Acta Biomed 2022; Vol. 93, N. 3: e2022243 DOI 10.23750/abm.v93i3.12643

Original article

Evolution of glucose-insulin homeostasis in children with β- thalassemia major (β -TM): A twenty-year retrospective ICET- A observational analysis from early childhood to young adulthood

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Abstract. *Background:* Thalassemia guidelines recommend oral glucose tolerance test (OGTT), starting from the age of 10 years, or earlier in the presence of iron overload. *Objective:* The aim of this retrospective study was to review and document the changes of glucose-insulin homeostasis from early childhood to young adulthood in β-thalassemia major (β -TM) patients with impaired fasting glucose (IFG) and normal OGTT. *Methods:* All data of the clinical patients' records of 18 β -TM patients from September 1983 to September 2021 were included in the study. Annual or biennial OGTT results, for a duration of 15-20 years, were available for all patients. *Results:* The main findings are: a) IFG in children with β -TM represents a risk factor for the development of glucose dysregulation (GD) at later age; b) fluctuations of glucose homeostasis during follow-up were observed mainly in β-TM patients with IFG at baseline; and c) the primary defect of GD appears to be a low degree insulin resistance (IR), as estimated by HOMA-IR, followed by an insulin secretion defect. *Conclusion:* These results are noteworthy as they revealed that firstly, the baseline IFG predicts future development of GD, and secondly, that almost half of patients with IFG at the outset had normal glucose handling 15 years later. Understanding the sequence of abnormalities in the progression from normal glucose homeostasis to GD and identifying the risk factors for the glycometabolic defects in thalassemic patients might help in the formulation of interventions.

Key words: β-thalassemia major, oral glucose tolerance test, impaired fasting glucose, glucose dysregulation, insulin resistance, insulin secretion defect, long-term follow-up

Introduction

Patients with β-thalassemia major (β -TM) require regular blood transfusions and appropriate iron chelation therapy throughout their lives, for survival. To manage iron overload (IOL), three iron chelators, desferrioxamine, deferasirox and deferiprone are currently available. However, the effectiveness of iron chelators in most cases is suboptimal and cannot achieve a normal iron balance. Therefore, IOL continues to be

a major challenge in patients with β -TM as it is associated with severe complications mainly in the heart, liver and endocrine glands (1,2).

Several biomarkers are available to assess the degree of IOL in thalassaemia patients of which serum ferritin (SF) is the most applicable and widely used. Besides SF, the evaluation iron content in several organs by non-invasive magnetic resonance imaging (MRI) has been established in recent years (3-5). Pancreatic iron content increase generally begins after the first decade of life and aggravates with age. In addition to accumulation of iron in the pancreas, fat infiltration is commonly seen in adult β -TM patients with overt diabetes, resulting from the progressive replacement of pancreatic parenchyma by inert adipose tissue after the death of β-pancreatic cells from the cytotoxic effects of iron (6).

To understand how rapidly iron overload develops, Berdoukas et al. (7) retrospectively reviewed the MRI in 125 chronically transfused children (median age: 6.0 years), for evaluation of liver, pancreatic, or cardiac iron overload. In 10% of β -TM patients the values of pancreatic MRI R2* were over 100 Hz, a value that was previously considered by Noetzli et al. (8) as predictive for glucose dysregulation (GD). MRI T2* may be converted to reciprocal R2* by the formula; R2*[Hz]=1000/T2*[ms] (9).

In subjects with β -TM, GD frequently starts during adolescence, while overt diabetes mellitus develops later in life (2). The prevalence of impaired fasting glucose (IFG), impaired glucose tolerance (IGT), and other endocrine disorders (mainly hypogonadotropic hypogonadism, hypothyroidism, and hypoparathyroidism) reported in a meta-analysis of 44 studies including 16,605 patients with β -TM was 17.2%, 12.4% and 43.9%, respectively (10). IFG and IGT reflect intermediate status of GD between euglycemia and diabetes. The World Health Organization (WHO) and the American Diabetes Association (ADA) utilise different cut-off values for IFG (WHO: 110-125 mg/dL = 6.1-6.9 mmol/L; ADA: 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) (11,12).

Published thalassemia guidelines recommend a 2-h oral glucose tolerance test (OGTT), preferably combined with insulin secretion determination, at 10, 12, 14, and 16 years of age, and annually thereafter if the patient has not already developed thalassemia related diabetes (Th-RD), or earlier (<10 years) if the patient has severe iron overload (13).

The aim of this retrospective study was to review and document the changes of glucose-insulin homeostasis, from early childhood to young adulthood, in β -TM children with IFG and normal oral glucose tolerance test (OGTT).

Patients, Design and Methods

This study is based on data collected during an ongoing retrospective longitudinal study on GD in patients with β -TM promoted by the International Network of Clinicians for Endocrinopathies in Thalassemia and Adolescent Medicine (ICET-A) in January 2021. β -TM patients were diagnosed based on their clinical and laboratory data (clinical history, hematological, biochemical and molecular findings). 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 (patients were transfused every 2–3 weeks when the hemoglobin (Hb) level dropped to 9.0–9.5 g/dl, at intervals of 15–20 days with packed red blood cells (14); b) age at first observation below 8 years; c) patients regularly followed by the same endocrinologist (VDS) for endocrine assessment (every 6–12 months) from early childhood to advanced adulthood, and d) availability of an annual or biennial OGTT (including plasma glucose and serum insulin at baseline and 30, 60, 90, 120 and 180 minutes after OGTTs). Exclusion criteria were: a) major chronic illness (other than β -TM); b) family history for type 1 diabetes mellitus; c) patients with thalassemia

intermedia, d) bone-marrow transplanted patients, e) patients with renal failure; e) presence of a genetic syndrome known to affect glucose tolerance, and f) treatment with medications known to affect insulin sensitivity (systemic steroids).

At baseline, the compliance to iron chelation therapy was graded using the "chelation index" that represents the ratio between the number of weekly recommended administrations of chelating agents and the actual daily number of chelating doses received by the patient during the week. These data were reported by parents in an *ad hoc* questionnaire and further confirmed through parents' interviews and were arbitrarily classified as: high (>90%), moderate (51-90%), poor (1% -50%) or non-compliant (0%).

Control subjects included 9 healthy prepubertal children (mean age: 5.1 ± 0.4; 5 males) referred for an assessment of short stature and 16 healthy volunteer adult subjects (mean age: 23.6 ± 3.5 years; 8 males). The adult group included brothers, sisters, or cousins of β -TM patients. None of them was a carrier for β-thalassemia or overweight (15).

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 (13).

Data Collection and Clinical Evaluation

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. Body weight 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 (16). We classified the patients using the Endocrine Society Clinical Practice Guidelines: a child was diagnosed as overweight if the BMI was at the 85 th percentile but less than the 95 th percentile for age and sex, and as obese if the BMI was at the 95 th percentile for age and sex (17). An adult patient was considered obese when BMI exceeded 30 Kg/m^2, overweight when BMI was 25 – 30 kg/m^2. Associated endocrine complications were assessed according to our previous report (13,16).

Analytical Methods and Assessment of Iron Overload

Serum concentrations of alanine aminotransferase (ALT) serum ferritin (SF) and hepatitis C virus seropositivity (HCV ab and HCV-RNA) were recorded as indices 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(14).

IOL was assessed by direct and indirect methods and was classified as mild [serum ferritin (SF):

< 1.000 ng/mL], moderate (SF: >1.000 ng/mL and < 2.000 ng/mL) and severe (SF: >2.000 ng/mL) (17). 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 in the last years by immunoradiometric assay and chemiluminescence immunoassay (14).

Starting between 2005-2008, hepatic and cardiac hemosiderosis were assessed by magnetic resonance imaging (MRI) T2* using a 1.5 T scanner (GE Signa/Excite HD, Milwaukee, WI, USA). Global cardiac T2* values were expressed in msec (ms), according to the following cut-off points: normal > 20 ms, mild: 14–20 ms, moderate: 10–14 ms, severe < 10 ms (18). Liver iron content (LIC) was quantified using the calibration curve introduced by Wood et al.(19). The

values were expressed in mg/g dry weight (mg/g. d.w.) and classified into mild (LIC: > 3.2 and < 7 mg/g. d.w.), moderate (LIC: > 7 and < 14 mg/g. d.w.) and severe overload (LIC: > 14 mg/g. d.w.) (20).

Plasma glucose was measured by the glucose oxidase method and plasma insulin levels were measured by radioimmunoassay using a double-antibody method.

Glucose tolerance categories were defined according to the American Diabetes Association (ADA) guidelines for diagnosis and classification of diabetes (12).

Surrogate Measures of Insulin Secretion and Insulin Sensitivity

To assess insulin sensitivity (IS), the Homeostatic Model Assessment index of insulin resistance (HOMA-IR) and Matsuda index (MI) were calculated with the following equations: HOMA-IR: fasting glucose x fasting insulin/405 (21) and MI 0–120 (MI 0–120): [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/mL)] (22).

HOMA-IR was used as indicators of hepatic IS and MI 0–120 as indicators of hepatic plus peripheral IS (21,22). Lower HOMA-IR values indicate greater IS, whereas higher HOMA-IR values indicate lower insulin sensitivity (insulin resistance).

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 (22).

Acute first-phase insulin secretion was calculated using the insulinogenic index (IGI): Δ insulin (0–30) in Δ U/mL divided by the Δ glucose (0–30) in mg/dL (24). The IGI is a proxy for acute phase serum insulin response and was used for the evaluation of the β-cell function.

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 the â-cell function (first-phase insulin secretion) and the peripheral insulin sensitivity (hepatic and peripheral tissue sensitivity to insulin) (25). The oDI is a better index than IGI or MI 0–120 for reflecting the decline of β cell function for the glycemic deterioration from normal to overt diabetes.

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

a. Clinical and OGTT characteristics at baseline

The demographic data of recruited β -TM patients are summarized in Table 1. The mean age of 18 patients was 5.4 ± 0.7 years (15 were males). Patients were assigned to 2 groups according to the FPG levels. A total 9 out of 18 (50 %) patients with NGT and normal FPG were assigned to group A, and the remaining 9 (50 %) with isolated IFG and NGT to group B. None had IGT or diabetes. All patients were on treatment with desferrioxamine given by slow subcutaneous (s.c.) daily pump infusion. The recommended daily dose was 20–40 mg/kg body weight, over 8-h s.c., six days per week.

No significant differences were found between the two groups regarding age, BMI, total number of blood transfusions, mean ALT and SF. None was overweight or obese. Severe IOL was present in two patients of Group A and in two of Group B. A better "chelation index" was present in patients

Table 1. Clinical, laboratory characteristics and OGTT at baseline in 9 β - thalassemia major (β -TM) patients with normal glucose tolerance (NGT; Group A) and in 9 with isolated impaired fasting glucose (IFG; Group B) during OGTT, compared to 9 healthy prepubertal controls. The values are expressed as mean ± SD.

Baseline variables	Children with β -TM and NGT (n: 9) Group A	Children with β -TM and isolated IFG (n: 9) Group B	Controls (n. 9) Group C	P-value A vs. B	P-value A vs. C	P-value B vs. C
Chronological age (yrs)	5.41 ± 0.72	5.59 ± 1.73	5.14 ± 0.4	NS	NS	NS
Gender (Males/Females)	4/5	6/3	5/4	-	-	-
BMI (Kg/m^2)	17.5 ± 2.9	18.1 ± 2.7	18.1 ± 3.1	NS	NS	NS
Serum ferritin (ng/mL)	1,867 ± 654.9	1,848 ± 307.0	-	NS	-	-
ALT (U/L)	117.3 ± 92.9	74.11 ± 46.67	-	NS	-	-
Fasting plasma glucose (mg/dL)	84.7 ± 7.3	108.4 ± 5.0	76.3 ± 7.4	**< 0.001**	NS	**< 0.001**
Plasma glucose 1-h after OGTT (mg/dL)	130.8 ± 24.6	130.3 ± 24.7	108.3 ±21.7	NS	NS	NS
Plasma glucose 2-h after OGTT (mg/dL)	105.6 ± 13.6	107.1 ± 18.6	86.0 ± 12.0	NS	**< 0.05**	**< 0.05**
Plasma glucose 3-h after OGTT (mg/dL)	81.4 ± 17.5	98.3 ± 27.0	73.8 ± 12.1	NS	NS	**<0.0 5**
Fasting insulin (μU/ml)	8.88 ± 1.96	10.5 ± 2.7	3.7 ± 2.7	NS	**< 0.001**	**< 0.001**
Insulin peak (μU/ml)	35.4 ± 14.9	40.1 ± 29.5	34.8 ± 14.	NS	NS	NS
Insulin 3-h after OGTT (μU/ml)	8.0 ± 1.56	7.6 ± 1.5	4.5 ± 2.4	NS	**< 0.005**	**< 0.005**
MATSUDA INDEX (MI 0-120)	6.70 ± 2.24	6.38 ± 2.28	17.11 ± 6.7	NS	**< 0.001**	**< 0.001**
HOMA-IR	1.92 ± 0.53	2.85 ± 0.77	0.72 ± 0.52	**< 0.05**	**< 0.001**	**< 0.001**
Insulinogenic Index (IGI)	0.53 ± 0.29	0.74 ± 0.68	0.75 ± 0.16	NS	NS	NS
Oral disposition Index (oDI)	3.45 ± 2.11	4.01 ± 3.82	12.92 ± 5.11	NS	**< 0.001**	**< 0.001**
Chelation index (%)	86 ± 5	68 ± 16	-	**< 0.01**	-	-

of Group A vs. Group B (86 ± 5 % vs. 68 ±16 % ; P = <0.01).

In patients with IFG (Group B) the incremental rise in PG concentrations at 60,120 and 180 minutes after OGTT was similar to that of β -TM patients with NGT (Group A) (Table 1), but their PG levels at 2-h and 3-h after OGTT were significantly higher compared to controls (Group C) (Table 1).

The first-phase of insulin secretion, measured by IGI, was not significantly reduced in both groups of β -TM patients compared to controls. Conversely, indices of insulin secretion and sensitivity (MI 0-120, HOMA-IR, oDI) were statistically different (P = < 0.001). Moreover, HOMA-IR was higher in patients of Group B vs. Group A (2.85 ± 0.77 vs. 1.92 ± 0.53; P= 0.006) (Table 1). A HOMA-IR value > 2.24, expression of an intermediate value of IR, was present in 2 patients (22.2%) of Group A and 6 patients (66.7%) of Group B.

No correlation was found between ALT and SF levels and between both these parameters with FPG, MI 0-120, HOMA-IR, IGI and oDI.

b. Clinical and OGTT characteristics during follow-up

Annual or biennial OGTT results were available for all patients. However, only the results collected at five-year intervals were included in the analysis.

Fluctuations of glucose homeostasis were observed during the follow-up mainly in patients of Group B. The results of OGTT at 5,10 and 15 years, and the SF and ALT levels are depicted in Tables 2 and 3. The mean values of SF at 5, 10, 15 and 20 years were not statistically different between the two groups of patients (P= 0.36).

c. Clinical and OGTT findings at last observation

The incidence of GD (IFG, IGT and IFG + IGT) at last OGTT was more frequent (about five times) in Group B patients compared to Group A (χ^2: 5.505; P = 0.01). There were no case of diabetes mellitus. In patients with baseline NGT, a small proportion (2/9 at 15 years and only 1/8 at 20 years) developed abnormal GD. On the other hand, 3 out of 9 patients with IFG at baseline had persistent IFG 5 years later, 1 had persistent isolated IFG and 3 developed IGT 10 years later, 5/9 had IFG 15 years later, and finally 6/8 with IFG at baseline had abnormal GD after 20 years. There was no data for one patient (no. 2) because she has not yet completed the 20 years of follow-up (Table 3).

In both groups the FPG levels and the PG values at 1, 2 and 3 hours after OGTT were significantly higher compared to the control group (Table 4). Moreover, the insulin peak (Figure 1 B) and the oDI, after OGTT, were statistically lower compared to controls (Table 4). A lower IGI was documented only in Group B patients compared to controls (Table 4).

All other variables of table 4 were not statistically significant with the exception of a higher mean value of insulin at 3-h after OGTT in both groups of patients compared to controls (Table 4).

The comparison of glucose and insulin levels after OGTT in both groups of patients, at presentation and last observation, are illustrated in **figure 1 A and B**.

In both groups of patients, no correlation was observed between SF, ALT and indices of IS and IR. Compared to baseline, a reduction of HOMA-IR was present in patients of Group A, at last observation, and was associated with a reduction of ALT levels (P = 0.024).

A normal LIC (< 3.2 mg Fe/g d.w.) was observed in 3/6 patients of Group A and 1/6 patients of Group B, a mild increase in LIC (> 3.2 - <7 mg Fe/g d.w.) in 3/6 patients of Group A and 4/6 patients of Group B. One patient from Group B had very severe LIC (21.3 mg Fe/g d.w.) that was associated with a low cardiac T2* (5.3 ms).

The number of β -TM patients with endocrine complications did not differ significantly between the two groups: four out of 7 (77.1 %) patients of Group B had two endocrinopathies (Table 3: no.2, 4, 5 and 9) and 3/9 (33.3%) patients of Group A one endocrinopathy (Table 2: no 4, 5 and 6). The commonest complication in both groups was primary or secondary hypogonadotropic hypogonadism, followed by central hypothyroidism and hypoparathyroidism.

Two female patients in Group B (1 with spontaneous puberty and 1 with hypogonadotropic hypogonadism) presented with short stature (< 3rd centile). The mean IGF-1 concentrations were significantly decreased in both groups of patients compared to controls; they were not significantly correlated with serum ALT values (P = 0.46; P = > 0.05).

In the β -TM patients without GD (Group A: no. 1, 2, 4-9 plus Group B: no. 2-4), the cardiac T2* MRI values were normal and compared to prediabetic patients with IFG, IGT and IFG + IGT (40 ± 14.3 ms vs. 24.6 ± 13.4 ms; P = 0.039).

Discussion

In recent years there has been a growing interest in GD in patients with β thalassemia especially in TDT patients The pathogenetic mechanisms of glucose dysregulation are is complex and multifactorial ; the three main factors, IOL, chronic hypoxia, and chronic liver disease, may coexist in virtually all patients, and it is difficult to isolate and evaluate the contribution of each factor separately (2).

Prediabetes is an intermediate state of hyperglycemia with glycemic parameters above normal but

Table 2. Follow-up of oral glucose tolerance test (OGTT), serum ferritin (SF) and alanine aminotransferase (ALT), at baseline and after 5 - 10 - 15 and 20 years, in 9 β - thalassemia major (β -TM) patients normal glucose tolerance (NGT; Group A).

Patient at baseline no., sex, age at baseline (yrs)	OGTT after: 5 yrs, SF (ng/mL) and ALT (U/L)	OGTT after: 10 yrs, SF (ng/mlL) and ALT (U/L)	OGTT after: 15 yrs, SF (ng/mL) and ALT (U/L)	OGTT after: 20 yrs, SF (ng/mL) and ALT (U/L)
1. F -5.6	2040 19	2575 14	1496 18	2184 23
2. M -5.11	1540 80	702 46	706 28	958 47
3. M – 4.5	5035 147	5300 104	3745 43	2811 39
4. F – 5.11	1040 82	424 22	694 15	401 27
5. F – 5.11	1430 25	504 14	507 20	548 26
6. M – 6.11	1255 59	1685 46	1241 52	1413 38
7. F -7.10	1371 13	1136 27	1315 34	X
8. F – 5.5	2600 19	971 19	1099 24	1126 28
9. M – 4,9	2655 197	1717 171	901 32	1519 86

Normal OGTT | IFG:Impaired fasting glucose | IGT: Impaired OGTT | IFG+ IGT

SF: serum ferritin

ALT: alanine aminotransferase

Table 3. Follow-up of oral glucose tolerance test (OGTT), serum ferritin (SF) and alanine aminotransferase (ALT)at baseline and after 5 - 10 -15 and 20 years, in 9 β - thalassemia major (β -TM) patients with isolated impaired fasting glucose (IFG; Group B).

Patient at baseline no., sex, age at baseline (yrs)	OGTT after: 5 yrs, SF (ng/mL) and ALT (U/L)	OGTT after: 10 yrs, SF (ng/mL) and ALT (U/L)	OGTT after: 15 yrs, SF (ng/mL) and ALT (U/L)	OGTT after: 20 yrs, SF (ng/mL) and ALT (U/L)
1. F - 7.9	1675 46	776 39	393 22	2998 85
2. M -3.10	1480 40	1605 67	1259 56	X
3. M – 3.11	529 33	1930 11	759 13	1843 15
4. F- 4.4	1914 163	1957 47	1937 58	2108 38
5. M – 6.1	2365 60	3000 73	2159 39	2479 41
6. F – 6.1	1910 60	2318 24	1930 21	948 31
7. M- 7.10	1175 22	802 110	1755 29	1086 38
8. M – 7.7	1405 43	625 29	664 39	363 35
9. M- 4.8	1108 15	523 11	695 18	632 30

Normal OGTT | IFG:Impaired fasting glucose (at baseline) | IGT: Impaired OGTT | IFG+ IGT

SF: serum ferritin

ALT: alanine aminotransferase

Table 4. Clinical, laboratory and glycemic data, at last observation, in the two groups of 18 β - thalassemia major (β -TM) patients [Group A= n 9 with normal glucose tolerance (NGT); Group B: n = 9 with isolated impaired fasting glucose (IFG) at baseline] and Group C of healthy adult controls (n= 16). The values are expressed as mean ± SD.

Variables at last observation	β -TM of Group A (n: 9)	β -TM of Group B (n: 9)	Controls Group C (n: 16)	P-value A vs. B	P-value A vs. C	P-value B vs. C
Chronological age (yrs)	24.8 ± 1.9	25.2 ± 2.5	23.6 ± 3.5	NS	NS	NS
BMI (Kg/m^2)	22.2 ± 2.9	22.5 ± 3.5	21.5 ± 2.0	NS	NS	NS
Family history of diabetes • **Type 1** • **Type 2**	4/9 - 4	3/9 - 3	0/16	-	-	-
Splenectomy (yes)	0/9	1/9	-	-	-	-
Cardiac T2* (ms)	(6/9) 40 ± 14.3	(8/9) 24.6 ± 13.4	-	**0.039**	-	-
Liver iron concentration (LIC: mg Fe/g dry weight)	(6/9) 5.2 ± 4.2	(7/9) 7.7 ± 6.9	-	NS		-
ALT (U/L)	38.6 ± 19.3	41.0 ± 19.7		NS	-	-
IGF-1 (ng/mL)	99.7 ± 41.6	88.4 ± 33.2	280.3 ± 60.4	NS	< **0.0001**	< **0.0001**
HCVAb positivity	7/9	6/9	-	-	-	-
HCV-RNA positivity	2/9	3/9	-	-	-	-
Iron chelation therapy: **Desferrioxamine (DFO)** **Deferiprone (DFP)** **Deferasirox (DFX)** **DFO + DFP**	- 7 1 1 0	- 2 4 1 2	-	-	-	-
Fasting plasma glucose (mg/dL)	91.8 ± 8.1	97.4 ± 7.5	83.5 ± 8.6	NS	< **0.05**	< **0.001**
Plasma glucose 1-h after OGTT (mg/dL)	124.6 ± 19.3	159.6 ± 28.8	100.3 ± 18.6	<**0.01** .	<**0.01**	< **0.0001**
Plasma glucose 2- h after OGTT (mg/dL)	118.6 ± 21.8	139.7 ± 27.6	89.9 ± 16.4	NS	<**0.01**	< **0.0001**
Plasma glucose 3- h after OGTT (mg/dL)	105.4 ± 17.5	103.5 ± 25.6	78.5 ± 23.6	NS	**0.001**	<**0.05**
Fasting insulin (μU/mL)	7.0 ± 4.4	6.7 ± 3.5	5.6 ± 3.4	NS	NS	NS
Insulin peak (μU/mL)	37.4 ± 19.2	38.4 ± 16.6	54.5 ± 16.3	NS	<**0.05**	<**0.05**
Insulin 3-h after OGTT (μU/mL)	18.1 ± 9.9	17.4 ± 13.4	9.2 ± 4.4	NS	<**0.01**	<**0.01**
MATSUDA INDEX (MI 0-120)	8.59 ± 2.96	6.80 ± 2.28	8.62 ±3.52	NS	NS	NS
HOMA-IR	1.63 ± 1.14	1.53 ± 0.84	1.2 ± 0.8	NS	NS	NS
Insulinogenic Index (IGI)	0.69 ± 0.29	0.56 ± 0.29	1.7 ± 1.5	NS	NS	< **0.05**
Oral disposition Index (oDI)	5.71 ± 2.85	3.91 ± 2.15	13.8 ±10.1	NS	< **0.05**	< **0.01**

(*Contiuned*)

Variables at last observation	β -TM of Group A (n: 9)	β -TM of Group B (n: 9)	Controls Group C (n: 16)	P-value A vs. B	P-value A vs. C	P-value B vs. C
Associated endocrine complications: 1. **HH** 2. **Secondary HH** 3. **Central HT** 4. **HPT**	3/9 (33.3%) 1 2 - -	7/9 (77.7%) 6 1 2 1	-	NS	-	-
Serum ferritin (ng/mL)	1,363 ± 759.0	1,524 ± 884.9	-	NS	-	-
Total number OGTTs	14.1 ± 2.8	14.1 ± 1.4	-	NS	-	-

Legend: **HH** = hypogonadotropic hypogonadism; **Central HT**= Central hypothyroidism; **HPT**= Hypoparathyroidism

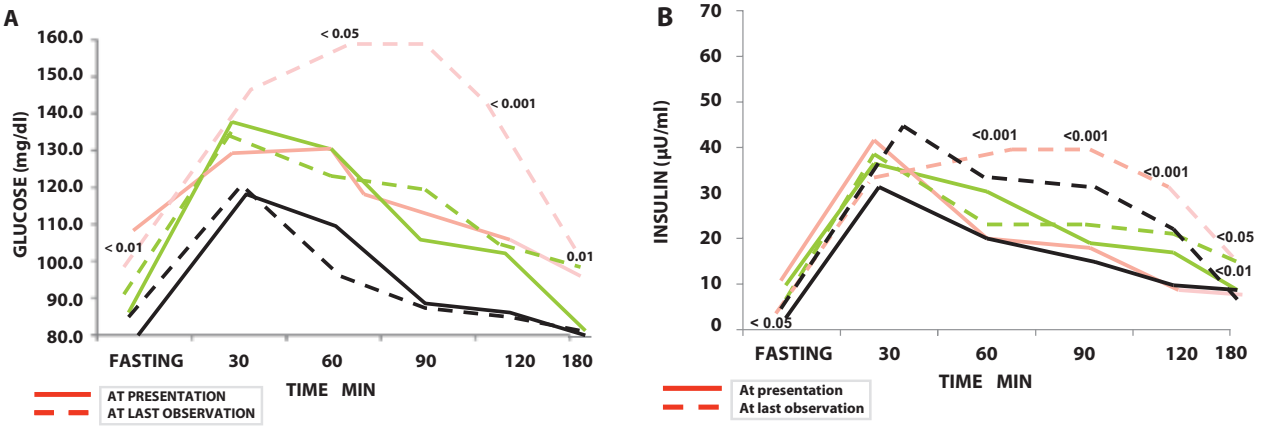

Figure 1. A and B. Glucose (A) and insulin (B) response curves during OGTT, at presentation and last observation (dotted lines), in β- TM patients of Group A with baseline NGT (green) and Group B with baseline IFG (pink). In black age matched control subjects (prepubertal and young adults; dotted lines). Only the statistically significant values comparing glucose and insulin levels, during OGTT, at presentation and last observation are reported in the figure.

below the diabetes threshold. It remains a state of high risk for developing diabetes in the general population, with an annual conversion rate of 5%-10%, with the same proportion converting back to normoglycaemia (27). Its progression to overt diabetes or regression to normal in β-TM patients is not straightforward.

The WHO and ADA propose 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 the ADA criteria, we have observed a prevalence of isolated IFG of 23.6 %, while using the higher threshold value of FPG of 110 mg/dL (6.1 mmol/L), according to WHO criteria, the prevalence was reduced to 15.3% (28).

In the general population, the two important pathophysiological mechanisms of diabetes, i.e. deficiency in islet β cell secretion and IS, are responsible for disorders of glycemic metabolism.

The present study demonstrates that: a) IFG in children with β -TM represents a risk factor for the development of GD at a later age; b) deterioration of glucose homeostasis developed gradually after a long period of time mainly in patients of Group B and was associated with fluctuations of glucose homeostasis; c) an early IR, (hepatic and peripheral tissue sensitivity

to insulin) as estimated by HOMA-IR and MI 0-120 followed by insulin secretion defect, assessed by IGI (Group B) and oDI; and d) compared to baseline, a reduction of HOMA-IR was present at last observation in patients of Group A, and was associated with a reduction of ALT levels (P = 0.024).

On the contrary, a significant reduction in the peak of insulin in response to arginine infusion and OGTT indicating a reduced insulin reserve before the development of significant IR or GD as reported by Soliman et al. (29) in 15 with β -TM children with a mean age of 2.8 ± 0.6 years, after a period of 3.1 ± 0. 6 years of high transfusions and intensive chelation. This observation supports the possibility of an additional pathophysiological mechanism responsible of GD in very young children with thalassemia.

Although a reduced oDI is considered an early marker of relationship between the â-cell function and the peripheral insulin sensitivity and is predictive of development of diabetes over 10 years (25), in our patients with NGT and reduced oDI, only 1 patient with IOL and serum ferritin of 1,843 ng/ml (Table 2, no.3) developed IFG after 20 years.

Understanding the pathophysiological changes that underlie IFG and IGT in TDT patients are important in developing additional intervention strategies to prevent the progression of one state to another, and eventually to diabetes. Patients with GD and IR, are most likely to benefit from lifestyle change and agents that improve hepatic insulin sensitivity, such as metformin. The development of IR may be due to: (a) a direct effect of iron overload and/or (b) hepatic dysfunction leading to reduced hepatic extraction of insulin resulting in an excess of glucose in the bloodstream (2,15). Moreover, an improvement of insulin secretion and glucose metabolism has been observed in patients with β-TM intensively treated with iron chelators in the early stages of dysglycemia (30).

Those patients with GD, who predominantly have muscle IR combined with impaired insulin secretion, are more likely to benefit from pharmacological agents that improve β-cell function, as reported by Osei et al. (31) and Abdul-Ghani et al. (32). Diabetes and insulin deficiency require immediate insulin implementation associated with intensive iron chelation therapy (2).

Our data on cardiac T2* values in patients with GD are in line with previous larger reports. Pepe et al. (33) systematically explored the link of pancreatic iron with glucose metabolism and cardiac complications in a cohort of 1,079 patients with β-TM patients. Patients with normal glucose metabolism showed significantly higher global pancreas T2* values than patients with IFG, IGT, and diabetes. A pancreatic T2* < 13.07 ms predicted an abnormal OGTT. Conversely, a normal pancreatic T2* value showed a 100% negative predictive value for disturbances of glucose metabolism and for cardiac iron. These data furtherly support a close link between pancreatic iron and heart disease and the need to intensify iron chelation therapy to prevent both impairment of glucose metabolism and cardiac and pancreatic iron accumulation.

The low IGF-1 level observed in our patients, compared to controls, suggests the potential role of impairment of liver function (increased ALT) and/or growth hormone insufficiency/ deficiency/ resistance (GHI/GHD/GHR) (33). There is increasing evidence that between 8% to 44% of adult patients with TM develop some degree of GHI/GHD (34). However, no correlation was observed between ALT and IGF-1 levels and no systematic studies were performed in our patients to assess the secretion of GH after stimulation tests.

Our study has several limitations. First, patients were enrolled from one centre and the sample size was small which might have affected the statistical analysis . On the other hand, there is the advantage of homogeneity of treatment with transfusions and, in particular, with chelation. It is to be noted that after 15-20 years follow-up, the IOL as expressed by SF was lower at the last evaluation compared to baseline, although the values were not statistically different. Hence, appropriately large cohorts of β-TM patients will be needed to solidify our results. Second, the assessment of IOL at baseline was made exclusively by SF as MRI was not available at that time.

Third, at baseline, we defined and validated cases with IFG on the basis of a single measurement, collected before OGTT, rather than using repeated measurement to confirm the diagnosis. Fourth, this retrospective study included patients who received, over time, different protocols for the treatment of

iron overload (though equally effective), therefore, the analysis of the effects of different iron chelators on glucose-insulin homeostasis was not possible. Lastly, the IS and IR are surrogate indices of pancreatic β-cell function. Nevertheless, to the best of our knowledges, the present study is the first to investigate the natural history of glucose-insulin homeostasis in young children with β -TM from early childhood to young adulthood.

In conclusion, these data are noteworthy for two reasons; for identifying, that baseline IFG predicts future development of GD, and, that almost half of patients with IFG at the outset had normal glucose handling 15 years later. One might question the need for an annual OGTT in patients with NGT and adequate iron chelation therapy since numerous OGTTs might raise patients' anxiety due to reproducibility problems, without necessarily indicating true deterioration of glucose handling. Therefore, it is difficult to conclude which is the optimal timing of OGTT. We believe that subjects with repeated normal FPG and no evidence of severe IOL could be monitored with biennial, rather than annual OGTT, until the age of 20 years. However, we have to keep in mind that our data concern a small group of children with β -TM and need confirmation. Understanding the pathogenetic mechanisms in the progression from normal glucose homeostasis to GD and identifying the risk factors for the glycometabolic defects in thalassemic patients might help the formulation of efficient interventions.

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.

A**uthor Contributions Statement:** VDS collected the de-identified data set, reviewed the current literature and prepared the first draft of manuscript. VDS, ATS, SD, ATS, PT, MK and CK partecipated actively to preparation of final version of manuscript. All the authors approved the final version of submitted manuscript.

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Received: 20 November 2021
Accepted: 5 December 2021
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