



Original Article

Can we Predict Incipient Diabetes Mellitus in Patients with Transfusion Dependent β -Thalassemia (β -TDT) Referred with a History of Prediabetes?

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Abstract. Background: Prediabetes and diabetes mellitus (DM) are complications in adult patients with transfusion-dependent β -thalassemia (β -TDT), with their incidence increasing with age. **Objective:** This retrospective observational study describes the glycemc trajectories and evaluates predictive indices of β -cell function and insulin sensitivity/resistance in β -TDT patients with prediabetes, both in a steady state and during 3-h oral glucose tolerance test (OGTT), in order to identify patients at high risk for incipient diabetes.

Setting: The study was mainly conducted at the Pediatric and Adolescent Outpatient Clinic, Quisisana Hospital, Ferrara (Italy), in collaboration³ with thalassemia referring centers across Italy.

Patients: The study included 11 β -TDT (aged 15.11- 31.10 years) with prediabetes.

Methods: The ADA criteria for the diagnosis of glucose dysregulation were adopted. Investigations included evaluating plasma glucose levels and insulin secretion, analyzing glycemc trajectories and indices of β -cell function, and insulin sensitivity/ resistance assessed in steady state and during OGTT.

Results: The duration of progression from prediabetes to DM, expressed in years, showed a positive direct correlation with corrected insulin response (CIR-30 = r: 0.7606, P: 0.0065), insulinogenic index (IGI 0-120 = r: 0.6121, P:0.045), oral disposition index (oDI = r: 0.7119, P:0.013), insulin growth factor-1 (IGF-1= r: 0.6246, P: 0.039) and an inverse linear correlation with serum ferritin (SF = r: -0.7197, P: 0.012). The number of patients with 1-hour post-load PG value ≥ 155 mg/dL (≥ 8.6 mmol/L) was at -4 years: 4/9 (44.4%); -3 years: 8/9 (88.8%); - 2 years: 7/10 (70 %) and at -1 year: 11/11 (100%) (PG range:162-217 mg/dL).

Conclusions: A progressive increase in 1-hour PG in response to OGTT is associated with progressive β -cell failure, peripheral resistance to insulin action, and reduced oDI and may be considered a relevant marker for incipient DM in β -TDT patients with prediabetes.

Keywords: Transfusion-dependent β -thalassemia; OGTT; Pancreatic β -cell function; Insulin sensitivity/resistance; Risk factors; Incipient diabetes.

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Introduction. Prediabetes and diabetes mellitus (DM) are common complications in adult patients with transfusion-dependent β -thalassemia (β -TDT), with their incidence increasing with age. However, there is a significant variability in the reported prevalence of glucose dysregulation (GD) rates between different studies. The major confounders are the number and the mean age of study cohorts and the study design.¹

Prediabetes status is defined as intermediate hyperglycemia that is characterized by the main pathophysiological changes of DM: β -cell dysfunction with impaired insulin secretion and decreased insulin tissue cell sensitivity that co-evolve in varying proportions simultaneously rather than sequentially.²

Individuals with prediabetes are a heterogeneous group as regards the pathophysiology and prognosis. Impaired fasting plasma glucose (IFG) and impaired glucose tolerance (IGT), both of which can appear in isolation or combination (IFG plus IGT), sometimes also associated with high HbA1c, define the subcategories of prediabetes in healthy individuals.^{3,4}

The progression from prediabetes to type 2 diabetes (T2D) in the general adult population is usually a gradual process that occurs over 5–10 years, depending on the characteristics of each population.⁵ The rate of progression to DM is higher in subjects with combined IFG-IGT (combined GI) (31.9%) or IGT (18.5%) than in those with isolated IFG (15.2%).⁶ In addition, the rate of progression from prediabetes to DM also depends on other factors, such as age, sex, body mass index (BMI), and ethnicity.^{3,5,6}

In contrast to the vast literature about metabolic predictors of deterioration of glucose tolerance in the general population, little is known about this process in patients with β -TDT.⁷ A selected group of 10 β -TDT patients with IFG was monitored from prepubertal age to adulthood for a long period (range:10.3 - 28.10 years). 9/10 (90%) exhibited further deterioration of glucose tolerance, while 2 female patients developed DM.⁸

Moreover, Kattamis et al.⁹ have reported that 12.4% of 263 patients (aged 11-30 years) with IGT developed DM within ten years.

In a recent long-term retrospective study of 58 β -TDT patients, 13 (22.4%) patients with combined GI developed diabetes after a median of 3 years, 13 (22.4%) patients reverted to normal glucose tolerance (NGT)

after a median of 3 years, and 32/58 patients (55.1%) remained stable at a state of prediabetes.¹

Understanding the natural history of prediabetes in β -TDT patients is essential for the early detection of glucose dysregulation (GD) to facilitate any effort to prevent progression to DM. Therefore, a retrospective observational study was promoted by the International Network of Clinicians for Endocrinopathies in Thalassemia and Adolescence Medicine (ICET-A) to provide valuable information for predicting the development of incipient diabetes in β -TDT individuals with prediabetes.¹⁰

The gold standard tests for determining insulin secretion and resistance are the hyperglycemic and euglycemic insulin clamp techniques, respectively.¹¹ However, these techniques are costly, labor intensive, and time-consuming, with limited availability in routine clinical practice. In recent years, simple surrogate indices, either derived from plasma glucose and insulin concentrations under fasting conditions (steady state) or in the postprandial state during an OGTT (dynamic state), have been used in study populations.¹¹ In clinical practice, these indices correlate closely with whole-body insulin sensitivity measured with the insulin clamp and have already been applied to studies in β -TDT, albeit in a limited number of subjects.^{1,7,8} The data presented here include insulin secretory and sensitivity parameters obtained by modeling OGTT data.

Subjects and methods

Study population and design. The records of β -TDT patients with prediabetes referred for consultation from January 2015 to December 2022 to the Pediatric and Adolescent Outpatient Clinic, Private Accredited Quisisana Hospital, Ferrara (Italy) were reviewed. Only those who progressed to DM during follow-up were selected for the present study. Exclusion criteria included: (a) non-transfusion dependent β -thalassemia patients (NTDT); (b) bone-marrow transplanted patients; (c) patients using drugs affecting glucose metabolism, and (d) subjects with other major chronic associated illnesses besides β -TDT, such as HIV positivity, chronic active hepatitis, and chronic kidney disease.

Patients' data, collected from the records at the time of referral, included age, sex, history of HCV infection and its treatment, age of splenectomy, type of iron

chelators, history of blood transfusion, data of clinical physical examination, and laboratory investigations (complete blood count, liver function tests, serum creatinine, HCV-antibodies, PCR for HCV-RNA and serum ferritin). β -TDT was diagnosed by the referring Centers using hemoglobin HPLC and/or molecularly characterized genotype.

A family history of DM was defined as at least one first-degree or second-degree relative having DM. We used the criteria previously described by the international network on endocrine complications in thalassemia to diagnose associated endocrine complications.¹²

Oral glucose tolerance test (OGTT) and analytical methods. Blood specimens were collected before (0 min), 30, 60, 90, 120, and 180 min after a 75 g oral glucose load to determine PG and insulin levels. PG was measured using the glucose oxidase method, and serum insulin was measured using a chemiluminescent immunoassay. Plasma for the assessment of insulin was collected in EDTA tubes on ice, separated after centrifugation, and stored at -60°C until the assays were performed. The OGTT was repeated every 6–12 months for dynamic assessment of glucose tolerance status.

The collected data were compared to 11 healthy adult volunteers of comparable age (23.8 ± 3.2 years), sex (5 males and 6 females), and body mass index (BMI) (<25 Kg/m²).

Classification of sub-phenotypes of prediabetes and DM. Diagnostic glycemic criteria for prediabetes and DM according to the American Diabetes Association (ADA) are as follows: (a) NGT, (b) IFG: fasting PG 100–125 mg/dL (5.6–6.9 mmol/L), (c) IGT: 2-h PG levels during OGTT between 140 and 199 mg/dL (7.8–11.0 mmol/L), (d) combined glucose intolerance (combined GI: IFG plus IGT) (e) DM without fasting hyperglycemia and (f) DM with fasting hyperglycemia (≥ 126 mg/dL or 7.0 mmol/L) (10). HbA1c was not assessed because its interpretation is still debated, particularly in non-transfused or poorly transfused patients with β -thalassemias.¹³

In addition, the 1-hour post-load PG value ≥ 155 mg/dl (8.6 mmol/L) was evaluated because large-scale population studies have shown that 1-hour PG above this cut-off value is a strong predictor of future development of DM, with a higher predictive value than FPG or 2-h PG levels.¹⁴

Selection of insulin secretion and insulin sensitivity/resistance indices. To assess β -cell function, we used 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: $\Delta\text{Ins}_{30-0}/\Delta\text{gluc}_{30-0}$) after the ingestion of glucose,^{11,15} and the corrected insulin response (CIR).¹⁶ IGI 0-120

($\Delta\text{Ins}_{0-120}/\Delta\text{gluc}_{0-120}$) was calculated as the mean of incremental insulin and glucose concentrations during the OGTT.

IGI is a proxy for acute phase and late serum insulin response and is used to evaluate the β -cell function; lower CIR suggests insulin hyposecretion for the glucose level, and higher CIR suggests insulin hypersecretion.

Insulin resistance was determined by static Quantitative Insulin Sensitivity Check Index (QUICKI)¹¹ and dynamic index [Matsuda-DeFronzo index (MI 0-120)].¹⁷

QUICKI is an empirically derived mathematical transformation of FPG and plasma insulin concentrations, providing a reliable, reproducible, and accurate insulin sensitivity index.

MI 0-120 combines both hepatic and peripheral tissue insulin sensitivity. Moreover, oral glucose insulin sensitivity (OGIS-180) correlates significantly with glucose clearance measured by the clamp technique (correlation coefficients ranging from 0.7 to 0.8). It is considered a more sensitive measure of insulin sensitivity because it reflects the glucose uptake by muscle tissue.^{18,19} In subjects with normal glucose tolerance, the mean normal value of OGIS-180 was 440 ± 16 mL/min/m².¹⁸

The oral disposition index (oDI)²⁰ was calculated as the product of the MI 0-120 and IGI, and insulin secretion-sensitivity index-2 (ISSI-2: $\text{AUC}_{\text{Ins}}/\text{AUC}_{\text{Glu}} \times \text{MI 0-120}$).²¹ ISSI-2 is a validated OGTT-derived measure of β -cell function analogous to the disposition index. ISSI-2 is defined as the product of insulin secretion, as measured by the ratio of AUC_{Ins} to AUC_{Glu} multiplied by the Matsuda index.²² Substantially, it refers to the relationship between insulin sensitivity and insulin secretion²⁰ and is considered a better index than IGI or MI 0-120 for assessing the decline of β cell function.²²

Areas under the glucose and insulin curves (AUC_{0-180}) were calculated by the trapezoid rule. Insulin secretion was evaluated with the area under the insulin concentration curve ($\text{AUC}_{\text{Ins } 0-180}$), whereas β -cell function (how β - β -cells respond to the glucose stimulus) was assessed as the ratio of $\text{AUC}_{\text{Ins } 0-180}$ to $\text{AUC}_{\text{Glu } 0-180}$, that is the area under the glucose concentration curve (AUC ratio: $\text{AUC}_{\text{Ins } 0-180}/\text{AUC}_{\text{Glu } 0-180}$). Substantially, the ratio is used as a surrogate index of insulin secretion during the OGTT.

Patients were considered to have IR in the presence of either of the following cut-off limits: QUICKI (25th percentile): < 0.3 and MI 0-120 (25th percentile): < 4.31 . These references refer to 130 selected Polish subjects, aged 18–31 years, with $\text{FPG} \leq 100$ ng/mL, $\text{BMI} < 25$ kg/m², and without metabolic syndrome.²¹

Overall laboratory evaluations and assessment of iron overload at first consultation. Alanine aminotransferase (ALT), free thyroxine-FT4 and thyrotropin-TSH for

thyroid function, basal serum cortisol, gonadotropins (LH and FSH), estradiol (E2), and insulin growth factor-1 (IGF-1) were measured by commercial immunoassay kits.

Iron overload (IOL) was assessed by serum ferritin (SF) and was arbitrarily classified as mild (SF: < 1,000 ng/mL), moderate (SF: >1,000 ng/mL and < 2,000 ng/mL) or severe (SF: >2,000 ng/mL).²³

Statistical analysis. Continuous variables are described by mean ± standard deviation (SD). All data were tested for normal distribution with the Shapiro–Wilk test and compared using the Student's test in case of a normal distribution or the Wilcoxon-Mann-Whitney test in case of non-normal distribution. Statistical correlation between two variables was evaluated with Pearson's or Spearman's correlation in the case of a normal or a non-normal distribution. A software program was used and validated for the statistical analysis, according to Alder and Roesser.²⁴ A P value < 0.05 was considered statistically significant.

Ethics. No ethical approval was needed because the study was a retrospective review of observational data/records, containing no identifiable private information, and only aggregated data were analyzed and presented.²⁵ Moreover, the patients underwent only routine diagnostic procedures according to the national Italian protocols and the International Guidelines.^{12,26,27} The study was developed in accordance with the Helsinki Declaration (www.wma.net), and all patients provided informed consent.

Results

Patient demographic and characteristics at first consultation. Eleven patients with β-TDT were enrolled in this study (5 males and 6 females). The mean age was 25.1 ± 5.7 years. All the patients were on regular packed red blood cell transfusions to keep pre-transfusional hemoglobin level > 9 g/dL and regular iron chelating agents with desferrioxamine (3 patients), deferiprone (3 patients), deferasirox (2 patients) and a combination of desferrioxamine plus deferiprone (3 patients). Ten out of 11 patients were splenectomized, 6 had a history of HCV infection (HCV Ab + and HCV-RNA -), and 5 had received successful antiviral treatment for HCV-RNA +. Two out of 11 patients had a personal history of acquired hypogonadotropic hypogonadism and received irregular hormone replacement therapy and 3 out of 6 females developed secondary amenorrhea at an age of 18-20 years. Three patients were on treatment with L-thyroxine. Short stature (≤ 3rd centile) for age and sex, compared to the Italian growth reference chart,²⁸ was documented in 3 females and 1 male.

The mean SF level was 1403.2 ± 674.7 ng/mL (range: 485-2,600) (Table 1). Mild iron overload (SF: < 1.000

ng/mL) was present in 4 patients with combined GI, moderate iron overload (SF: >1.000 ng/mL and < 2.000 ng/mL) in 5 patients (2 with IGT and 3 with combined GI), and severe iron overload (SF: >2.000 ng/mL) in 2 patients (1 with IGT and 1 with combined GI).

Table 1. Clinical and laboratory characteristics at first endocrine consultation in 11 β-TDT patients with prediabetes. Data are expressed as mean ± SD, number (n.), and range.

Variables	Results
Age (yrs), range	25.1 ± 5.7 (range: 15.11- 31.10)
Males/Females	5/6
Positive family history (n.)	5/11
History of splenectomy (n.)	10/11
Body Mass Index (BMI: kg/ m ²)	21.1 ± 1.5 (range: 19.5-23.6)
Serum ferritin (ng/mL),	1403.2 ± 674.7 (range: 485-2,600)
History of HCV treatment (n.)	5/11
HCV Ab + and HCV-RNA - (n.)	6/11
ALT (normal values: < 40 IU/L)	42.4 ± 19.6 (range: 14-79)

Abbreviations: ALT: alanine aminotransferase; HCV: Hepatitis C virus.

Glucose metabolism and insulin response during OGTT at first consultation

At first consultation. At the first consultation for prediabetes, IGT and combined GI were documented in 3 and 8 β-TDT patients, respectively (Figure 1).

Of note, the first documentation of glucose dysregulation reported by the referring Centers (IFG: 3 patients; IGT: 2 patients and combined GI: 6 patients) was reported 5.3 ± 2.6 years earlier (range 2-10 years), at a mean age of 17.6 ± 3.3 years (Figure 1).

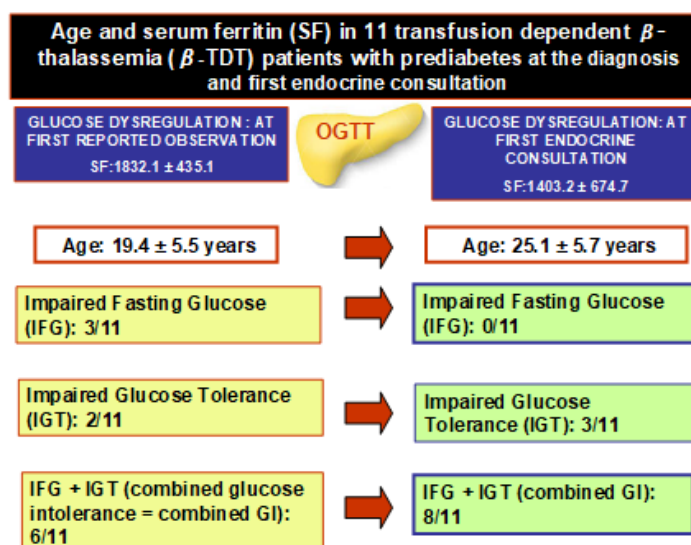


Figure 1. Diagnosis of glucose dysregulation (prediabetes) at first observation (reported by referring Centers) and at the first endocrine consultation.

The PG and insulin concentrations, at baseline and during the 3-h OGTT, in β -TDT patients with prediabetes compared to controls are reported in **Figures 2 and 3**.

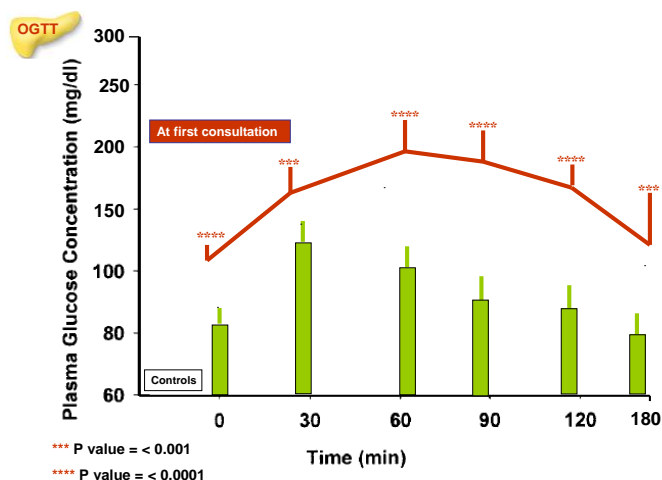


Figure 2. Plasma glucose levels before and during 3-h oral glucose tolerance test (OGTT) in 11 transfusion-dependent β -thalassemia (β -TDT) patients with prediabetes compared to 11 healthy controls (green bars)

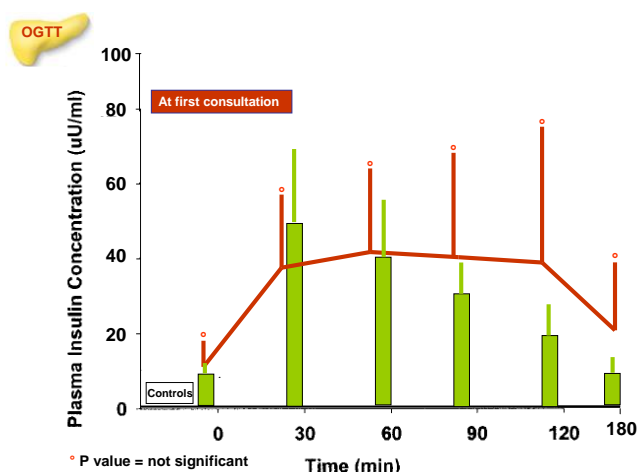


Figure 3. Plasma insulin levels were assessed before and during a 3-h oral glucose tolerance test (OGTT) in 11 transfusion-dependent β -thalassemia (β -TDT) patients with prediabetes compared to 11 healthy controls (green bars)

The WHO and ADA have proposed different cut-off values for the diagnosis of IFG. In this retrospective study, we adopted the latter criteria of 100-125 mg/dL (5.6 -6.9 mmol/L) to increase the sensitivity for early detection of GD. Using WHO criteria, only 2/11 patients (18.1%) with glucose dysregulation had an FPG \geq 110 mg/dL (5.6 mmol/L), while the percentage was substantially higher (8/11, 72.7%) as per the ADA criteria.

All patients had a PG level at 1-h during OGTT \geq 155 mg/dL (mean: 192.3 ± 21.1 mg/dL; range: 162-233 mg/dL) and a mean PG level at 2-h during OGTT of 162.4 ± 14.8 mg/dL (range: 142-187 mg/dL).

Seven of 11 patients (63.6 %) had a plasma insulin

peak between 90 and 120 minutes after glucose load.

Overall, indices of insulin secretion (IGI and CIR), sensitivity/resistance (QUICKI, OGIS-180), oDI, ISSI-2, and $AUC_{Glu\ 0-180}$ (mg/dL) were statistically different from controls (**Table 2**).

At last observation. DM was diagnosed after a mean interval of 12.4 ± 4.7 months (6-22 months) following the first consultation. The comparison of PG and insulin levels during OGTT and indices of insulin secretion, sensitivity/resistance, and oDI compared to the first consultation are reported in **Figures 4 and 5** and **Table 3**. Four patients with "borderline" PG level at 2-h during OGTT (range: 202-213 mg/dL) underwent a second OGTT test, which confirmed the diagnosis of DM.

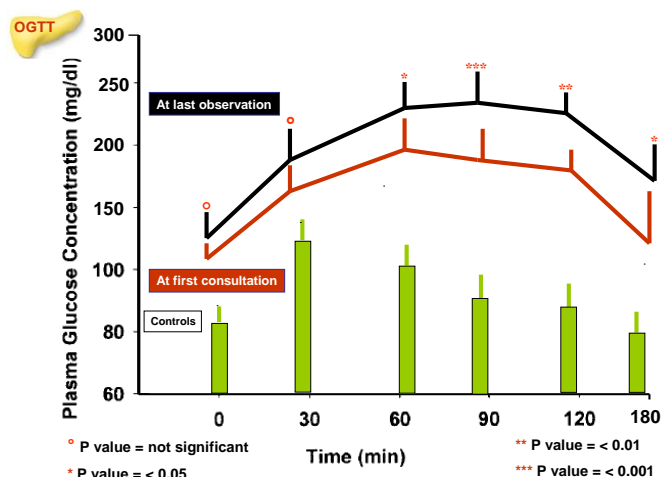


Figure 4. Plasma glucose levels before and during oral glucose tolerance test (OGTT) in 11 transfusion-dependent β -thalassemia (β -TDT) patients with DM compared to the first consultation carried out 12.4 ± 4.7 months earlier. Green bars refer to 11 healthy controls.

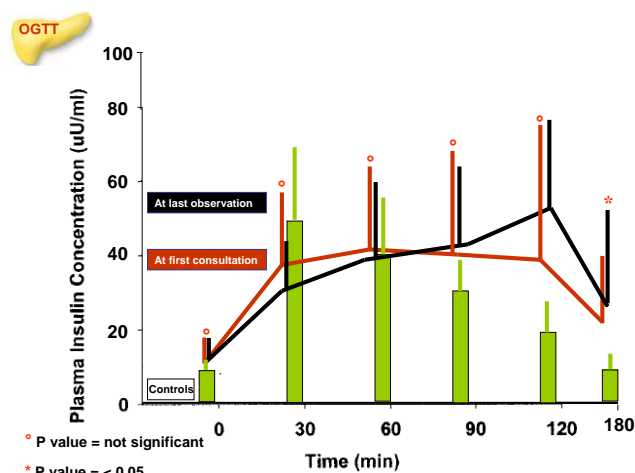


Figure 5. Plasma insulin levels before and during oral glucose tolerance test (OGTT) in 11 transfusion-dependent β -thalassemia (β -TDT) patients with DM compared to the first consultation carried out 12.4 ± 4.7 months earlier. Green bars refer to 11 healthy controls.

A further increase of PG level at 1-h after OGTT was observed compared to the first consultation (224.7 ± 40.2 mg/dL vs. 192.3 ± 21.1 mg/dL; $P: 0.027$) and $AUC_{Glu\ 0-180}$ (mg/dL) results were significantly higher compared

Table 2. Comparison, at first endocrine consultation of various indices derived from 3-h oral glucose tolerance test (OGTT) in 11 β -TDT versus 11 healthy control subjects. Data are expressed as mean \pm SD.

Variables	11 β -TDT patients with prediabetes	11 healthy control subjects	P value
Age (yrs) and range	25.1 \pm 5.7	23.8 \pm 3.2	0.53
PG level at 1-h during OGTT (mg/dL)	152.0 \pm 35.6	109.8 \pm 23.9	0.0052
Insulinogenic index (IGI) 0-30	0.52 \pm 0.38	1.54 \pm 0.99	0.0046
Insulinogenic index (IGI) 0-120	0.39 \pm 0.22	0.69 \pm 0.40	0.044
Corrected insulin response (CIR) 30	0.12 \pm 0.06	0.34 \pm 0.17	0.0007
Corrected insulin response (CIR) 120	0.15 \pm 0.15	0.30 \pm 0.19	0.057
QUICKI	0.34 \pm 0.03	0.37 \pm 0.02	0.015
Matsuda index 0-120	7.15 \pm 2.67	8.71 \pm 2.85	0.21
OGIS-180	427.0 \pm 74.6	537.3 \pm 73.2	0.016
Oral disposition index (oDI)	3.14 \pm 3.21	12.11 \pm 6.55	0.0007
ISSI-2	120.0 \pm 39.1	266.6 \pm 99.6	0.0002
AUC _{Glu} 0-180 (mg/dL)	469.2 \pm 23.1	337.5 \pm 58.3	< 0.0001
AUC _{Ins} 0-180 (μ U/mL)	93.2 \pm 73.2	92.9 \pm 44.8	0.99
Ratio AUC _{Ins} 0-180/AUC _{Glu} 0-180	0.18 \pm 0.10	0.22 \pm 0.11	0.39

Abbreviations: PG: plasma glucose; QUICKI: Quantitative Insulin Sensitivity Check Index; OGIS-180: oral glucose insulin sensitivity; ISSI-2: insulin secretion-sensitivity index- 2.

Table 3. Comparison of indices derived from 3-h oral glucose tolerance test (OGTT) at the diagnosis of DM (last observation) in 11 β -TDT patients compared to the first endocrine consultation carried out 12.4 \pm 4.7 months earlier. Data are expressed as mean \pm SD.

Variables	11 β -TDT patients with prediabetes	11 β -TM patients with diabetes	P value
Age (yrs)	25.1 \pm 5.7	26.6 \pm 6.0	=
Body Mass Index (kg/m ²),	21.1 \pm 1.5	21.7 \pm 1.8	0.40
SF (ng/mL), range	1403.2 \pm 674.7 485-2,600	1224.5 \pm 675.2 519-2,580	0.020
ALT (normal values: < 40 IU/L)	42.4 \pm 19.6	53.6 \pm 32.8	0.012
Insulinogenic index (IGI) 0-30	0.52 \pm 0.38	0.38 \pm 0.23	0.28
Insulinogenic index (IGI) 0-120	0.39 \pm 0.22	0.39 \pm 0.25	0.42
Corrected insulin response (CIR) 30	0.12 \pm 0.067	0.095 \pm 0.059	0.036
Corrected insulin response (CIR) 120	0.15 \pm 0.15	0.097 \pm 0.054	0.16
QUICKI	0.34 \pm 0.030	0.34 \pm 0.032	1
Matsuda index 0-120	7.15 \pm 2.67	5.11 \pm 2.93	0.18
OGIS -180	427.0 \pm 74.6	353.0 \pm 56	0.026
Oral disposition index (oDI)	3.14 \pm 3.21	1.63 \pm 0.94	0.061
ISSI-2	120.0 \pm 39.1	99.0 \pm 42.6	0.056
AUC _{Glu} 0-180 (mg/dL)	469.2 \pm 23.1	578.9 \pm 58.3	0.003
AUC _{Ins} 0-180 (μ U/mL)	93.2 \pm 73.2	106.7 \pm 59.9	0.28
Ratio AUC _{Ins} 0-180/AUC _{Glu} 0-180	0.19 \pm 0.15	0.18 \pm 0.10	0.92
IGF-1 (ng/mL)	==	60.6 \pm 34.9	=

Abbreviations:ALT: alanine aminotransferase; SF: serum ferritin; QUICKI: Quantitative Insulin Sensitivity Check Index; OGIS-180: oral glucose insulin sensitivity; ISSI-2: insulin secretion-sensitivity index- 2; IGF-1: Insulin growth factor-1.

to first consultation (P: 0.003) (**Table 3**).

Interestingly, the mean insulin response during the OGTT was not statistically different compared to the first endocrine consultation. However, 6 out of 11 patients (54.5 %) had a plasma insulin peak between 120 and 180 minutes after glucose load.

Indices of insulin secretion, sensitivity/resistance,

and oDI documented a significant difference in mean CIR-30 value (P: 0.036), OGIS -180 (P: 0.026), and AUC_{Glu} 0-180 (P: 0.003) (**Table 3**).

The trajectories of PG levels at baseline, 60 and 120 minutes after OGTT, reported by the referring Centers in the 3 years preceding the first endocrine consultation, are illustrated in **Figure 6**. We set the year of diabetes

diagnosis as year 0, and we traced PG at 0, 60, and 120 minutes after OGTT backward and in the following two

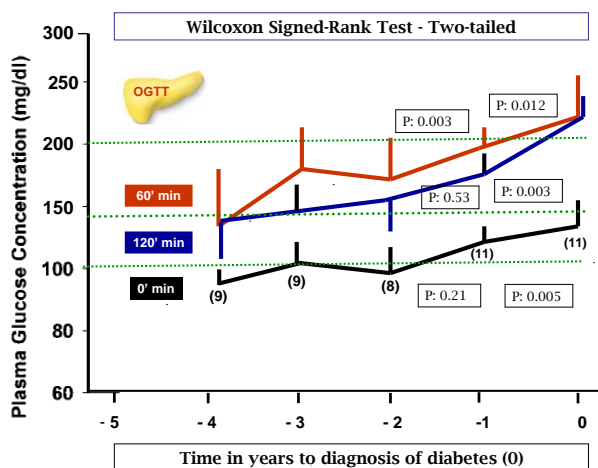


Figure 6. Time trajectories of glycemic levels at baseline (black line), 60' minutes (red line), and 120' (blue line) minutes before and after OGTT. The green dotted lines indicate the definition limits for IFG, IGT, combined GI, and diabetes.

years after the first endocrine consultation.

The number of patients with FPG level ≥ 100 mg/dL (≥ 5.6 mmol/L) at -4 years was: 1/9 (11.1%); -3 years: 5/9 (55.5%); -2 years: 4/9 (44.4%) and at -1 year: 11/11 (100%) (PG range: 94-123 mg/dL).

The number of patients with 1-hour post-load PG value ≥ 155 mg/dL (≥ 8.6 mmol/L) was at -4 years: 4/9 (44.4%); -3 years: 8/9 (88.8%); -2 years: 7/10 (70%) and at -1 year: 11/11 (100%) (PG range: 162-217 mg/dL).

Correlations

At first consultation. A strong positive correlation was observed between 1-hour post-load PG value ≥ 155 mg/dL (8.6 mmol/L) and $AUC_{Glu\ 0-180}$ ($r: 0.934$; $P: 0.000026$) and a negative correlation with oDI ($r: -0.6686$; $P: 0.024$). Moreover, QUICKI was strongly correlated to $MI\ 0-120$ ($r: 0.8601$; $P: 0.00068$) and oDI and ISSI-2 ($r: 0.91116$; $P: 0.0001$). The ratio $AUC_{Ins\ 0-180}/AUC_{Glu\ 0-180}$ was negatively correlated with $MI\ 0-120$ ($r: -0.67124$; $P: 0.023$).

At last consultation. An inverse linear correlation ($r: -0.7197$, $P: 0.012$) was observed between SF and progression duration from prediabetes to diabetes (mean: 6.57 ± 2.89 years; range 2.8-12.2 years). No correlation was observed with age at the first diagnosis of prediabetes carried out with OGTT ($r: -0.2046$, $P: 0.54$). Of note, the progression duration (in years) from prediabetes to diabetes had a direct correlation with $CIR-30$ ($r: 0.7606$, $P: 0.0065$), $IGI\ 0-120$ ($r: 0.6121$, $P: 0.045$), oDI ($r: 0.7119$, $P: 0.013$) and $IGF-1$ ($r: 0.6246$, $P: 0.039$).

Discussion. The prognosis of patients with β -TDT has improved over the last decades with the advent of increased blood transfusion safety measures, efficient

new oral iron chelators, and non-invasive methods to assess organ iron overload before the appearance of clinical complications. Nevertheless, a subset of patients continues to develop endocrine complications, mainly affecting glucose homeostasis and the hypothalamic-pituitary-gonadal axis, because certain tissues are particularly susceptible to excess iron toxicity and are highly sensitive to oxidant-generating substances.^{7,29,30-33}

All β -TDT patients are recommended to undergo annual screening for GD from the age of 10 years, including annual OGTT after the age of 16 years old.^{12,26,27} The simultaneous measurements of glucose and insulin during standard OGTT provide an excellent opportunity to establish, in a single test, not only the stage of glucose tolerance but also β -cell function. Therefore, when feasible, measurement of insulin and C-peptide can add valuable information beyond glucose tolerance, e.g., measurement of parameters related to insulin secretion, β -cell function, quantification of insulin sensitivity, and insulin clearance.

In contrast to the vast literature about metabolic predictors of deterioration of glucose tolerance in the general population, very few studies have been focused on this aspect in β -TDT patients.

In the present long-term retrospective study, we followed the progression from the first documentation of prediabetes (3 patients with IGT and 8 with combined GI) to the selected endpoint of DM in β -TDT patients. The mean interval period was 6.57 ± 2.89 years (range 2.8-12.2 years). Duration of progression, expressed in years, showed a direct correlation with $CIR-30$ ($r: 0.7606$, $P: 0.0065$), $IGI\ 0-120$ ($r: 0.6121$, $P: 0.045$), oDI ($r: 0.7119$, $P: 0.013$), $IGF-1$ ($r: 0.6246$, $P: 0.039$) and an inverse linear correlation with SF ($r: -0.7197$, $P: 0.012$). Therefore, it seems likely that reducing insulin secretion and peripheral resistance to insulin action is a prerequisite for developing incipient DM.

Our data further confirm a correlation of GD with SF level and the contribution of $IGF-1$ to worsening insulin resistance, leading to dysglycemia in adult patients with β -TDT, as reported in a previous study.³⁴ Moreover, a significant negative correlation was found between SF levels and progression duration from prediabetes to diabetes.

During the last 2 years of observation, there was a significantly sharp increase in the trajectories of FPG and 1-h PG during OGTT between years -2 and -1, and -1 and 0 (0.003 and $P: 0.012$, respectively). Interestingly, 8/10 β -TDT patients (80%) presented a 1-hour post-load PG value ≥ 155 mg/dL (8.6 mmol/L) three years before the diagnosis of DM.

The main limitation of our study was the small sample size, which may not be representative of other β -TDT populations since there is marked heterogeneity across different ethnic backgrounds with variable genotypes and treatment efficiency, especially of chelation.

Another weakness was the potential confounding from the effect of different iron chelators on glucose-insulin homeostasis since individuals received a variety of therapies for iron overload during the study.

The key strength of this retrospective study is the four-year longitudinal analysis of trajectories of 1-hour post-load PG values and the analysis of several indices of insulin sensitivity and β -cell function collected after the first endocrine consultation in β -TDT patients with prediabetes.

Conclusions. A progressive increase in 1-hour PG in response to OGTT is associated with progressive β -cell

failure, peripheral resistance to insulin action, and reduced oDI. It may be considered a relevant marker for incipient DM in β -TDT patients with prediabetes. Nevertheless, it remains to be seen whether the current study's findings will be replicated in larger cohorts of β -TDT patients.

Finally, taking into consideration the differences between DM in β -TDT patients with type T1-DM and T2-DM, we propose to use the terminology of β -TDT related diabetes (β -TDT-RD) in order to emphasize its presence as a distinct type of DM with distinct pathophysiological and clinical features.⁸

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