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Original article

Long-term retrospective study on the progression of prediabetes to diabetes mellitus in transfusion-dependent β-thalassemia (β-TDT) patients: The experience in Oman and Italy

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Abstract. *Background:* Prediabetes in transfusion-dependent β-thalassemia (β-TDT) patients is a significant risk factor for development of diabetes mellitus (DM), with increasing incidence with age. *Objectives:* This retrospective study aimed to compare the progression from prediabetes to DM in β-TDT patients who initiated iron chelation therapy (ICT) before age 6 and between ages 6 and 10. Additionally, we sought to characterize the trajectories of fasting and post-glucose load insulin sensitivity and secretion (HOMA2-%S and HOMA2-% β) in a β-TDT subgroup, monitored annually for four years preceding DM diagnosis. Research Design and Methods: We retrospectively reviewed annual oral glucose tolerance test (OGTT) data for 18 β-TDT patients from Oman and Italy, from the onset of prediabetes to DM diagnosis. *Results:* The mean age at prediabetes diagnosis was 17.5 ± 4.2 years, and at DM diagnosis was 21.6 ±5.2 years. A direct correlation was observed between the age at prediabetes and DM diagnosis (r: 0.6925, P: 0.0014). Three years before DM diagnosis, a notable increase in baseline, 1-hour, and 2-hour post-load plasma glucose levels during OGTT was observed, alongside a decrease in HOMA2-% β. *Conclusions:* Elevated 1-hour post-load plasma glucose levels and a declining HOMA2-% β during the transition to DM are indicative markers for identifying β-TDT patients with high likelihood for imminent progression to DM. (www.actabiomedica.it)

Key words: β-thalassemia major, prediabetes, diabetes,OGTT, HOMA2- IR, HOMA2-% β, HOMA2-%S

Introduction

Prediabetes is an intermediate stage between normal glycemia and diabetes and is highly prevalent, especially in older age groups and obese individuals (1). The prevalence of prediabetes varies depending on the population studied; African American, Native American, South Asian and Hispanic have all been shown to have an increased risk of having prediabetes compared with their Caucasian (2). Moreover, it is well known that the prevalence of prediabetes in some chronic diseases like thalassemia is higher compared to the general population (3-5).

The definitions and screening criteria for prediabetes differ between guidelines published by different organizations (1). For the American Diabetes Association (ADA), prediabetes is defined by two specific parameters, impaired fasting glucose (IFG), defined as

fasting plasma glucose (FPG) of 100 to 125 mg/dL, and impaired glucose tolerance (IGT), defined as 2-h plasma glucose of 140-199 mg/dL, measured after a 75-g oral glucose load or a combination of both (IFG plus IGT) and/or glycated hemoglobin level (HbA_{1C}) of 5.7%-6.4% (6). The World Health Organization (WHO) considers a higher cut-off value for IFG (110-125 mg/dL) (7).

Prediabetes will progress to overt type 2 diabetes (T2DM) in approximately 25% of subjects within 3–5 years, and as many as 70% of individuals with prediabetes will develop overt diabetes within their lifetime (8). Prediabetes is likely to progress to overt type 2 diabetes (T2DM) in approximately 25% of individuals within 3–5 years. Moreover, up to 70% of those with prediabetes may develop overt diabetes over their lifetime (8). However, prediabetes may be reversible, through the implementation of lifestyle modification programmes and increased levels of physical activity (9).

An Italian study conducted over 11.5 years revealed that diabetes developed in 9.1% of patients with isolated impaired fasting glucose (IFG) and in 44.4% of subjects with both IFG and impaired glucose tolerance (IGT) (10). A higher risk of diabetes has also been reported in transfusion-dependent β-thalassemia (β-TDT) patients who exhibit both IFG and IGT (3-5). In these patients, glucose dysregulation (GD) may manifest at an earlier stage, with the incidence increasing with age (11).

Due to the insidious onset of DM, annual OGTT in all β-TDT patients, starting from the age of 10 years, and even at an earlier age in severely iron overloaded patients, is crucial for the prompt diagnosis and identification of high-risk subjects (5). Additional screening methods such as urine glucose testing, random plasma glucose measurements, fructosamine testing, and monitoring of hemoglobin A1c levels are not recommended due to their low sensitivity (12,13).

Diabetes Mellitus (DM) in β-TDT patients is characterized by qualitative and quantitative defects in insulin secretion, often accompanied by insulin resistance (IR). IR can result from reduced glucose uptake in muscle tissue (peripheral insulin resistance) and from impaired suppression of hepatic glucose production (hepatic insulin resistance). These conditions are primarily attributed to iron overload, a consequence of chronic blood transfusions, and to a lesser extent, from dietary iron absorption (3-5).

Recently, our research demonstrated that a progressive increase in 1-hour post-glucose-load plasma glucose (PG) during a 3-hour oral glucose tolerance test (OGTT) is linked to progressive β-cell failure, increased peripheral IR, and a reduction in the oral disposition index (oDI). This finding suggests that it could be a significant marker for emerging DM in β-TDT patients with prediabetes (14). However, such comprehensive evaluations are time-consuming and present logistical challenges for both patients and healthcare providers (15).

The first objective of this retrospective study was to evaluate and compare the natural course during OGTT (PG at baseline and 2-h) of prediabetes to DM, using the current definitions of ADA (6) in patients who started iron chelation therapy (ICT) before the age of 6 years and between 6 and 10 years, and the second outcome was to characterize the trajectories of fasting and post glucose load, insulin sensitivity and insulin secretion (HOMA2-%S and HOMA2-% β) in a subgroup of β-TDT patients assessed annually for 4 years before the diagnosis of DM.

Material and methods

Study design and patient population

The retrospective study included β-TDT patients followed in the last four decades in Italy (2 Centers; Ferrara and Siracusa) and Oman (1 Center; Muscat). The follow-up study included patients with isolated IFG, isolated IGT and IFG plus IGT. Only those who progressed to DM during the course of follow-up were selected for the present study.

β -TDT was diagnosed by the referring Centers using hemoglobin HPLC and/or molecularly characterized genotype.

Information on age, sex, family history of diabetes in siblings or parents, medical history and age at starting of ICT were collected. Height and weight were measured using standard techniques. Body mass index (BMI) was calculated as body weight divided by

the square of height and categorized as underweight (<20 kg m^2), normal weight (20–24.9 kg m^2), overweight (25–29.9 kg m^2) and obesity (≥30 kg m^2).

Eligibility criteria

Inclusion criteria were: (a) β-TDT patients regularly subjected to OGTT screening every 12 months and (b) β-TDT patients with full data including annual 2h- OGTT (at baseline and 120' min); and (c) outcome progression from the diagnosis of prediabetes to diabetes. The exclusion criteria included: (a) non-transfusion dependent β-thalassemia patients (NTDT); (b) bone-marrow transplanted patients; (c) β-TDT patients using drugs affecting glucose metabolism; (d) presence of associated chronic illnesses besides β-TDT, such as HIV positivity and chronic kidney diseases; (e) β-TDT patients pharmacologically treated for prediabetes, and (f) patients with lack of clinical and OGTT data.

Classification of sub phenotypes of prediabetes and DM

The criteria of American Diabetes Association (ADA) (15) were followed for the diagnosis of: (a) normal glucose tolerance (NG); (b) isolated IFG: fasting PG 100–125 mg/dL (5.6–6.9 mmol/L); (c) isolated IGT: 2-h PG levels during OGTT between 140 and 199 mg/dL (7.8-11.0 mmol/L); (d) combined glucose intolerance (IFG plus IGT) and DM. Moreover, patients with diabetes were divided into two categories: without fasting hyperglycemia (DM FH^-: ≤ 126 mg/dL or ≤ 7.0 mmol/L) and with fasting hyperglycemia (DM FH^+ ≥ 126 mg/dL or ≥ 7.0 mmol/L).

Venous plasma blood samples were taken after 8-10 hours of fasting before undergoing a standard 2-hour OGTT. Blood glucose was measured using glucose oxidase method (16).

Estimation of trajectories of fasting glucose, 2-hour glucose, HOMA2-%β and HOMA2-%S

In a subset of patients who initiated iron chelation therapy (ICT) at a mean age of 5.7 ± 2.0 years (ranging from 2.8 to 9.7 years), we also evaluated the trajectories of fasting plasma glucose (FPG), 2-hour plasma glucose (PG), HOMA 2 insulin sensitivity (HOMA 2-%S), and HOMA 2 β-cell function (HOMA 2-% β) over a 4-year annual follow-up period leading up to the diagnosis of diabetes mellitus (DM). These evaluations were conducted using the HOMA2 calculator v2.2 (17,18). Insulin levels were measured either by radioimmunoassay or chemiluminescent immunoassay.

Laboratory evaluations and assessment of iron overload

Iron overload (IOL) was assessed by serum ferritin (SF) and was arbitrarily classified as mild (SF: < 1,000 ng/mL), moderate (SF: >1,000 ng/mL and < 2,000 ng/mL) or severe (SF: >2, 000 ng/mL).

HCV-antibodies, PCR for HCV-RNA, free thyroxine (FT4), serum thyrotropin (TSH), plasma cortisol, gonadotropins (LH and FSH), estradiol (E2) or total testosterone (TT) values were also collected.

Statistical analysis

Qualitative variables are expressed as mean ± standard deviation (SD), standard error (ES) median and percentages. All data were tested for normal distribution with the Shapiro–Wilk test and were compared using the Student's test in case of a normal distribution or using the Wilcoxon-Mann-Whitney test in case of non-normal distribution. Statistical correlation between two variables was evaluated with Pearson's or with Spearman's correlation in case of a normal or a non-normal distribution, respectively. For the statistical analysis, a software program was used and validated, according to Alder and Roesser (19). Two-tailed *P*-values were used and were considered as significant if < 0.05.

Ethics

No ethical approval was needed because the study was a retrospective review of observational data/records, containing no identifiable private information and only aggregated data were analyzed and presented (20). Moreover, the patients underwent only routine diagnostic procedures according to the national Italian protocols and the International Guidelines

(4,5, 21,22). The study was developed in accordance with the Helsinki declaration (www.wma.net) and all patients provided informed consent.

Results

A total 18 out of 57 (31.5%) β-TDT patients with DM were eligible to be included in the study. The prevalence of DM was 13.9% in Oman and 11.5% in Italy (Figure 1).

Patients' characteristics at diagnosis of prediabetes

The mean age at the diagnosis of prediabetes was 17.5 ±4.2 years (Oman: 16.8 ±4.1 years, Italy: 18.2 ±4.5 years; P: 0.50). Seven (38.8%) were females and 11 (61.1%) were males. A family history of DM was reported in 4/14 patients (28.5%). In 4 patients, information about family history were not available. Eleven patients (61.1%) had a normal BMI; 5 patients (27.7%) were underweighting and 2 (11.1%) were classified as obese (both were females). HCV positivity was present in 12/18 patients (66.6%) (9/18 HCV-Ab^{+} and 3 HCV RNA^{+}). The mean SF in the whole cohort at the time of commencing iron chelation therapy (9 patients < 6 years of age [*Group A* = mean: 4.5 ±0.9 years] and 9 patients between 6-10 years [*Group B* = mean: 7.8 ± 1.4 years] was 2,963 ±1,797 ng/mL. Detailed information on pretransfusion hemoglobin level, history of splenectomy, ICT, and SF at starting of ICT and at the diagnosis of prediabetes are reported in Table 1.

Progression of prediabetes to diabetes mellitus

For the whole cohort, the time interval from diagnosis of prediabetes to development of DM was

4.8 ±3.6 years (range: 0.10-13 years). The median time interval was shorter in Group B vs. Group A (3.5 vs. 6 years), but the difference between the two groups was not statistically significant (P: 0.13) (Table 2). However, the age at the diagnosis of prediabetes was directly correlated with the age at diagnosis of DM (r: 0.6925, P: 0.0014).

No significant difference in the progression of prediabetes to diabetes in relation to patients' gender

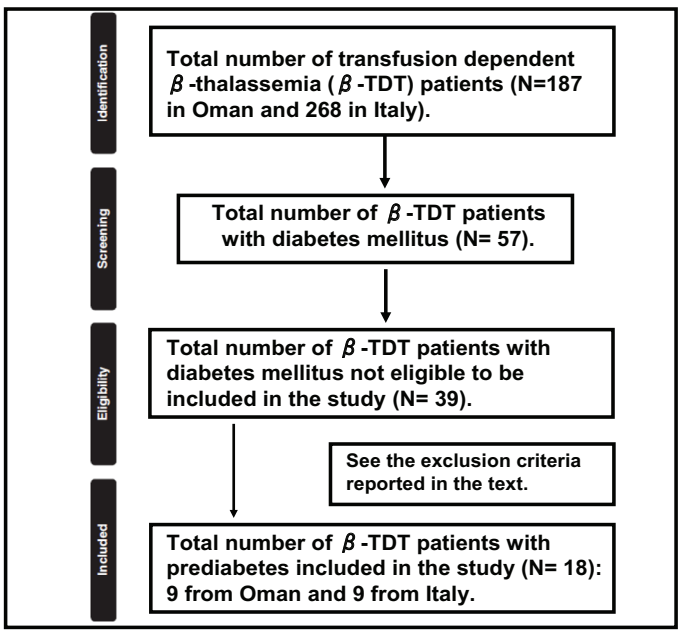

Figure 1. PRISMA flow chart for the inclusion criteria of β-TDT patients with natural history from prediabetes to diabetes mellitus.

Table 1. Demographic characteristics, biochemical and diagnostic parameters (mean and standard deviation) in 18 β-TDT patients at the diagnosis of prediabetes (PD). Patients are subdivided in 2 groups in relation to the age at starting iron chelation therapy (Group A: < 6 years and Group B: from 6 to 10 years).

Variables	Group A: < 6 years	Group B: from 6 to 10 years	P value
Number of β-TM patients	9	9	=
Age (yr)	15.8 ±3	17.1±4.7	0.17
Sex (M/F)	2/7	5/4	=
Body Mass Index (Kg/m^2)	21.2 ±4.4	22.3 ±5.8	0.65
Family history of diabetes: **Yes (n)** **Not available**	= 1/7 2/9	= 3/7 2/9	= 0.27 =
Splenectomy (n. and %)	4/9	5/9	=
Mean pre-transfusion Hb level (g/dL)	9.0 ±0.2	9.1 ±0.4	0.51
Age (yr) at starting iron chelation therapy	4.5 ±0.9	7.8 ±1.4	**< 0.0001**
Serum ferritin (ng/mL) at starting chelation therapy	3,265 ±1,134	4,872 ±1,085	**0.0073**
Serum ferritin (ng/mL) in the year of prediabetes	2,399 ±1,547	3,740 ±2,520 (*)	0.20
Iron chelation therapy at the diagnosis of PD **Desferrioxamine (DFO) given s.c. (n)** **Deferiprone (DFP) (n)** **Deferasirox (DFX) (n)** **DFO + DFP (n)**	 5/9 1/9 2/9 1/9	 6/9 1/9 1/9 1/9	 = = = =
Age at diagnosis of prediabetes	15.8 ±3	17.1 ±4.7	0.49
Diagnosis of prediabetes: **1. isolated impaired fasting glucose (i-IFG) (n)** **2. isolated impaired glucose tolerance (i-IGT) (n)** **3. IFG+IGT (n)**	 1/9 3/9 5/9	 0/9 2/9 7/9	 0.31 0.60 0.33

Abbreviations: (*): 8 patients; PD: prediabetes; s.c.: subcutaneously.

was found (P:0.64). The mean age at the diagnosis of DM was 21.6 ±6.3 years (Oman) vs. 21.5 ±4.1 years (Italy) (P:0.96).

The incidence in percentage of diabetes in relation to years of disease progression is reported in Figure 2. During this time, treatment recommendations included lifestyle modifications, regular physical activity along with diet modifications and intensification of ICT.

Detailed information, at diagnosis of diabetes, on pre-transfusion Hb level, BMI, ICT, and SF and associated endocrine complications are reported in Table 2. Diabetes mellitus without fasting hyperglycemia (DM FH$^-$) was reported in 1/19 patients (5.2%). Primary and acquired idiopathic hypogonadotropic hypogonadism were the most commonly reported endocrine complication (Table 2).

The SF at the diagnosis of DM was in 6/18 patients on DFO treatment: 2,385 ±1,920 ng/mL; in 6/18 patients on oral chelation monotherapy: 2,933 ±2,889 ng/mL and in 6/18 patients on combined therapy (DFO+DFP): 2,266 ±2,620 ng/mL.

Trajectories of plasma glucose progression before the diagnosis of diabetes mellitus

Trajectories of PG levels at baseline, 60' and 120' minutes after OGTT in a subgroup of 9 β-TDT

Table 2. Demographic characteristics, biochemical and diagnostic parameters (mean and standard deviation) in 18 β-TDT patients at the diagnosis of diabetes mellitus (DM). Patients are subdivided in 2 groups in relation to the age at starting iron chelation therapy (Group A: < 6 years and Group B: from 6 to 10 years).

Variables	Group A	Group B	P value
Number of β-TM patients	9	9	=
Age (yr)	22.1 ±4.6	20.7 ±6.2	0.59
Body Mass Index (Kg/m^2)	21.3 ±4.2	22.8 ±6.1	0.55
Mean pre-transfusional Hb level (g/dL)	9.3 ±0.2	9.5 ±0.4	0.19
Serum ferritin (ng/mL) at the diagnosis of DM	1,432 ±662.6	4,039 ±2,891	**0.017**
Progression from prediabetes to DM (yr)	6.0 ±3.4	3.5 ±3.8	0.13
Iron chelation therapy at the diagnosis of DM			
Desferrioxamine (DFO) (n)	2/9	4/9	0.15
Deferiprone (DFP) (n)	1/9	19	=
Deferasirox (DFX) (n)	2/9	2/9	=
DFO + DFP (n)	4/9	1/9	0.27
Associated endocrine complications:			
1. Delayed puberty (n and %)	0/9	1/5 (*)	=
2. HH in males (n.)	2/2	3/5	=
3. HH in females (n.)	5/7	3/4	=
4. Secondary amenorrhea	1/7	0/0	=
5. Primary hypothyroidism (n.)	1/7	1/4	=
6. Hypoparathyroidism (n.)	1/9	1/9	=
7. Hypocortisolism (n.)	0/9	0/9	=

Abbreviations: HH: hypogonadotropic hypogonadism; (*): 1 male patient had spontaneous pubertal development (SF at the diagnosis of DM:1,340 ng/mL).

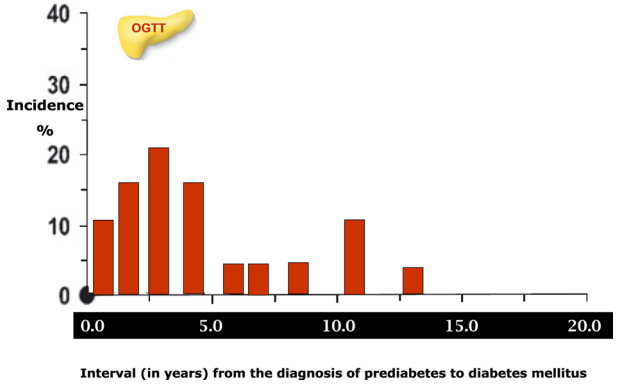

Figure 2. Incidence of diabetes (%) in relation to the interval (in years) from the diagnosis of prediabetes to diabetes mellitus.

Italian patients was assessed for the 4 years prior to the diagnosis of DM (Figure 3). During this observational period there was a significantly progressive increase in the trajectories of PG at 0', 1-h and 2-h before and during OGTT (Figure 3). Interestingly, 8/10 β-TDT patients (80%) presented 1-hour post-load PG value ≥155 mg/dL (8.6 mmol/L) three years before the diagnosis of DM.

The number of patients with 1-hour post-load PG value ≥ 155 mg/dL (≥ 8.6 mmol/L) was at -4 years: 2/7 (28.5%); -3 years: 8/9 (88.8%); - 2 years: 7/9 (77.7%) and at -1 year: 7/9 (77.7%) (PG range:148-276 mg/dL).

The calculated HOMA2-IR did not change significantly during the 4 years that preceded the diagnosis of DM (Figure 4). One overweight female patient had a HOMA 2-IR value above the mean of 75 th percentile reported in 191 healthy subjects, aged 18–31 years (23). A progressive and significant decline of HOMA 2-β cell % function was observed starting from 2 years before the diagnosis of diabetes (Figure 5). No significant variations were found in HOMA 2- insulin % sensitivity (Figure 6). The normal values reported in the literature in subjects aged 20-45 years, with BMI between 18.5-24.9 Kg/m^2, without diabetes or prediabetes and not taking medications are: HOMA2-% β 89.9 (range: 76.9-110.5) and HOMA2-%S 151.8 (range: 111.8-209.2) (24).

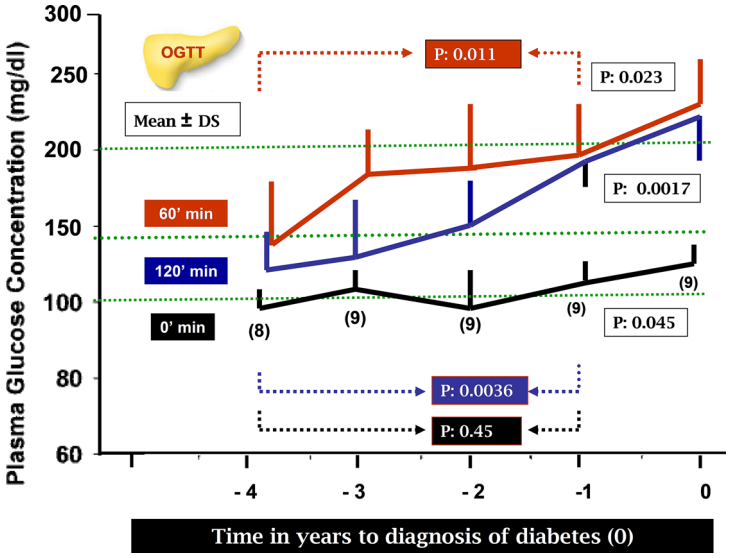

Figure 3. Time trajectories of glycemic levels at baseline (black line), 60' minutes (red line) and 120' (blue line) minutes (blue line) after OGTT before and at diagnosis of diabetes. The green dotted lines indicate the definition limits for IFG, IGT and diabetes.

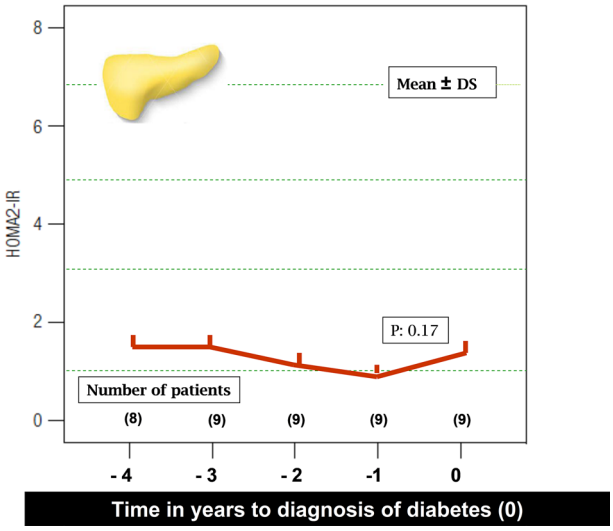

Figure 4. Homeostasis model assessment of insulin resistance (HOMA2-IR) values, expressed as mean and standard deviation (DS), before and at the diagnosis of diabetes.

Correlations

The age at the diagnosis of prediabetes was directly correlated with the age at diagnosis of DM (r: 0.6925, P: 0.0014) but no other statistically significant correlations were observed between all the variables included in the study and SF. The limited number of patients with i-IFG and i-IGT did not permit a comparison of disease progression versus patients with IFG plus IGT.

A significant direct correlation at the diagnosis of diabetes was found between SF and HOMA2-IR (r: 0.7363, P:0.023) and an inverse correlation with HOMA 2-IS% (r: -0.75, P:0.019).

Discussion

The mechanisms underlying glucose dysregulation (GD) in β-thalassemia major patients (β-TDT) are subject to intense investigation. The Oral Glucose Tolerance Test (OGTT) is useful for screening prediabetes and detecting the presence of GD in β-TDT patients, aiding in the prevention or delay of diabetes mellitus (DM) in high-risk individuals. In β-TDT patients with DM, insulin deficiency is the primary defect, but insulin resistance (IR) also contributes. As for the pathophysiology of prediabetes in the general population, individuals with isolated impaired fasting

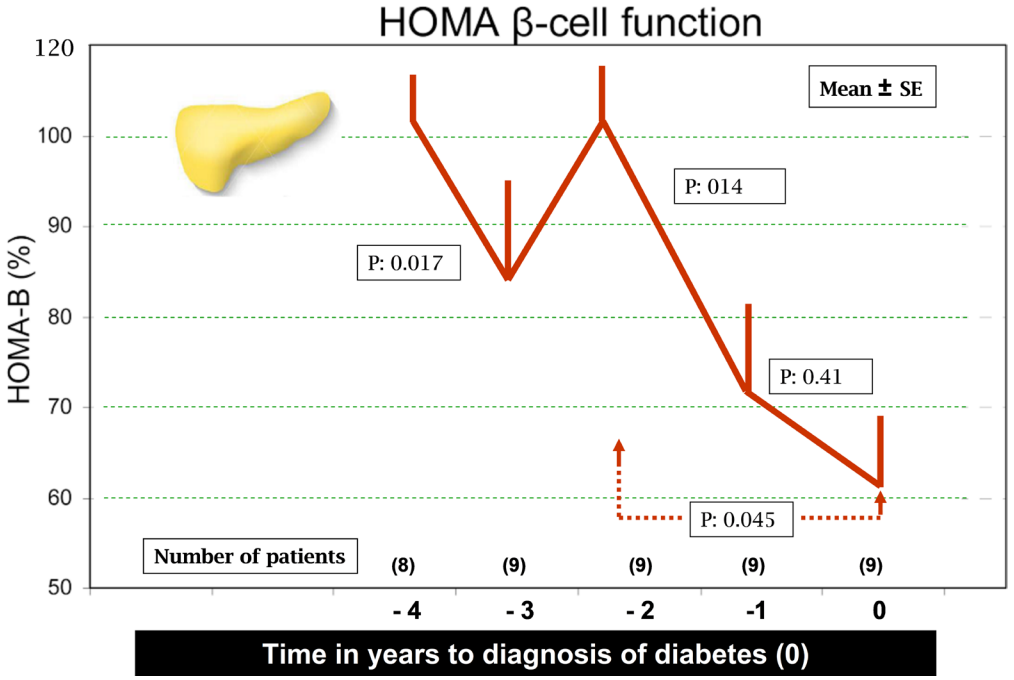

Figure 5. Homeostasis model assessment of β-cell function percentage (HOMA 2 β-cell function %), expressed as mean and **standard error** (SE), before and at the diagnosis of diabetes.

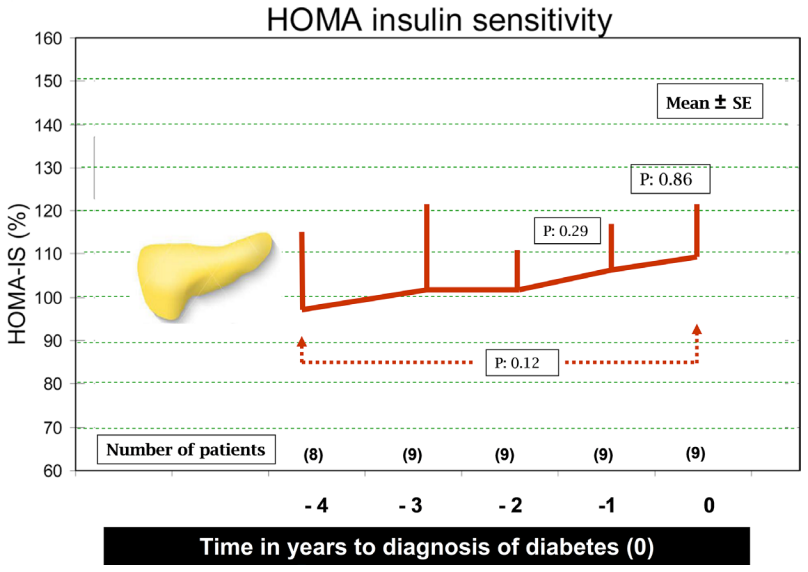

Figure 6. Homeostasis model assessment of insulin sensitivity percentage (HOMA 2-S%), expressed as mean and **standard error** (SE), before and at the diagnosis of diabetes.

glucose (i-IFG) predominantly exhibit hepatic IR, while maintaining normal muscle IR. β-Cell function is compromised, showing a diminished insulin secretory response in the initial 30 minutes, which normalizes in the later stages (60 to 120 minutes). Those with isolated impaired glucose tolerance (i-IGT) display normal or slightly reduced hepatic insulin sensitivity, alongside moderate to severe muscle IR (25).

In β-TDT patients receiving regular blood transfusion, ICT is required to decrease the iron burden and to prevent and/or delay long-term complications associated with iron deposition in tissues. Regular adherence to treatment regimens throughout a lifetime is recommended, as even short periods of interruption to treatment can have damaging effects. Most guidelines agree that the indication to initiate ICT should start after the cumulative transfusion of 10–20 units of packed red blood cells (pRBC), when SF is greater than 1,000 ng/mL and/or transferrin saturation (TS) level is ≥70% (9,10). However, iron overload in patients with β-TDT may occur even earlier (26) and before they received 10 times transfusion, as reported by Susanah et al. (27). However, in very young children, iron chelation therapy requires a delicate balance between prevention of iron toxicity while at the same time minimizing the risk of iron depletion that is critical for neurodevelopment and other rapidly proliferating cells.

Combined therapy (use of two chelators) has been shown to be beneficial in severe iron overloaded subjects. The choice of the most appropriate chelation regimen depends on the iron burden, patient preference, adherence to treatment, toxicity of ICT, costs and availability of chelating agent. Due to differences in organ-specific iron transport, the rate of iron loading and unloading is much faster in the liver than in the heart and endocrine organs (28).

Our retrospective study provides interesting information on GD in β-TDT patients who started ICT between the ages of 2.8 -10 years (mean: 6.2 years, median: 5.8 years). In particular:

a. The mean age at the diagnosis of prediabetes (mainly IFG plus IGT) was not statistically different in patients who started ICT at a mean age of 4.5 years versus those who started at a mean age of 7.8 years. In the latter group, the mean SF level was higher at diagnosis of prediabetes compared to the former. Therefore, early chelation therapy has been suggested to reduce saturation of transferrin (TSAT) and the appearance of LPI, which is considered the main form of iron accumulation in organs that include the pancreas, heart, and pituitary gland (29).
b. The age at which prediabetes is diagnosed directly correlated with the age at which DM was diagnosed in patients with β-TDT (r: 0.6925, P: 0.0014), indicating that the transition from prediabetes to diabetes occurs over a shorter period in younger β-TDT patients. This age-related variation may be attributable to an accelerated decline in β-cell secretion among adolescents with β-TDT compared to their young adult counterparts, a trend also observed in individuals without thalassemia (1).
c. Within 3 years of being diagnosed with prediabetes, 12 out of 19 (63.1%) patients with β-TDT developed DM. This rapid progression to DM in over half of the cases highlights that some individuals with β-TDT advance more quickly than others. Consequently, distinguishing between patients likely to progress rapidly versus those who may progress more slowly is crucial. Such differentiation could be instrumental for the early detection of β-cell function decline and the timely initiation of intervention strategies.
d. The long-term follow-up study on the pathophysiology of potential risk factors responsible for the development of DM in β-TDT patients with prediabetes is limited. Therefore, our objective was also to elucidate the trajectories of PG, IR, β-cell function and insulin sensitivity assessed annually for 4 years prior the diagnosis of DM. Transition from prediabetes to DM was accompanied by a progressively rapid PG rise at baseline, 1-h and 2-h during OGTT from -2 to 0 year (at diagnosis of DM) and was associated with a decline of HOMA2-% β. HOMA2-IR was increased in only 1 patient, and insulin sensitivity was not found to be a

significant predictor of diabetes development, as reported in another our studies (14).

e. At diagnosis of DM, severe iron overload (SF: >2, 000 ng/mL) was found in 2/9 (22.2%) patients who started ICT < 6 years of age (1 pt. was treated with DFO and 1 pt. with DFX) versus 5/9 (55.5%) patients who started ICT between 6-10 years of age (2 pts. were treated with DFO, 2 pts. with DFO+DFP and 1 pt. with DFX). Although it was not possible to assess in our retrospective study the patients ' adherence to iron chelation therapy, it is possible that the worsening of iron burden in β-TDT patients who started late ICT required more aggressive treatment, such as increased dosage of chelating agent, combinations of chelators and additional clinical and diagnostic evaluations that associated to psychological, social, and financial factors have favored the non-adherence to ICT.

It is worth mentioning that the time interval for progression from prediabetes to DM was numerically shorter at 3.5 years in the subgroup of patients who started iron chelation after the age of 6 years old in comparison to a latency period of 6 years in those who started iron chelation before the age of 6. Although this difference did not reach statistical significance, the small sample size of subgroups suggests a potentially true difference in time interval for this progression which should be explored in future studies with larger populations. Additionally, the significantly higher iron burden in patients who started iron chelation after the age of 6 years might be the key contributor to their potentially more rapid progression to deterioration of dysglycemia. It remains to be seen to which extent the age of starting iron chelation as well as the total exposure and magnitude to iron overload determine the rate and speed of progression to worse categories of dysglycemia.

Our study is subject to several notable limitations. Firstly, the relatively small sample size may limit the generalizability of our findings. Secondly, the measurement of plasma glucose (PG) concentrations was based on a single OGTT, which is subject to within-subject variability. Thirdly, our study did not assess the validity and reliability of the Static Sensitivity Index (SSI) in estimating β-cell function, insulin sensitivity (IS), and insulin resistance (IR) against reference standards from direct methods such as the hyperinsulinemic-euglycemic glucose clamp and the insulin suppression test, or indirect methods like the frequently sampled intravenous glucose tolerance test (FSIVGTT). However, Romo-Romo et al. (24) reported a significant correlation of HOMA2-%β and HOMA2-%S with the acute insulin response to glucose and the insulin sensitivity index from the FSIVGTT (P:< 0.01). Fourthly, the use of different insulin assays in our patients could introduce variability at both preanalytical and analytical levels. Finally, our study did not draw comparisons between patients who progressed from prediabetes to diabetes and those who did not progress.

Despite the above limitations, we believe that our results have important clinical value. We have reported information on the conversion rate from prediabetes to DM in β-TDT patients followed over a median time period of 3.5 years (ranging from 10 months to 13 years) to elucidate the patterns of change over time. The trajectories of PG and the evolution of simple surrogate indices of insulin secretion and sensitivity may represent an easy and inexpensive tool for any clinician to recognize patients at increased risk for developing DM. Moreover, this is the first study that assessed surrogate indices of glucose metabolism based on a single blood sample for a better understanding of pathophysiology of prediabetes progression and for elucidating the patterns of insulin secretion over time as a very practical alternative tool to more complex study analyses.

In brief, additional studies are necessary to better understand the respective roles of the progressive reduction of insulin secretion and the variation of insulin sensitivity.

In conclusion, our results indicate that a younger age at the diagnosis of prediabetes, progressive increase of PG at baseline and during OGTT and/or progressive decline of HOMA 2- β % during the transition from prediabetes to DM, could have a negative impact on glucose homeostasis. Finally, our preliminary data support that, in some β-TDT patients, DM seems to be predominantly characterized by an insulin deficiency state.

Conflict of Interest Statement: 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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