



**Original Article**

**Fasting Plasma Glucose Levels within the High Normal Range are Associated with a Significantly Increased Risk of Future Dysglycemia in Transfusion-Dependent  $\beta$  Thalassemia: A Decade-Long Multicenter Retrospective Analysis**

Vincenzo De Sanctis<sup>1</sup>, Forough Saki<sup>2</sup>, Mehran Karimi<sup>3</sup>, Mohammad Faranoush<sup>4</sup>, Ihab Elhakim<sup>5</sup>, Ashraf T. Soliman<sup>6</sup>, Shahina Daar<sup>7</sup> and Ploutarchos Tzoulis<sup>8</sup>.

<sup>1</sup> Coordinator of ICET-A Network (International Network of Clinicians for Endocrinopathies in Thalassemia and Adolescent Medicine), Pediatric and Adolescent Outpatient Clinic, Quisisana Hospital, Ferrara, Italy.

<sup>2</sup> Shiraz Endocrinology and Metabolism Research Center, Shiraz, Iran.

<sup>3</sup> Pediatric Hematology Oncology Department, American Hospital, Dubai, UAE.

<sup>4</sup> Pediatric Growth and Development Research Center, Institute of Endocrinology, Iran University of Medical Sciences, Tehran, Iran.

<sup>5</sup> Emeritus Professor of Pediatric Nephrology, Ain Shams University; Head of the Department of Pediatrics, BUC (Badr University in Cairo), Cairo, Egypt.

<sup>6</sup> Department of Pediatric Division of Endocrinology, Hamad General Hospital, Doha, Qatar.

<sup>7</sup> Department of Haematology, College of Medicine and Health Sciences, Sultan Qaboos University, Muscat, Sultanate of Oman.

<sup>8</sup> Department of Diabetes and Endocrinology, Whittington Hospital, University College London, London, UK.

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**Abstract. Objectives:** The primary objective was to evaluate the risk of developing glucose dysregulation and diabetes mellitus over 10 years among transfusion-dependent  $\beta$ -thalassemia ( $\beta$ -TDT) patients with varying levels of fasting plasma glucose (FPG) within the normoglycemic range. The secondary objective was to identify which baseline variables were associated with a higher risk of developing abnormal fasting glucose levels in the future.

**Setting:** This retrospective observational study included  $\beta$ -TDT patients followed from January 2014 to January 2025 in three thalassemia centers: Tehran and Shiraz in Iran, and Ferrara in Italy.

**Patients and results:** A total of 238  $\beta$ -TDT patients (age range: 10–41.9 years; 96 males and 142 females) were included in the study. Patients were categorized into three subgroups according to their fasting glycemic status during the 10 year follow-up [Group A: 93/238  $\beta$ -TDT patients (39.1%) with persistent normal FPG according to the American Diabetes Association (ADA) criteria; Group B: 67/238 patients (28.1%) developed persistent impaired fasting glucose (IFG), and Group C: 78/238 patients (32.8%) developed thalassemia-related diabetes mellitus (Th-RDM)]. To determine the optimal cutoff for the risk of progressing to impaired fasting glucose (IFG) and Th-RDM at 10-year follow-up, ROC curve analyses and respective areas under the curve were analyzed. The FPG cutoff value for optimal specificity and sensitivity was established at 87.5 mg/dL. Almost all (76/78) patients who developed Th-RDM (97.4%) were diagnosed in Shiraz. At the diagnosis of Th-RDM, the multivariate linear regression model documented an association of FPG with serum ferritin level (t-stat: 2.9873; P: 0.0041) but not with the other investigated variables: age, gender, body mass index, pre-transfusional hemoglobin level, oral iron chelating agents, serum ferritin, history of splenectomy and positive family history for T1 DM and

**T2 DM reported at baseline. These results reinforce the main role of chronic iron burden in the etiopathogenesis of Th-RDM in  $\beta$ -TDT patients.**

**Conclusions:** FPG levels at the upper end of the normal range (defined as  $< 100$  mg/dL) in  $\beta$ -TDT patients with severe iron overload are associated with a significantly increased risk for developing either IFG or Th-RDM over a 10-year observational period. The identification of an FPG cutoff (87.5 mg/dL), above which the risk for future dysglycemia increases significantly, could be useful in the routine practice, urging clinicians to intensify iron chelation and monitor these patients more closely.

**Keywords:** Transfusion-dependent  $\beta$ -thalassemia; higher normal fasting plasma glucose; glucose dysregulation; fasting plasma glucose trajectories; risk factors; long-term follow-up; ICET-A Network.

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Correspondence to: Vincenzo De Sanctis, Coordinator of ICET-A Network (International Network of Clinicians for Endocrinopathies in Thalassemia and Adolescent Medicine) and Pediatric and Adolescent Outpatient Clinic, Quisisana Hospital, Ferrara, Italy. Tel: +39 3284852374. E-mail: [vdesanctis@libero.it](mailto:vdesanctis@libero.it)

**Introduction.** Multiple studies have demonstrated that glucose dysregulation (GD) is one of the commonest complications in patients with transfusion-dependent  $\beta$ -thalassemia ( $\beta$ -TDT), particularly among those with inadequate iron chelation.<sup>1,2</sup> The wide variability in the reported prevalence of GD and diabetes mellitus (DM) — more accurately referred to as thalassemia-related diabetes mellitus (Th-RDM) — has been attributed mainly to the degree of iron overload (IOL), the efficacy of chelation therapy, and adherence to treatment protocols.<sup>1-4</sup> The transition from normoglycemia to GD and ultimately to Th-RDM is typically a gradual process, involving intermediate stages of dysglycemia over a period of several years.

Oral glucose tolerance test (OGTT), fasting plasma glucose (FPG) and glycosylated hemoglobin (HbA1c) have been recommended by the WHO<sup>5</sup> and American Diabetes Association (ADA)<sup>6</sup> as methods to diagnose diabetes, with FPG and HbA1c being widely used due to their convenience, whereas OGTT is less often utilised due to several practical drawbacks, including the need for dedicated staff, the lengthy duration of the test itself, and patients' discomfort and/or refusal.<sup>7</sup> In patients with  $\beta$ -TDT, FPG is more accurate than HbA1c for the diagnosis of Th-RDM and, therefore, should be adopted as the diagnostic test of choice.<sup>8,9</sup>

Notably, several studies in children and adults have reported that higher FPG levels, while still within the normal range, are strong and consistent predictors of diabetes risk.<sup>10-14</sup> Moreover, Noetzi et al.<sup>15</sup> found, in 59  $\beta$ -TDT patients, aged  $23.3 \pm 9.8$  years (range 10 to 49 years), that FPG  $\geq 97$  mg/dL and serum insulin  $\geq 9$   $\mu$ U/mL (a fasting insulin cutoff of 17  $\mu$ U/mL was

considered normal) accurately identified an abnormal OGTT with a sensitivity of 89% and a specificity of 90%. Therefore, they recommended that  $\beta$ -TDT patients with glucose or insulin values outside these ranges should be referred for a confirmatory OGTT.

The primary outcome of this 10-year retrospective study, promoted by the International Network of Clinicians for Endocrinopathies in Thalassemia and Adolescent Medicine (ICET-A Network), was to evaluate the association of different baseline FPG levels within the normoglycemic range with the rate of progression to GD and Th-RDM. The secondary objective was to identify which baseline risk variable was associated with a higher risk of developing future alterations in glucose homeostasis.

## Materials and Methods

### Research design.

A. Study setting and study period design. Two Institutes of Endocrinology (Iran -Tehran: 44 patients and Shiraz: 148 patients) and the Outpatient Pediatric and Adolescent Endocrine Clinic (Italy-Ferrara: 47 patients) performed a retrospective observational study, between January 2013 and January 2025, to evaluate the performance of different high-normal FPG levels to predict the development of GD and Th-RDM among  $\beta$ -TDT patients from age 10 years to adulthood.

B. Eligibility criteria. Eligible criteria for patients' inclusion were: (a)  $\beta$ -TDT patients receiving routine blood transfusions and iron chelation therapy; (b) patients' age  $\geq 10$  years; (c) patients with FPG level  $< 100$  mg/dL at baseline, and (d) FPG trajectories available for 10 consecutive years. Patient-year follow-

up was calculated as the period between the first entry and the last confirmed follow-up. The main exclusion criteria included: (a) fluctuations of FPG concentrations; (b) non-transfusion-dependent thalassemia (NTDT) patients; (c) bone marrow transplanted patients; (d) history of recent viral hepatitis; (e) pregnancy at any time during the 10-year follow-up; (f) intake of medications affecting glucose metabolism (such as: thiazide diuretics, beta-blockers and corticosteroids) and (g) patients taking insulin or oral antidiabetic agents.

C. Patients' sample size at baseline. A total of 238 patients were recruited in the study, including 96 males and 142 females, with a male-to-female ratio of 1:1.4.  $\beta$ -TDT was diagnosed using complete blood count and hemoglobin HPLC, and molecular characterization of genotype in 81/238 (34%) patients.

*Methods and data collection.* Height and weight were measured according to international recommendations. Body mass index (BMI) was calculated by dividing the weight (Kg) by the square of the height ( $m^2$ ), according to the WHO criteria.<sup>16</sup> Children and adolescents, with BMI from the 5<sup>th</sup> to the 85<sup>th</sup> percentile, were defined as normal weight.

A BMI at or above the 85<sup>th</sup> percentile but below the 95<sup>th</sup> percentile was considered diagnostic for overweight, and a BMI at or above the 95<sup>th</sup> percentile was considered for age and sex diagnostic for obesity. Severe obesity class 2 was defined as a BMI  $>35 \text{ Kg/m}^2$  and  $< 40 \text{ Kg/m}^2$ . In patients above the age of 18 years, BMI was classified as: underweight ( $< 18.5 \text{ kg/m}^2$ ), normal weight ( $18.5\text{--}24.9 \text{ kg/m}^2$ ), overweight ( $25.0\text{--}29.9 \text{ kg/m}^2$ ), and obesity ( $\geq 30 \text{ kg/m}^2$ ).<sup>16</sup>

A. Biochemical analysis. Fasting plasma glucose (FPG) samples were collected in the morning in citrate-containing tubes after an overnight fast of at least 8 hours. FPG concentrations were measured using the glucose oxidase method using a Beckman Glucose Analyzer (Beckman Instruments, Fullerton, CA). Results for other markers of glycemia, such as OGTT or HbA1c, were not available.

All biochemical parameters were determined by standard methods. The level of alanine aminotransferase (ALT) was determined by an automated analyzer, and iron overload (IOL) was assessed by serum ferritin (SF). IOL was arbitrarily classified as mild (SF: between 500 and  $800 \mu\text{g/L}$ ), moderate (SF:  $\geq 800 \mu\text{g/L}$  and  $< 1,500 \mu\text{g/L}$ ), high (SF:  $\geq 1,500 \mu\text{g/L}$  and  $< 3,000 \mu\text{g/L}$ ) and severe (SF:  $\geq 3,000 \mu\text{g/L}$ ). SF was measured by chemiluminescence immunoassay. The 50<sup>th</sup> centile of reported normal values is  $105 \mu\text{g/L}$  in males and  $35 \mu\text{g/L}$  in females.<sup>17</sup>

*Statistical analysis.* Standard computer program SPSS for Windows, release 18.0 (SPSS Inc., USA) was used for data entry and analysis. Data are summarized in

tables using mean  $\pm$  standard deviation (SD), median, count, percent (%), and 95% confidence interval (CI) for categorical variables. The Kolmogorov-Smirnov was used to verify the normality of distribution of variables. Longitudinal trends in fasting plasma glucose (FPG) over the 10-year follow-up were assessed using repeated-measures ANOVA for continuous variables, and non-normally distributed data were evaluated using the Kruskal-Wallis test, a non-parametric method for comparing independent samples. Relationships between variables were determined by Pearson linear correlation for normally distributed variables,

Spearman Rho for quantitative variables abnormally distributed. According to Swinscow, the correlation coefficient was considered as follows:  $<0.4$  as weak, from  $\geq 0.4$  to  $0.59$  moderate, from  $\geq 0.6$  to  $0.79$  strong, and  $\geq 0.8$  very strong.<sup>18</sup> The categorical data were analyzed using the chi-square ( $\chi^2$ ) test. Furthermore, time-dependent receiver operating characteristic (ROC) curve analyses and respective areas under the curve, by plotting sensitivity (true positive rate) on the y-axis against  $1 - \text{specificity}$  (false positive rate) on the x-axis for the various FPG values were tabulated to evaluate the performance of FPG measurements ( $<100 \text{ mg/dL}$ ) for the incident risk of impaired fasting plasma glucose (IFG) and Th-RDM at 10-year follow-up. An AUC value of 0.5 denotes a differentiation ability while an AUC of 1.0 represents a value with significant superior discriminatory power.<sup>19</sup> Multiple linear regression analyses were employed to evaluate the relationship between the dependent variable FPG at the diagnosis of Th-RDM or at last follow-up in normoglycemic patients and the potential associated risk factors: age, gender, BMI, pre-transfusional hemoglobin level, oral iron chelating agents, SF, history of splenectomy, and positive family history for T1 DM and T2 DM reported at baseline. In the analyses, male and female patients were combined to increase statistical power and to simplify the presentation. For all tests, a probability (P value - 2-tailed) less than 0.05 was considered significant.

*Ethics.* All participants or parents gave informed consent in accordance with the principles of the Declaration of Helsinki and its later amendments in 2020 ([www.wma.net](http://www.wma.net)) after a detailed explanation of the procedures for performing the OGTT, as well as the nature and purpose of the test. Moreover, in our retrospective observational study, patients underwent only routine diagnostic procedures according to the current recommendations or guidelines.<sup>20-22</sup> No additional interventions were provided. The personal data of patients were not disclosed during the study. The study was approved by the local institutional review boards or was waived in accordance with the local legislation and institutional requirements.<sup>23</sup>

## Results

**Patient characteristics at start of the 10-year follow-up study.** A total of 238  $\beta$ -TDT patients were included in the retrospective observational study. The cohort comprised 96 males and 142 females, with a mean age of  $23.0 \pm 8.1$  years (range: 10.1–41.9 years). Of these, 71 patients (29.8%) were under 18 years of age. Genotypic data were available for 77 patients, with the majority (70/77; 90.9%) being homozygous for  $\beta^0$ -thalassemia and 7 patients (9.1%) having a compound heterozygous  $\beta^0/\beta^+$  genotype.

The mean annual pre-transfusion Hb level was  $8.2 \pm 0.7$  (range: 6.5–10 g/dL; median: 8.25 g/dL).

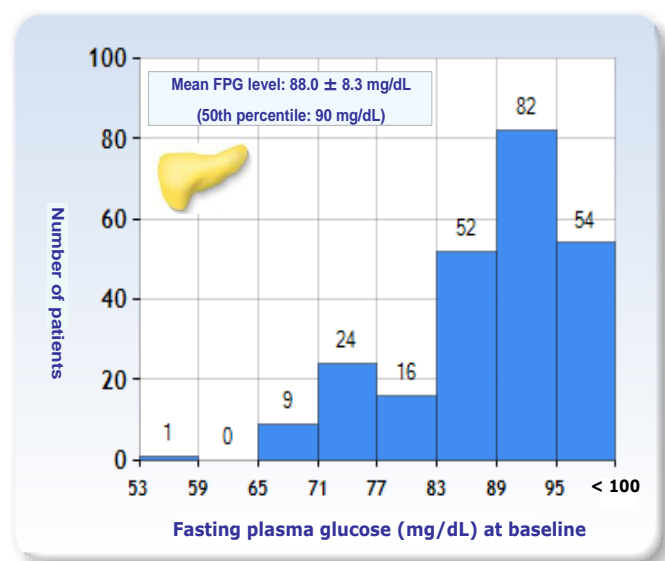
Among the 71 children and adolescents, 12 (16.9%) were classified as overweight or obese, while 5 (7.0%) were underweight. In patients over 18 years of age ( $n = 167$ ), 4 (2.3%) were overweight and 11 (6.5%) were underweight.

A positive family history for diabetes type 1 or 2 diabetes was reported in 95/238 (39.9%) patients, and a history of splenectomy was present in 75/238 (31.5%)  $\beta$ -TDT patients.

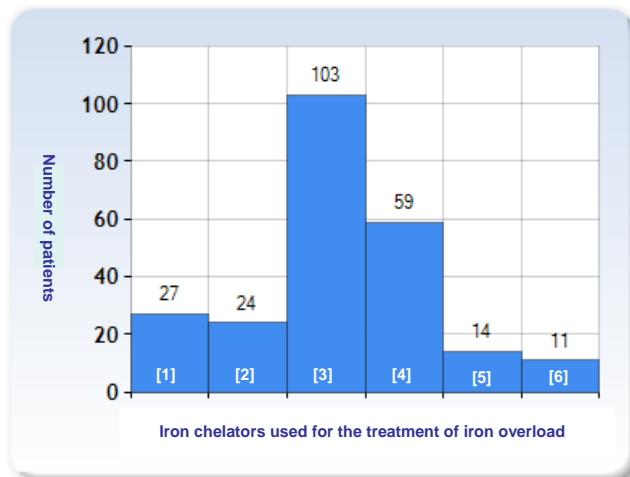
At baseline, the total mean FPG level was  $88.0 \pm 8.3$  mg/dL (50<sup>th</sup> and 75<sup>th</sup> percentiles: 90 mg/dL and 94 mg/dL, respectively) (**Figure 1**) and the mean serum ferritin (SF) level was  $2,080 \pm 2,072 \mu\text{g/L}$  (median:  $1,368 \mu\text{g/L}$ ). In 108/238 (45.3%) patients, the SF levels were classified as high or severe. The mean alanine aminotransferase level, available in 201/238 patients (84.4%), was  $29.0 \pm 25.8$  IU/L (median: 43 IU/L).

For the treatment of iron overload different iron chelators were used: [1] desferrioxamine (DFO); [2] deferiprone (DFP); [3] deferasirox (DFX); [4] DFO+DFP; [5] DFP+DFX, and [6] DFO +DFX.

Deferasirox (DFX) monotherapy was the most commonly used oral iron chelating agent (103/238;



**Figure 1.** Distribution of fasting plasma glucose (FPG: mg/dL) levels at baseline.



**Figure 2.** Distribution of iron chelators used for the treatment of iron overload. Legend: [1] desferrioxamine (DFO); [2] deferiprone (DFP); [3] deferasirox (DFX); [4] DFO+DFP; [5] DFP+DFX, and [6] DFO +DFX.

43.2%), followed by desferrioxamine (DFO: 27/238; 11.3%) and deferiprone (DFP: 24/238; 10.0%). 84/238 patients (35.2%) were on different combined chelating therapies (**Figure 2**).

**Evolution of fasting glucose status during 10-year follow-up.** The principal demographic and clinical characteristics of patients enrolled in the retrospective study are summarized in **Table 1**. Based on the trajectory of their FPG over the 10-year study period, adult  $\beta$ -TDT patients (age range: 20.3–51 years) were classified as having persistent normoglycemia (Group A), prediabetes (IFG; Group B), or diabetes (Group C,) according to the ADA criteria.<sup>6</sup> Normoglycemia ( $n = 93$ ) was defined as FPG level  $< 100$  mg/dL; prediabetes (IFG;  $n = 67$ ) as FPG levels of 100–125 mg/dL and diabetes ( $n = 78$ ) as FPG levels equal or above 126 mg/dL.

Trajectory stability was based on a minimum of five consecutive FPG measurements in a 2-year interval within the 10 years of follow-up. FPG trajectories were also used to calculate the interval between the patients' entry (baseline) and the diagnosis of IFG or Th-RDM. Their clinical and laboratory characteristics are summarized in **Table 1** and their FPG trajectories, during 10-year follow-up, are illustrated in **Figure 3**.

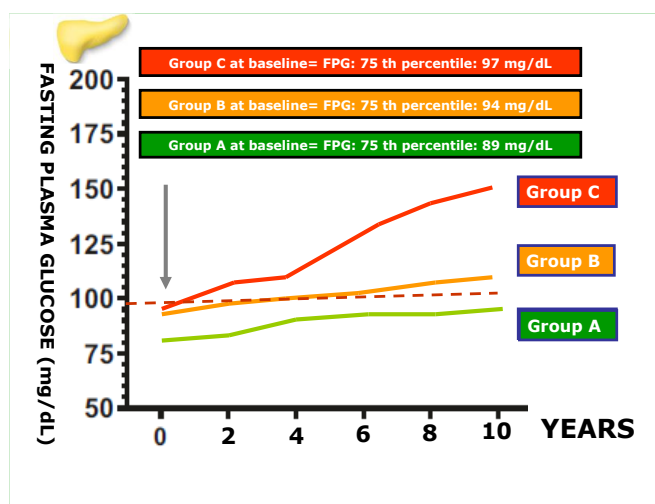
Substantially, FPG concentrations remained stable  $< 100$  mg/dL in 93/238  $\beta$ -TDT patients (39.0%), 67/238 (28.1%) patients developed persistent IFG, and 78/238 (32.8%) patients developed Th-RDM.

The first detection of IFG and Th-RDM was registered at  $5.7 \pm 2.3$  years and  $6.6 \pm 2.6$ , respectively, after the baseline. The mean time interval for conversion from IFG to Th-RDM was  $3.3 \pm 2.3$  years (95% 2.790 - 3.810). Their mean FPG level was  $159.5 \pm 29.0$  mg/dL (10<sup>th</sup> centile: 129.2 mg/dL and 90<sup>th</sup> centile: 189.8 mg/dL). At baseline, the SF levels in 50 out of 78 (64.1%) patients with Th-RDM were classified as high or severe.

**Table 1.** Summary of clinical and laboratory characteristics (mean  $\pm$ SD, median, and %) and 10-year follow-up in 238 normoglycemic transfusion-dependent  $\beta$ -thalassemia patients ( $\beta$ -TDT).

Variables	GROUP A (n: 93)	GROUP B (n:67)	GROUP C (n: 78)	P- value A vs. B / A vs. C	P- value B vs. C
Mean age at baseline (yrs)	21.3 $\pm$ 8.5	22.6 $\pm$ 7.5	25.5 $\pm$ 7.4	0.32/ <b>0.0011</b>	<b>0.024</b> =
Age range and median at last observation (yrs)	20.3-51 (31)	20-47 (32)	20-50 (35)	=	=
FPG at baseline and median (mg/dL)	83.3 $\pm$ 7.7 (85)	89.8 $\pm$ 6.3 (92)	92.0 $\pm$ 7.8 (94)	< <b>0.00001</b> / < <b>0.00001</b>	<b>0.0022</b> =
95% CI	81.6-84.9	88.2-91.3	90.2 - 93.7	=	=
Gender (Males/Females)	41/52	29/38	30/48	0.91/0.53	0.61
Positive family history for T1DM and T2DM (N. /%)	33 (35.4%)	42 (62.6%)	68 (87.1%)	<b>0.0008</b> / < <b>0.00001</b>	<b>0.0008</b> =
History of splenectomy (N. /%)	36 (38.7%)	15 (22.3%)	24 (30.7%)	<b>0.038</b> / 0.33	0.26 =
Pre-transfusional Hb level (g/dL)	8.59 $\pm$ 0.66	8.15 $\pm$ 0.78	8.0 $\pm$ 0.67	<b>0.0002</b> / < <b>0.00001</b>	0.25 =
Median (g/dL)	(8.8)	(8.3)	(7.9)	=	=
SF ( $\mu$ g/L) at baseline and	1525.5 $\pm$ 1457.0	1560.0 $\pm$ 1339.1	3188.5 $\pm$ 2674.1	0.87/ < <b>0.00001</b>	<b>0.00011</b> =
SF > 1,500 $\mu$ g/L (%)	33.3%	40.2%	64.1%	=	=
ALT (IU/L) at baseline	42.6 $\pm$ 23.3	44.7 $\pm$ 23.0	61.6 $\pm$ 26.9	0.59/ <b>0.000014</b>	<b>0.0004</b> =
	(82/93 pts.)	(57/67 pts.)	(62/78 pts.)	=	=

**Legend = Group A:** patients with persistently normal FPG; **Group B:** patients who developed IFG; **Group C:** patients who developed Th-RDM; **T1 DM and T2DM:** diabetes mellitus type 1 or 2; **Hb:** hemoglobin; **SF:** Serum ferritin; **ALT:** alanine aminotransferase (normal values: <40 mU/ml); **FPG:** fasting plasma glucose; **95% CI:** Confidence Interval; **P -values in bold** represent significant results.



**Figure 3.** Trajectories of mean fasting plasma glucose (mg/dL) during 10 years of follow-up in the 3 subgroups of transfusion-dependent  $\beta$ -thalassemia patients ( $\beta$ -TDT): Group A: stable FPG, Group B: patients progressing to IFG, and Group C: patients who developed diabetes (Th-RDM). The red dotted line corresponds to the normal cutoff of FPG, established by ADA criteria.

The commonest recommended ICT was DFO + DFP: 27/78 (34.6%) patients, DFX: 26/78 (33.3%) patients, and DFO: 9/78 (11.5%) patients.

**Correlations and ROC analysis.** Correlation analysis between all variables and FPG at baseline and after 10-year follow-up was performed in the whole group of  $\beta$ -TDT patients.

At baseline, in the total group of 238 patients, no correlation was observed of FPG with age, gender, history of splenectomy and family history of diabetes

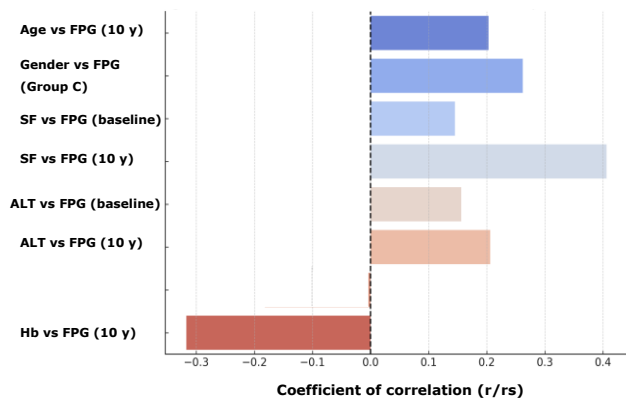
T1DM and T2DM [ $r_s$ : 0.05303,  $P = 0.41$ ;  $r_s$ : 0.12,  $P (2\text{-tailed}) = 0.064$ ;  $r_s$ : 0.04152,  $P = 0.52$ , and  $r_s$ : -0.07402,  $P = 0.25$ , respectively]. Nevertheless, a significant, but weak, correlation was documented between age and FPG after 10-year follow-up ( $r_s$ : 0.20332,  $P = 0.0016$ ).

A significant, but weak, correlation was found between SF and FPG at baseline and a moderate correlation at 10-year follow-up ( $r$ : 0.1448,  $P = 0.025$  and  $r$ : 0.4057,  $P = < 0.00001$ , respectively), while a significant, but weak, correlation was also recorded between ALT vs FPG (at baseline =  $r$ : 0.1559,  $P = 0.027$  and at 10-year follow-up =  $r$ : 0.2057,  $P = 0.0035$ ). On the other hand, an inverse moderate correlation was documented between Hb vs FPG at 10-year follow-up ( $r$ : -0.317,  $P = < 0.00001$ ) and a weak correlation was documented between age and FPG after 10-year follow-up ( $r_s$ : 0.20332,  $P = 0.0016$ ).

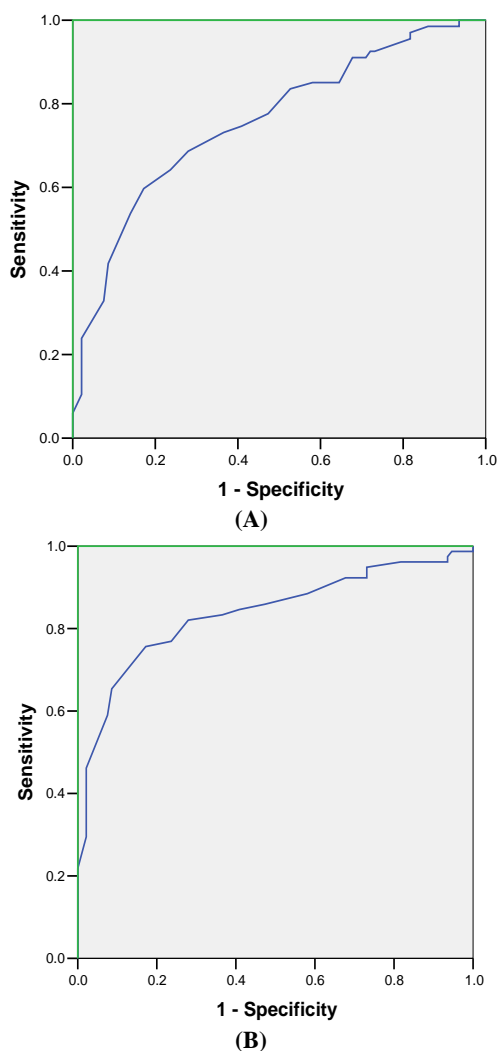
In  $\beta$ -TDT of Group A, a significant, but weak, inverse correlation was present between Hb vs SF ( $r_s$ : -0.28907,  $P = 0.0049$ ) and Hb vs FPG ( $r_s$ : -0.27995,  $P = 0.0065$ ). No significant correlations were observed in  $\beta$ -TDT of Group B and a positive direct weak correlation was present between basal SF vs FPG at the diagnosis of diabetes in patients of Group C ( $r_s$ : 0.23308,  $P = 0.040$ ) (**Figure 4**). Moreover, a significant, but weak, correlation was also observed in Group C between FPG and gender (female =  $r_s$ : 0.26249,  $P = 0.020$ ).

Of note, an inverse weak correlation was found between baseline FPG and time to prediabetes development ( $r_s$ : -0.340,  $P = 0.0048$ ), while no correlation was found at onset of Th-RDM ( $r_s$ : -0.055,  $P = 0.63$ ).

Almost all (76/78) patients who developed Th-RDM



**Figure 4.** Correlations between FPG and clinical parameters in  $\beta$ -TDT patients.



**Figure 5.** Receiver operating characteristics (ROC) curves for normal FPG at baseline and at 10-year follow-up for the prediction of IFG (A) and Th-RDM (B), according to ADA criteria. The optimal cutoff for the test is the point closest to the upper-left corner of the graph, which corresponds to an FPG measurement of 87.5 mg/dL. Plots are based on the results of the ROC curve test and areas under the ROC curve.

(97.4%) were diagnosed in Shiraz. A linear correlation was documented with severity of iron overload, family

history for T1 DM and T2 DM, older age, lower pre-transfusion of splenectomy transfusion hemoglobin levels, past history of splenectomy, and elevated liver enzyme levels (Group C versus A, **Table 1**). However, at the diagnosis of Th-RDM, the multivariate linear regression model confirmed only an association of FPG with SF level (t-stat: 2.9873; P: 0.0041) and not with the other investigated variables. Of note, at the last observation, in  $\beta$ -TDT Iranian patients with persistent normal FPG, none of the investigated variables were significantly associated with FPG. These results reinforce the main role of chronic iron burden in the etiopathogenesis of Th-RDM in  $\beta$ -TDT Iranian patients.

The receiver operating characteristics (ROC) curves for normal fasting plasma glucose (FPG) at baseline and after 10-year follow-up for the risk of IFG and Th-RD are reported in **Figure 5** (A and B). The area under the A curve for the incidence of IFG was 0.758, and for Th-RDM was 0.837.

In brief, FBG at baseline was very effective in differentiating patients who developed IFG from those who remained normoglycemic/dL with a sensitivity of 73.1% and a specificity of 63.4% at the cutoff value of 87.5 mg/dL. Moreover, FBG at baseline was very effective in differentiating patients who progressed to Th-RDM from those who did not, with a cutoff value of 87.5 mg/dL, a sensitivity of 83.8%, and a specificity of 63.4%. These results prove that FPG in the normal range is useful in identifying  $\beta$ -TDT patients at risk for IFG and Th-RDM.

**Strengths and Limitations.** A key strength of the present study is: (a) the large sample size of subjects with  $\beta$ -TDT followed for 10 years, which enhances the statistical power and allows for more reliable subgroup analyses; (b) the clinical relevance for early identification of at-risk  $\beta$ -TDT patients, and (c) the identification of an FPG threshold lower than ADA cutoffs, with potential implications for monitoring. Nevertheless, the study has several important limitations that should be considered when interpreting the findings.

First, the diagnosis of Th-RDM was made based on the results of FPG and did not include oral glucose tolerance test (OGTT). According to ADA criteria 6, diabetes may be diagnosed based on hemoglobin A1c (A1C  $\geq 6.5\%$ ) or plasma glucose criteria, either the fasting plasma glucose (FPG:  $\geq 126$  mg/dL) value or 2-h glucose (2-h PG:  $\geq 200$  mg/dL) value during a 75-g oral glucose tolerance test (OGTT), or random glucose value  $\geq 200$  mg/dL accompanied by classic hyperglycemic symptoms (e.g., polyuria, polydipsia, and unexplained weight loss) or hyperglycemic crises. Although the FPG is a more reproducible test, increasing data suggest that the diagnostic value of  $\geq 126$  mg/dL has low sensitivity.<sup>24,25</sup> The validity of using this FPG cutoff value in diagnosing diabetes in  $\beta$ -TDT patients remains

to be established. Nevertheless, a preliminary ongoing study, promoted by ICET-A Network, in 72  $\beta$ -TDT patients, followed in Teheran, Muscat, and Ferrara (mean age  $25.6 \pm 10.4$  years; 38 females), on the concordance rate among FPG (between 126 mg/dL and 200 mg/dL) and 2-hour post-challenge glucose test (2-hr PG:  $\geq 200$  mg/dL) for the diagnosis of Th-RDM has documented a percentage rate of 77.7% that increase with the elevation of FPG levels (De Sanctis V et al., preliminary observations). Second, although FPG is more practical and less expensive compared with OGTT, the latter is useful for the screening of impaired glucose tolerance (IGT). However, given the large sample size, the consequences of this limitation could be expected to be negligible. In a previous study including 234  $\beta$ -TDT patients (aged 5-40 years) with FPG  $< 100$  mg/dL, the prevalence of isolated impaired glucose tolerance and Th-RDM, after OGTT, was 4.2% and 1.2%, respectively.<sup>26</sup> Third, pre-analytical factors such as glycolysis can further compromise FPG measurements, as glucose levels in whole blood samples can decline by 5%-7% per hour if not processed promptly.<sup>26</sup> Finally, further studies are needed to understand the mechanisms that regulate higher normal FPG concentrations.

**Discussion.** Normal fasting plasma glucose (FPG) level reflects the body's ability to maintain adequate basal insulin secretion in combination with hepatic insulin sensitivity sufficient to control hepatic glucose output.<sup>27</sup> Because of the convenience and low cost, FPG rather than OGTT is frequently utilised in various healthcare settings as an easy screening test for evaluation of glycemic status.

In the past, several studies in the general population have reported that higher FPG levels within the normoglycemic range are a predictor for developing future prediabetes and diabetes.<sup>10-14</sup> Our retrospective study confirmed this finding in a population of  $\beta$ -TDT patients, reporting that the higher, but still within normal range, the FPG is, the greater the likelihood of developing IFG or Th-RDM in the future.<sup>28</sup>

To determine the associated risk for future glucose dysregulation and diabetes, we calculated the ROC curves for normal fasting plasma glucose (FPG) at baseline and at 10-year follow-up (**Figure 5**). The FPG cutoff value for optimal specificity and sensitivity for developing IFG and Th-RDM was established at 87.5 mg/dL, which is significantly lower than the cutoff suggested by Noetzly et al.<sup>15</sup> and Pepe et al.,<sup>29</sup> who had reported in a large number of  $\beta$ -TDT patients that an FPG value of 98 mg/dL predicted the presence of an abnormal OGTT with a sensitivity of 60.4% and a specificity of 95.9%.

Our study confirms that a relationship exists between FPG levels in the high normal range and the development of GD and Th-RDM, and draws the clinicians' attention

to the significant risk of progression in  $\beta$ -TDT patients with FPG exceeding 87.5 mg/dl. However, ethnic and genetic variations in metabolism would suggest that a single cutoff value may not be applicable to all  $\beta$ -TDT patients.

The mechanisms by which higher normal FPG reflects a negative effect on glucose homeostasis are not entirely clear and require further investigation. Putative factors could be an increased hepatic insulin resistance, impaired insulin secretion and action, and decreased insulin clearance.<sup>30</sup>

In children with FBG between 90 and 100, a significant decline ( $\sim 23\%$ ) of  $\beta$ -cell function has been reported compared to those with FPG below 90 mg/dL.<sup>31</sup>

Subgroup linear correlation analysis revealed important nuances. In Group A (patients with normal FPG), inverse correlations between hemoglobin and both serum ferritin and FPG suggest a protective metabolic profile with lower iron load and better glycemic control. Conversely, in Group C (patients with Th-RDM), a significant positive correlation between baseline SF and FPG at diabetes diagnosis reinforces the role of chronic iron burden in diabetes pathogenesis. In contrast, Group B (patients with persistent IFG) showed no significant correlations, possibly reflecting a heterogeneous transitional state or the influence of other unmeasured factors, such as genetic factors, a history of splenectomy, and lower pre-transfusional Hb levels that could exacerbate iron overload and metabolic complications.

Pancreatic  $\beta$ -cells are particularly susceptible to excess iron because they are rich in mitochondria and are highly sensitive to oxidant-generating substances. The iron-mediated damage is further exacerbated by the impaired ability of the pancreas to handle oxidative stress, which results in tissue fibrosis and inflammation, contributing to both endocrine and exocrine dysfunction.<sup>32,33</sup>

Additional factors include chronic hypoxia due to anemia, which may potentiate the toxicity of iron deposition in endocrine glands and other organs, as well as viral infections. Moreover, recent studies, including one by Rujito et al.,<sup>34</sup> have identified mutations in diabetes related genes (HNF4A, PTPN, KCNJ11, and PPAR gamma) in thalassemia patients, suggesting a susceptibility to diabetes and exacerbation of iron deposition in the pancreas, impacting glucose homeostasis.

The association between ALT and FPG supports this mechanistic link, as elevated ALT reflects hepatocellular injury and may reflect hepatic insulin resistance. Interestingly, hemoglobin levels showed a persistent inverse correlation with FPG, both at baseline and follow-up. Lower hemoglobin levels may reflect ineffective erythropoiesis or increased transfusion burden, which, in turn, could exacerbate iron overload and metabolic complications. This observation warrants

further investigation, as optimizing transfusion regimens may have metabolic benefits beyond hematologic correction.

**Conclusion.** Higher FPG levels within the normoglycemic range are associated with an increased risk of future glucose dysregulation. This study identified a basal FPG level of 87.5 mg/dL as a significant cutoff for the risk of progression to IFG or Th-RDM in  $\beta$ -TDT patients with severe iron overload. Therefore, these patients should be under close monitoring, necessitating 3-4 monthly measurements of FPG concentration, while intensification of chelation therapy and lifestyle interventions should also be considered. Future research should evaluate the accuracy of high normal FPG across different settings and ethnicities and understand the clinical implications of FPG at lower thresholds to optimize resource use efficiently.

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