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Original article

A retrospective study of glucose homeostasis, insulin secretion, sensitivity/resistance in non- transfusion-dependent β-thalassemia patients (NTD- β Thal): reduced β-cell secretion rather than insulin resistance seems to be the dominant defect for glucose dysregulation (GD)

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Abstract. *Aims:* Non-transfusion - dependent β-thalassemias (NTD-βThal) can cause iron overload and serious iron-related organ complications as endocrine dysfunction, including glucose dysregulation (GD). *Patients and methods:* We retrieved data of all NTD- β Thal patients referred consecutively to a single Outpatient Italian Clinic from October 2010 to April 2023. All patients underwent a standard 3-h oral glucose tolerance test (OGTT) for analysis of glucose homeostasis, insulin secretion and sensitivity/resistance (IR), using conventional surrogate indices derived from the OGTT. The collected data in NTD- β Thal patients were compared to 20 healthy subjects. *Results:* Seventeen of 26 (65.3 %) NTD- β Thal patients (aged: 7.8 -35.1 years) had normal glucose tolerance, 1/26 (3.8 %) had impaired fasting glucose (IFG), 5/26 (19.2 %) impaired glucose tolerance (IGT), 1/26 (3.8%) IFG plus IGT and 2/26 (7.6%) plasma glucose (PG) level ≥155 mg/dL 1-h after glucose load. GD was observed exclusively in young adult patients; none of them had diabetes mellitus (DM). These findings were associated with a low insulinogenic index (IGI) and oral disposition index. HOMA-IR and QUICKI were not significantly different compared to controls. Interestingly, in young adult patients, ISI-Matsuda index was statistically higher compared to the control group, suggesting an increased insulin sensitivity. *Conclusions:* This study reported a high prevalence of GD in young adults with NTD- β Thal. The documented reduction of IGI rather than the presence of IR, indicates reduced insulin secretory capacity as the pathophysiological basis of dysglycemia that may represent a novel investigational path for future studies on the mechanism(s) responsible for GD in NTD- β Thal patients. (www.actabiomedica.it)

Key words: non–transfusion-dependent thalassemia (NTDT), oral glucose tolerance test (OGTT), insulin secretion, insulin sensitivity/resistance

Introduction

Non–transfusion-dependent thalassemia (NTDT), also known as thalassemia intermedia (TI) encompasses three clinically distinct forms: β-thalassemia intermedia (NTD-β-Thal), hemoglobin E/β-thalassemia (mild and moderate forms), and α-thalassemia intermedia (hemoglobin H disease) (1).

The main drivers underlying the pathophysiology of NTDT are chronic anaemia, due mainly to ineffective erythropoiesis and chronic hemolysis from peripheral destruction of red blood cells leading to marrow expansion, extramedullary hematopoiesis and iron overload (IOL) due to increased intestinal iron absorption caused by chronic anemia (1,2).

Diagnosis and management of these patients is particularly challenging for clinicians because they can present with a wide variety of clinical manifestations and complications which vary in severity. Therefore, an accurate differentiation from transfusion-dependent thalassemia (TDT) is essential for developing an appropriate management plan for each patient, which may be complex at presentation (1,2) and should be tailored to the patient's genotype severity, as well as to clinical characteristics (such as, severity of chronic anemia, reduced growth/year, presence of skeletal deformities and progressive splenomegaly). Usually, patients with a good proportion of HbA maintaina hemoglobin (Hb) level between 7 and 10 g/dL. They may require occasional transfusions (during pregnancy, intercurrent illnesses, surgery) or even frequent transfusions in later life to prevent or treat late complications (such as pulmonary hypertension and extensive medullary expansion) (1-3).

The accumulation of iron from intestinal absorption in NTDT patients is slower than that observed in TDT. Liver iron concentration (LIC) is the gold standard for estimating iron load and can accurately predict total body iron (1,2). The TIF recommends starting iron chelation therapy (ICT) in patients with NTDT aged ≥10 years if LIC is ≥ 5 mg Fe/g dry weight (d.w.) and to discontinue the treatment when the level is < 3 mg Fe/g d.w. (3). If LIC measurement is inaccessible, iron chelation therapy should be initiated at ferritin level (SF) ≥ 800 ng/mL and interrupted at <300 ng/mL, to avoid over-chelation (3).

The prevalence of endocrine complications is lower than that in TDT patients and varies greatly with time, depending on the number of subjects who have been investigated and the design of the study, the number of blood transfusions, the administration of iron chelation therapy (ICT) and the iron absorption from the gastrointestinal tract; the latter is heavily influenced by the severity of ineffective erythropoiesis and chronic anemia (4-14).

The clinical presentation of glucose dysregulation (GD) encompasses a wide spectrum, ranging from impaired glucose tolerance (IGT) to overt diabetes mellitus (DM). The prevalence of GD varies between different study groups (5-14), is more common in TDT than in NTDT (31.0 % vs. 12.1%) and usually manifests after the second or third decade of life (12). Risk factors for IGT in NTDT patients are older age (≥ 24 years) and SF concentration ≥ 2,500 ng/mL (9).

The main data available in literature on glucose homeostasis in patients with NTD-β-Thal have been focused on the prevalence of GD in different countries (4-14). For a better understanding of pathophysiological changes of various indices of insulin secretion, sensitivity/resistance, obtained from dynamic testing, we undertook a retrospective study in 26 patients with NTD- β Thal and compared the results with a group of healthy peer subjects.

Patients and methods

a. Study design

We retrieved data of all NTD-β-Thal patients consecutively referred for an endocrine evaluation to a single Italian Outpatient Clinic experienced in Endocrinopathies of Thalassemias from October 2010 to April 2023 totalling 26, (10 children and 16 young adults). Additionally, 8 of the16 young adult patients with endocrine complications were followed, every 12-18 months, after the first consultation.

This retrospective study was mainly focused on glucose homeostasis, insulin secretion, sensitivity/resistance during 3-h OGTT on the year of reference (baseline). For each patient, we collected at baseline the following data: age, gender, mean hemoglobin

level during the previous 12 months, height, weight, frequency of blood transfusions in previous years, past history of relevant endocrine disorders, family history of diabetes, systemic treatment, if any, and history of splenectomy.

b. Oral Glucose Tolerance Test (OGTT)

All participants underwent a standard 3-h OGTT (75 g of glucose in 250–300 mL water) after an overnight fast (8-10 h). Blood samples were collected at 0, 0.5-h, 1-h, 2-h and 3-h post-glucose load for the determination of plasma glucose (PG) and insulin levels.

Glucose tolerance was classified in accordance with the American Diabetes Association criteria (15). A fasting plasma glucose (FPG) of less than 100 mg/dL and 2-h glucose of less than 140 mg/dL indicated normal glucose tolerance (NGT). Impaired fasting glucose (IFG) was diagnosed in the presence of FPG levels between 100 and 126 mg/dL. IGT was defined by 2-h PG between 140–200 mg/dL, with a FPG <126 mg/dL. DM was defined by FPG ≥ 126 mg/dL or 2-h PG ≥ 200 mg/dL during an OGTT.

Additional data assessed were: (a) PG levels ≥155 mg/dL at 1-h (H-NGT) (16); (b) 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 (17) and (c) the presence of hypoglycemia ≤ 55 mg/dL at any time during the OGTT with or without symptoms of hypoglycemia (18).

c. Measurement of insulin secretion and determination of insulin resistance status

Early-phase insulin secretion, an index of β -cell function, was estimated by the insulinogenic index (IGI) calculated from the ratio of increments of serum insulin to glucose measured at 30 min by the follows: ΔI30/ΔG30=(Ins30-Ins0)/(Glu30-Glu0) (19).

Insulin resistance was estimated by homeostasis model assessment of insulin resistance (HOMA-IR) (20), Quantitative Insulin sensitivity Check Index (QUICKI) (21), and insulin sensitivity index-Matsuda (ISI-Matsuda), which encompasses both hepatic and peripheral tissue insulin sensitivity (20, 22).

Patients were considered to have IR in the presence of one or more of the following cut-off limits: HOMA-IR (75 thpercentile) > 2.78, QUICKI (25th percentile) < 0.3 and ISI-Matsuda (25th percentile) < 4.31 (23). These references refer to 130 selected Polish subjects, aged 18–31 years, with FPG ≤ 100 mg/dL, BMI <25 kg/m^2, and without metabolic syndrome (23).

d. Assessment of β-cell compensation

The oral disposition index (oDI) was calculated as the product of the IGI and ISI-Matsuda index. This index reflects the relationship between β-cell function (early-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 GD (24,25)

The collected data in 26 NTD- β Thal patients were compared to 20 healthy subjects of comparable age reported in our previous studies (26,27). No control subject had β-thalassemia trait or was overweight/obese. Body mass index (BMI) was calculated by dividing the weight (kg) by the square of the height (m^2). Patients were classified according to BMI as underweight (BMI < 18.5 kg/m^2), normal weight (BMI 18.5–24.9 kg/m^2), overweight (BMI 25–29.9 kg/m^2), or obese (BMI ≥ 30 kg/m^2), and patients ≤ 20 years old were classified according to BMI Z-score, using the WHO and CDC growth charts for children from birth to 20 years (Available online: https://www.cdc.gov/ nccdphp/ dnpa/ growthcharts/resources/ growth chart.pdf; accessed on 1 February 2023). Overweight and obesity for children and adolescents aged 5–19 was defined as follows: overweight was defined as weight-for-height greater than 1 standard deviation above the growth reference standard median and obesity was defined as weight-for-height greater than 2 standard deviations above the growth reference standard.

e. Analytical assays

Plasma glucose was measured using an automated glucose oxidase reaction (Glucose Analyser, Ames). Plasma insulin levels were determined by a commercial

immunoassay technique (Diagnostic Products Corporation, Los Angeles, CA).

The level of serum alanine aminotransferase (ALT) was determined by an automated analyzer (normal range 0–40 mU/L). SF was measured periodically (2-4 times per year) by immunoassay in the different referring Centers and the reported values are the mean values registered in the year preceding the consultation. The 90th percentile of reported normal values in females and males are 201 and 243 ng/ mL, respectively (28).

Statistical analysis

All numeric variables were expressed as mean, ± standard deviation (SD). Different variables in the two groups were compared using unpaired student t-test and Mann-Whitney test for normal and non-parametric variables, respectively. Continuous variables were also compared using a one-way analysis of variance (ANOVA). Chi-square ($\chi 2$) test was used to compare the frequency of qualitative variables among the different groups. Pearson's and Spearman's correlation tests (2-tailed) were used to study correlations between variables with parametric and non-parametric distributions, respectively. A P-value < 0.05 was considered statistically significant. For the statistical analysis, a software program was used and validated, according to Alder and Roesser (29).

Ethics

All participants gave informed consent in accordance with principles of the Declaration of Helsinki and its later amendments in October 2013 (www.wma.net) after a detailed explanation of the procedures for performing the OGTT test, the nature and purpose of the study, and the patient's benefits for collecting such information. Ethics approval was not required because the OGTT is considered to be part of optimal diagnostic procedures according to current guidelines (3), no identifiable private information was collected, and an anonymized dataset was analyzed (30).

Results

a. Clinical characteristics of NTD- β Thal patients: main results

The retrospective, non-interventional, study group consisted of 26 NTD- β Thal patients; 15 (57.6%) were males and 11 (42.3%) were females. At baseline, the mean age of patients was 22.1 ± 8.4 years (range 7.7–35.8 years) and the mean hemoglobin level was 8.1 ± 0.7 g/dL (range 7.1– 9.5 g/dL). Eleven (42.3%) patients were splenectomized. Regarding patient treatment, 5/26 (19.3 %) had never been transfused, 18 (69.2 %) had been occasionally transfused, and 3 (11.5%) adult patients (mean age: 33.7 years) had been transfused fairly often (6-8 times/year).

Eighteen patients (64.2 %) were on chelation therapy [desferrioxamine (DFO), deferiprone (DFP), deferasirox (DFX) or DFP in combination with DFO]; 3 (11.5 %) patients were on treatment with hydroxyurea. Compliance to iron chelation therapy was graded arbitrarily as: high (> 90%), moderate (51-90%), poor (1% -50%) or non-compliant (0%). Compliance was moderate in 12 patients (63.1%) and poor in 7 patients (26.9%). Eight of 26 patients (30.7%) had a history of associated endocrinopathies (Table 1).

NTD- β Thal patients were divided in 2 groups: children and adolescents (10 patients; aged: 7.8-15.4 years) and young adults (16 patients; aged:19.6-35.8 years). Three children and adolescents and 1 young adult were mildly underweight. None was overweight.

b. Analysis of glucose levels and insulin secretion during OGTT

In the entire study population 17/26 (65.3 %) patients had NGT. GD was observed exclusively in young adult NTD-β Thal patients (Table1). The youngest was a female 24.8 years old (Table1).

Asymptomatic hypoglycemia was documented at 180 minutes after glucose load in one prepubertal boy (12.11 years; PG: 43 mg/dL) and in a young man (30.4 years; PG: 40 mg/dL) (Table 1).

Table 1. Baseline clinical laboratory and diagnostic data in 10 children and adolescents and 16 young adult NTD- β Thal patients. Data are expressed as mean ± SD, percentage and range.

Variables	Children and adolescents NTD- β Thal patients	Young adult NTD- β Thal patients
Number of patients	10	16
Age (yrs), range	13.1 ± 2.47 7.8-15.4	27.7 ± 5.2 19.6-35.11
Males/females	8/2	7/9
Mean Hb level in the last year (g/dL), range	8.4 ± 0.57 7.1-8.9	7.9 ± 0.80 6.2 **(*)** - 9.5
Number of splenectomized patients	2	9
Assessment of Body Mass Index	3 mildly underweight. None overweight	1 mildly underweight. None overweight
Iron chelation therapy **Treatment with hydroxyurea**	3 1	16 2
Serum ferritin (ng/mL), mean and range values reported in the year preceding the consultation	663.2 ± 162.5 457- 1,010	1,314 ± 855.1 548 -3,200
ALT (normal values: < 40 IU/L)	29.6 ± 13.5	38.9 ± 11.7
Assessment OGTT: **1. Isolated IFG (n and %)** **2. H-NGT (n and %)** **3. IGT (n and %)** **4. IFG plus IGT (n and %)** **5. INDET (n and %)** **6. Diabetes mellitus (n and %)** **Hypoglycemia (3-h after glucose load)** **History of endocrine complications:** • **DP (n and %)** • **Acquired HH (n and %)** • **Secondary amenorrhea (n and %)** • **Other endocrine complications**	- 0 0 0 0 0 0 1 (10%) == 1/4 (25 %) **(**)** - 0 0	- 1 (6.2 %) 2 (12.5%) 5 (31.2 %) 1 (6.2 %) 0 0 1 (6.2%) = 4 (25 %) 1 (6.2 %) 2 (12.5%) 0

Abbreviations: Hb: hemoglobin; (*): due to hypersplemism; ALT: alanine aminotransferase; IFG: impaired fasting glucose; IGT: impaired glucose tolerance; H-NGT: PG levels ≥155 mg/dL at 1-h; INDET: indeterminate glucose tolerance; DP: delayed puberty (** ≥ 13 years in females and ≥14 years in males); Acquired HH: hypogonadotropic hypogonadism.

In the 9 adult β-TI patients with GD, LIC was assessed by MRI. The mean value was: 3.9 ± 2.6 mg Fe/g d.w. (range:1.2- 9.4 mg Fe/g d.w.).

A detailed description of PG and insulin levels registered during OGTT in the two groups of NTDT-β Thal patients, compared to a group of healthy peers of comparable age, is summarized in tables 2 and 3. In young adult NTDT- β Thal patients, PG level at 1-h and 2-h after oral glucose load and the insulin secretion during the first 60 min of OGTT were significantly lower compared to controls, as was the insulin peak after the glucose load. These findings were associated with low IGI and oDI indices (Tables 2 and 3).

c. OGTT-derived indices of insulin sensitivity/resistance and β-cell compensation

The surrogate indices for insulin sensitivity/resistance (HOMA-IR and QUICKI) were not different compared to controls and references reported in the current scientific literature (23,24). A detailed presentation of derived indices of insulin secretion,

Table 2. Oral glucose tolerance test and derived indices of insulin sensitivity/resistance, secretion and β-cell function in children and adolescent NTD- β Thal patients versus controls.

Variables	Children and adolescents NTD- β Thal patients (n.10)	Controls (n.10)	P-value
Fasting plasma glucose (mg/dL)	79.0 ± 7.3	83.8 ± 11.0	0.26
30 min glucose (mg/dL)	112.7 ± 28.5	115.9 ± 20.5	0.77
1-h glucose (mg/dL)	109.6 ± 28.0	101.1 ± 26.0	0.49
2-h glucose (mg/dL)	84.6 ± 22.3	83.5 ±17.0	0.90
3-h glucose (mg/dL)	69.7 ± 17.4	85.6 ± 17.1	0.054
Fasting insulin (μU/mL)	6.2 ± 5.2	6.1 ± 1.9	0.95
30 min insulin (μU/mL)	24.8 ± 18.8	34.9 ± 17.1	0.99
1- h insulin (μU/mL)	23.6 ± 16.9	28.2 ± 12.8	0.50
2 -h insulin (μU/mL)	12.5 ± 10.6	19.3 ± 8.2	0.12
3- h insulin (μU/mL)	8.2 ± 8.9	10.7 ± 9.2	0.56
Insulin- peak (μU/mL)	33.2 ± 18.9	40.2 ± 13.0	0.34
Insulinogenic index (IGI)	0.76 ± 0.65	1.0 ± 0.91	0.50
HOMA-IR	1.29 ± 1.17	1.25 ± 0.44	0.92
QUICKI	0.39 ± 0.05	0.37 ± 0.02	0.25
ISI-Matsuda	17.3 ± 13.0	9.6 ± 2.8	0.08
Oral Disposition Index (oDI)	9.0 ± 5.55	9.44 ± 7.56	0.89

Table 3. Oral glucose tolerance test and derived indices of insulin sensitivity/resistance, secretion and β-cell function in young adult NTD- β Thal patients versus controls.

Variables	Young adult NTD- β Thal patients (n.16)	Controls (n.10)	P-value
Fasting plasma glucose (mg/dL)	84.0 ± 14.9	86.4 ± 7.2	0.64
30 min glucose (mg/dL)	125.6 ± 27.5	118.8 ± 18.8	0.49
1-h glucose (mg/dL)	139.1 ± 32.8	95.4 ± 18.0	**0.0008**
2-h glucose (mg/dL)	122.6 ± 26.8	84.6 ± 18.0	**0.0006**
3-h glucose (mg/dL)	94.3 ± 28.0	77.4 ± 14.4	0.09
Fasting insulin (μU/mL)	3.5 ± 2.5	7.0 ± 3.0	**0.0037**
30 min insulin (μU/mL)	19.4 ± 13.9	46.2 ± 25.3	**0.0018**
1- h insulin (μU/mL)	20.8 ± 14.7	37.0 ± 17.6	**0.018**
2 -h insulin (μU/mL)	18.3 ± 11.6	24.1 ± 12.2	0.23
3- h insulin (μU/mL)	8.4 ± 6.7	10.7 ± 7.7	0.42
Insulin- peak (μU/mL)	26.7 ± 15.1	52.9 ± 26.6	**0.0037**
Insulinogenic index (IGI)	0.36 ± 0.21	1.54 ± 0.99	**0.0001**
HOMA-IR	0.79 ± 0.62	1.16 ± 0.76	0.18
QUICKI	0.42 ± 0.04	0.39± 0.05	0.10
ISI-Matsuda	16.8 ± 8.8	8.71 ± 2.85	**0.010**
Oral Disposition Index (oDI)	6.4 ± 5.2	12.11 ± 6.55	**0.021**

sensitivity/resistance, and β-cell function in comparison to 20 health control subjects is reported in tables 2 and 3.

Interestingly, in the young adult patients, ISI-Matsuda index was statistically higher compared to the control group despite having lower IGI and oDI indices (Table 3).

d. Significant correlations

Correlation analysis between the collected variables including age, Hb, ALT, SF, PG and insulin during OGTT, indices of insulin secretion, sensitivity/resistance and β-cell function was performed in the whole group of NTDT- β Thal patients. No

Table 4. Correlation between different variables in 26 NTD- β Thal patients.

Variables	Age	PG 1-h during OGTT	QUICKI	ISI-Matsuda
Hb	**N.S.**	**N.S.**	**R= - 0.4369; P:0.025**	**N.S.**
Serum ferritin	**R= 0.3891; P: 0.049**	**R= 0.437; P: 0.0023**	N.S.	N.S.
PG at baseline	N.S.	N.S.	**R=- 0.6494; P:0.0003**	N.S.
PG at 2-h during OGTT	**R= 0.6346; P: 0.0004**	**R=0.587; P: 0.0016**	N.S.	N.S.
Insulin peak during OGTT	N.S.	N.S.	**R= - 0.4428; P:0.023**	N.S.
Insulinogenic index (IGI)	N.S.	**R= -0.3866; P: 0.055**	N.S.	N.S.
HOMA-IR	N.S.	N.S.	**R= -0.908; P: < 0.00001.**	**R= -0.6681; P: 0.0001**
ISI-Matsuda	N.S.	N.S.	**R= 0.7754; P: < 0 .00001**	-
Oral Disposition Index (oDI)	N.S.	**R= -0.5356; P: 0.0048**	N.S.	N.S.

Abbreviations: Hb: hemoglobin; PG: plasma glucose; OGTT: oral glucose tolerance test; HOMA-IR: homeostasis model assessment of insulin resistance; QUICKI; Quantitative Insulin sensitivity Check Index; ISI-Matsuda: Insulin Sensitivity Index-Matsuda.

correlations were found between ALT levels and glucose homeostasis and insulin secretion. The significant correlations found in the study are reported in Table 4. Of note, SF correlated with the patient's age and PG 1-h value assessed during OGTT.

Discussion

OGTT assesses an individual's ability to clear circulating glucose after glucose load. It is widely used in clinics to diagnose impaired glucose tolerance (IGT) and/or type 2 diabetes mellitus (T2D), despite the notorious poor reproducibility of the test (31). Nevertheless, β-cell function estimated using OGTT presents a more physiological pattern of glucose, insulin and incretin changes compared to the hyperglycemic clamp or an intravenous glucose tolerance test (32). The prevalence and underlying pathology of glucose homeostasis was studied in 211 NTDT Chinese patients aged 4–63 years (79 β -TI patients, 114 Hb H disease patients and 18 Hb E/β thalassemia patients). OGTT was performed on all patients, according to the diabetes diagnostic criteria of the World Health Organization (33), using a 2-h OGTT including 3 sample points (0, 30 min and 2-h). 149 NTDT patients had NGT (70.6%), 25 (11.84%) had IGT, 4 (1.89%) had DM and 33 (15.6%) had hypoglycemia (9). None had IFG.

The results of the Chinese study showed that in patients with SF ≥ 2,500 ng/mL or LIC ≥15 mg Fe/g d.w., the prevalence of hyperglycemia was significantly higher than in patients with SF < 300 ng/mL or LIC <3 mg Fe/g d.w. Moreover, during OGTT, a significant correlation was observed between 2-h PG levels and age, HOMA-IR index, insulin sensitivity index, total insulin secretion area under the curve, LIC and ALT levels (9). The authors attributed the GD to excessive iron deposition in the pancreas, leading to decreased pancreatic β-cell secretion, and excessive iron deposition in the liver and muscles with impaired insulin-mediated glucose uptake and peripheral utilization (9).

In our present study we used an extended 3-h OGTT, with 8 sample points, in 26 exclusively NTDT-β Thal patients aged 7.8 -35.11 years to assess PG levels, insulin secretion and insulin sensitivity/resistance.

The mean Hb level at baseline was: 8.0 ± 1.0 g/dL (range: 6.2-9.3 g/dL) and the mean SF was:1,387.4 ± 899.9 (range:600-3,200 ng/mL).

The first finding was a high prevalence of GD in 9/16 (56.2%) young adult patients. None of the children or adolescents manifested changes of glucose homeostasis and insulin secretion after oral glucose load. The mean LIC level in patients with GD was: 3.9 ± 2.6 mg Fe/g d.w. (range: 1.2- 9.4 mg Fe/g d.w.). The higher prevalence of GD compared to other studies could be partially attributed to the inclusion of the additional diagnostic sensitive index H-NGT (PG levels ≥155 mg/dL at 1-h) for the assessment of glucose tolerance (16).

The PG level at 2-h during OGTT was strongly correlated with age (R= 0.6346; P: 0.0004) and PG level at 1-h during OGTT (R= 0.587; P: 0.0016). Moreover, a negative correlation was also observed between PG level at 1-h during OGTT versus IGI (R= -0.3866; P: 0.055) and oDI (R= -0.5356; P: 0.0048).

The second finding was a direct significant correlation between SF and H-NGT (R= 0.437; P: 0.0023). As reported in our previous study, an isolated H-NGT during OGTT could represent an additional sensitive biomarker to detect high-risk patients with chronic liver disease and/or IOL, who need periodic glycemic surveillance (16). In the general population, it has also been reported that patients with NGT and 1-hour blood glucose ≥155 mg/dL during OGTT, are at higher risk of T2D (34).

Of note was one of two young adult male patients (7.69%) with isolated H-NGT (1-hour PG post-load:162 and 172 mg/dL) who manifested asymptomatic hypoglycemia 3-h after OGTT, leading to the hypothesis of an early defect of β-cell and/or of counter-regulatory hormones. This defect may involve deficient glucagon secretion, as documented in a previous study (35) and/or a sympathoadrenal response, requiring further study. The highest prevalence of hypoglycemia (15.6%) using a 2-h OGTT has been reported by Luo et al. (9). Its long-term consequences are not fully defined.

A third point was that many metabolic markers and indices, most of them calculated by fasting and stimulated levels of glucose and insulin, have been used in previous studies to assess β-cell function, insulin sensitivity and resistance. Interestingly, in our NTD- β thal patients, GD was associated with normal HOMA-IR and QUICKI indices and decrease of IGI and oDI. These findings suggest that the progressive deterioration of β-cell function over time is the prominent event in the natural history of glyco-metabolic status in NTD- β Thal patients and that the effects of this event may be only partly counteracted by an increased insulin sensitivity, as documented by a higher ISI-Matsuda index compared to the healthy adult controls (Table 3; R= 0.7754; P: < 0.00001). However, it is noteworthy to remember that ISI-Matsuda index contains the product of glucose and insulin concentrations (fasting and during the OGTT) in the denominator. Therefore, a fall in plasma insulin levels due to β-cell dysfunction will incorrectly increase the calculated sensitivity value if the reduction in insulin is not directly accompanied by an increase in PG levels (36). This may result in overestimation of insulin sensitivity using OGTT-based indices and may be a confusing factor for the analysis of results. Therefore, caution is required for the interpretation of OGTT-derived insulin sensitivity values in clinical research.

Finally, a negative correlation was observed between Hb level and QUICKI index (R= - 0.4369; P:0.025); the latter index was negatively correlated with the basal PG level (R=- 0.6494; P:0.0003) and insulin peak level during OGTT (R= - 0.4428; P:0.023). Little is known about the reduction of red blood cells, hemoglobin and hematocrit in relation to glucose metabolism (37). Therefore, understanding the relationship between Hb and glucose homeostasis, independent of iron status, could be beneficial in identifying patients at risk for GD.

In a study of fifty children and adolescents with β-thalassemia/HbE disease, regularly transfused with packed red cells, ISI-Matsuda index and HOMA-IR tended to increase shortly after acute blood transfusion, although not reaching statistical significance (38). Therefore, in the absence of any new high-quality evidence, the current guidelines approach on the transfusion practice for NTDT still stand. Prospective trials are necessary to validate the current recommendations or to evaluate possible alternatives.

The major weaknesses of the current study are the small sample of recruited NTD- β Thal patients, with variability in the severity of clinical phenotype

and probably genotype, the diagnosis of GD was performed by a single OGTT, the lack of longitudinal assessment of glucose tolerance and inefficient compliance to ICT in some patients. Prospective studies are required to further corroborate our findings and to evaluate their implications for the clinical course of glucose homeostasis. Molecular characterization of NTD β-Thal genotypes is also needed.

In conclusion, young adults with NTDT- β Thal had significantly lower insulin levels at various time points following glucose load in comparison to controls, suggesting diminished insulin secretory capacity as the main cause of GD in NTDT patients. The documented reduction of IGI rather than the presence of IR may represent a novel investigational path for future studies on the mechanism(s) responsible for GD in NTDT- β Thal patients.

Conflict of Interest statement: Each author declares that he or she has no commercial associations (e.g. consultancies, stock ownership, equity interest, patent/licensing arrangement etc.) that might pose a conflict of interest in connection with the submitted article.

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