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Original article

A study of isolated hyperglycemia (blood glucose ≥155 mg/dL) at 1-hour of oral glucose tolerance test (OGTT) in patients with β-transfusion dependent thalassemia (β-TDT) followed for 12 years

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Abstract. *Objective:* Subjects with normal glucose tolerance (NGT) but 1-hour post-load plasma glucose (1-h OGTT) ≥ 155 mg/dl (8.6 mmol/L; H-NGT) have an increased risk for developing Type 2 diabetes mellitus (T2DM), determining a new risk factor category with deeper metabolic impairment. The aim of this study was to evaluate the H-NGT as a diagnostic predictor of future dysglycemia in β-transfusion dependent thalassemia (β-TDT). Indices of insulin secretion and insulin sensitivity derived at baseline from OGTTs, were also reviewed. *Study design and methods:* OGTT and indices of insulin secretion and insulin sensitivity, derived at baseline during OGTT, in 17 β-TDT with H-NGT and 29 β-TDT with normal OGTT (NGT) and without H-NGT followed for 12 years were studied. *Results:* H-NGT was associated with decreased insulin sensitivity and progressive deterioration of glucose tolerance. At baseline, serum ferritin and serum alanine aminotransferase (ALT) levels were higher in patients with H-NGT compared to patients with NGT. A strong correlation was observed between ALT and 1-hour plasma glucose value during OGTT in the total group of 36 patients . Compliance to iron chelation therapy was poor in β-TDT patients with H-NGT. An inverse correlation was found between 1-hour plasma glucose value during OGTT and insulin secretion-sensitivity index-2 (ISSI-2) (r: -0.3298; p: 0.025), between ISSI-2 and ALT (r: -0.3262; p: 0.027), and between 1-hour plasma glucose value and ISSI-2 (r: -0.537; p: 0.005) in the whole group of β-TDT patients enrolled in our study. *Conclusions:* It is of paramount importance to screen early β-TDT patients at increased risk for glucose dysregulation. This retrospective study displayed that finding an isolated high 1-hour post-load glucose level (≥155 mg/dL; H-NGT) during the OGTT may serve as a simple biomarker to detect high-risk patients, with chronic liver disease and/or iron overload, who need periodic glycemic surveillance. Measuring the ISSI 2 represented another valuable predictive marker in the assessment of glycemia in these patients.

Keywords: β-transfusion dependent thalassemia, oral glucose tolerance test, 1-Hr plasma glucose. Prediabetes, diabetes.

Introduction

The hemoglobinopathies (mainly thalassemias and sickle-cell disease) are the most frequent inherited genetic disorders worldwide, with some 300,000 infants born annually with major hemoglobinopathies. Approximately 80% of these births occur in low- and middle-income countries where the control and management of hemoglobinpathies is extremely poor (1).

At present, thalassemias are classified into transfusion-dependent thalassemia (TDT) and non-transfusion-dependent thalassemia (NTDT) based on severity of clinical phenotype, and evaluation of whether they require regular blood transfusions to survive or not (2).

The recommended treatment of β-TDT consists of regular transfusions (every 2-4 weeks) and iron chelation therapy to remove the excess iron accumulation resulting from transfusions. Iron overload constitutes the most important complication of transfusions in β-TDT, and is thus the major focus of clinical management. The predominant mechanisms driving the process of iron loading include increased iron burden secondary to transfusion therapy in TDT and enhanced intestinal absorption secondary to ineffective erythropoiesis and hepcidin suppression in NTDT (3).

Apart from iron overload, other factors responsible for organ damage include chronic hypoxia due to anemia, that may potentiate the toxicity of iron deposition in endocrine glands and other organs. In addition, viral infections, as well as individual susceptibility to iron overload, have been implicated in causing endocrine dysfunctions (4).

Glucose tolerance abnormalities and diabetes mellitus (DM) are common complications in β-TDT patients. In these patients, pancreatic iron loading begins after the first decade of life and the incidence and severity increases with age. Glucose intolerance frequently starts during adolescence, while diabetes mellitus (DM) develops later in life, usually related to iron overload of pancreas and liver as well as chronic liver disease (5-8). Elevated serum ferritin (SF) concentrations and hepatitis C infection have long been considered as important factors for the development of abnormal glucose tolerance in β-TM patients (5, 8).

Because of the insidious onset of glucose abnormalities, the current standard of care guidelines recommend annual screening of glucose homeostasis in patients with β- TDT, using the 2-h oral glucose tolerance test (OGTT) starting from age 10 (9,10). Subjects undergoing OGTT are categorized as having impaired glucose tolerance (IGT) for 2-hour glucose 140-199 mg/dL (7.8–11.0 mmol/L). DM is diagnosed by fasting glycemia ≥126 mg/dL (7.0 mmol/L.) on two separate days, or plasma glucose ≥200 mg/dL (11.1 mmol/L) two hours after a 75-g oral glucose load.

Impaired fasting glucose (IFG) and impaired glucose tolerance (IGT) are the intermediate metabolic states between normal and diabetic glucose homeostasis. These conditions are thought to be the precursors of diabetes mellitus (DM), but the progression to overt disease is not straightforward.

The prevalence of IFG in β-TM is higher in patients with SF > 2500 ng/mL, persistant serum alanine aminotransferase (ALT) levels > 50 IU/L and cardiac MRI T2* <20 ms (11). Moreover, a pancreatic T2* < 13.07 ms has been reported as a predictor of an abnormal OGTT (12).

Recent studies suggest that plasma glucose (PG) levels ≥155 mg/dL at 1 h (8.6 mmol/L;H-NGT) during an OGTT can help to distinguish those with reduced β-cell function prior to progression to prediabetes and diabetes among individuals with normal OGTT (NGT) (13-15).

Based on these observations, we performed a 12- year retrospective study to assess the validity of isolated 1-hour OGTT as a diagnostic predictor of dysglycemia among NGT subjects with β-TDT. Indices of insulin secretion and insulin sensitivity, derived at baseline from OGTTs, are also reported.

Patients and Methods

a) Setting, research design, and definitions

This retrospective study included 170 β-TDT patients followed annually or bi-annually, by the same physician (VDS), at the Pediatric and Adolescent Outpatient Clinic of Ferrara from January 1984 to September 2010. The study was completed by the same physician at the end of June 2020 at the Quisisana Pediatric and Adolescent Outpatient Clinic of Ferrara.

Inclusion criteria were: a) a confirmed hematologically and molecularly diagnsis of β-TDT; b) age above 10 years; c) patients with isolated H-NGT (defined as high 1 hour plasma glucose and otherwise normal fasting and 2 hour glucose values on OGTT); d) annual assessment of OGTT, during the follow-up (not less than 5 years).

β-TDT patients with normal OGTT, according to the American Diabetes Association (ADA) criteria (16), followed annually with an OGTT during the same follow-up period, served as controls.

Exclusion criteria were: a) patients with NTDT; b) patients with acute illness in the previous month of OGTT; c) renal insufficiency; d) bone marrow transplanted patients; e) HIV positivity; f) patients taking medications affecting glucose tolerance, and g) patients with incomplete data.

The following data were collected for each patient: demographic data, age at first transfusion, interval between transfusions, weight, height, body mass index (BMI), type of iron chelation therapy and compliance to treatment, associated endocrine complications.

BMI was calculated as body weight in kilograms divided by height in meters squared. A subject was considered obese when BMI exceeded 30 Kg/m ^{2}, overweight when BMI was 25 - 30 kg/m^2, of normal weight when BMI was 18.5-25 kg/m^2, and underweight when the BMI was < 18.5 kg/m^2.

Self-reported questionnaires (SRQs), patients or care providers' interviews and random urinary iron excretion were used to assess the degree of compliance with chelation therapy as high (> 90%), moderate (51-90%), poor (1% -50%) or non-compliant (0%) (17).

The level of serum alanine aminotransferase (ALT) was determined by an automated analyzer

(normal range 0–40 mU/L). HCV antibodies had been tested annually since 1991. SF was measured by immunoassays. The 90th percentile of reported normal values is 201-243: ng/mL (18).

b) Glucose tolerance at baseline and during the annual follow-up

The OGTT (1.75 g/kg, max 75 g) was performed in the morning after an overnight fast. Blood samples were collected from the venous catheter at 0, 30, 60, 90, 120 and 180 minutes for plasma glucose and insulin measurements. Plasma glucose was measured using an automated glucose oxidase reaction (Glucose Analyser, Ames). Plasma insulin was determined by a commercial solid phase radioimmunoassay technique (Coat-A-Count insulin kit, Diagnostic Products Corporation, Los Angeles, CA) with intra- and inter-assay coefficients of variance of 3.3% and 2.5%, respectively.

c) Calculations of insulin secretion and sensitivity indices

Various indices of insulin secretion and sensitivity were calculated, including: Insulinogenic index (IGI), Homeostasis Model Assessment of Insulin Resistance (HOMA1-IR), Quantitative Insulin sensitivity ChecK Index (QUICKI), which is a reciprocal logarithmic transformation of the HOMA-IR, Matsuda insulin sensitivity index, and Insulin Secretion-Sensitivity Index-2 (ISSI-2) (19-21).

The insulinogenic index was calculated as the incremental change in insulin concentration during the first 30 min of the OGTT divided by the incremental change in glucose during the same time period (22).

d) Statistical analysis

Data are presented as means ± standard deviation (SD). Statistical comparison between parameters

was made using the paired "t" test. Simple linear regression tested the correlations between variables. 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.

e) 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. Informed consent was obtained from all patients after detailed explanation of the nature and purpose of

the study and the likely risks and benefits associated with study participation.

Results

a) Clinical characteristics

All β-TDT patients were of Italian ethnic origin. At baseline, 29 β-TDT subjects had NGT and 17 H-NGT (Table 1). The patients' demographic data and other parameters are shown in Table 1. All patients were on regular blood transfusions and iron chelation therapy with desferrioxamine (DFO) monotherapy. None was overweight or obese. A BMI < 18.5 kg/m² was present in 4 patients with NGT (2 males and 2 females).

All patients were transfused every 2–3 weeks. At baseline, their mean annual pre-transfusional Hb level was 8.7 g/dL. No statistical difference was observed between the two selected groups of patients (Table 1).

Thirty-six patients (78.2%) had undergone splenectomy because of increased transfusion requirements of packed red cells (> 200 ml/kg/year) and/or for the presence of other signs of hypersplenism such as leukopenia, thrombocytopenia, and/or splenomegaly.

Based on transfusional iron input and serum ferritin level all β-TDT patients were on treatment with DFO (25-55 mg/kg body weight) given subcutaneously by pump for 7 to 8 hours per night, for 5 to 6 days a week (Table 1). Ascorbic acid was added orally at a dose of 2–5 mg/kg (maximum dose 200 mg) in a selected group of patients.

The mean SF level at baseline was significantly higher in the H-NGT group versus the NGT group. A SF level > 1,000 ng/mL was present in 15/17 β-TDT patients with H-NGT (88.2%) and 18/29 β-TDT patients with NGT (62.0%). The difference and the compliance to iron chelation therapy between the two groups, expressed in %, was statistically significant (Table 1).

The mean level of ALT was consistently higher in patients with H-NGT compared to those with NGT

Table 1. Clinical, laboratory data, and treatment compliance in transfusion-dependent β-thalassemia (β-TDT) patients at baseline.

Variables	β-TDT patients with 1-h OGTT ≥ 155 mg/dL (H-NGT)	β-TDT patients with normal OGTT	P value
Number of patients	17	29	
Age (yr)	17.6 ± 4.9	18.1 ± 5.3	NS
Sex (M/F)	7/10	15/14	
Body Mass Index (kg/m2)	21.4 ± 2.6	21.8 ± 1.9	NS
Splenectomy (n and %)	13/17 (76.4)	23/29 (79.3)	NS
Mean pre-transfusion annual Hb level one year before OGTT (g/dL)	8.7 ± 0.2	8.7 ± 0.3	NS
Chelation therapy with DFO (mg/kg b.w. given subcutaneously)	43.0 ± 17.8	33.3 ± 12.1	0.0331
Mean compliance to chelation therapy (%)	67.3 ± 39.5	90.5 ± 20.1	0.0097
Serum ferritin (ng/mL) at baseline **Serum ferritin level > 1,000 ng/ mL**	3009.5 ± 1914.6 15/17 (88.2%)	1507.1 ± 1036.3 18/29 (62.0%)	0.0012 0.0597
ALT (U/L)	77.7 ± 31.4	41.7 ± 22.7	< 0.0001
Fasting glucose (mg/dL) **1 h glucose (mg/dL)** **2 h glucose (mg/dL)**	85.6 ± 8.5 169.4 ± 14.6 112.6 ± 14.3	86.8 ± 9.1 112.9 ± 25.0 106.1 ± 12.0	NS < 0.0001 NS
Fasting insulin (μU/mL) **1 h insulin (μU/mL)** **2 h insulin (μU/mL)**	10.7 ± 2.8 59.7 ± 31.7 27.5 ± 15.6	9.4 ± 4.5 41.6 ± 34.1 27.1 ± 13.9	NS 0.0816 NS

Variables	β-TDT patients with 1-h OGTT ≥ 155 mg/dL (H-NGT)	β-TDT patients with normal OGTT	P value
Insulinogenic index	0.6 ± 0.5	1.1 ± 0.6	NS
HOMA-IR	2.2 ± 0.7	2.0 ± 1.0	NS
QUICKI	0.34±0.02	0.35±0.02	NS
Matsuda IR Index	0.2±0.07	0.2±0.1	NS
ISSI-2	167.6±56.5	210.8±41.6	0.0048

Legend = Normal insulin values (µU/mL) before and during OGTT= 0'**:7 ± 3; **60'**: 37 ± 17.6; **120'**: 24.1 ± 12.2 **µU/mL** (From: De Sanctis et al. Postgrad Med J. 1985; 61: 963-967). **Normal values of Homeostasis Model Assessment of Insulin Resistance (HOMA-IR)**: 2.31 (2.21–2.46); **Quantitative Insulin Sensitive Check (QUICKI) :** 0.34 (0.33–0.34); **Insulin secretion-sensitivity index-2 (ISSI-2):** 304 (290–327); (From: Płaczkowska et al. Ann Agric Environ Med. 2020;27:248-254). **Matsuda IR index:** normal values < **0.33.

(p: < 0.0001) and the ISSI- 2 values were lower in β-TDT patients with H-NGT compared to β-TDT patients with NGT (p:0.0048) (Table 1). Insulinogenic index, HOMA-IR, QUICKI, and Matsuda IR index values were not significantly different between the two groups of patients **(**Table 1).

ISSI-2 values in 15 β-TDT patients who developed dysregulation of glucose homeostasis were lower compared to 12 β-TDT patients with NGT at baseline who developed dysregulation of glucose homeostasis during the follow-up (170.0 ± 59.0 vs. 210.0 ± 36.6; p: 0.029). In this group of patients the 25th percentile of ISSI-2 was 110 and 149, respectively).

b) Correlations

An inverse correlation was found between 1-hour plasma glucose value, during OGTT, and ISSI-2 (r: -0.3298; p: 0.025), between ISSI-2 and ALT (r: -0.3262; p: 0.027) and between 1-hour plasma glucose value and ISSI-2 (r: -0.537; p: 0.005) in the whole group of β-TDT patients. An inverse correlation was found between HOMA-IR and ISSI-2 in patients with H-NGT (r: -0.629; p: 0.006). A significant direct correlation was observed between ALT and HOMA-IR in the whole group of patients (r: 0.4608; p:0.001).

Correlations between age, SF levels, ALT and 1-hour plasma glucose value during OGTT were not statistically significant as well as between SF and insulin secretion and sensitivity markers.

c) Retrospective follow-up

The mean duration of follow-up in the two groups was 7.2 ± 2.1 years in H-NGT and 7.9 ± 1.7 years in NGT (p:NS). The duration range in the two groups was similar in the two groups (from 5 to 12 years) (Table 2).

Most β-TDT patients in the study (34 out of 46) were HCV-ab or HCV-RNA positive. Polymerase chain reaction (PCR) was performed for genotyping HCV-RNA β-TDT patients. Four different HCV genotypes: 1b (32.3 %), 2 (26.4%), 1b/2 (2.9%), and 3a (2.9% %) were identified (Table 2).

During the follow-up, depending on the results of the OGTT, β-TDT patients were classified into different subgroups of glucose tolerance according to the American Diabetes Association (ADA) criteria (16):

- Normal Glucose Tolerance (NGT): Fasting plasma glucose (FPG) < 100 mg/dL and 2-h PG < 140 mg/dL;
- Impaired Fasting Glucose (IFG): FPG between 100 and 125 mg/dL.
- Impaired Glucose Tolerance (IGT) IGT: 2-h PG between 140 and 199 mg/dL.
- Diabetes Mellitus (DM): FPG ≥ 126 mg/dl or 2-h PG ≥ 200 mg/dL.

β-TDT patients with H-NGT developed earlier and more consistently changes of glucose homeostasis compared to those with NGT (p: < 0.0001 and 0.0019, respectively) **(**Table 2, figures 1 and 2).

Table 2. Relevant clinical and laboratory data during follow-up in the two groups of transfusion-dependent β-thalassemia (β-TDT) patients with H-NGT and NGT.

Variables	β-TDT patients with 1-h OGTT ≥ 155 mg/dL (H-NGT)	β-TDT patients with normal OGTT	P value
Number of β-TDT patients	**17**	**29**	-
Duration of follow-up (year) **Range:**	7.2 ± 2.1 (5 -12)	7.9 ± 1.7 (5 - 12)	NS
HCV-ab positive/RNA negative (n) **HCV–RNA positive (n)** **Prevalence of HCV genotypes:** **Genotype 1b** **Genotype 2** **Genotype 1b/2** **Genotype 3**	5/13 (38.4) 8/13 (61.5) 4/8 4/8 0/8 0/8	7/21 (33.3) 14/21 (66.6) 7/21 5/21 1/21 1/21	NS NS - NS NS NS NS
Chelation therapy at last observation: **DFO (n)** **DFP (n)** **DFO + DFP (n)** **DFX (n)**	 10/17 4/17 0/17 3/17	 22/29 4/29 1/29 2/29	 NS NS NS NS
Interval in years (mean ± SD) and range before the first detection of glucose abnormality	5.0 ± 2.5 (1 -11)	7.8 ± 1.7 (5 - 9)	< 0.0001
Number, sex (M/F) and % of patients with glucose abnormalities registered during the follow-up	15/17 7/6 (88.2)	12/29 8/4 (38.6)	0.0019
Hypogonadotropic hypogonadism (n) **Arrested puberty (n)** **Secondary amenorrhea (n)** **Secondary hypogonadotropic hypogonadism (n)** **Total (n and %)**	4/17 3/17 1/17 0/17 8/17 (47.0)	6/29 2/29 1/29 1/29 10/29 (34.4)	NS NS NS NS NS
Subclinical hypothyroidism (n) **Overt hypothyroidism (n)** **Central hypothyroidism (n)**	- 1/17 -	1/29 - 2/29	- - -
Short stature (< 3rd centile) (n and %)	4/17 (23.5)	7/29 (24.1)	NS
Hypoparathyroidism (n)	1/17	0/29	NS
Adrenal insufficiency (n)	0/17	0/29	-

Legend: DFO: *desferrioxamine;* ***DFP:*** *deferiprone;* ***DFX:*** *deferasirox.*

The oral chelator deferiprone (DFP) had been available since 1995; it was given at a dose of 75 mg/kg B.W. to patients over the age of 11 years. In the following years, combined therapy with daily DFP and s-c DFO 3–6 days/week, was given to patients with severe iron overload and high iron input. In 2007, the new oral chelating agent deferasirox (DFX) was introduced at a dose of 25–30 mg/kg B.W. for patients in whom treatment with DFO was contraindicated or inadequate.

At the last observation, 22 patients were on treatment with DFO, 8 were on oral chelator DFP, 5 were on oral chelator DFX, and 1 patient on DFO plus DFP (Table 2).

The development of associated endocrine complications observed in the two groups at the last observation are summarized in table 2. The first and most frequent endocrine complication was hypogonadotropic hypogonadism followed by short stature.

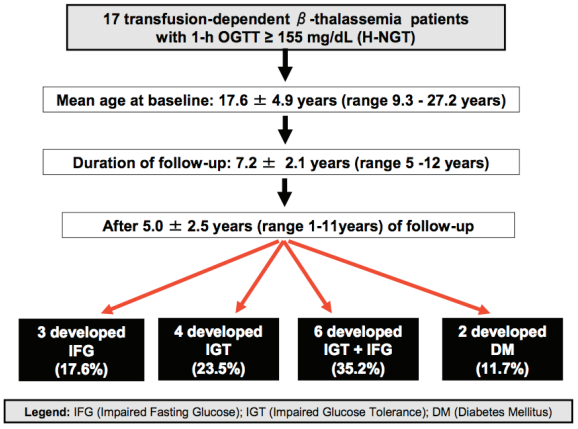

Figure 1. Outcome of 17 β-TDT patients with isolated hyperglycemia (≥155 mg/dL) at 1-hour during oral glucose tolerance test (OGTT).

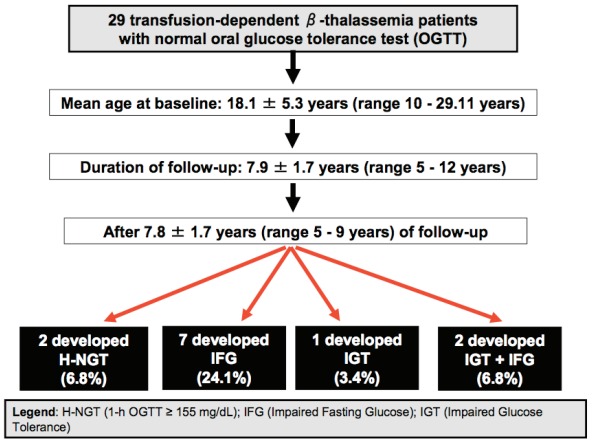

Figure 2. Outcome of 29 β-TDT patients with normal oral glucose tolerance test (OGTT).

Discussion

Prediabetes state (intermediate hyperglycemia) is represented by two glucose abnormalities, the impaired fasting glucose (IFG) and the impaired glucose tolerance (IGT) detected by a standardized 75-gram oral glucose tolerance test (OGTT) (25).

An interesting study pointed out that recognition of IFG among children may be treated as a prelude to occurrence of diabetes type 2 during adolescence (26).

In addition, considerable evidence suggests that a 1-hour post-load plasma glucose value ≥155 mg/dl (8.6 mmol/L) may identify individuals with reduced β-cell function prior to progressing to prediabetes and diabetes, and is also more predictive for those likely to progress to diabetes than HbA_{1c} or 2-hour post-load glucose values (27).

Our β-TDT patients with H-NGT, defined as 1-h-PG >155mg/dL (>8.6mmol/L) but with normal 2-h-PG < 140 mg/dL (<7.8 mmol/L), had a significantly increased risk, compared to those with NGT, to develop prediabetes or DM during the twelve-year follow up period. At baseline, SF and ALT levels were consistently higher in patients with H-NGT compared to β-TDT with NGT. Moreover, compliance to iron chelation therapy was poor in β-TDT patients with H-NGT.

The interplay between liver siderosis and active hepatitis C virus (HCV) infection facilitates the progression to DM, at least in adulthood (28). This potential effect seems to be related to different hepatitis C virus (HCV) genotypes. The specific mechanisms by which HCV infection leads to diabetes are not fully understood, but it seems that an increase of insulin resistance associated with both steatosis and the overproduction of pro-inflammatory cytokines could play a crucial role (29).

Insulin Resistance (IR) defines the state in which the body does not respond to the action of insulin, and, therefore, the normal regulatory mechanisms of glucose are impaired. Indices derived from measurements of fasting plasma glucose and insulin concentrations (HOMA and QUICKI) primarily reflect hepatic insulin resistance.

In our patients, the percentage of hepatitis C virus (HCV) infection in the 2 groups of patients was not different. However, a significant correlation was observed between ALT and HOMA-IR (r: 0.4608; p:0.001), endorsing an important role of hepatic pathology on the development of IR and subsequent deterioration of the glucose dysregulation (IFG, IGT and DM).

ISSI-2 is defined as 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 dysregulation of glucose homeostasis. Substantially, it refers to the

relationship between insulin sensitivity and insulin secretion (21,30).

An inverse correlation was found between 1-hour plasma glucose value during OGTT and ISSI-2 (r: -0.3298; p: 0.025). Moreover, in the whole group of β-TDT patients, significant negative correlations were detected between ISSI-2 and ALT (r: -0.3262; p: 0.027) and between 1-hour plasma glucose value and ISSI-2 (r: -0.537; p: 0.005).

In support of our view, Karadas et al. (31) evaluated the impact of pancreas R2* MRI in combination with ISSI-2 index to identify β-TDT patients at the highest risk for developing glucose dysregulation (GD). These patients showed a significant lower ISSI-2 index (p: < 0.001) as well as the Stumvoll index and Stumvoll first and second phase indices compared to patients with NGT (p: < 0.001). All patients with GD also had a pancreas MR R2* value >50 Hz. The authors suggested that pancreas R2* MRI combined with ISSI-2 index were valuable parameters to identify patients at the highest risk for developing GD.

In summary, based on the hyperbolic relationship between insulin secretion and insulin sensitivity, the lower values of ISSI-2 in H-NGT group support the hypothesis of a lower insulin secretory capacity of βcells to adapt to insulin resistance.

The finding of higher SF in the group with H-NGT (and lower compliance to chelation) who developed more significant dysglycemia versus the NGT (relatively better compliance to chelation) support the important role of iron toxicity in the development of dysglycemia**.** Non-compliance with iron chelation therapy is a big threat to effective treatment and one of the most common problems encountered in clinical practice. The motivation of these patients to comply poorly with chelation therapy was not available. However, in our long-term personal experience with this group of patients, compliance was influenced by several factors, such as age, socio-economic status, lack of family support, concern or fear from side effects, chronicity, severity of the disease, lack of immediate benefit and presence or absence of complications. Furthermore, multiple drug therapy and complex treatments that interfered with daily life were also additional reasons for non-compliance. These findings support the notion that healthcare workers must look beyond the individual when examining non-compliant behaviour and direct attention to the external factors, such as family dynamics and socioeconomic status (32).

The validity of the study is potentially limited by the small sample size recruited from a single centre. Other potential limitations are those of any retrospective study, the absence of a modern evaluation of iron overload (MR of liver and pancreas) and the absence of HbA_{1c} or fructosamine data inclusion at baseline.

In conclusion, this retrospective study covers an unexplored area of research in β-TDT patients.

Identifying patients with isolated 1-hour post-load glucose level during the 75-g OGTT may serve as a simple biomarker to detect high-risk patient population that warrants periodic surveillance.

It would seem reasonable to include a 1 hour post-load glucose measurement in every β-TDT patient when OGTT is performed. A wide variety of techniques are available for assessing IR and they all have their own advantages and limitations. Based on our results, reduced ISSI- 2 value is an additional predictive marker that can be valuable in the calculation of conventional OGTT.

Conflicts of interest: 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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