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Original article

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)

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Abstract. *Background:* Patients with transfusion dependent β-thalassemia (TDT) are at high risk for developing, over the time, a form of diabetes distinct from type 1 and type 2 diabetes, but with similarities to both. *Aims of study:* The aim of this study is to describe the clinical and laboratory data, and the insulin secretion and sensitivity, in TDT patients recently diagnosed with diabetes mellitus (DM). *Materials and Methods:* The medical records of 25 TDT patients with DM, diagnosed by standardized oral glucose tolerance test (OGTT) and insulin secretion, were analysed; data were compared to TDT patients without diabetes and to a group of healthy subjects. Natural history of glucometabolic status before the diagnosis of DM was also reviewed. *Results:* On average, the TDT patients with DM were younger compared to TDT patients without diabetes. The mean age at diagnosis of DM in female and male TDT patients was 24.0 ± 7.1 years and 31.9 ± 5.6 years, respectively (P: 0.007). Serum alanine aminotransferase values, basal insulin levels and prevalence of hypogonadism were consistently higher in TDT patients with DM compared to those without diabetes. Decreased insulin secretion and increased insulin resistance was observed in patients with DM. *Conclusion:* The natural history of glucometabolic status in TDT patients is characterized by a deterioration of glucose tolerance over time. Iron overload and liver dysfuction are the main factors responsible for glucose disturbances (GD) in TDT patients. The therapeutic approach must be individualized and followed by a multidisciplinary team. (www.actabiomedica.it)

Keywords: β-thalassemia, thalassemia- related glucose disturbances, diabetes, insulin deficiency, insulin resistance, treatmen

Introduction

The maintenance of normal glucose homeostasis involves the simultaneous and coordinated roles of insulin release from the pancreatic β-cells and insulin action on peripheral tissues, primarily muscle (1,2). Diabetes comprises disorders characterized by hyperglycaemia. The diagnosis of diabetes is based on (at least) one of the following criteria: a fasting plasma glucose (FPG) ≥ 126 mg/dl (7.0 mmol/L), a random plasma glucose ≥ 200 mg/dl (11.1 mmol/L) in the presence of symptoms, a 2-h plasma glucose during the 75-g oral glucose tolerance test (OGTT) ≥ 200 mg/dl (11.1 mmol/L) and/or hemoglobin A1c (HbA_{1c}) ≥ 6.5% (3).

In the general population, approximately 95% of adults diagnosed with diabetes have type 2 diabetes (T2DM), and 5% type 1 diabetes (T1DM) (4,5). The etiology of T1DM and T2DM is multifactorial in which genetic predisposition plays a key role. The distinction between T1DM and T2DM has been based historically on four factors: age at onset, degree of loss of β-cell function, degree of insulin resistance (IR), and presence of diabetes-associated autoantibodies (4,5). In T1DM, hyperglycemia is due to complete or near-complete destruction of insulin-producing β-cells in the pancreatic islets. In T2DM, hyperglycemia is initially characterized by impaired insulin sensitivity and subsequently by inadequate compensatory insulin secretion.

In addition to T1DM and T2DM, other less common types of diabetes exist that can present diagnostic challenges and require individual management and care planning (5,6). A typical example is the diabetes of transfusion dependent β-thalassemia (TDT) patients which has not yet been clearly classified and differs from that of T1DM and T2DM in healthy people, although it has similarities with both. The diagnosis of diabetes in TDT patients is made using the criteria of the other forms of diabetes, including a diabetic response to an OGTT, fasting hyperglycemia, or random glucose elevation in the presence of classic symptoms of diabetes. HbA_{1c} levels are

considered unreliable in transfused patients with hemoglobinopathies (7).

Because diabetes in thalassemia has been recognized as a specific form of GD and advances in understanding the mechanisms leading to hyperglycaemia in the subtypes forms of diabetes are relevant for a better classification of diabetes, personalized therapeutic intervention and treatment (8), the International Network on Endocrine Complications in Thalassaemia (ICET-A) planned a study on patients with TDT recently diagnosed with diabetes mellitus.

In the present study, we report the clinical characteristics, laboratory data and natural history of glucometabolic status in the five years prior to the diagnosis of DM in 25 patients with TDT. In these patients, in addition to standardized OGTT, we also evaluated the insulin secretion and sensitivity during OGTT to investigate the role of insulin resistance in the pathophysiology of recently diagnosed DM patients with TDT. The collected data were compared to a group of non-diabetic TDT patients and to healthy controls.

Patients and Methods

a. Study population and design

The study included 25 TDT patients who were consecutively referred for consultation or second opinion to an Italian center for GD over the last decade (June 2014 and March 2021). Group A:10 patients with DM [FPG ≥ 126 mg/dL (≥7.0 mmol/L) and 2-h after OGTT ≥ 200 mg/dL (≥11.1 mmol/L)], Group B:13 patients with IFG [FPG between 100 and 125 mg/dL (5.6-6.9 mmol/L)], and Group C: 2 patients with hypogonadism, secondary to hypothalamic/pituitary deficiency, normal FPG and PG 2-h after OGTT ≥ 200 mg/dL (≥11.1 mmol/L).

The following data were retrieved from the medical records: age, gender, ethnicity, life style, anthropometry [weight, height, body mass index (BMI), pubertal status], natural history of glucose tolerance in the previous five years before the diagnosis of DM, age at first transfusion, interval between transfusions, compliance to iron chelation therapy, age at splenectomy and presence of associated cardiac, hepatic and endocrine complications. All patients had been on regular blood transfusions from the first 14 months of life and started iron chelation by the age of 2 years.

b. Procedures

Height and weight were measured according to international recommendations. Body weight was measured, wearing minimal underclothes, to the nearest 100 g on properly calibrated scales (9). 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 75^{th} and 95^{th} percentile, and obese when the BMI was equal to or above the 95^{th} percentile (10). Puberty was assessed using the standards of

Tanner and Marshall for breast development in girls (using inspection and palpation), genital development in boys, and pubic hair in boys and girls (11).

To evaluate the liver status, the concentrations of serum alanine aminotransferase (ALT) and positivity for hepatitis C infection (HCVAb and HCV-RNA) and liver iron content were collected.

Iron overload was assessed by serum ferritin and classified as mild (serum ferritin < 1.000 ng/mL), moderate (serum ferritin >1.000 ng/mL and < 2.000 ng/mL) and severe (serum ferritin >2.000 ng/mL) (12). Global myocardial and liver iron overload data, assessed by magnetic resonance imaging (MRI), less than 6 months before the first consultation, were also included. Cardiac MRI T2* values were expressed in msec (T2* normal values: < 20 msec) (13). Liver iron content (LIC) was quantified using the calibration curve introduced by Wood et al. (13). 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) (14).

c. Oral glucose tolerance test (OGTT) and insulin secretion

In TDT patients without fasting hyperglycaemia, an OGTT (1.75 g/kg, max 75 g) after an overnight fast was required. 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. Plasma glucose was measured using an automated glucose oxidase reaction. Plasma insulin levels were determined by a commercial immunoassay technique. According to Crofts et al. (15), the insulin secretory capacity was defined as reduced if all insulin values during OGTT were ≤ 30 μU/mL. Moreover, the integrated insulin secretion was measured as the area under the curve (AUC-INS 0–120 min), calculated by the trapezoid rule. The ADA criteria were used for the diagnosis of normal glucose tolerance (NGT), impaired fasting glucoser (IFG) and impaired glucose tolerance (IGT) (3).

Sixteen healthy volunteer adult subjects (mean age: 23.6 ± 3.5 years; 8 males, 50%) and 12 TDT patients with normal glucose tolerance test (NGT; 3 males, 25%) served as controls (16). None of them was carrier for β-thalassemia or overweight/obese (Table 1).

d. Calculation of variables

1) 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 time period (Δ Ins 30–0/ Δ Gluc 30-0) (17). The IGI is a proxy for the acute phase serum insulin response and was used for the evaluation of the β-cell function.

2) Insulin sensitivity indices

To assess insulin sensitivity, the Homeostatic Model Assessment index of insulin resistance (HOMA-IR) and Matsuda index were calculated using the following equations: HOMA-IR: fasting

glucose x fasting insulin/405 (18) 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)] (19). The HOMA index estimates IR from fasting glucose and insulin concentrations, but primarily reflects hepatic IR rather than peripheral IR. The whole-body insulin sensitivity of MI combines both hepatic and peripheral tissue insulin sensitivity.

3) β-cell function index

To evaluate β-cell function adjusted for insulin sensitivity, disposition index (DI) was calculated as

the product of the IGI and MI. 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 (20,21). Basically, the DI shows the failure of pancreatic β-cell to compensate for IR in subjects at high risk for developing T2DM and IFG. Low DI implies intrinsic β-cell dysfunction and may be associated with irreversible damage.

Statistical analysis

All numeric variables were expressed as mean ± standard deviation (SD). Comparison of different variables in the two groups was made using the unpaired

student t-test and Mann-Whitney test for normal and nonparametric variables respectively. Continuous variables were also compared

using 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. For the statistical analysis, a software program was used and validated, according to Alder and Roesser (22). A p value < 0.05 was considered statistically significant.

Ethics

All procedures were in accordance with the 1964 Helsinki declaration and its later amendments. According to Italian regulations, ethics approval by the local Ethics Committee was not required for the following reasons: no identifiable private information was collected; patients underwent only routine diagnostic and therapeutic procedures according to current guidelines (23,24); and an anonymized data set was analyzed. Informed consent was obtained from all patients after detailed explanation of the nature and purpose of the study and the likely risks and benefits associated with study participation.

Results

a. Patients' characteristics and laboratory findings

Twenty-five TDT subjects (aged 24.1 ± 6.6 years, range 14.6- 40.4 years;10 males, 40%) were diagnosed with DM, according to ADA criteria (5). The age distribution is illustrated in figure 1. The mean age at diagnosis of DM in female and male TDT patients was 24.0 ± 7.1 years and 31.9 ± 5.6 years respectively (P: 0.007).

The natural history of glucometabolic status in the five years that preceded the diagnosis of DM in the 3 groups of TDT patients (A, B and C) is presented in tables 1 and 2. Interestingly, in 6 of 25 patients (24%) the screening revealed a high variability of glucose tolerance (Tables 1 and 2), mainly in group B and C patients. Moreover, the recommended regular annual screening had been implemented in only 3 of 25 patients (12%).

A detailed description of clinical characteristics, biochemical data, image parameters, and treatments with iron chelation therapy at diagnosis of DM is presented in table 3.

On average, the TDT patients with newly diagnosed diabetes were younger compared to TDT patients without diabetes. Their ALT values, basal insulin levels and prevalence of hypogonadism were consistently higher compared to TDT patients with normal glucose tolerance (69.08 ± 45.54 U/L vs. 32.9 ±19.6 U/L, P :0.01; 11.6 ± 6.5 μU/ml vs. 5.7 ± 3.1 μU/ml, P: 0.005 and 92% vs. 41.6%, P: 0.008, respectively). The other clinical and biochemical parameters, including gender, BMI, splenectomy, serology for markers of hepatitis C infection, chelation therapy, serum ferritin levels and cardiac T2* values, showed no significant differences between the two TDT groups (Table 3).

Moreover, when we compared all these variables of group A patients with the 13 group B patients, a statistical difference was found in regard to age (19.5 ± 5.1 yr vs. 27.1 ± 5.9 yr , P: 0.003), ALT: (90.2 ± 51.0 U/L vs. 55.6 ± 35.1 U/L, P: 0.048), plasma glucose 2-h after OGTT (248.7 ± 37.7 mg/dL vs. 214.4 ± 8.9, P: 0.004) and HOMA-IR (4.8 ± 3.0 vs. 2.7 ± 1.67, P: 0.044).

Three of 10 group A patients (30%) had severe iron overload (serum ferritin mean value: 4.144 ± 1.223 ng/mL) and a reduced insulin secretory capacity, according to the cut-off level reported by Crofts et al. (15).

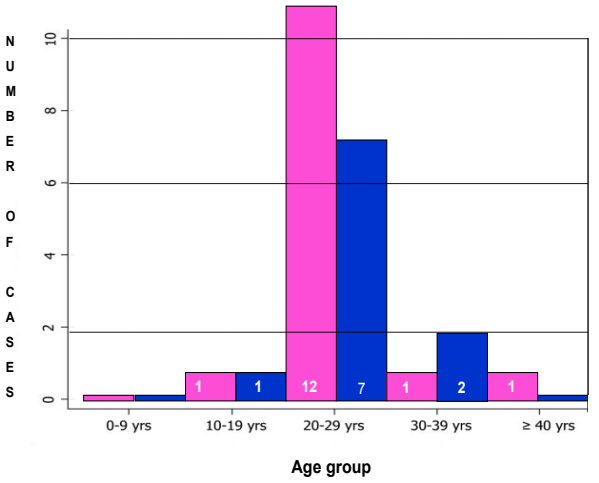

Figure 1. Age distribution of TDT patients with diabetes mellitus (pink bars: females; blue bars: males)

Table 1 Natural history of glucometabolic status in the five years before the diagnosis of DM in TDT patients of group A.

Group A No.	AGE/GENDER	-1 years	-2 years	-3 years	-4 years	-5 years
1	**18.1/F**	ND	ND	ND	IFG +IGT	ND
2	**25.2/M**	IFG	IFG +IGT	IFG	ND	ND
3	**14.6 /F**	ND	ND	ND	ND	ND
4	**16.11/F**	IFG+IGT	NGT	NGT	ND	ND
5	**32.5 /F**	IGT	NGT	ND	IFG+IGT	ND
6	**16.0/F**	IFG+IGT	ND	ND	ND	ND
7	**17.4/M**	ND	ND	ND	ND	ND
8	**17.4/M**	IFG+IGT	IFG+IGT	NGT	ND	ND
9	**21.11/F**	IFG+IGT	IFG+IGT	IFG+IGT	IFG+IGT	ND
10	**17.10/F**	IFG+IGT	IFG+IGT	IFG	ND	ND

Legend: ND: not done; NGT: normal glucose tolerance; IFG: impaired fasting glucose; IGT: impaired glucose tolarance; DM: diabetes mellitus.

Table 2. Natural history of glucometabolic status in the five years before the diagnosis of DM in TDT patients of groups B and C .

Group B No.	AGE/GENDER	-1 years	-2 years	-3 years	-4 years	-5 years
1	**32.4/ M**	NGT	IFG+IGT	NGT	NGT	NGT
2	**33.0/M**	IFG	IFG	IFG	IGT	NGT
3	**22.11/M**	IFG+IGT	IFG+IGT	IGT	IGT	ND
4	**20.5/ F**	IGT	ND	ND	ND	ND
5	**24.5/M**	NGT	NGT	NGT	NGT	IGT
6	**28.6/M**	IFG+IGT	IFG+IGT	NGT	IFG+IGT	IFG+IGT
7	**40.4/ F**	IFG+IGT	IFG	ND	ND	ND
8	**31.5/F**	ND	ND	IFG+IGT	ND	IFG
9	**28.8/F**	IFG+IGT	NGT	IFG	IGT	NGT
10	**25.11/F**	IGT	IGT	IGT	NGT	ND
11	**22.7/F**	NGT	ND	ND	IFG+IGT	NGT
12	**26.4/F**	NGT	NGT	NGT	NGT	NGT
13	**17.11/M**	ND	NGT	NGT	ND	ND
Group C No.						
1	**29.1/F**	IGT	IGT	IGT	IGT	NGT
2	**27.0/M**	NGT	IGT	IGT	IGT	IGT

Legend: ND: not done; NGT: normal glucose tolerance; IFG: impaired fasting glucose;IGT: impaired glucose tolarance.

Totally, six patients were on treatment with L-thyroxine due to primary hypothyroidism (associated with hypoparathyroidism in 3 cases). Short stature (height below the 3rd percentile for age and sex) was present in 7 patients (28%).

c. Insulin secretion and insulin sensitivity

Interestingly, in fifteen of the 25 patients with NDM (60%), the time required to reach peak insulin level after OGTT was delayed (120 min) compared to TDT patients with normal glucose tolerance tolerance and healthy control subjects (30-60 min). No correlation was observed between IGI and PG level at 1-h and 2-h during OGTT.

Comparing the 25 TDT patients with NDM to 28 individuals with NGT (12 TDT and 16 healthy control subjects), a statistical difference was found in the following indices of hepatic and peripheral tissue insulin sensitivity, and β-cell function adjusted for insulin sensitivity: IGI after OGTT, Matsuda index and DI, respectively (Table 4).

Table 3. Demographic, clinical, biochemical, and image parameters in 25 TDT patients recently diagnosed with diabetes mellitus (DM: Group A) compared to 12 TDT patients with normal glucose tolerance (NGT: Group B) and 16 healthy controls.

Variables	TDT patients with DM after OGTT (n. 25) Group A	TDT patients with NGT (n. 12) Group B	Control subjects (n.16)	P value Group A vs. Group B	P value Group A vs. Group C
Chronological age (yrs)	24.1 ± 6.6	29.6 ± 4.4	23.6 ± 3.5	**0.01**	0.78
Gender (Males/Females)	10/15	3/9	8/8	0.16	-
BMI (Kg/m ^{2})	23.4 ± 4.4	21.9 ± 2.8	21.5 ± 2.0	0.28	0.11
Family history of diabetes	9	4	2	-	-
- Type 1	2	1	0	-	-
- Type 2	7	3	2	-	-
Splenectomy (yes)	15/25 (60%)	4/12 (33.3%)	-	0.13	-
Serum ferritin (ng/mL)	1955.7 ± 1749.9	1033.3 ± 607.1	-	0.08	-
ALT (U/L)	69.08 ± 45.54	32.9 ±19.6	-	**0.01**	-
HCVAb positivity	22 (88%)	12 (100%)	-	0.21	-
HCV-RNA positivity	16 (64%)	4 (33.3%)	-	0.08	-
Fasting plasma glucose (mg/dL)	123.8 ± 20.4	89.5 ± 5.4	83.5 ± 8.6	**< 0.0001**	**< 0.0001**
Plasma glucose 2 h after OGTT (mg/dL)	229.8 ± 29.7	122.8 ± 13.1	89.9 ± 16.4	**< 0.0001**	**< 0.0001**
Fasting insulin (µU/ml)	11.6 ± 6.5	5.7 ± 3.1	5.6 ± 3.4	**0.005**	**0.001**
Insulin peak (µU/ml)	61.4 ± 52.2	52.9 ± 26.6	54.5 ± 16.3	0.59	0.61
Iron chelation therapy					
DFO	16 (64.0%)	7 (58.3 %)	-		-
DFP	5 (20.0%)	3 (25.0 %)	-		-
DFO+DFP	4 (16%)	2 (16.6 %)	-		-
DFX	0 (0 %)	0 (0 %)	-		-
Liver iron concentration (LIC: mg Fe/g dry weight)	6.9 ± 4.1 (n:10)	6.4 ± 6.6 (n:10)	-	0.84	-
Cardiac T2* **Number of patients**	22.06 ± 11.7 (n.10)	30.9 ± 16.1 (n: 9)	-	0.11	-
Hypogonadism	21/25 (84%)	5/12 (41.6%)	-	**0.008**	-
Insulinogenic Index (IGI)	0.35 ± 0.27	0.95 ± 1.0	1.7 ± 1.5	**0.007**	**0.0001**
HOMA-IR	3.59 ± 2.50	1.27 ± 0.76	1.2 ± 0.8	**0.003**	**0.0007**
MATSUDA INDEX 0-120 (MI)	4.26 ± 2.90	7.46 ± 3.4	8.62 ± 3.52	**0.005**	**0.0001**
Oral disposition index (DI)	1.08 ± 0.73	4.89 ± 2.9	13.8 ± 10.1	**< 0.0001**	**< 0.0001**

Legend: BMI = body mass index; ALT: alanine aminotransferase: normal values: <40 mU/ml; SF: serum ferritin, 50th centile: 105 ng/mL in males and 35 ng/mL in females; OGTT: Oral glucose tolerance test; DM: diabetes mellitus; NGT: normal glucose tolerance test; DFO: desferrioxamine; DFP: deferiprone: DFX: deferasirox; LIC: Liver iron content.

d. Correlations

The significant linear correlations between the different variables is shown in table 4.

Discussion

In recent years there has been a growing interest in the clinical impact of GD in TDT patients.

The estimated prevalence of DM in TDT patients varies from 9.7% to 29% (25). This variability partly relates to the introduction and application of effective iron chelation therapy and to patients' age, with lower rates in younger patients (26). The mean age at diagnosis of DM in 29 of 448 patients (6.5%) with TDT, attending seven Italian centers, was 17 years (range 11-24)(27).

There are specific issues to consider in the pathogenesis and management of diabetes in transfused

Table 4. Significant correlations observed in the group of 25 TDT patients with DM

Dependent Variable	Parameter	Estimate R	P value
Fasting plasma glucose	Plasma glucose at 2 hr	0.6047	0.001
Fasting plasma glucose	HOMA IR	0.5538	0.0040
Plasma glucose at 2- h	HOMA IR	0.553	0.0041
Basal insulin	HOMA IR	0.9496	< 0.00001
Basal insulin	Matsuda index	- 0.810	< 0.00001
Insulin peak	HOMA IR	0.553	0.041
Matsuda index	HOMA IR	- 0.703	0.00008

Legend= DM: diabetes mellitus; HOMA-IR: Homeostatic Model Assessment index of insulin resistance

thalassemia patients. First, diabetes in TM is distinct from T1DM and T2DM in normal individuals, though there are similarities. In contrast to T1DM of healthy individuals, in which insulin production declines rapidly and has an abrupt and symptomatic onset, in TDT patients, the insulin production declines gradually, and diabetes may be asymptomatic (28). Second, microvascular complications may occur (29-31), but they are not as frequent or as severe as in other types of diabetes (32). Third, macrovascular complications (cerebrovascular disease and peripheral vascular disease) are rare (31). Lastly, international thalassaemia management guidelines recommend screening with annual oral glucose tolerance test, starting from the age of 10 years (28,33), although the ability of OGTT alone to detect early abnormalities in blood glucose regulation has been questioned (34,35).

From recent studies, GD in TDT patients can be didactically classified as follows: 1) impaired fasting glucose (IFG); 2) high-normal glucose tolerance (H-NGT) plasma glucose levels ≥155 mg/dL at 1-h (8.6 mmol/L) during OGTT; 3) impaired glucose intolerance (IGT), glucose levels at 2-h during OGTT between 140 mg/dL and 199 mg/dL (7.8-11.0 mmol/L); 4) diabetes without fasting hyperglycemia; 5) diabetes with fasting hyperglycemia (16,28). The prevalence and characteristics of the latter two forms of glucose dysregulation have not been fully reported before.

In this group of 25 TDT patients, 10 (40%) had diabetes with fasting hyperglycemia, 13 (52%) had diabetes without fasting hyperglycemia and 2 (8%) had diabetes with normal FPG. A moderate iron overload, assessed by serum ferritin (>1.000 ng/mL and < 2.000 ng/mL), was present in 7 patients (28%; 4 with fasting hyperglycemia); severe iron overload (>2.000 ng/mL) in 12 patients (48%; 6 with fasting hyperglycemia) and mild iron overload (< 1.000 ng/mL)in 6 patients (24%) with diabetes without fasting hyperglycemia. Moreover, 19 out of 25 TDT patients (76%) with DM had increased liver enzymes and in 7/25 (28%) the ALT levels were double the normal range (0-40U/L).

None of the TDT patients with diabetes and fasting hyperglycemia had specific manifestations of T1DM (polyuria, polydipsia, weight loss) while four patients with diabetes without fasting hyperglycemia were overweight/obese. More importantly DM was characterized by qualitative and quantitative defects of insulin secretion and by peripheral IR, assessed through surrogate indexes, such as HOMA-IR, Matsuda index and disposition index measured by the combined evaluation of changes of plasma glucose and insulin secretion during OGTT applied in this study.

Interestingly, in two patients with normal fasting plasma glucose, the IGI, Matsuda and DI indexes were significantly lower compared to TDT with NGT and healthy controls, indicating a significant decrease in the maximal insulin secretory capability in response to OGTT and β-cell dysfunction despite a normal FPG level. Therefore, it is possible that DI measurement could be a useful index to predict the subsequent development of DM in TDT-NGT patients. However, the cut off value for DI below which there is a risk for developing GD should be determined after conducting a large prospective trial, and longitudinally following TDT patients with NGT over time.

Although no correlation was observed in our patients between iron overload (assessed by serum ferritin levels), liver enzymes (assessed by ALT), and insulin parameters before and after OGTT, 76% had moderate to severe iron overload and increased liver enzymes.

Thus, it could be assumed that in TDT in the early stages of GD, insulin levels tend to be higher to compensate for insulin resistance. Later with disease progression, the chronic stimulation of insulin secretion will gradually lead to secondary β-cell failure, as

reported in T2DM. In patients with iron overload, excess iron starts to deposit primarily in the liver, followed by the pancreas and the heart. These tissues take up plasma iron from transferrin or the free, non-transferrin-bound iron (NTBI), which appears in the plasma when the capacity of transferrin is exceeded, as in hemochromatosis and transfused β-thalassemia. NTBI is rapidly cleared by the liver (36-38) and to a lesser extent by the pancreas, and heart (36-40).

Cells take up transferrin in proportion to the number of the available transferrin receptors (TR) located at the cell surface. After transferrin binds to TR, the complex enters endosomes, which become acidified, dissociating iron from transferrin. The liberated ferric iron is then reduced to ferrous iron and transported across the endosomal membrane into the cytosol (41). TR in the normal human pancreas are expressed predominantly in the islets (41) and are presumed to be a physiologic means by which transferrin-bound iron enters islet cells. The transport of iron out of the endosome is mediated by divalent metal-ion transporter-1 (DMT1). The abundant expression of DMT1 in islet cells suggests that DMT1 also plays an important role in iron uptake by β-cell leading to β-cell damage (42).

An adjunctive pathway of cellular iron uptake may involve ZIP14, a member of the ZIP family of metal-ion transporters (43). The abundant presence of ZIP14 in liver, pancreas, and heart (43) have led to the hypothesis that ZIP14 transports NTBI into these organs (44,45).

The IR could be attributed to a number of factors, including: iron overload (46), chronic liver infection due to hepatitis C (47-49), hyperglucagonemia (50,51) and vitamin D deficiency (52) .

Prolonged severe iron overload results in autophagy defects through mTORC1-UVRAG inhibition with accumulation of dysfunctional autolysosomes and loss of free lysosomes in skeletal muscle, resulting in IR and glucose intolerance (53,54). Furthermore, cytokines such as tumor necrosis factor alpha (TNF- α), which are increased in patients with liver damage, and iron overload could contribute to peripheral IR (55).

The major general recommendations for the management of diabetes in the reported group of TDT patients included strict adhesion to iron chelation therapy either as monotherapy or as a combination treatment with deferoxamine plus deferiprone in severely iron overloaded patients. A second OGTT test to confirm the diagnosis of diabetes in patients with NFG or IFG was recommended since very little information is available on the reproducibility of OGTT in TDT patients. Moreover, an individualized patient-tailored therapeutic approach was recommended with subcutaneous insulin therapy or antihyperglycemic drugs, after careful consideration of drug contraindications and interactions. Therefore, the individualized therapeutical approach should be focused on the clinical and severity aspects of each case, taking their hyperglycaemia into account considering also treatment burden, lifestyle and psychological factors. Education, awareness, parental and patients' co-operation are important elements in the establishment of mutual trust, with the objective of treating the disease early and appropriately.

Although our study has some limitations, due to the relatively small group of patients which may affect its statistical power, the use of surrogate indices for assessing iron overload, and non-use of magnetic resonance imaging (MRI) estimation of pancreatic iron content, it has the strength of detailed documentation of clinical data and natural history of glucometabolic status, and evaluation of insulin sensitivity and insulin release in TDT patients recently diagnosed with DM. Moreover, we observed that not all patients had undergone to an annual OGTT test and the analysis of glucose tolerance revealed variable results over time.

Conclusion

In conclusion: (i) the natural history of glucometabolic status in TDT patients is characterized by a gradual deterioration of glucose tolerance over time; (ii) appropriate resources should be invested into timely detection of GD in TDT patients; (iii) the mechanisms of GD are complex and multifactorial, and thus their management poses clinical challenges; (iv) based on the clinical and laboratory patients' characteristics that combine decreased insulin secretion and increased insulin resistance, we believe that a more appropriate terminology of diabetes in TDT patients could be "Thalassemia-Related Glucose Disturbances (TRGD)".

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