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Original Article

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

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Abstract. ***Background:*** **Glucose dysregulation (GD), including prediabetes and diabetes mellitus (DM), is a common complication of transfusion-dependent β-thalassemia (TDT) patients. The prevalence increases with age and magnitude of iron overload, affecting a significant proportion of patients. According to the international guidelines, the development of GD is frequently asymptomatic. Therefore, an early diagnosis requires an annual oral glucose tolerance test (OGTT) in all TDT patients aged ten years or older.**

Purpose: **This retrospective study aims to evaluate the prevalence of GD in a homogenous population of prepubertal TDT patients and to enhance understanding of the pathogenesis and progression of glucose homeostasis in this group of patients.**

Methods: **A selected group of 28 TDT patients was followed for at least 10.3 years (range: 10.3 - 28.10 years) from prepubertal age (mean 11.0 ± standard deviation 1.1 years) to adulthood (28.7 ± 3.7 years). Glucose tolerance and insulin response to OGTT were assessed, and indices of β-cell function, insulin sensitivity, and insulin secretion were calculated.**

Results: **At baseline, 18 TDT patients had normal glucose tolerance (NGT) and 10 had isolated impaired fasting glycemia (IFG), according to the American Diabetes Association (ADA) criteria. Compared to 18 healthy prepubertal controls (mean ± SD age: 10.9 ± 1.1 years), the fasting plasma glucose (FPG), basal insulin level, and Homeostatic Model Assessment for Insulin Resistance (HOMA-IR) index were significantly higher in the group of TDT patients (p= 0.001, 0.01 and 0.012, respectively). At the last observation, 7/18 patients (38.8%) with NGT and 9/10 (90%) with IFG at baseline deteriorated; 3 female patients developed type 2 DM (1 from the NGT group and 2 from the IFG group). Compared to adult controls, TDT patients with NGT had a reduced oral disposition index (DI) (p= 0.006) but no significant difference in HOMA-IR and Matsuda index. Conversely, all insulin indices (HOMA-IR, MI, and DI) but one [insulinogenic index (IGI)] were statistically different in TDT patients with GD compared to controls.**

Conclusion: **This study underlines the concept that the spectrum of glucose tolerance in TDT patients represents a continuum of glucose homeostasis disturbances and that prepubertal patients with IFG are at higher risk of developing a further deterioration of glucose metabolism with time. Moreover, it appears that one-third of adult TDT patients with normal fasting glucose may develop GD in the second-third decade of life. Thus, early intervention could help to prevent an expected further decline of glucose tolerance.**

Keywords: Thalassemia; Iron overload; Oral glucose tolerance test; Type 2 diabetes mellitus; Insulin secretion and sensitivity indices; Long-term follow-up.

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Introduction. Over the past 40 years, the improved life expectancy of patients with transfusion-dependent thalassemia (TDT) has led to the emergence of new complications, such as glucose dysregulation (GD), including prediabetes and diabetes mellitus (DM).[1] The progression from normoglycemia to DM takes several years and involves intermediate stages of dysglycemia. The wide variation in GD prevalence in TDT patients has been attributed to a number of factors: the patient's age, the total and annual blood consumption, degree of iron load, and the efficacy of chelation therapy based on the type of chelators and compliance to treatment. Higher serum ferritin levels (SF) and increased levels of liver enzymes may adversely affect glucose homeostasis.[1-4] Therefore, early detection of GD is expected to play an important preventive role in its deterioration; at present this is an area of considerable research interest for TDT patients.

Although GD has become a well-recognized complication of older children with TDT,[3,4] data are lacking on the natural history of this condition.

The aim of this retrospective study was to evaluate the progression of GD in a homogenous population of prepubertal TDT patients followed closely for a long period.

Patients and methods. The records of 28 prepubertal TDT patients followed annually for at least 10.3 years (range: 10.3 - 28.10 years) from prepubertal age (mean 11.0 ± standard deviation 1.1 years) to adulthood (28.7 ± 3.7 years) were reviewed. Glucose tolerance and insulin response to the oral glucose tolerance test (OGTT) were analyzed, and indices of β-cell function, insulin sensitivity, and insulin secretion were calculated. All TDT patients were of Italian ethnic origin.

Eighteen healthy prepubertal children (mean age: 10.9 ± 1.1 years; 10 males) and 16 healthy volunteer adult subjects **(**mean age: 23.6 ± 3.5 years; 8 males) served as controls. All were brothers, sisters, or cousins of TDT patients. None of them was a carrier for β-thalassemia or overweight.

Data Collection and Clinical Measurements. Data collection included: demographic characteristics, gender, age at first transfusion, the interval between transfusions, compliance to iron chelation, anthropometry (weight, height, BMI, pubertal status), and endocrine complications. Height and weight were measured according to international recommendations. Bodyweight was measured, wearing minimal underclothes, to the nearest 100 g on properly calibrated scales. BMI was calculated by the following formula: weight in Kg/ height in m^2. An adult patient was considered obese when BMI exceeded 30 Kg/m^2, overweight when BMI was 25 - 30 kg/m^2. A child or an adolescent (< 18 years) was defined as overweight when the BMI was between the 75th and 95th percentile and obese when the BMI was equal to or above the 95th percentile. A subject was defined as underweight when the BMI value was below the 5th percentile for age and sex in children and adults (>18 years) when BMI was < 19 kg/m^2.[5]

Laboratory methods and assessment of iron overload. Serum concentrations of alanine aminotransferase (ALT) and hepatitis C virus seropositivity (HCV ab and HCV-RNA) were recorded to evaluate liver status. The level of ALT was determined by an automated analyzer (normal range 0–40 U/L). HCV antibodies had been tested annually since 1991.

Serum ferritin (SF) was measured in the early years by radioimmunoassay at a serum dilution of 1:1000 and in the last few years by electrochemiluminescence immunoassays. The 90th percentile of reported normal values in females and males are 201 and 243 ng/ml respectively.[6]

To adequately discriminate between poorly chelated and well chelated patients, a cut-off point at SF1000.0 ng/mL was used.[7]

Cardiac and hepatic hemosiderosis were assessed by magnetic resonance imaging (MRI) T2* using a 1.5 T scanner (GE Signa/Excite HD, Milwaukee, WI, USA).[17] Global cardiac T2* values were expressed in msec, according to the following cut-off points: normal > 20 ms, mild: 14–20 ms, moderate: 10–14 ms, severe < 10 ms.[8] Liver iron content (LIC) was quantified using the calibration curve introduced by Wood et al..[9] The values were expressed in mg/g dry weight (d.w.) and classified into mild (LIC > 3 and < 7), moderate (LIC > 7 and < 14) and severe overload (LIC > 14).[10]

Testing procedure and interpretation of OGTT.

Glucose tolerance at baseline and during annual follow-up. The OGTT (1.75 g/kg, max 75 g) was performed in the morning, after an overnight fast, in subjects clinically stable and without a history of acute infection in the previous 3 weeks. In patients with IFG, two baseline measurements of plasma glucose (PG) were collected before OGTT. In addition, blood samples were collected from a venous catheter at 0, 30, 60, 90, and 120 minutes following oral glucose administration to measure plasma glucose and insulin. During the test, subjects remained at rest, either seated or lying. Plasma glucose was measured using an automated glucose oxidase reaction. Plasma insulin levels were determined by a commercial immunoassay technique.

Interpretation of plasma glucose levels after OGTT. Depending on the results of the OGTT, patients were classified into different subgroups of glucose metabolism according to the American Diabetes Association (ADA) criteria:[11]

- *Normal Glucose Tolerance* (NGT): Fasting plasma glucose (FPG) < 100 mg/dL (< 5.6 mmol/L) and 2-h PG < 140 mg/dL (< 7.8 mmol/L),
- *Impaired Fasting Glucose* (IFG): FPG between 100 and 125 mg/dL (5.6-6.9 mmol/L),
- *Impaired Glucose Tolerance* (IGT): 2-h PG between 140 mg/dL and 199 mg/dL (7.8-11.0 mmol/L),
- *Diabetes Mellitus* (DM): FPG ≥ 126 mg/dL (≥7.0 mmol/L) or 2-h PG ≥ 200 mg/dL (≥11.1 mmol/L).

When OGTT was diagnostic of DM in asymptomatic patients, it was repeated after 4-6 weeks.

Calculations of variables:

a) Insulin secretion index: For the evaluation of acute-phase serum insulin response, during OGTT, the insulinogenic index (IGI) 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 period (Δ Ins 30–0/ Δ Gluc 30-0).[12] The IGI is a proxy of the acute phase serum insulin response and was used for the evaluation of the β-cell function.

b) Insulin sensitivity indices: To assess insulin sensitivity, the Homeostatic Model Assessment index of insulin resistance (HOMA-IR) and Matsuda index were calculated with the following equations: HOMA-IR: fasting glucose x fasting insulin/405[13] and Matsuda index 0-120 (MI): [10,000/√ [(FPG 0 (mg/dL) x insulin 0 (μU/L)] x [(mean plasma glucose 0-120 (mg/dL) x mean insulin 0-120 (μU/L)].[14] The whole-body insulin sensitivity of MI combines both hepatic and peripheral tissue insulin sensitivity.

c) B-cell function index: To evaluate β-cell function adjusted for insulin sensitivity, the authors calculated the disposition index (DI) as the product of the IGI and MI (0-120 minutes during OGTT). The index reflects the relationship between the β-cell function and the peripheral insulin sensitivity, as the ability of β-cells to compensate for alterations in insulin sensitivity.[15,16] Substantially, the DI shows the failure of pancreatic β-cells to compensate for insulin resistance (IR) in subjects at high risk for developing type 2 diabetes and IFG.

Statistical analysis: All numeric variables were expressed as mean, ± standard deviation (SD). Comparison of different variables in the two groups was made using unpaired student t-test and Mann-Whitney test for normal and non-parametric variables, respectively. Continuous variables were also compared using a one-way analysis of variance (ANOVA). Chi-square (χ^2) test was used to compare the frequency of qualitative variables among the different groups. Pearson's and Spearman's correlation tests (2-tailed) were used to study correlations between variables with parametric and non-parametric distributions, respectively. A p-value < 0.05 was considered statistically significant. For the statistical analysis, a software program was used and validated, according to Alder and Roesser.[17]

Ethics: All procedures were in accordance with the 1964 Helsinki declaration and its later amendments in October 2013 (www.wma.net). The protocol was approved by the institutional board with the agreement of the Thalassemia Patients' Association (protocol number: 6/2018). Informed consent was obtained from parents and each TDT patient after a detailed explanation of the procedures for performing the OGTT test, the nature and purpose of the study, and the patient's benefits for collecting such information.

Results. At baseline, 18 TDT patients had normal glucose tolerance (NGT) and 10 had isolated impaired fasting glycemia (IFG), according to the American

Diabetes Association (ADA) criteria. The FPG in 7 out of 10 ten patients with isolated IFG were between 100-109 mg/dL (mean 103.8 ± 3.4 mg/dL) and between 110 -125 mg/dL (mean 117.3 ± 5.5 mg/dL) in the remaining 3 patients.

Compared to 18 healthy prepubertal controls (mean ± SD age: 10.9 ± 1.1 years), the fasting plasma glucose (FPG), basal insulin level, and Homeostatic Model Assessment for Insulin Resistance (HOMA-IR) index were significantly higher in the group of TDT patients (p= 0.001, 0.01 and 0.01, respectively) (**Table 1**).

A significant linear correlation was observed between FBG and HOMA- IR (r = 0.42749, p = 0.023), and an inverse correlation between HOMA- IR and MI (r = -0.615, p = 0.00049) and between 2-h PG and DI (r = -0.378, p = 0.047) in the prepubertal TDT group. No correlation was observed between basal FPG in patients with IFG and plasma glucose level at 2-h after OGTT (r: 0.2356, p= 0.51).

At first detection of glucose tolerance deterioration registered during the annual follow-up and mean age at the peak of serum ferritin level. During the follow-up, 11 out of 18 patients with NGT (61.1%) had deterioration of glucose homeostasis: 2 developed isolated IFG, 8 IGT, and one a combination of IFG and IGT. Of the 10 TDT patients with isolated IFG at baseline, 2 developed IGT, 7 IFG plus IGT. One patient with a SF of 1055.0 ng/mL reverted to NGT.

The mean age at the first detection of glucose deterioration was 23.0 ± 4.9 yrs in patients with NGT at baseline, and 17.2 ± 4.9 yrs in patients with isolated IFG (p = 0.042). The time interval from baseline to deterioration of glucose homeostasis in the two groups of patients (NGT vs. IFG) was 12.1 ± 6.1 yrs and 7.3 ± 5.7 yrs (p= 0.044), respectively. At 20 yrs of age, 36% of 28 TDT patients were identified by annual OGTT as having GD. Eight patients were females, and 12 were males.

A SF peak of 2900.5 ± 1128.5 ng/mL was registered at a mean age of 15.6 ± 5.9 years.

The mean SF level at the first appearance of GD in the entire group of 20 TDT patients who developed GD was 2031.4 ± 1291.8 ng/mL, and the mean ALT level was 53.3 ± 56.4 mU/mL.

Interestingly, the mean ALT level was higher and statistically different in TDT patients with isolated IFG at baseline compared to TDT patients with NGT at baseline (60 ± 33.5 mU/mL vs. 29.3 ± 13.7 mU/mL, p= 0.011). Still, no significant difference was found between the SF levels in the two groups of patients (2288.4 ± 1420.3 ng/mL vs. 1821.1 ± 383.2 ng/mL, p = 0.44).

All patients but one tested after 1990's for HCV antibodies were seropositive. HCV-RNA positivity was present in 10/28 patients (35.7%). Three different HCV genotypes, 1b (61.1%), 2a (22.2%), and 3a (16.6%) were identified.

At last observation.

a. Clinical characteristics: The mean age of our study cohort of 28 TDT patients at the last observation was 29.0 ± 4.7 yrs. There was no significant difference between patients with NGT and GD regarding age, BMI, family history of diabetes, and splenectomy. Two

Table 1. Clinical and laboratory characteristics of 18 TDT patients with normal glucose tolerance (NGT) and 10 TDT patients with isolated impaired fasting glucose (IFG) after OGTT compared to 18 healthy prepubertal controls. The values are expressed as mean ± SD.

Variables at baseline	Prepubertal TDT patients (28 patients)	Controls (18 subjects)	P value
Age	10.6 ± 1.3	10.9 ± 1.1	N.S.
Gender (Males/Females)	12/16	10/8	N.S.
BMI (Kg/m 2)	17.8 ± 3.0	18.3 ± 3.7	N.S.
Serum ferritin (ng/mL)	2418.3 ± 1216.8	39.7 ± 10.6	**< 0.0001**
ALT (U/L)	40.5 ± 24.1	10.1 ± 2.0	**< 0.0001**
Fasting plasma glucose (mg/dL)	94.1± 13.1	81.2 ± 10.6	**0.001**
Plasma glucose 2 h after OGTT (mg/dL)	63.2 ±56.6	88.5±15.6	N.S.
Fasting insulin (µU/ml)	9.1 ± 4.2	6.0±3.1	**0.01**
Insulin peak (µU/ml)	57.9 ± 29.1	50.1±36.9	N.S.
MATSUDA INDEX (MA)	8.8 ± 3.0	9.6±5.1	N.S.
HOMA-IR	1.8 ± 0.80	1.2±0.7	**0.01**
Insulinogenic Index (IGI)	1.67 ± 2.22	1.6±1.4	N.S.
Oral disposition Index (DI)	10.3 ± 11.5	12.8±9.6	N.S.

patients with IFG at baseline became overweight and obese, and 4 became underweight (3 in the patients with NGT at baseline).

b. Glucose dysregulation (GD): At the last observation, the occurrence of GD in the total group of TDT patients (29.0 ± 4.7 yrs) was significantly higher compared to baseline. Seven out of 18 patients (38.8 %) with NGT at baseline and 9/10 (90%) with IFG developed deterioration of glucose homeostasis (**Figure 1**).

However, a regression/improvement of GD was observed at the last observation in two patients (one patient with NGT at baseline who developed IGT during the follow-up and one patient with IFG at baseline).

The mean duration between two consecutive OGTTs was 1.16 ± 0.34 yrs in the 12 patients with NGT and 1.14 ± 0.28 yrs in the group of 16 patients who developed GD (**Table 2).**

c. Indices of insulin secretion, insulin sensitivity and pancreatic β-cell function: Compared to adult healthy control subjects, TDT patients with NGT had a reduced DI (p= 0.006), but no significant difference in HOMA-IR and MI indices. Conversely, 3 insulin indices (HOMA-IR, MI and DI) differed significantly between TDT patients with GD and controls. IGI did not differ between the two groups (**Table 2**). In TDT patients, no correlation was detected between MI and DI and the patient's age, BMI, SF, and ALT. An inverse correlation was found between HOMA IR and ID (r: -0.542, p = 0.030). Finally, comparing the 4 insulin indices (IGI, HOMA IR, MI, and DI), a significant reduction was found at baseline and at the last examination in the IGI and DI indices (p = 0.005 and 0.004 respectively).

d. Assessment of iron overload and iron chelation therapy: At last observation, 6/12 patients (50 %) with NGT had a SF < 1000 ng/mL (568.5 ± 188.8 ng/mL) and 7/18 patients (38.8%) in those with IFG (548.2 ± 210.8 ng/mL). Moreover, a global cardiac T2* value from 10 to 14 ms (values < 20 ms indicate cardiac iron overload that is severe in patients with a level < 10 ms) was observed in 3/10 patients (30%) with NGT and in 5/12 patients (41.6%) with GD. The quantification of LIC, assessed by MRI, was reported as moderate (> 7 and < 14 mg Fe/g d.w.) in 1 female patient with GD and severe (> 14 mg Fe/g d.w.) in 3/8 patients (37.5%) with NGT (2 females) and 3/14 patients (21.4%) with GD (2 females). Thirteen (46.4%) of the total group of TDT patients were on treatment with DFO. Most of them (7/12; 58.3 %) were in the group of patients with NGT.

e. Liver and endocrine associated complications: Alanine aminotransferase (ALT) values above the normal range (40 U/L) were present in 4 patients with GD and none with NGT. The prevalence of HCVAb and HCV- RNA positivity in both groups is reported in **Table 2.**

The commonest endocrine complications in the 12 patients with NGT were primary amenorrhea (in 1 patient, associated with growth hormone deficiency) and secondary amenorrhea (affecting 4 patients, one of whom also exhibited severe short stature). In the group of 16 patients with GD, the commonest endocrine complications were hypogonadotropic hypogonadism in

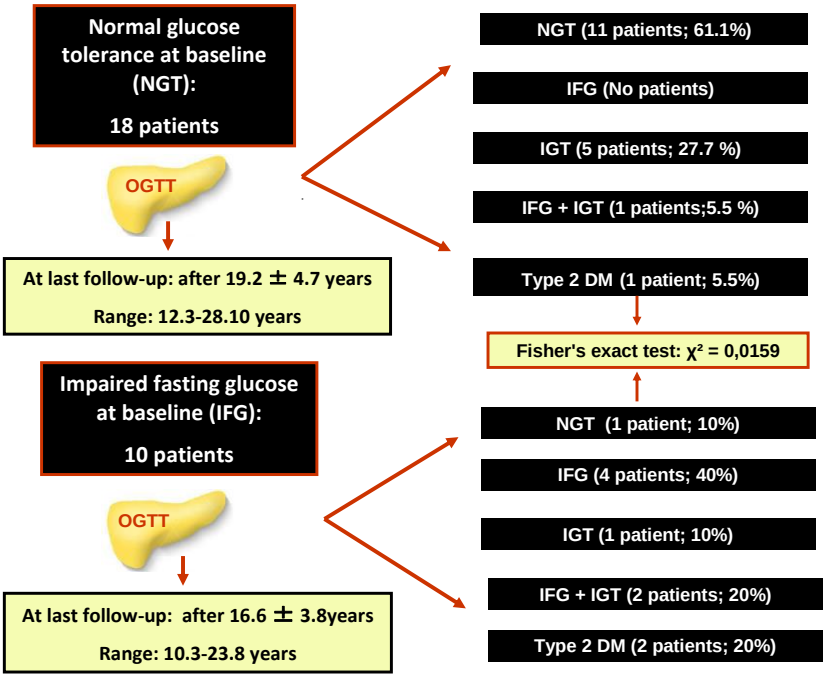

Figure 1. Glucose abnormalities, after OGTT, at last observation. Legend: NGT: normal glucose tolerance; IFG: impaired fasting glucose; IGT: impaired glucose tolerance; Type 2 DM: type 2 diabetes mellitus.

Table 2. Clinical and laboratory characteristics in 12 TDT patients with normal glucose tolerance (NGT) and glucose dysregulation (GD), after OGTT, at last observation compared to adult controls. The values are expressed as mean ± SD.

Variables at last observation	TDT patients with NGT (n: 12) Group A	TDT patients with glucose abnormalities (n:16) Group B	Controls (n.16) Group C	P-value A vs. B	P-value B vs. C
Chronological age (yrs)	29.6 ± 4.4	29.3 ± 5.3	23.6 ± 3.5	N.S.	**0.0005**
Gender (Males/Females)	3/9	9/7	8/8	N.S.	-
BMI (Kg/m ^{2})	21.9 ± 2.8	21.5 ± 3.4	21.5 ± 2.0	N.S.	N.S.
Family history of diabetes	4	6	2	N.S.	-
- Type 1	1	1	0	N.S.	-
- Type 2	3	5	2	N.S.	-
Splenectomy (yes)	4/12	8/16	-	N.S.	-
Serum ferritin at last observation (ng/mL)	1033.3 ± 607.1	1370.6 ± 1106.1	-	N.S.	-
ALT (U/L)	32.9±19.6	55.0 ± 42.7	-	N.S.	-
HCVAb positivity	12	16	-	-	-
HCV-RNA positivity	4	6		-	-
Liver iron concentration (LIC: mg Fe/g dry weight)	6.42 ± 6.69	8.38 ±10.27	-	N.S.	-
	(n:10)	(n:14)			
Fasting plasma glucose (mg/dL)	89.5 ± 5.4	103.3 ± 15.1	83.5 ± 8.6	**0.005**	**0.0001**
Plasma glucose 2 h after OGTT (mg/dL)	122.8 ± 13.1	161.3 ± 35.3	89.9 ± 16.4	**0.001**	**0.00001**
Fasting insulin (µU/ml)	5.7 ± 3.1	6.9 ± 3.3	5.6 ± 3.4	N.S.	N.S.
Insulin peak (µU/ml)	52.9 ± 26.6	57.5 ± 16.8	54.5 ± 16.3	N.S.	N.S.
Iron chelation therapy:					
Desferrioxamine (DFO)	7 (58.3 %)	6 (37.5 %)	-	-	-
Deferiprone (DFP)	3 (25.0 %)	4 (25.0 %)	-	-	-
DFO + DFP	2 (16.6 %)	4 (25.0 %)	-	-	-
Deferasirox (DFX)	0 (0 %)	2 (12.5 %)	-	-	-
Cardiac T2*	30.9 ± 16.1	27.4 ± 12.8	-	N.S.	-
Number of patients	(n: 9)	(n: 12)			
Associated endocrine complications	5/12 (41.6%)	10/16 (62.5%)	-	N.S.	-
Insulinogenic Index (IGI)	0.95 ± 1.0	1.0 ± 2.2	1.7 ± 1.5	N.S.	N.S.
HOMA-IR	1.27 ± 0.76	2.1 ± 1.3	1.2 ± 0.8	0.059	**0.025**
MATSUDA INDEX 0-120 (MI)	7.46 ± 3.4	5.48 ± 3.3	8.62 ± 3.52	N.S.	**0.014**
Oral disposition Index (DI)	4.89 ± 2.9	2.4 ± 1.6	13.8 ± 10.1	**0.0074**	**0.0001**
Total number OGTTs	14.9 ± 2.3	15.8 ± 3.9	-	N.S.	-
Mean interval between OGTTs (yr)	1.16 ± 0.34	1.14 ± 0.28	-	N.S.	-

Legend: **N.S.**= not significant.

8 patients (6 females), of whom one had concomitant primary hypothyroidism.

Discussion. In the last few decades, along with the significant increase in life expectancy of patients with TDT, new complications have emerged. GD is frequent

among TDT patients on conventional treatment with regular blood transfusions and chelation treatment. Because of the insidious onset of GD, the current international guidelines recommend annual screening for GD in all TDT patients from the age of ten years (or earlier in the presence of iron overload), using the 2-h OGTT.[18]

Hemoglobin A1c (HbA1c) is not routinely used for screening because of its low sensitivity in this population.[19,20]

The recommended annual screening is based on the evidence that pancreatic iron loading in TDT patients starts in early childhood[22-23] and that an efficient chelation regimen with DFO alone[24] or in combination with DFP,[23] in the early stages of dysglycemia, can prevent GD.

Prediabetes is a type of glucose dysregulation representing an intermediate stage between NGT and DM. According to ADA criteria, it consists of two subcategories: IFG, defined as an FPG concentration of 100–125 mg/dL, and IGT, defined as a 2-h PG concentration, after OGTT, of 140–199 mg/dL. The two dysglycemic conditions have different underlying pathophysiological patterns. Subjects with IFG exhibit a hepatic IR and impaired early insulin secretion during OGTT and subjects with IGT have muscle IR and impairment of late-phase insulin secretion.[25-27]

Early diagnosis of prediabetes is essential for the prompt identification of high-risk individuals who will benefit from intensive iron chelation therapy and lifestyle modification. However, it is still unclear whether the ADA diagnostic criteria[24] or higher thresholds, as suggested by WHO[28] should be used in TDT patients to define IFG. The WHO defines a subject with IFG when the FPG corresponds to 110-125 mg/dL (6.1 mmol/L- 6.9 mmol/L) compared to the ADA lower criteria of FPG levels (100 mg/dL-125 mg/dL = 5.6 mmol/L- 6.9 nmol/L). The criteria of WHO and ADA for the definitions of IGT and DM are the same.

In the present retrospective study, using the ADA criteria, the prevalence of isolated IFG was 18.3%, while increasing the threshold value of FPG to 110 mg/dL (6.1 mmol/L), according to WHO criteria decreased the prevalence to 5.7%. Interestingly, the current study found a progressive deterioration of glucose tolerance after OGTT, using ADA criteria, in 9 out of 10 prepubertal TDT patients with IFG at baseline.

Focusing on the progression of GD in TDT patients with NGT at baseline, 5 out 18 patients (27.7%) developed, at last observation, an IGT (27.7%) and 1 patient a DM (5.5%). Moreover, a reduced oral disposition index (DI) of 2.4 ± 1.6 was observed in TDT patients with GD compared to control group (13.8 ± 10.1) and to TDT patients with NGT at the last observation (4.89 ± 2.9) (p: 0.0001 and 0.0074, respectively). These observations would explain the real role of performing a periodic OGTT in clinical practice. Although this recommendation especially refers to patients with iron overload, it is noteworthy that 6 TDT patients (3 males and 3 females, aged 32.8 ± 5.1 years) with NFG and IGT or DM, after OGTT, had a mean SF level of 1026.8 ± 575.6 ng/mL (range 506 - 2221 ng/mL).

The limitations of this study include: 1) the relatively small sample size of patients recruited from a single center; 2) the formulas used in our study because none of the OGTT indices reveal exactly the same information as those obtained during hyperinsulinemic-euglycemic clamps and hyperglycemic clamps; 3) all measures of insulin sensitivity or response do not necessarily follow a hyperbolic pattern; 4) the insulinogenic index that we used (ΔI 0–30/ΔG0–30) included only two insulin measurements, and finally 5) a modern evaluation of iron overload in the pancreas by magnetic resonance imaging was not done. However, we believe that these limitations were unlikely to have had an important effect on the validity of the long-term follow-up findings.

Conclusions. Our study underlines the concept that the spectrum of glucose tolerance in TDT patients represents a continuum of glucose homeostasis disturbances and that prepubertal patients with IFG are at higher risk of developing a further deterioration of glucose metabolism with time. Moreover, it appears that one-third of adult TDT patients with normal fasting glucose may develop GD in the second-third decade of life (mean age: 32.8 ± 5.1 years). Thus, early intervention could help to prevent an expected further decline of glucose tolerance.

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