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Original article

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

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Abstract. *Background:* Advances in β- thalassemia major (β-TM) care have transformed a disease which had previously led to an early childhood death into a chronic condition. With increased lifespan, comorbidities associated with the disease have become more common, among them glucose dysregulation (GD) which develops insidiously, aggravating prognosis and patients' quality of life. *Objectives:* The objectives of this study were to retrospectively review the extent to which β-TM patients, having combined impaired fasting glucose (IFG) and impaired glucose tolerance test (IGT) on oral glucose tolerance test (OGTT), progressed to diabetes and to analyze the potential determinants inducing this progression, or regression to normal glucose tolerance test (NGT). *Research design and method:* Data of 58 β-TM patients, followed for a mean duration of 7.7 years (range: 1-20 years) with annual or biennial OGTT, were retrieved. Insulin release and insulin sensitivity (IS) were also analyzed. *Results:* During the follow-up, FPG and 2-h PG levels after OGTT reverted to NGT in 13 patients (22.4%), deteriorated in 13 patients (22.4%) who developed diabetes mellitus, and did not change in the remaining 32 patients (55.2%). A significant correlation was observed between FPG and ALT level (r: 0.3158; P:0.01) and an inverse correlation was found between chronological age and serum ferritin (SF) level (r: -0.321; P:0.014). Finally, SF and ALT, both at the baseline and at the time of last observation, were independent predictors of evolution to diabetes mellitus. *Conclusion:* The combination IFG/IGT in β-TM patients with severe iron overload constitutes a high-risk state for developing diabetes. (www.actabiomedica.it)

Key words: β- thalassemia major, prediabetes, combination IFG/IGT, follow-up, iron overload, liver enzymes

Introduction

Life-long red blood cell transfusions, regular iron chelation therapy and new diagnostic techniques available for evaluating iron overload have led to improved survival of patients with β-thalassemia major (β-TM), transforming the disease into a chronic condition (1). In parallel with the increased lifespan, the comorbidities associated with the disease have become more prevalent. Glucose dysregulation (GD) is a common finding that develops insidiously and may aggravate the patients' quality of life and prognosis (2).

In December 2020, the International Network on Endocrine Complications in Thalassaemia (ICET-A) promoted a preliminary multi-country survey with the aim of assessing the prevalence of GD in 2,252 patients with β-TM followed in 14 centers of the ICET-A Network. Isolated impaired fasting glucose (i-IFG) was reported in 141 patients (6.2 %) and IFG with impaired glucose tolerance (IGT) in 59 (2.6 %) patients. 195 patients (8.6%) were diagnosed as having β-TM related diabetes mellitus and 138 (6.1%) patients type 2 diabetes (T2DM) (3), according to the World Health Organization (WHO) or the American Diabetes Association (ADA) criteria (4,5). The WHO and ADA utilise different cut-off values for IFG (WHO: $IFG_{high\text{-}range}$ 110-125 mg/dL = 6.1-6.9 mmol/L; ADA: $IFG_{low\text{-}range}$ 100-125 mg/dL = 5.6-6.9 mmol/L) but the same cut-off values for IGT (140-199 mg/dL = 7.8-11.0 mmol/L) (4,5).

Screening with measurements of fasting plasma glucose (PG) or glycated hemoglobin A_{1c}, alone or in combination, does not reliably identify GD in β-TM patients, as compared with the 2-h PG value measured during an OGTT (6).

Prediabetes, defined as the presence of IFG, IGT, or both, can progress with an enhanced risk of 5%–10% for development of T2DM in adults, (8) with a similar proportion converting to normoglycaemia (7,8).

i-IFG is more closely associated with reduced hepatic insulin sensitivity (IS), stationary β-cell dysfunction and inappropriately elevated glucagon secretion. Conversely, in the prediabetic state, i-IGT is characterised by reduced peripheral IS, near-normal hepatic insulin sensitivity, progressive loss of β-cell function, and inappropriately elevated glucagon secretion (9). Obesity and a high fat diet may contribute to the development of both IR and insulin secretory dysfunction in susceptible individuals (10). Strategies that improve IR and enhance early insulin secretion may prevent the progression from IGT to diabetes (11,12).

Diabetes mellitus in thalassemia represents a distinct clinical condition with pathophysiological and clinical differences which require a specific approach for diagnosis and management (13). Because the WHO and ADA classifications are based on the pathogenesis of the disease and not on its treatment, and diabetes mellitus in β-TM patients is considered a distinct clinical entity caused either by a variable IR along with a progressive decrease in the circulating insulin levels, the ICET-A Network have proposed the term "β-thalassemia major -related diabetes (β-TM-RD)" to classify subjects with diabetes mellitus (3).

The recognition of GD in β-TM patients is an important aspect of care in these patients because early diagnosis and treatment with intensive chelation regimen (monotherapy or combined) can improve insulin secretion and glucose metabolism (14,15).

The objective of this study was to retrospectively review the extent to which β-TM patients with combined IFG/IGT developed β-TM-RD during early adulthood and to analyze the potential determinants inducing progression to diabetes mellitus, or regression to normal glucose tolerance (NGT). As a secondary aim, we evaluated the most appropriate diagnostic/prognostic IFG criteria in high risk β-TM patients with iron overload.

Patients and Methods

Study population and design

This study is based on data collected during an ongoing retrospective longitudinal study on GD in patients with β -TM promoted by ICET-A in January 2021. The de-identified data of patients followed by the same endocrinologist (VDS) from the diagnosis of GD at Pediatric Endocrinology and Adolescent Medicine Outpatient Clinic of St. Anna Hospital of Ferrara (September 1983-September 2010) and Pediatric Endocrinology and Adolescent Medicine Outpatient Clinic of Quisisana Hospital of Ferrara (October 2010-September 2021) were considered for the study.

Eligible subjects included: (a) β -TM patients receiving routine blood transfusion and chelation treatment; (b) patients with combined IFG/IGT, based on results of OGTT and (c) availability of an annual or biennial OGTT (including plasma glucose and serum insulin at baseline, 30, 60, 90, 120 and 180 minutes after OGTTs. Exclusion criteria were: (a) patients already diagnosed with β-TM-RD; (b) patients

receiving anti-diabetic agents; (c) major chronic illness other than β -TM; (d) patients with non-transfusion dependent thalassemia; (e) bone-marrow transplanted patients, and (f) use of drugs affecting glucose metabolism.

The medical records of 58 β-TM patients with combined IFG/IGT were retrieved and the following data were collected: age, gender, ethnicity, anthropometry [weight, height, body mass index (BMI), pubertal status], age at first transfusion, interval between transfusions, iron chelation therapy, presence of splenectomy, results of OGTT and associated endocrine complications.

β -TM patients were diagnosed on the basis of clinical and laboratory data, including the measurement of red blood cell indices by automatic hematology analyzer, Hb analysis, and molecular characterization of genotypes (16).

Anthropometric assessments

Height and weight were measured using a standard technique. BMI was calculated as weight in kilograms divided by the square of height in meters. Height and weight were measured according to international recommendations (17). An adult patient was considered obese when BMI exceeded 30 Kg/m^2, overweight when BMI was 25 – 30 kg/m^2. Short stature was defined as height 2 SD below the mean height for age and sex. Associated endocrine complications were defined according to our previous reports (18,19).

Laboratory parameters, assessment of iron overload and analytical methods

Serum alanine aminotransferase (ALT) concentrations and serum ferritin (SF) determinations were collected. Both determinations were assessed before blood transfusions. The highest value of SF registered after the beginning of iron chelation therapy and before the diagnosis of combined IFG/IGT was also collected and classified as SF peak. Results of screening assays for hepatitis C virus seropositivity (HCV-ab and HCV-RNA) were also collected from1991.

The level of ALT was determined by an automated analyzer (upper normal limit: < 40 U/L). Iron overload (IOL) was assessed by indirect methods and was arbitrarily classified as: mild, SF: < 1,000 ng/mL, moderate SF: >1,000 ng/mL and severe < 2,000 ng/mL (19).

SF was measured in the early years by radioimmunoassay at a serum dilution of 1:1000 (normal values ± SD: males 108 ± 68 ng/mL, females 32 ± 25 ng/mL) and later by immunoradiometric and chemiluminescence immunoassay.

OGTT: Method and definitions

Oral glucose (1.75 g/kg, max 75 g) was given in the morning after an overnight fast. Subjects were clinically stable and without history of acute infection in the previous 3 weeks. Blood samples were collected from a venous catheter at 0, 30, 60, 90,120 and 180 minutes following oral glucose administration, to measure PG and insulin.

Glucose tolerance status was classified according to the ADA criteria by a single OGTT. Patients with FPG between 100 to 125 mg/dL (5.6-6.9 mmol/L) were classified as IFG and those with 2 h-PG values in OGTT between 140–199 mg/dL (7.8–11.0 mmol/L) as IGT. Patients with IFG were subdivided in two groups according to FPG level: "low range" when FPG was between 100 and 109 mg/dL and "high range" between 110 and 125 mg/dL. Newly diagnosed diabetes (defined as " β-TM - related diabetes) were those with FPG ≥ 126 mg/dL or 2 h-PG ≥ 200 mg/dL (FPG: ≥7.0 mmol/L or 2h- PG: ≥11.1 mmol/L).To obtain more precise evaluation of changes in OGTT, annual or biennial results were evaluated.

The control group consisted of the data reported in a previous study in eleven healthy volunteer adult subjects (mean age: 23.8 ± 3.2 years) (20). No control was a carrier for β-thalassemia or was overweight/obese.

Plasma glucose was measured using an automated glucose oxidase reaction (Glucose Analyser, Ames). Plasma insulin levels were determined by a commercial immunoassays techniques [Dow Lepetit, Milan, Italy; Immulite 1000 Bayer (ADVIA Centaur Insulin) and Coat-A-Count insulin kit, Diagnostic Products Corporation, Los Angeles, CA]. All samples were tested in duplicate and values expressed in μU/ml.

Surrogate measures of insulin secretion and insulin sensitivity

Insulin resistance was calculated by the homeostasis model assessment of insulin resistance (HOMA-IR) (21) and Matsuda index (MI 0-120) (22). Early-phase insulin secretion (IGI) was calculated as the ratio between the incremental plasma insulin and glucose concentrations during the first 30 min of the OGTT (ΔI0–30/ΔG0–30).

Although HOMA-IR and MI 0-120 cut-off points for diagnosis of IR have not been fully defined for children and adolescents, the following values were considered indicative of IS and IR: a) IS: < 2.24; b) intermediate IR: > 2.24 and ≤ 3.59; and c) severe IR: > 3.59 (23). MI 0-120 Index ≤ 2.5 indicates the presence of IR (24).

Finally, the oral disposition index (oDI) was calculated as the product of the IGI and the MI 0-120. The index reflects the relationship between β-cell function (first-phase insulin secretion) and peripheral insulin sensitivity (hepatic and peripheral tissue sensitivity to insulin) (25). The oDI provides a measure of pancreatic β-cell function adjusted for insulin sensitivity and is predictive for deterioration from normal to overt diabetes (25).

Ethics

All procedures were in accordance with the 1964 Helsinki declaration and its later amendments in October 2013 (www.wma.net). The study protocol was approved by the Unit Board and two Thalassemia Associations (Ferrara and Rovigo) at the beginning of study (September 1983). Informed consent was obtained from the patients or guardians, if applicable. In the course of retrospective study the de-identified data set was analyzed, no identifiable private information was collected, patients underwent only routine diagnostic procedures according to the national Italian protocols and following International Guidelines (18).

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. 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 (26).

Results

Characteristics of β-TM patients with combined IFG/IGT at baseline

The demographic and laboratory data of recruited β -TM patients are summarized in Table 1. Regular subcutaneous desferioxamine mesylate (DFO) infusion was started in 1978 in patients older than 2 years.

Table 1. Clinical and laboratory findings in 58 β-thalassemia major (β-TM) patients at diagnosis of combined impaired fasting glucose (IFG)/impaired glucose tolerance (IGT).

Variables	β -TM patients (n: 58) at diagnosis of combined IFG/IGT
Chronological age (yrs)	
< 10 yrs (n. and %)	2 (3.4%)
10-15 yrs (n. and %)	9 (15.5%)
16-20 yrs (n. and %)	23 (39.6%)
21-30 yrs (n. and %)	20 (34.4%)
> 31 yrs (n. and %)	4 (6.8%)

(*Continued*)

Variables	β -TM patients (n: 58) at diagnosis of combined IFG/IGT
Gender (Males/Females)	32/26
BMI category (Kg/m^2) <25 (n. and %) 25–30 (n. and %) > 30 (n. and %)	 51 (87.9%) 4 (6.8%) 3 (5.1%)
Family history of prediabetes or diabetes mellitus None Yes: grandparent, uncle, aunt or cousin (n. and %) Yes: biological father, mother or sibling (n. and %)	 44 (75 %) 13 (22 %) 2 (3 %)
Splenectomy Yes (n. and %) No (n. and %)	 38 (65.5%) 20 (34.4%)
Serum ferritin (ng/mL) - Mean and SD < 1,000 ng/mL (n. and %) > 1,000 - < 2,000 ng/mL (n. and %) > 2,000 - < 3,000 ng/mL (n. and %) > 3,000 ng/ml (n. and %)	2215.3 ± 1760.9 13 (22.4 %) 22 (37.9%) 10 (17.2%) 13 (22.4%)
ALT (U/L) < 40 U/L (n. and %) > 40 - < 80 U/L (n. and %) > 80 U/L (n. and %)	 22 (37.9%) 21 (36.2%) 15 (25.8%)
HCV-ab negative (n. and %) **HCV-ab positive** (n. and %) **HCV –RNA positive** (n. and %)	8/58 (13.7%) 50/58 (86.2%) 27/50 (54.0%)
Fasting plasma glucose (FPG: mg/dL) - Mean and SD 1. FPG: 100-109 mg/dL 2. FPG: 110-125 mg/dL	108.3 ± 6.8 39 (67.3 %) 19 (32.7 %)
Oral glucose tolerance test (OGTT)	**Mean and SD**
Plasma glucose (mg/dL) after 1 h	159.5 ± 34.4
Plasma glucose (mg/dL) after 2 h	154.3 ± 13.6
Plasma glucose (mg/dL) after 3 h	114.9 ± 25.0
Fasting insulin (μU/mL)	9.5 ± 4.9
Insulin peak (μU/mL)	47.5 ± 28.9
Plasma insulin (μU/mL) after 3 h	18.8 ± 14.1
HOMA-IR- Mean and SD IS: < 2.24 (n. and %) Intermediate IR: > 2.24 and ≤ 3.59 (n. and %) Severe IR: > 3.59 (n. and %)	2.56 ± 1.38 30 (51.7%) 21 (36.2%) 7 (12.0%)

Initially, the recommended DFO dose was 20 mg/kg BW administered daily at night, by infusion pump over 10 hours. Based on transfusional iron input the dose was increased to 40 mg/kg BW in 1982 and up to 60 mg/kg BW in 1984. The oral chelator deferiprone (DFP) has been available since 1995; it was first given to some patients as monotherapy at a dose of 75 mg/kg BW and later as combined therapy with daily DFP and subcutaneous DFO for 3-6 days/week in patients with severe iron overload and high iron input. In 2007, the new oral chelating agent deferasirox (DFX) was introduced at a dose of 25-30 mg/kg BW for patients in whom treatment with DFO was contraindicated or inadequate.

Thirty-eight patients (65.5%) had undergone splenectomy because of increased blood transfusion requirements and/or for the presence of other signs of hypersplenism such as leukopenia, thrombocytopenia, and/or massive splenomegaly.

In 39 of 58 (67.2%) β-TM patients, the mean value of the FPG was 104.4 ± 3.0 mg/dL in patients with $IFG_{low\text{-}range}$ and in 19 patients with $IFG_{high\text{-}range}$ was 117.0 ± 4.5 mg/dL.

At baseline, a significant correlation was observed between FPG and ALT levels (r: 0.3158; P:0.01) and an inverse correlation between chronological age and SF levels (r: -0.321; P:0.014) (Figures 1 and 2).

Follow-up after initial diagnosis of prediabetes (combined IFG/IGT) and predictors of progression to β-TM-RD or reversion to NGT

The mean duration of follow-up since diagnosis of combined IFG/IGT was 7.7 ± 4.8 years (range:1-19 years). Thirteen β-TM patients (Group A), after 4.8 ± 4.1 years (range: 1-10 years; median: 3 years) developed β-TM-RD (8/13 had FPG level < 126 mg/dL and PG 2-h after OGTT > 200 mg/dL, and 5/13 had a basal FPG level ≥ 126 mg/dL and PG 2-h after OGTT > 200 mg/dL). Interestingly, one female patient had an FPG of 86 mg/dL and a 2-h PG after OGTT equal to 248 mg/dL.

β-TM-RD was preceded, in the previous 1 to 3 years, by NGT in 5 patients, i-IFG in 3 patients and i-IGT in 3 patients. No information was available for the remaining 2 patients. In 5 patients (4 females) the development of β-TM-RD was observed within 1 year from the diagnosis of combined IFG/IGT. In 4 patients a family history of diabetes was present (2 cases with T1DM and 2 cases with T2DM). Moreover, those who progressed from combined IFG/IGT to β-TM-RD were more often female (8 vs. 5; $\chi^2 = 1.334$; P: NS). The number of patients was too small to calculate the conversion rate per year to β-TM -RD (13 patients) or reversion rate per year to NGT (13 patients).

Thirteen patients (Group B;8 females), after 8.6 ± 4.0 years (range: 3-17 years: median: 3 years), reverted to NGT. At baseline, 9/13 patients had a $IFG_{low\text{-}range}$ (FPG:104.8 ± 2.8 mg/dL and 2-h PG after OGTT: 158.5 ± 13.7 mg/dL).

After 8.6 ± 4.7 years (range: 3-20 years; median 10 years), 32 patients (Group C), remained prediabetic (IFG, IGT or had combined IFG/IGT) (Table 2). At baseline, 22/32 had a $IFG_{low\text{-}range}$ (FPG: 104.6 ± 2.6 mg/dL and 2-h PG:152.7 ± 11.8 mg/dL). The clinical

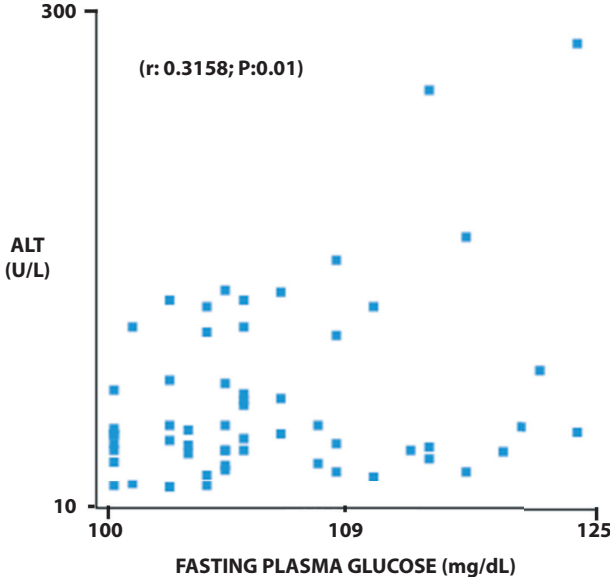

Figure 1. Linear correlation between fasting plasma glucose (mg/dL) and alanine aminotransferase (ALT: U/L) at baseline.

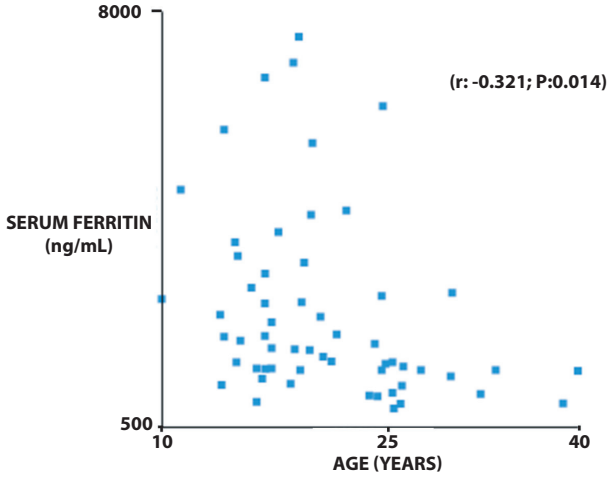

Figure 2. Inverse linear correlation between chronological age (years) and serum ferritin level (ng/mL) at baseline.

Table 2. Clinical and laboratory parameters reported at the diagnosis of β-TM-RD and at the last visit in the 3 groups of β-TM patients.

Patient's groups	Age (yrs) [*]	SF (ng/mL) [*]	ALT (U/L) [*]	SF peak (ng/mL)	Duration of follow-up (yrs)	SF (ng/mL) at last obs.	ALT (U/L) at last obs.	BMI (kg/m^2) at last obs.
Group A: From IFG + IGT to β-TM-RD (13 patients)	21.0 ± 7.5	3467.7 ± 2089.2	104.1 ± 82.0	4676.5 ± 1758.8	4.8 ± 4.1	2519.1 ± 1641.6	78.7 ± 49.9	23.9 ± 5.4
Group B: From IFG + IGT to NGT (13 patients)	21.3 ± 6.4	1319.6 ± 608.8	59.3 ± 31.7	2858.6 ± 1163.8	8.6 ± 4.0	1219.3 ± 802.7	41.3 ± 20.4	23.1 ± 2.7
Group C: From IFG/ IGT to PP (32 patients)	19.8 ± 5.8	2071.8 ± 1646.2	54.7 ± 36.6	4071.4 ± 1451.2	8.8 ± 5.0	1251.7 ± 835.5	55.6 ± 37.5	22.5 ± 2.6
Group A vs. B: P value	NS	0.001	NS	0.004	0.02	0.017	0.01	NS
Group A vs. C: P value	NS	0.021	0.01	NS	0.01	0.001	NS	NS
Group B vs. C: P value	NS	NS	NS	0.01	NS	NS	NS	NS

Abbreviations = [*]: *at diagnosis of IFG+IGT;* ***SF:*** *Serum ferritin;* ***ALT:*** *Alanine aminotransferase;* ***BMI:*** *Body Mass Index;* ***Obs:*** *observation;* ***PP:*** *persistent prediabetes (IFG, IGT, combined IFG/IGT).*

and laboratory parameters in the 3 groups of β-TM patients are reported in table 2.

During the long-term follow-up, a fluctuation of PG levels during OGTT was observed in 17 (29.3%) patients of Group B and C.

Comparison between groups of GD patients and correlations

No correlation was observed between BMI and PG at baseline and at last observation (r: 0.1833; P: 0.1 and r: 0.0677; P: 0.6, respectively) in any patient.

At baseline, SF and ALT levels were the most significant statistical parameters when we compared Group A to Groups B and C. Patients with combined IFG/IGT who reverted to NGT (Group B) had a lower SF peak value when compared to those who remained in the prediabetic state at the last visit (Group C: P: 0.01) (Table 2).

A significant correlation was also observed between SF and fasting PG at diagnosis of β-TM-RD and at the last visit in patients of Groups B and C (r: 0.3478; P:0.007). In the latter group of patients, no correlation was observed between SF and PG at 2- h after OGTT (r: 0.2504; P: 0.057).

In addition, it is worth mentioning that individuals who progressed to β-TM-RD had higher levels of SF and ALT, both at the baseline and at the time of last observation, compared to those who either remained stable or improved their glycemic status (Table 2).

Plasma glucose and insulin responses during OGTT in the 3 groups of patients

The plasma glucose and insulin responses during OGTT are depicted in figure 3 A and B. A delay in peak insulin concentration (between 60' and 180' minutes) was found in 12 of 13 (92.3%) patients of Group A, and in 6 of 13 (46.1%) patients (between 60' and 120' minutes) of Group B, and in 13 of 32 (40.6%) patients (between 60' and 120' minutes) of Group C, while in control subjects the maximal insulin concentration was reached 30 minutes after the oral glucose uptake (Figure 3- A). Insulin levels and secretory kinetics during OGTT did not differ statistically between the 3 groups of patients (Figure 3- B).

All the selected indices used for assessing insulin secretion and sensitivity in β-TM patients who developed β-TM-RD (Group A) were statistically significant when compared to Group B patients, who

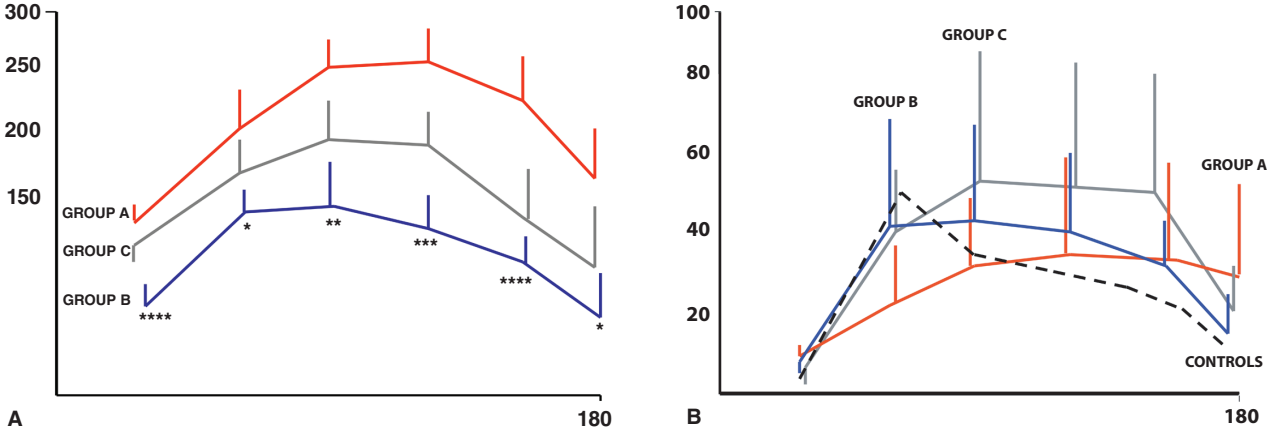

Figure 3. A and B. Plasma glucose (Panel A) and insulin (Panel B) concentrations during the OGTT (at baseline, 30, 60,90,120 and 180 minutes) in the 3 Groups of β- TM patients at the diagnosis of TM-RD and at the last visit. Group A: patients who developed diabetes (β-TM-RD), Group B: patients who reverted to normal glucose tolerance and Group C: patients who remained prediabetic. Plasma glucose concentration (A): Group B vs. C (* P: < 0.05; **P:< 0.005; ****P: < 0.0001). Plasma insulin concentration (B): Group A vs B and C; P : not significant at all times. Dotted line: normal insulin values (μU/mL) before and during OGTT= 0' :7 ± 3; 30': 46.2 ± 25.3; 60': 37 ± 17.6; 90': 35.2 ± 12.2; 120': 24.1 ± 12.2, and 180'; 10.7 ± 7.7 μU/mL (From: De Sanctis et al. Postgrad Med J. 1985; 61: 963-7).

reverted to NGT (Table 3). Surprisingly, IGI and oDI indices, expression of impaired β-cell function and insulin IR, were lower in patients who reverted to NGT (Group B) compared to controls (Table 3).

Short stature and endocrine complications in relation to chelation therapy at last observation

The number of subjects with short stature and associated endocrine complications in the three groups of patients and the type of iron chelation therapy at the last observation are reported in table 4. We found that the total number of endocrine complications in two groups of patients who had similar duration of follow-up (Group B: 8.8 ± 4.0 vs. Group C: 8.8 ± 5.0 years) were more common in group C (29 complications; 90.6%) compared to 9 complications (69.2%) in Group B, but the difference between the two groups was not statistically significant (χ^2: 3.147; P:0.07). Interestingly, short stature (< 2 SD for age and sex) was more common in Group B than in Group A, but the difference was not statistically significant (χ^2: 3.348; P: 0.067) (Table 4).

Table 3. Comparison of surrogate indices of insulin secretion and insulin sensitivity at baseline and diagnosis of β-TM RD (Group A) and regression of combined IFG/IGT (Group B) at last visit

	HOMA-IR	**MI 0-120**	**IGI**	**oDI**
***Group A* at the diagnosis of combined IGF/IGT (13 patients)**	3.26 ± 2.08	5.42 ± 2.63	0.85 ± 1.06	4.71 ± 7.53
***Group A* at the diagnosis of β-TM- RD (13 patients)**	3.15 ± 2.08	5.06 ± 2.94	0.28 ± 0.30	1.24 ± 1.45
P value	**NS**	**NS**	**NS**	**NS**
***Group B* at the diagnosis of combined IGF/IGT (13 patients)**	2.44 ± 1.15	4.74 ± 3.0	0.98 ± 0.53	3.89 ± 1.42
***Group B* at last visit of NGT (13 patients)**	1.32 ± 0.47	7.22 ± 2.00	0.70 ± 0.35	4.90 ± 2.25
P value	**0.003**	**0.02**	**NS**	**NS**
***Group A* at the diagnosis of β-TM-RD vs. *Group B* at last visit of NGT (P value)**	**0.005**	**0.03**	**0.003**	**< 0.0001**
***Controls* (11 subjects)**	1.16 ± 0.76	8.71 ± 2.85	1.54 ± 0.99	12.11 ± 6.55
***Controls* (no.11) *vs. Group B* (no.13) at last visit of NGT (P value)**	**NS**	**NS**	**< 0.01**	**0.0012**

Table 4. Short stature (< 2 SD for age and sex) and endocrine complications in the 3 groups of β- TM patients and the type of iron chelation therapy at last visit.

Patient's groups	**Standing height (< 3rd centile)**	**P-HT**	**C-HT**	**HH**	**S-HH**	**HPT**	**Iron chelation therapy**
Group A (13 β-TM pts.)	1 (7.6%)	2 (15.3%)	1 (7.6%)	4 (30.7%)	6 (46.1%)	1 (7.6%)	DFO: 8 pts DFP: 3 pts DFO+DFP:2 pts
Group B (13 β-TM pts.)	5 (38.4%)	1 (7.6%)	0 -	3 (23.0%)	5 (38.4%)	0 -	DFO: 11 pts DFP: 2 pts
Group C (32 β-TM pts.)	9 (28.1%)	5 (15.6%)	2 (6.2%)	13 (40.6%)	8 (25%)	1 (3.1%)	DFO: 21 pts DFP: 6 pts DFO + DFP: 3 pts DFX: 2 pts

Abbreviations = P-HT: *Primary hypothyroidism;* ***C-HT:*** *Central hypothyroidism;* ***HH:*** *Hypogonadotropic hypogonadism;* ***S-HH:*** *Acquired hypogonadotropic hypogonadism;* ***HPT:*** *Hypoparathyroidism.* ***Note:*** *No clinical cases of adrenal insufficiency were reported.*

Discussion

In the present retrospective study, conducted under the framework of an ongoing study promoted by ICET-A, we reviewed 58 prediabetic β-TM patients followed from 1983 to 2021 (mean: 20.4 ± 6.4 years). Changes in glucose tolerance during OGTT were evaluated in relation to BMI, SF and ALT levels on an annual or biennial basis.

Prediabetes (IFG, IGT or combined IFG/IGT) is an intermediate state of glycemia control which implies a high risk of developing diabetes in the following years. In TDT patients the course of i-IFG or i-IGT is variable and difficult to predict because different rates of progression may reflect different genetic and environmental factors. However, individuals with prediabetes have an increased risk of developing T2DM and other associated complications (27,28). Subjects with combined IFG/IGT can develop diabetes at twice the rate of individuals who manifest a single abnormality (i-IFG or i-IGT) (29). An Italian study spanning 11.5 years found that 44.4% of subjects with combined IFG/IGT developed diabetes compared to 9.1% of patients with only i-IFG (30).

Understanding the natural history of prediabetes in β-TM patients is essential for early detection of GD and to try to interrupt the progression to β-TM-RD. However, limited longitudinal data are available to support this assumption. A selected group of 10 β-TM patients with i-IFG was followed for at least 10.3 years (range: 10.3 - 28.10 years) from prepubertal age to adulthood. 9/10 (90%) had a further deterioration of glucose tolerance and 2 female patients developed β-TM-RD (31). Moreover, 12.4% of 263 patients (aged 11-30 years) with i-IGT developed diabetes within a period of 10 years, as reported by Kattamis et al. (32). In the present long-term retrospective study, 13 patients (22.4%) with combined IFG/IGT developed β-TM-RD after a median of 3 years, 13 patients (22.4%) reverted to NGT after a median of 3 years, and about half of them (32/58 patients; 55.1%) remained prediabetic.

Several interesting aspects have emerged from our study:

(a) ADA and WHO recommend different cut-off points for the diagnosis of IFG. Such discrepancies leave physicians in a conundrum as to which diagnostic criteria should be used for a specific population and when to recommend repeat testing or further management (33,34). Even though there are still a number of controversies as to the ideal cut-off point for IFG, in our study we observed that 39 of 58 β-TM patients (67.2%) had a FPG level in the low-range ($FPG_{low\text{-}range}$:100 to 109 mg/dL) at baseline. Therefore, we can argue that by using the WHO classification alone, we could miss more than half of IGT cases. Such findings suggest that in high-risk β-TM patients, the lower FPG cut-off point may act as a better diagnostic index. However, it is recommended that a larger population is studied to further evaluate this finding;

(b) iron overload (measured by SF concentration) and impaired liver status (as assessed by serum ALT levels) were strong risk factors for GD and were the two variables found to be closely linked with the evolution of GD either towards the direction of diabetes or to restoration of normal glycemic status;

(c) our study did not fully portray the rate of compliance to chelation therapy. However, a presumed low adherence to iron chelation therapy, assessed indirectly by SF, was observed mainly during the adolescent years, as supported by an inverse linear correlation between chronological age and SF level (Figure 2). Many different factors could potentially impact on adherence to treatment, such as demographic, familial, socioeconomic, personal, therapeutic regimens (multiple drug therapy and complex treatments) and the relationship with health care professionals (35,36). Thus, evaluation of adherence should be an important part of follow up;

(d) although SF levels <1,000 ng/mL are considered a value associated with a lower probability of morbidity and mortality (33,34). 9/25 β-TM patients (36.0 %) with SF level <1,000 ng/mL (mean DF: 611.3 ± 258.6 ng/mL) presented, in the course of follow-up, a new endocrine complication (primary or secondary hypogonadotropic hypogonadism associated in 2 cases with primary subclinical hypothyroidism or central hypothyroidism), unfortunately the assessment of pituitary siderosis (37) by magnetic resonance imaging (MRI) was not available;

(e) at baseline, 28/58 patients (48.2 %) presented with an increased HOMA-IR index associated with a defect in β-cell secretion (IGI), indicating an insufficient compensation for IR. The latter insulin secretory

defect persisted during follow-up and was associated with a reduction of oDI, reflecting the decline of pancreatic β-cell function in β-TM-RD patients;

(f) at last observation, patients of Group B with NGT had a reduced index of IGI and oDI compared to controls, indicating a significant decrease in the maximal insulin secretory capability in response to OGTT and β-cell dysfunction. These findings support the need for regular follow-up of glucose homeostasis in β-TM patients who revert to NGT.

Our study has some limitations. Firstly, the sample size was small and might have had an effect on the study results and interpretation. Secondly, the data were retrospectively collected from TDT patients at high risk for GD, and probably do not fully reflect the prevalence and patterns in the current generation of young patients with β-TM. Although more accurate methods of evaluating tissue iron load are available today, (eg MRI), only serum ferritin was available throughout the length of this study. However, SF may be influenced by a number of factors, such as inflammatory states and hepatitis, which may show abnormally high SF levels (38), and vitamin C deficiency, with abnormally low SF levels (38). Nevertheless, serial assessments of SF levels are considered valuable for monitoring iron overload and evaluating the efficacy of iron chelation (38). Finally, serum insulin was measured over the time by three different radioimmunoassay techniques, which have a relatively high degree of cross reactivity with proinsulin. Moreover, the estimation of HOMA-IR values from different insulin assays has documented limitations and should be carefully considered in subjects with a lower β-cell function and high FPG (39,40). Currently, all assays are standardised with the same reference preparation (IRP 66/304); however, results still vary by up to a factor of 2 (39).

The above may have reduced the power of study; however, we do not think that the final conclusions would be affected much as several studies have documented IR after euglycemic insulin-clamp technique or during OGTT in patients with β-TM before the development of insulin deficiency (41-44). Although the euglycemic-hyperinsulinemic clamp is the "gold standard" for measurement of IS (45), we used OGTT data because it was routinely performed in our patients and easier to perform widely.

Conclusion

In conclusion, understanding the natural history of β-TM-RD is essential for timely diagnosis and treatment(s) in this vulnerable population of patients. We have documented for the first time the importance of the potential role of SF peak, in peripubertal and pubertal ages, for the development of β-TM-RD or persistence of prediabetes in β-TM patients. Many different factors could potentially impact adherence to iron chelation therapy, such as age, familial status, socioeconomic conditions, hesitancy, therapeutic regimens (multiple drug therapy and complex treatments) and the relationship with healthcare professionals. Thus, evaluation of adherence should be an important part of follow-up. The combination IFG/IGT in β-TM patients who are severely iron overloaded constitutes a high-risk state for developing diabetes. In less severely iron overloaded patients, prediabetes may be reversible through the implementation of a regular iron chelation therapy, associated with lifestyle modification programmes based on the adoption of healthier diet and increased levels of physical activity.

Conflicts of interest: Each author declares that he or she has no commercial associations (e.g. consultancies, stock ownership, equity interest, patent/licensing arrangement etc.) that might pose a conflict of interest in connection with the submitted article.

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