T2*, the overall prevalence of GD at the final observation remained statistically unchanged (P = 0.75). Over the course of the 4.2-year follow-up, two males developed hypogonadotropic hypogonadism and two females developed secondary amenorrhea.

A normal iron level is essential for proper β-cell function; however, its excess plays a catalytic role in producing reactive oxidant species (ROS) and free radicals, leading to oxidative damage. This has a direct toxic effect on pancreatic β-cells, causing insulin deficiency and the development of glucose dysregulation (GD). Iron excess can also induce β-cell loss through ferroptosis, a non-apoptotic cell death mechanism characterized by lipid ROS accumulation due to glutathione (GSH) depletion and consequent inhibition of glutathione peroxidase-4 (GPX4) (33). Furthermore, iron overload (IOL) primarily accumulates in tissues with high levels of transferrin receptor (TR), which are predominantly expressed in the islets of the normal human pancreas. The transport of iron out of the endosome is mediated by divalent metal-ion transporter-1 (DMT1), and the abundant expression of DMT1 in islet cells suggests a significant role in iron uptake by β-cells, leading to their damage (33). When pancreatic β-cells succumb to the cytotoxic effects of iron, the pancreatic parenchyma is progressively replaced by adipose tissue (34). However, GD or diabetes mellitus (DM) occurs only when more than 70% of β-cells have died (35).

Significantly, at the last observation, there was an improvement in the Homeostatic Model Assessment 2-%S (HOMA2-%S) compared to baseline and healthy controls. However, the insulin secretion capacity (HOMA2-%β) deteriorated further compared to baseline. Moreover, the additional decline in the oral Disposition Index (oDI) among β-TDT patients, relative to healthy controls, indicates the inability of pancreatic β-cells to compensate for the degree of insulin resistance.

Therefore, it remains to be determined whether the observed changes in glucose handling during the period of intensifying ICT, for example normalization of fasting glucose in 3 out of 6 patients with isolated impaired fasting glucose (i-IFG) at the baseline, could be attributed to a reversal/improvement in glucose homeostasis related to reducing IOL, or simply represent random fluctuations in glucose tolerance, as reported in a previous study (25).

The normalization of thyroid function observed in three patients with primary hypothyroidism, along with the development of hypogonadotropic hypogonadism in two males and secondary amenorrhea in two females, further support the variable response rates of endocrine glands to intensified ICT. Therefore, a more aggressive ICT approach and/or a prolonged follow-up of patients with GD will be necessary before drawing final conclusions. Poggi et al. (8). have reported that a SF level below 200 ng/mL predicted the reversal of existing endocrinopathies. However, this clinical observation was not supported by statistically significant evidence (P = 0.147). Furthermore, the feasibility of maintaining iron levels near 'normal' without unacceptable toxicity risks has yet to be established.

The strength of our study is the detailed analysis of ICT intensification on glucose homeostasis, indices of insulin secretion/resistance and action, derived from 3-h OGTT. Nevertheless, certain limitations should be recognized. It was a single site study, which reduces the generalizability of the results. There was no monitoring of the effect of ICT intensification on the pancreatic MRI and plasma levels of trace elements such as zinc. The number of patients enrolled in the study was small and it was not possible to compare in depth the effects of two oral chelators on glucose homeostasis.

Nevertheless, in terms of MRI-monitored reversal of the pancreatic siderosis during chelation therapy, recent prospective research data by Meloni et al. demonstrated that the removal of IOL from the pancreas is challenging, and the three iron chelators (deferrioxamine, deferiprone or deferasirox) have equivalent efficacy when used alone (36).

Conclusion

This retrospective study aimed to evaluate the impact of intensified ICT on glucose homeostasis and related endocrine disorders in a group of 19 adolescents and very young adults with β-TDT and moderate to severe iron overload. Despite the intensive ICT and consequent significant reduction in SF levels and iron overload in the liver and heart, the overall prevalence of GD remained unchanged, after a median follow-up period of 4.2 years. Despite reversal of i-IFG to NGT in 15.7% of patients and improvement in post-glucose load values in individual cases, the study found that intensive ICT was not uniformly effective in improving glucose regulation. In addition, this study reported a variable effect of intensive ICT on the function of other endocrine organs, as evidenced by a combination of normalization of thyroid function in some patients and development of hypogonadotropic hypogonadism and secondary amenorrhea in other cases.

Therefore, future studies with larger cohorts and longer follow-up are warranted to examine the impact of ICT intensification on different endocrine organs and determine the optimal duration and magnitude of iron chelation required to improve endocrine status.

Besides strengthening the evidence towards the necessity of sustained adherence to ICT, this study highlights the need for coordinated research efforts in order to advance our understanding of the long-term effects of ICT on endocrine and metabolic outcomes. Finally, intensification of ICT, albeit potentially improving certain endocrine dysfunctions, is not a panacea, particularly for established iron-related organ toxicity.

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Correspondence:
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Vincenzo De Sanctis, MD
Coordinator of ICET-A Network (International Network of Clinicians for Endocrinopathies in Thalassemia and Adolescent Medicine) and Pediatric and Adolescent Outpatient Clinic, Quisisana Hospital, Ferrara, Italy
E-mail: vdesanctis@libero.it