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Original article

The credibility of intermediate hyperglycemia (IH) at one hour of OGTT in 30 β-thalassemia patients (β-thal) with persistent low iron overload

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

[1]UOSD Thalassemia, Umberto I° Hospital, Siracusa, Italy; [2]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; [3]Department of Pediatrics, Division of Endocrinology, Hamad General Hospital, Doha, Qatar; [4]Department of Hematology, College of Medicine and Health Sciences, Sultan Qaboos University, Muscat, Sultanate of Oman; [5]Thalassemia Unit, First Department of Pediatrics, National Kapodistrian University of Athens, Greece

Abstract. *Background*: Intermediate hyperglycemia (IH) at 1-hour during oral glucose tolerance test (OGTT) has received attention as a useful early biomarker of dysglycemia. *Objective*: The main objective of this study was to gain further insight into IH in 30 β-thalassemia (β-thal) patients with a spectrum of genotypes and persistent low iron load (serum ferritin; SF:<1,000 μg/L), related to effective treatment with oral iron chelation monotherapy. *Methods:* OGTT was performed according to the American Diabetes Association (ADA) guidelines. Venous blood samples for PG and insulin assays were collected at 0, 60, and 120 minutes after glucose load for PG and insulin assays. *Results:* Based on the OGTT results, 13 out of 30 patients (43.3%) had normal glucose tolerance (NGT), and 4 out of 32 (12.5%) patients impaired fasting glucose (IFG). Interestingly, isolated IH (i-IH at 1-hour post-OGTT: PG: ≥ 155 mg/dL) and type 2 diabetes mellitus at 1-hour post-OGTT (PG: ≥ 209 mg/dL) were detected in 5 (16.6%) and 3 (10%) out of 30 patients, respectively. IH associated with IFG and impaired glucose tolerance (IGT) was detected in 3 (10%) and 2 (6.6%) out of 30 patients, respectively. β-TDT patients were divided into two subgroups: those with NGT and those with IH. A statistically significant difference between the two groups was observed only for the HOMA 2 - % β index (82.6 ± 23.5 vs. 64.8 ± 25.4; P = 0.042). *Conclusion:* Intermediate hyperglycemia at 1-hour during OGTT in β-thal is a sensitive index to identify early glucose dysregulation even in patients with NGT. (www.actabiomedica.it)

Key words: 1-hour plasma glucose post- glucose load, OGTT, *iron chelation*, genotype, β-*thalassaemia*, ICET-A

Introduction

In recent years, the glucose homeostasis of patients with thalassemia has been mainly investigated for its relationship to total iron load and to the amount of iron deposition in different organs mainly liver, heart and pancreas, assessed by magnetic resonance imaging (MRI) using R2- (T2) or R2*- (T2*) relaxometry (1-5), and to a lesser extent with the impact of patients' genotype (6) and oral iron chelation therapy (1,4,5,7). Moreover, the glucose homeostasis assessed by glucose tolerance test (OGTT) has taken into consideration only the plasma glucose (PG) levels and plasma insulin at fasting and PG 2-hours after the glucose load (1,4,5).

In the last decade, several studies have shown that 1-hour PG concentration post-load (PG: ≥ 155 mg/dL) is a useful early biomarker of dysglycemia. In 2024, the Position Statement of International Diabetes Federation (IDF) has stated that 1-hour post-load PG concentration ≥ 209 mg/dL should be considered compatible with the diagnosis of diabetes type 2 (T2DM), if 1-hour PG level is confirmed by a second OGTT (8). Intermediate hyperglycaemia (IH) is considered by IDF as an indicator of prediabetes in addition to impaired glucose tolerance (IGT) and impaired fasting glucose (IFG), although determination of 1-h-PG values is not recommended by the current American Diabetes Association (ADA) as criteria for identifying adults at increased risk of T2DM (9).

Although the determination of the optimal method for early identification of β-thal patients at risk for deterioration of glucose homeostasis is still challenging, a recent retrospective study by our group has confirmed the utility of 1-hour PG value as a simple biomarker to detect high-risk β-thal patients that warrants periodic surveillance (10,11).

Thus, the main objective of this study, designed by the International Network of Clinicians for Endocrinopathies in Thalassemia and Adolescence Medicine (ICET-A), was to gain further insight on IH in a group of β-thal patients with a wide spectrum of severity of genotypes and clinical phenotypes in parallel with a persistent mild iron overload, assessed by serum ferritin (SF). As a secondary endpoint, the study aimed to evaluate fasting markers of insulin secretion and sensitivity/resistance in patients with normal glucose tolerance (NGT) versus those with IH.

Patients and methods

a) Setting, research design, and definitions

In this cross-sectional study, we collected data on OGTT, performed between February 2019 and March 2024, and other biochemical parameters, in 30 individuals selected out of 103 β-thal patients (29.1%) regularly followed at the outpatient Thalassemia Center of Umberto I° Hospital, Siracusa (Italy); the selected patients had low SF (< 1,000 μg/L) during the 5 years preceding the OGTT. In 14 out of 30 patients (46.6%) the OGTT was assessed in the last 12 months.

The exclusion study criteria included patients with: (a) diabetes mellitus; (b) Hb variants, such as HbS, C, Lepore and E; (c) bone marrow transplantation; (d) recent intake of medications affecting glucose metabolism; and (e) patients lacking clinical and OGTT data.

β-thal was diagnosed using complete blood count and hemoglobin HPLC. From 1995 the molecular characterization of genotype was also available. Genomic DNA was extracted from peripheral blood (buffy coat) and amplified by a multiplex polymerase reaction (PCR) with subsequent reverse hybridization to characterize the commonest Mediterranean β-thalassemia mutations. Reverse hybridization technique and direct gene sequencing were used to characterize β-thalassemia mutations (HBB MIM*141900). The α-thalassemia deletions and nondeletions were identified by allele-specific oligonucleotide hybridization and automatic sequencing (12).

Patients were regularly transfused with packed red blood cells (PRBC) and treated in the last decades with oral chelation therapy, namely: deferiprone (DFP) in tablet form and deferasirox (DFX), initially as a dispersible tablet and more recently as a film-coated tablet. DFP has been available since 1995 and was administered at a dose of 75 mg/kg body weight. DFX was introduced in 2007, at a dose of 25–30 mg/kg body weight, for patients for whom treatment with deferoxamine mesylate (DFO) was contraindicated or inadequate. According to current guidelines, when the SF level was < 500 μg/L in two successive tests (13), the dose of the oral iron chelator was reduced and discontinued temporarily when SF was ≤ 300 μg/L to avoid adverse events due to treatment (14).

Data collected at the time of OGTT included: age, gender, anthropometric measurements (standing height, weight and body mass index), medical history, biochemical parameters and associated endocrine complications. Body mass index (BMI) was used to define overweight and obesity, with overweight classified as a BMI 25–29.9 kg/m^2 and obesity as BMI of ≥ 30 kg/m^2 in adults. The ICET-A criteria previously described were used to diagnose associated endocrine complications (15). All biochemical and endocrine parameters were determined by standard methods.

The study was approved by the local institutional review boards and was performed in accordance with the Declaration of Helsinki. Informed consent was obtained from all patients.

OGTT and definition criteria for the classification of glucose abnormalities

OGTT was performed using a standard dose of 1.75 g of glucose/kg of body weight (max 75 g) according to the American Diabetes Association (ADA) guidelines. Venous blood samples were collected at 0', 60' and 120' minutes after glucose load, for PG and insulin assay. Based on PG results on OGTT, each patient's glycemic status was classified according to the ADA criteria (16). Moreover, PG level at 1-hour post glucose load was assessed and defined as IH if PG was ≥155 mg/dL and as T2DM if ≥ 209 mg/dL (8). A PG level ≤ 55 mg/dL, at any time during the OGTT with or without symptoms, was defined as hypoglycemia.

PG was measured using glucose oxidase method and insulin was assayed by a highly selective and sensitive chemiluminescence immunoassay (CLIA) with fully automated analyzer (Beckman Coulter Diagnostics).

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 (17).

Statistical analysis

Variables are presented as the mean ± standard deviation and percentages. Continuous variables were tested for normality using the Kolmogorov-Smirnov test. The differences in the mean value of different parameters were assessed using Student *t*-test for parametric measures and Wilcoxon's signed rank test for continuous values with no normal distribution. Correlation analysis was performed using Pearson's test or Spearman's test where appropriate. All analyses were performed using STATA 12 (StataCorp LP). In all tests, a 2-tailed probability value < 0.05 was considered statistically significant.

Results

30 β-thal patients (16 men and 14 women; mean age; 40.4 ± 9.0 years at the time OGTT) were enrolled in the study and 2 patients were excluded because of lacking OGTT data. Their mean age of 30 β-thal patients at the first blood transfusion was 11.8 ± 8.6 months (range: 2-38 months). The last mean annual pre- transfusion Hb level was 9.7 ± 0.30 g /dL (range: 9.4-10.2 g/dL). Up to 1995, the main criteria for starting PRBC were persistent (i.e. more than 2 weeks) hemoglobin level ~7 g/dL in the absence of infections, significant symptoms of anemia and poor growth/failure to thrive (18,19).

The frequency of different genotypes in 29 out of 30 patients was substantially characterized by homozygous β^{+} (N = 9; 31.0%), compound heterozygous β^{0}/β^{+} (N =13;44.8%), and homozygous β^{0} (N =6; 20.6 %). Of note, in one patient the genotype was characterized by $\beta^{++}/\alpha\alpha\alpha$. The most frequent genotypes were IVS-I-6 and IVS-I-110.

DFX was the most commonly used oral iron-chelating agent (22/30; 73.3%) followed by DFP (8/30; 26.6 %). The mean SF level, at the assessment of OGTT, was 462.0 ± 246.5 μg/L (range:102-980). The remaining clinical and laboratory data are summarized in Table 1. Oral iron chelation therapy was generally well-tolerated, with mild adverse events reported.

Based on the OGTT results, 13/30 patients (43.3%) had NGT, isolated impaired fasting PG (i-IFG- PG level of 100–125 mg/dL and 2-hour PG <140 mg/dL) was reported in 4/30 (13.3%) patients.

Interestingly, an isolated IH (i-IH at 1-hour post OGTT: PG ≥ 155 mg/dL and i-T2 DM (1-hour post OGTT: PG ≥ 209 mg/dL) were diagnosed in 5/30 (16.6 %) and in 3/30 (10 %) patients, respectively. An IH associated with IFG and IGT was detected in 3/30 (10 %) and 2/30 (6.6%) patients, respectively (Table 1). The mean PG at 1-hour post OGTT in the 13 patients with IH was: 195.7 ± 25.7 mg/dL (range: 161-228 mg/dL). Surprisingly, primary hypogonadotropic hypogonadism was more common in males compared to female patients [15/16 male (93.7 %) patients 6/14 female (28.5%); P: 0.0003].

Table 1. Summary of clinical and laboratory characteristics/comorbidities in 30 transfusion-dependent β-thalassemia patients (β-TDT) with 5 years persistent mild serum ferritin (< 1,000 μg/L) at the time of OGTT

Variable	Results
Mean age (yrs)	40.4 ± 9.0
Gender distribution	Males: 16 ; Females: 14
Positive family history for diabetes type 1 or 2	6 out of 30 (20 %)
History of splenectomy	12/30 (40 %)
Mean Body Mass Index (BMI: Kg/m²)	23.4 ± 3.7
BMI: > 25 kg/m²	6/30 (20 %); mean 28.3 ± 2.8 Kg/m²; 4 males and 3 females
Pretransfusional hemoglobin level (g/dL)	9.7 ± 0.30
Mean serum ferritin (μg/L) at OGTT	462.0 ± 246.5 (Range:102-980)
Mean alanine aminotransferase (ALT)	25.3 ± 14.9 (Range:9-89)
ALT > 40 IU/L	3/30 (10 %)
Iron chelation therapy at the time of OGTT	- DFP monotherapy: 8/30 (26.6 %)
	- DFX monotherapy: 22/30 (73.3 %)
2-h Oral Glucose Tolerance Test [OGTT: plasma glucose (PG) and insulin at 0', 60 and 120' min.]	Normal plasma glucose (PG) levels at 0' and 120' min. during OGTT: 13/30 (43.3%)
	Isolated impaired fasting plasma glucose (i-IFG): 4/30 (13.3%)
	Isolated intermediate hyperglycemia at 1-h post-load PG (i-IH: ≥ 155 mg/dL and i-type 2 DM: ≥ 209 mg/dL): 5/30 (16.6 %) and 3/30 (10 %), respectively
	- IFG + IH: 3/30 (10 %)
	- Impaired glucose tolerance (IGT) + IH: 2/30 (6.6%)
Hypogonadotropic hypogonadism	- 21/30 (70%); 6/14 female (28.5%) and 15/16 male (93.7%) patients
Primary hypothyroidism	- 1/30 (3.1%) associated to HH in a male patient

Asymptomatic hypoglycemia (PG: 51 mg/dL) was documented, at 120 min after glucose load, in one male patient, aged 40 years, with NGT.

The genotype analysis of β-TDT patients with NGT, IFG and isolated or combined IH is reported in Table 2. Two β-thal patients with IH were overweight and one patient obese. No difference was found in the distribution of oral chelation regimens in the 3 groups of β-thal patients

Of interest, a direct positive correlation was documented between PG 1- hour vs PG 2-h (r: 0.4109; P: 0.024- Figure 1) and pre-transfusional Hb level vs HOMA 2- % β (r: 0.3932; P: 0.031). An inverse correlation between pre-transfusional Hb level vs. HOMA-2 % S (r: -0.3734; P: 0.035) and between BMI vs. HOMA-2 % S was also observed, but the latter was not statistically significant (r: -0.3493; P: 0.0502). For the assessment of fasting HOMA- 2 % β (a marker of insulin secretion), HOMA- 2 % S and HOMA-2 IR (markers of insulin sensitivity/resistance), β-TDT patients were divided in two subgroups: β -TDT with NGT and with IH. A statistically significant difference between the two groups was observed only for the HOMA 2 -% β index (82.6 ± 23.5 vs 64.8 ±25.4; P: 0.042).

Discussion

OGTT is currently considered the gold standard reference test for the diagnosis of GD, although several critical issues related to analytical variables have challenged its reproducibility and accuracy (20). A number of surrogate indices, derived from PG and insulin levels at a fasting state or after oral glucose load, have been proposed to estimate the pancreatic

Table 2. Genotype analysis in 29 β-thal patients with normal glucose tolerance (NGT), impaired fasting glucose (IFG) and intermediate hyperglycemia [IH: isolated or associated to IFG (3 patients) and impaired glucose tolerance (IGT: 2 patients)]

Mutations in 12 β-thal patients with NGT	Type	Mutations in 4 β-thal patients with isolated IFG	Type	Mutations in 13 β-thal patients with isolated or combined IH	Type
IVS-I-1/IVS-I-6	β^{0}/β^{++}	CD 39/IVS-I-110	β^{0}/β^{+}	IVS-I-1/IVS-I-110	β^{0}/β^{+}
IVS-I-6/IVS-I-6	β^{++}/β^{++}	CD39/CD39	β^{0}/β^{0}	CD39/IVS-II-745	β^{0}/β^{+}
IVS-I-2/IVS-I-6	β^{0}/β^{++}	IVS-I-6/IVS-I-110	β^{++}/β^{+}	CD 39/IVS-I-6	β^{0}/β^{++}
IVS-I-2/IVS-I-6	β^{0}/β^{++}	IVS-I-110/IVS-I-110	β^{++}/β^{+}	CD39/CD39	β^{0}/β^{0}
IVS-I-110/IVS-I-110	β^{+}/β^{+}	=	=	IVS-I-6/IVS-I-110	β^{++}/β^{+}
CD39/CD39	β^{0}/β^{0}	=	=	CD 39/IVS-I-6	β^{0}/β^{++}
CD 39/IVS-I-6	β^{0}/β^{++}	=	=	IVS-I-6/αααα	$\beta^{++}/$αααα
CD 39/IVS-I-6	β^{0}/β^{++}	=	=	CD39/IVS-I-1	β^{0}/β^{0}
CD 39/IVS-I-110	β^{0}/β^{+}	=	=	CD39/IVS-I-6	β^{0}/β^{++}
CD 39/IVS-I-110	β^{0}/β^{+}	=	=	IVS-I-110/IVS-I-5	β^{+}/β^{+}
IVS-I-6/IVS-I-110	β^{++}/β^{+}	=	=	IVS-I-110/IVS-I-110	β^{+}/β^{+}
CD39/IVS-I-2	β^{0}/β^{0}	=	=	CD39/IVS-I-1	β^{0}/β^{0}
=	=	=	=	CD 39/IVS-I-6	β^{0}/β^{++}

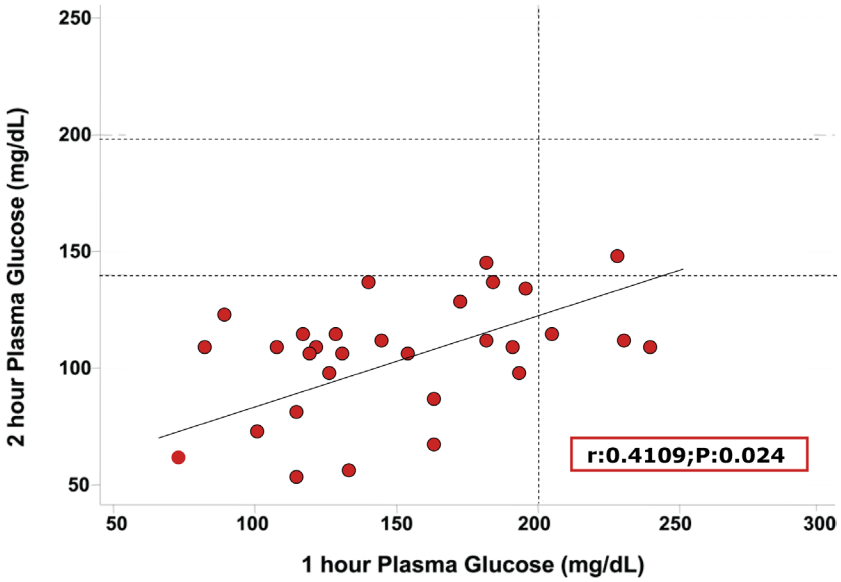

Figure 1. 1-hour plasma glucose level (PG), post oral glucose tolerance test (OGTT) related to 2-hours PG (mg/dL) level.

β-cell response, and the ability of β-cells to compensate for changes of insulin sensitivity by modulating insulin secretion (disposition index). These indices can detect subtle disturbances of glucose metabolism and may help in understanding the sequences of pathophysiology underlying glucose dysregulation in β-TDT patients (21).

Pancreatic iron loading in β-thal patients begins in early childhood (22) and increases with age. It is associated with progressive fat infiltration, resulting from the replacement of pancreatic parenchyma by inert adipose tissue. Notably, fatty replacement of the pancreas in adult β-TDT patients is not linked to a metabolic syndrome, is irreversible with intensive

iron chelation therapy and most likely represents severe or end stage pancreatic disease (3,4,7). A global pancreatic iron T2* overload T2* <13.73 ms (23) and a pancreatic T2* fat fraction > 15.3% were reported as cut-off levels for predicting co-existence of abnormal OGTT (4). An association between genotype and levels of pancreatic IOL has been reported. Patients with homozygous β^0 genotype and patients with β^0/β^+ genotype, when compared to patients with the homozygous β^+ genotype, had a risk for developing pancreatic IOL more rapidly, probably because of the high annual blood consumption and relative annual iron input (6).

In addition to IOL, the degree of anemia related to severity of genotypes, the presence of chronic liver disease particularly chronic active hepatitis C infection, the clinical history of splenectomy, obesity, hypogonadism and the association with zinc deficiency are all well-established contributing factors to the development of glucose dysregulation (GD) (20, 24).

Meloni et al. (6), starting from the assumption that the knowledge of molecular background can play a key role in the understanding the factors affecting the diverse clinical manifestations of β-thal patients, evaluated 68 subjects (mean age:11.9 ± 3.6 years). 33 different genotypes were identified, with the most common β-thal gene mutations being CD39 and IVS-I-110. The mean age at first blood transfusion in homozygous β^0 patients (n.25) was 12.4 ± 11.8 months, in β^0/β^+ patients (n.24) was 19.7 ± 17.6 months and in homozygous β^+ patients (n.19) it was 20.4 ± 29.7 months. The difference between the 3 groups was not statistically significant (P: 0.339). The three groups were homogeneous for age, sex, and hemato-biochemical parameters. Their mean SF level was 1.886 ± 1.805 μg/L in homozygous β^0 patients, 1.655 ± 1.518 μg/L in β^0/β^+ patients and 1.684 ± 1.276 μg/L in homozygous β^+ patients (P: 0.901). No patients were diagnosed with diabetes. Delayed puberty/hypogonadism, hypothyroidism, growth hormone deficiency (GHD) and hypoparathyroidism were reported, respectively, in 4, 3, 3 and 3 homozygous β^0 and β^0/β^+ thal patients. Of interest, two patients with in homozygous β^+ patients developed hypothyroidism and GHD, respectively, and 2-hour PG data after glucose load were not reported.

Therefore, to reinforce the importance of OGTT and to justify furtherly the use of the 1-hour PG index as an adjunctive sensitive diagnostic criterion for the diagnosis of GD in β-thal patients, we planned and applied the present study in 30 β -thal patients with a wide spectrum of genotypic severity with co-existing persistent low iron load (SF:< 1,000 μg/L), effectively treated with oral iron chelation monotherapy. Our results showed that an isolated IH (PG 1-h post OGTT: ≥ 155 mg/dL and PG 1-h post OGTT: ≥ 209 mg/dL, classified as type 2 DM) was detected in 8/30 (26.6 %) TDT patients with mild iron overload.

In population-based observational studies the prevalence of an elevated 1-hour PG level ranges from 11–16%, and in high-risk cohorts, with at least one cardiovascular risk factor, from ~25-42%. Moreover, the prevalence increases to > 50% in subjects with combined IFG and IGT, as glucose tolerance deteriorates, and above 90% in newly diagnosed T2 DM (25,26).

In brief, these observations further emphasize the importance of 1-hour PG during OGTT for the surveillance of GD in thalassemia.

The pathophysiologic mechanisms underlying 1-hour postload IH are not fully known. In a previous report, we documented an inverse correlation between 1-hour PG value and insulin secretion-sensitivity index-2 (ISSI-2; r: -0.3298; p: 0.025) in β-thal patients (11). ISSI-2 represents the ratio of the area under the insulin curve to the area under the glucose curve, multiplied by the Matsuda index. It constitutes a surrogate measure of insulin secretion relative to insulin sensitivity and emphasizes the pivotal role of impaired insulin secretion in the development of GD (11).

Unfortunately, in the present study it was only possible to assess the fasting β-cell function: HOMA- 2 % β (as a marker of insulin secretion) and HOMA- 2 % S and HOMA 2- IR (as markers of insulin sensitivity/resistance). Despite these limitations, in the entire group of patients, we observed an inverse linear correlation between pre-transfusional Hb level vs. HOMA- 2 % S (r:- 0.3734; P: 0.035) and a tendency for a significant correlation between BMI vs. HOMA-2 % S (r:- 0.3493; P: 0.0502). Both indices were compensated by a higher HOMA 2- % β (r: 0.3932; P: 0.031). Nevertheless, a statistically significant difference between β-thal subjects with

NGT and IH was observed (HOMA 2 - % β index: 82.6 ± 23.5 vs. 64.8 ± 25.4; P: 0.042), suggesting β-cell dysfunction as a plausible factor responsible over time for the development of elevated 1-hour PG concentrations.

This study has some limitations due to its retrospective cross-sectional nature. Firstly, the small number of selected patients necessitates larger prospective studies to confirm our findings. Secondly, we assessed the patients' IOL using SF concentrations, an indirect method for measuring iron deposits in the body. Although other more accurate modalities for diagnosing and monitoring IOL in patients with β-thal are available, SF determination is cheaper, more affordable, widely available, and recommended by most guidelines for making decisions about iron chelation therapy (ICT). Fourth, serum zinc concentrations were not assessed. β-thal patients with marginal zinc deficient had lower secretion of insulin and an impaired glucose response curve following OGTT (27). Increased utilization of zinc due to oxidative stress, increased urinary zinc excretion and sequestration in the liver related to increasing iron overload, and chelation therapy may account for the zinc deficiency in β-thal patients (27-30). Nevertheless, more studies are needed to unravel the exact role of zinc ions in the pancreatic β-cell and in islet cell-to-cell communication in relation to ICT. Lastly, patients' OGTT follow-up was poor, although an annual OGTT screening is recommended for all β-thal patients older than 10 years. β-thal patients tend to delay testing for many reasons, such as: the difficulty to schedule an OGTT appointment separate from clinic visit, the prolonged periods of fasting before performing the OGTT, and probably the insufficient awareness of the importance of regular glucose homeostasis screening.

Despite these limitations, the data enabled us to draw the following conclusions/considerations: (a) OGTT screening with fasting and 2-hour PG levels is relatively insensitive for detecting early dysglycemia, even in β-thal patients with low SF and NGT; (b) the observational nature of this study does not allow conclusions on the predictive value for future glucose dysregulation (GD) in β-TDT patients with intermediate hyperglycemia (IH), but it does justify the use of 1-hour PG concentrations during OGTT as an additional diagnostic criterion for evaluating glucose metabolism and the efficacy of recent novel iron chelation therapies in preventing or delaying the progression of GD in β-thal patients; (c) the complexity of the pathophysiologic mechanisms underlying GD in patients with β-thal necessitates that clinicians possess specific and up-to-date knowledge in order to perform rigorous diagnostic evaluations and correctly interpret relevant data in relation to the efficacy of different ICT regimes.

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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
ORCID: 0000-0002-6131-974X