Mediterranean Journal of Hematology and Infectious Diseases

Original Article

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

Vincenzo De Sanctis[1], Ashraf T Soliman[2], Shahina Daar[3], Ploutarchos Tzoulis[4] and Christos Kattamis[5].

[1] Coordinator of ICET-A Network (International Network of Clinicians for Endocrinopathies in Thalassemia and Adolescent Medicine) and Pediatric and Adolescent Outpatient Clinic, Quisisana Hospital, Ferrara, Italy.
[2] Department of Pediatrics, Division of Endocrinology, Hamad General Hospital, Doha, Qatar.
[3] Department of Haematology, College of Medicine and Health Sciences, Sultan Qaboos University, Muscat, Sultanate of Oman.
[4] Department of Diabetes and Endocrinology, Whittington Hospital, University College London, London, UK.
[5] Thalassemia Unit, First Department of Paediatrics, National Kapodistrian University of Athens 11527, Greece.

Competing interests: The authors declare no conflict of Interest.

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 glycemic 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 collaboration 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 glycemic 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.

Citation: De Sanctis V., Soliman A.T., Daar S., Tzoulis P., Kattamis C. Can we predict incipient diabetes mellitus in patients with transfusion dependent β-thalassemia (β-TDT) referred with a history of prediabetes? Mediterr J Hematol Infect Dis 2024, 16(1): e2024005, DOI: http://dx.doi.org/10.4084/MJHID.2024.005

Published: January 01, 2024 **Received: September 11, 2023** **Accepted: December 12, 2023**

Correspondence to: Vincenzo De Sanctis. 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.

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/ m^2).

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: $\Delta Ins_{30-0}/\Delta gluc_{30-0}$) after the ingestion of glucose, [11,15] and the corrected insulin response (CIR).[16] IGI 0-120 ($\Delta Ins_{0-120}/\Delta gluc_{0-120}$) 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/m^2.[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: AUC_{Ins}/AUC_{Glu} x 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_{Gluc} multiplied by the Matsuda index.[22] Substantially, it refers to the relationship between insulin sensitivity and insulin secretion[20] 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 ($AUCs_{0-180}$) were calculated by the trapezoid rule. Insulin secretion was evaluated with the area under the insulin concentration curve ($AUC_{Ins\ 0-180}$), whereas β-cell function (how β -β-cells respond to the glucose stimulus) was assessed as the ratio of $AUC_{Ins\ 0-180}$ to $AUC_{Glu0-180}$, that is the area under the glucose concentration curve (AUC ratio: $AUC_{Ins\ 0-180}/AUC_{Glu\ 0-180}$). 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 (25^{th} percentile): < 0.3 and MI 0-120 (25^{th} percentile): < 4.31. These references refer to 130 selected Polish subjects, aged 18–31 years, with FPG ≤ 100 ng/mL, BMI <25 kg/m^2, 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**).

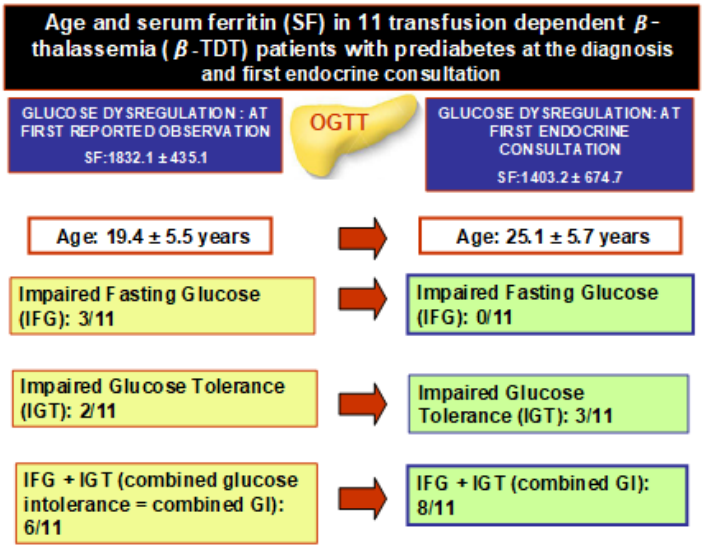

Figure 1. Diagnosis of glucose dysregulation (prediabetes) at first observation (reported by referring Centers) and at the first endocrine consultation.

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**.

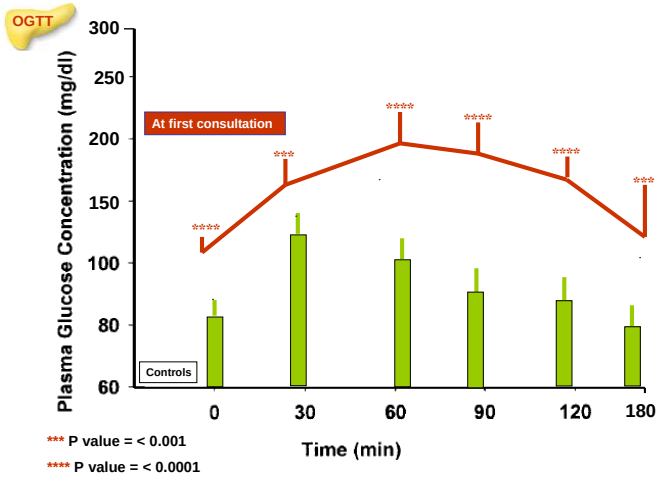

Figure 2. Plasma glucose levels before and during 3-h oral glucose tolerance test (OGTT) in 11 transfusion-dependent β-thalassemia (β-TDT) patients with prediabetes compared to 11 healthy controls (green bars)

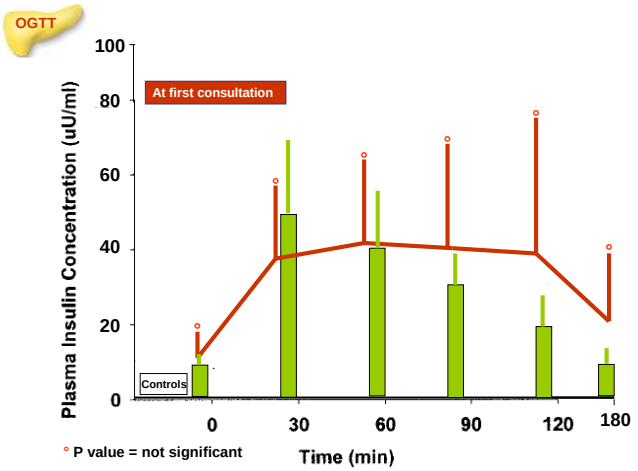

Figure 3. Plasma insulin levels were assessed before and during a 3-h oral glucose tolerance test (OGTT) in 11 transfusion-dependent β-thalassemia (β-TDT) patients with prediabetes compared to 11 healthy controls (green bars)

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 ≥ 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 ≥ 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 $AUC_{Glu\ 0\text{-}180}$ (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.

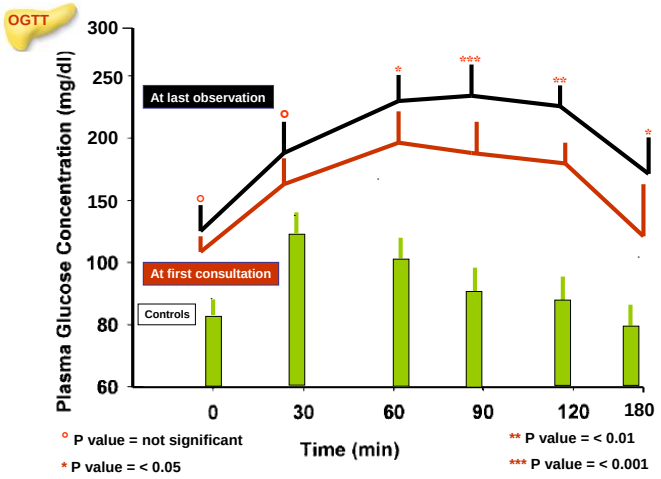

Figure 4. Plasma glucose levels before and during oral glucose tolerance test (OGTT) in 11 transfusion-dependent β-thalassemia (β-TDT) patients with DM compared to the first consultation carried out 12.4 ± 4.7 months earlier. Green bars refer to 11 healthy controls.

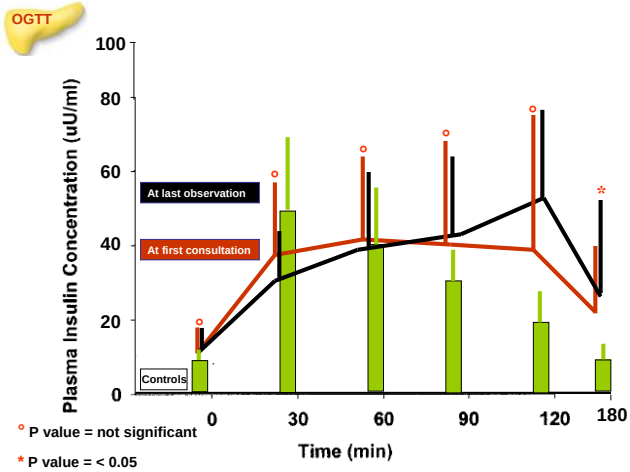

Figure 5. Plasma insulin levels before and during oral glucose tolerance test (OGTT) in 11 transfusion-dependent β-thalassemia (β-TDT) patients with DM compared to the first consultation carried out 12.4 ± 4.7 months earlier. Green bars refer to 11 healthy controls.

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 $AUC_{Glu\ 0\text{-}180}$ (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 ± SD.

Variables	11 β-TDT patients with prediabetes	11 healthy control subjects	P value
Age (yrs) and range	25.1±5.7	23.8 ± 3.2	0.53
PG level at 1-h during OGTT (mg/dL)	152.0 ± 35.6	109.8 ± 23.9	**0.0052**
Insulinogenic index (IGI) 0-30	0.52 ± 0.38	1.54 ± 0.99	**0.0046**
Insulinogenic index (IGI) 0-120	0.39 ± 0.22	0.69 ± 0.40	**0.044**
Corrected insulin response (CIR) 30	0.12 ± 0.06	0.34 ± 0.17	**0.0007**
Corrected insulin response (CIR) 120	0.15 ± 0.15	0.30 ± 0.19	0.057
QUICKI	0.34 ± 0.03	0.37 ± 0.02	**0.015**
Matsuda index 0-120	7.15 ± 2.67	8.71 ± 2.85	0.21
OGIS-180	427.0 ± 74.6	537.3 ± 73.2	**0.016**
Oral disposition index (oDI)	3.14 ± 3.21	12.11 ± 6.55	**0.0007**
ISSI-2	120.0 ± 39.1	266.6 ± 99.6	**0.0002**
$AUC_{Glu\ 0-180}$ (mg/dL)	469.2 ± 23.1	337.5 ± 58.3	**< 0.0001**
$AUC_{Ins\ 0-180}$ (µU/mL)	93.2 ± 73.2	92.9 ± 44.8	0.99
Ratio $AUC_{Ins\ 0-180}$ /$AUC_{Glu\ 0-180}$	0.18 ± 0.10	0.22± 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 ± 4.7 months earlier. Data are expressed as mean ± SD.

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

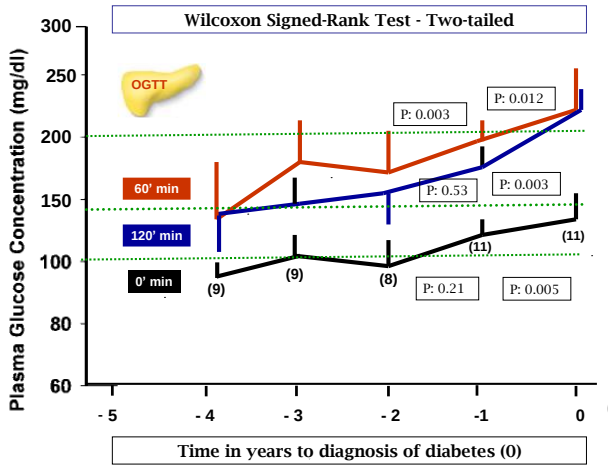

Figure 6. Time trajectories of glycemic levels at baseline (black line), 60' minutes (red line), and 120' (blue line) minutes (blue line) before and after OGTT. The green dotted lines indicate the definition limits for IFG, IGT, combined GI, and diabetes.

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 $AUC_{Glu\ 0\text{-}180}$ (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 $AUC_{Ins\ 0\text{-}180}$ /$AUC_{Glu\ 0\text{-}180}$ 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]

References:

1. De Sanctis V, Daar S, Soliman AT, Tzoulis P, Yassin MA, Kattamis C. Evolution of combined impaired fasting glucose and impaired glucose tolerance in β-thalassemia major: Results in 58 patients with a mean 7.7-year follow-up. Acta Biomed. 2022; 93(3): e2022242. https://doi.org/10.1530/endoabs.81.EP262
2. Barbu E, Popescu MR, Popescu AC, Balanescu SM. Phenotyping the Prediabetic Population-A Closer Look at Intermediate Glucose Status and Cardiovascular Disease. Int J Mol Sci. 2021; 22(13):6864. https://doi.org/10.3390/ijms22136864 PMid:34202289 PMCid:PMC8268766
3. American Diabetes Association. Classification and Diagnosis of Diabetes: Standards of Medical Care in Diabetes - 2020. Diabetes Care. 2020; 43(Suppl.1): S14-S31. https://doi.org/10.2337/dc20-S002 PMid:31862745
4. Guo F, Moellering DR, Garvey WT.Use of HbA1c for Diagnoses of Diabetes and Prediabetes: Comparison with Diagnoses Based on Fasting and 2-Hr Glucose Values and Effects of Gender, Race, and Age. Metab Syndr Relat Disord. 2014;12(5): 258-268. https://doi.org/10.1089/met.2013.0128 PMid:24512556 PMCid:PMC4088353
5. Nichols GA, Hillier TA, Brown JB. Progression from newly acquired impaired fasting glucose to type 2 diabetes. Diabetes Care. 2007; 30: 228-233. https://doi.org/10.2337/dc06-1392 PMid:17259486 PMCid:PMC1851903
6. Kim YA, Ku EJ, Khang AR, Hong ES, Kim KM, Moon JH, Choi SH, Park KS, Jang HC, Lim S. Role of various indices derived from an oral glucose tolerance test in the prediction of conversion from prediabetes to type 2 diabetes. Diabetes Res Clin Pract .2014;106(2):351-359. https://doi.org/10.1016/j.diabres.2014.08.014 PMid:25245975
7. De Sanctisv, Soliman A, Tzoulis P, Daar S, Karimi M, Yassin MA, Pozzobon G, Kattamis C. Clinical characteristics, biochemical parameters and insulin response to oral glucose tolerance test (OGTT) in 25 transfusion dependent β-thalassemia (TDT) patients recently diagnosed with diabetes mellitus (DM).Acta Biomed 2021;92 (6): e2021488.
8. De Sanctis V, Soliman AT, Tzoulis P, Daar S, Di Maio S, Fiscina B, Kattamis C. Glucose Metabolism and Insulin Response to Oral Glucose Tolerance Test (OGTT) in Prepubertal Patients with Transfusion-Dependent β-thalassemia (TDT): A Long-Term Retrospective Analysis. Mediterr J Hematol Infect Dis 2021;13(1):e2021051. https://doi.org/10.4084/MJHID.2021.051 PMid:34527203 PMCid:PMC8425353
9. Kattamis C, Ladis V, Tsoussis D, Kaloumenou I, Theodoridis C. Evolution of glucose intolerance and diabetes in transfused patients with thalassemia. Pediatr Endocrinol Rev 2004;2 (Suppl 2):267-271. PIMD:16462709.
10. De Sanctis V, Soliman AT. ICET-A an Opportunity for Improving Thalassemia Management. J Blood Disord. 2014;1(1): 2.
11. Gutch M, Kumar S, Razi SM, Gupta KK, Gupta A. Assessment of insulin sensitivity/resistance. Indian J Endocr Metab. 2015;19:160-4. https://doi.org/10.4103/2230-8210.146874 PMid:25593845 PMCid:PMC4287763
12. De Sanctis V, Soliman AT, Elsedfy H, Skordis N, Kattamis C, Angastiniotis M, Karimi M,Yassin MA, El Awwa A,Stoeva I, Raiola G, Galati MC, Bedair EM, Fiscina B, El Kholy M. Growth and endocrine disorders in talassemia. The international network on endocrine complications in thalassemia (I-CET) position statement and guidelines. Indian J Endocrinol Metab. 2013;17:8-18. https://doi.org/10.4103/2230-8210.107808 PMid:23776848 PMCid:PMC3659911
13. Chen Z, Shao L, Jiang M, Ba X, Ma B, Zhou T. Interpretation of HbA1c lies at the intersection of analytical methodology, clinical biochemistry and hematology (Review). Exp Ther Med. 2022; 24(6):707. https://doi.org/10.3892/etm.2022.11643 PMid:36382101 PMCid:PMC9634344
14. Abdul-Ghani MA, Abdul-Ghani T, Ali N, Defronzo RA. One-hour plasma glucose concentration and the metabolic syndrome identify subjects at high risk for future type 2 diabetes. Diabetes Care. 2008; 31(8): 1650-1655. https://doi.org/10.2337/dc08-0225 PMid:18487478 PMCid:PMC2494641
15. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC.Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia. 1985;28(7):412-419. https://doi.org/10.1007/BF00280883 PMid:3899825
16. Sluiter WJ, Erkelens DW, Reitsma WD, Doorenbos H. Glucose tolerance and insulin release, a mathematical approach I. Assay of the β-cell response after oral glucose loading. Diabetes. 1976; 25:241-244. https://doi.org/10.2337/diab.25.4.241 PMid:773721
17. Matsuda M, DeFronzo RA. Insulin sensitivity indices obtained from oral glucose tolerance testing: comparison with the euglycemic insulin clamp. Diabetes Care. 1999;22(9):1462-1470. https://doi.org/10.2337/diacare.22.9.1462 PMid:10480510
18. Mari A, Pacini G, Murphy E, Ludvik B, Nolan JJ. A model-based method for assessing insulin sensitivity from the oral glucose tolerance test. Diabetes Care. 2001;24:539-548.PIMD:11289482. https://doi.org/10.2337/diacare.24.3.539 PMid:11289482
19. Pacini G, Mari A. Methods for clinical assessment of insulin sensitivity and β-cell function. Best Pract Res Clin Endocrinol Metab 2003;17:305-322. https://doi.org/10.1016/S1521-690X(03)00042-3 PMid:12962688
20. Utzschneider KM, Prigeon RL, Faulenbach MV, Tong J,Carr DB, Boyko EJ, Leonetti DL, McNeely MJ, Fujimoto WY, Kahn SE. Oral disposition index predicts the development of future diabetes above and beyond fasting and 2-h glucose levels. Diabetes Care. 2009;32(2):335-341. https://doi.org/10.2337/dc08-1478 PMid:18957530 PMCid:PMC2628704
21. Płaczkowska S, Pawlik-Sobecka L, Kokot I, Piwowar A. Estimation of reference intervals of insulin resistance (HOMA), insulin sensitivity (Matsuda), and insulin secretion sensitivity indices (ISSI-2) in Polish young people. Ann Agric Environ Med. 2020;27:248-254. https://doi.org/10.26444/aaem/109225 PMid:32588601
22. Goedecke JH, Dave JA, Faulenbach MV, Utzschneider KM, Lambert EV,

West S, Collins M, Olsson T, Walker BR, Seckl JR, Kahn SE, Levitt NS. Insulin response in relation to insulin sensitivity: an appropriate beta-cell response in black South African women. Diabetes Care. 2009; 32: 860-865.
https://doi.org/10.2337/dc08-2048
PMid:19196884 PMCid:PMC2671086

23. De Sanctis V, Elsedfy H, Soliman AT, Elhakim IZ, Kattamis C, Soliman NA, Elalaily R. Clinical and biochemical data of adult thalassemia major patients (TM) with multiple endocrine complications (MEC) versus TM patients with normal endocrine functions: a long-term retrospective study (40 years) in a tertiary care center in Italy. Mediterr J Hematol Infect Dis 2016: 8(1): e2016022.
https://doi.org/10.4084/mjhid.2016.022
PMid:27158435 PMCid:PMC4848017
24. Alder R, Roesser EB. Introduction to probability and statistics. WH Freeman and Company Eds. Sixth Edition. San Francisco (USA), 1975.
25. De Sanctis V, Soliman AT, Daar S, Tzoulis P, Fiscina B, Kattamis C, International Network of Clinicians for Endocrinopathies in Thalassemia and Adolescence Medicine (ICET-A). Retrospective observational studies: Lights and shadows for medical writers. Acta Biomed. 2022;93(5):e2022319.
26. Farmakis D, Porter J, Taher A, Cappellini MD, Angastiniotis M, Eleftheriou A. 2021 Thalassaemia International Federation Guidelines for the Management of Transfusion-dependent Thalassemia. Hemasphere.2022;6(8):e732.
https://doi.org/10.1097/HS9.0000000000000732
PMid:35928543 PMCid:PMC9345633
27. De Sanctis V, Daar S, Soliman AT, Tzoulis P, Karimi M, Di Maio S, Kattamis C. Screening for glucose dysregulation in β-thalassemia major (β-TM): An update of current evidences and personal experience. Acta Biomed. 2022;93(1)1: e2022158.
28. Cacciari E, Milani S, Balsamo A, Spada E, Bona G, Cavallo L, Cerutti F, Gargantini L, Greggio N, Tonini G, Gargantini L.Italian cross-sectional growth charts for height, weight and BMI (2 to 20 yr). J Endocrinol Invest. 2006; 29:581-593.
https://doi.org/10.1007/BF03344156
PMid:16957405
29. Papanikolaou G, Pantopoulos K. Iron metabolism and toxicity. Toxicol Appl Pharmacol. 2005;202:199-211.
https://doi.org/10.1016/j.taap.2004.06.021
PMid:15629195
30. Voskou S, Aslan M, Fanis P, Phylactides M, Kleanthous M. Oxidative stress in β-thalassaemia and sickle cell disease. Redox Biol. 2015; 6: 226-239.
https://doi.org/10.1016/j.redox.2015.07.018
PMid:26285072 PMCid:PMC4543215
31. Goldberg EK, Lal A, Fung EB. Nutrition in Thalassemia: A Systematic Review of Deficiency, Relations to Morbidity, and Supplementation Recommendations. J Pediatr Hematol Oncol. 2022; 44(1): 1-11.
https://doi.org/10.1097/MPH.0000000000002291
PMid:34486568 PMCid:PMC8732300
32. Soliman A, De Sanctis V, Yassin M. Vitamin D status in thalassemia major: an update. Mediterr J Hematol Infect Dis. 2013;5:e2013057.
https://doi.org/10.4084/mjhid.2013.057
PMid:24106607 PMCid:PMC3787712
33. Huang J ,Shen J ,Yang Q, Cheng Z, Chen X, Yu T, Zhong J, Su Y, Biling Liang HG, Quantification of pancreatic iron overload and fat infiltration and their correlation with glucose disturbance in pediatric thalassemia major patients. Quant Imaging Med Surg. 2021; 11(2): 665-675,
https://doi.org/10.21037/qims-20-292
PMid:33532266 PMCid:PMC7779935
34. De Sanctis V, Soliman A, Daar S, Tzoulis P, Yassin MA, Di Maio S, Kattamis C. Insulin-like growth factor -1 (IGF-1) and glucose dysregulation in young adult patients with β-thalassemia major: causality or potential link? Acta Biomed. 2022; 93 (6): e2022331.