

**Scientific Letters****Concordance Rate of Fasting Plasma Glucose (PG) and 2-hour PG post Oral Glucose Tolerance Test (OGTT) in Patients with New Diagnosis of Thalassemia-Related Diabetes Mellitus (Th-RDM): Implications for Clinical Practice**

**Keywords:** Thalassemia-Related Diabetes Mellitus; Fasting plasma glucose  $\geq 126$  mg/dL; OGTT concordance rate;  $\beta$ -cell dysfunction; Insulin sensitivity/resistance; ICET-A.

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**To the editor.**

In patients with transfusion-dependent  $\beta$ -thalassemia ( $\beta$ -TDT), several forms of dysglycemia have been reported at various stages of the disease. Although diabetes mellitus (DM) in patients with  $\beta$ -TDT shares many characteristics with type 2 DM (T2DM) and fewer with type 1 DM, it is a distinct type of diabetes, known as thalassemia-related diabetes mellitus (Th-RDM), with different outcomes and monitoring. The estimated prevalence of Th-RDM varies from 9.7% to 29%.<sup>1</sup>

Current standards of care for  $\beta$ -TDT patients recommend an annual 2-h oral glucose tolerance test (OGTT) starting at age 10 (or earlier if the patient has severe iron overload).<sup>2</sup> Recommendations do not include simultaneous measurement of insulin response during OGTT, while glycated hemoglobin (HbA1c) is considered an unreliable biomarker for long-term glucose control in  $\beta$ -TDT.<sup>2</sup>

The diagnostic criteria for DM of the American Diabetes Association (ADA) are based on fasting plasma glucose (FPG)  $\geq 126$  mg/dL or 2-h PG  $\geq 200$  mg/dL, after glucose load, or random venous plasma glucose (PG)  $\geq 200$  mg/dL associated with clinical symptoms of hyperglycemia or hyperglycemic crisis, and/or HbA1c  $\geq 6.5\%$ .<sup>3</sup>

To the best of our knowledge, no studies have evaluated the diagnostic accuracy of FPG  $\geq 126$  mg/dL in patients with  $\beta$ -TDT. Thus, the ICET-A promoted a multicenter survey to establish: (a) the concordance rate of criteria used to diagnose diabetes between FPG and 2-hour PG post OGTT,

and (b) to evaluate the optimal cutoff value of FPG to establish confirmation of DM diagnosis.

**Patients and Methods.** We retrospectively selected 71  $\beta$ -TDT patients with FPG  $\geq 126$  mg/dL, who were followed at three Thalassemia centers (Iran, Italy, and Oman) and underwent annual or biannual 2-h OGTTs over the last 30 years. The main exclusion criteria included: (a) non-transfusion-dependent thalassemia (NTDT); (b) bone marrow transplanted patients; (c) pregnancy; (d) patients on treatment with medications influencing glucose metabolism; (e) patients with incomplete data, and (f) positive history of recent surgery, or illness, or current use of oral hypoglycemic agents. Data collected at OGTT screening included: age, gender, anthropometric measurements [standing height, weight and body mass index (BMI), according to Center for Disease Control and Prevention],<sup>4</sup> and patients' medical data (age at first transfusion, age of splenectomy, type and dose of chelating drugs, family history of diabetes, relevant biochemical and hematological parameters). Serum alanine aminotransferase (ALT) was measured by an automated analyzer (normal range 0–40 mU/L), and active HCV infection was determined by a qualitative HCV-RNA assay according to the manufacturer's instructions. Iron overload (IOL) was assessed by serum ferritin (SF) in  $\mu$ g/L. IOL was arbitrarily classified as mild (SF:  $< 800$   $\mu$ g/L), moderate (SF:  $\geq 800$   $\mu$ g/L and  $< 1,500$   $\mu$ g/L), high (SF:  $\geq 1,500$   $\mu$ g/L and  $< 3,000$   $\mu$ g/L), and severe (SF:  $\geq 3,000$   $\mu$ g/L).

OGTT was performed after a 10-h fast using 75 g dextrose monohydrate, and results were evaluated according to the current ADA criteria. Plasma glucose (PG) concentrations were measured by an automated glucose oxidase reaction. Data on insulin secretion, sensitivity, and resistance were available for 27 patients followed at a single Center (Ferrara). Serum insulin concentrations were assessed by a commercial immunoassay technique (Diagnostic Products Corporation, Los Angeles, CA) at 0', 30', 60', and 120' minutes after OGTT. The following surrogate indices were calculated: homeostasis model assessment (HOMA-IR), insulinogenic index (IGI), Matsuda Whole Body Insulin Sensitivity Index (Matsuda Index), and oral disposition index (oDI). The oral disposition index (oDI) was determined as the product of the Matsuda index and the IGI obtained during the OGTT.<sup>5</sup>

Statistical analysis was conducted using STATA (v12.1, College Station, TX). Numeric variables are expressed as mean  $\pm$  standard deviation (SD) and 95% confidence intervals (CI). The Kolmogorov-Smirnov test was used to verify the normality of the distributions of the variables. Normally distributed continuous variables were compared using a one-way analysis of variance (ANOVA), and non-normally distributed variables were calculated using the Kruskal-Wallis test. Relationships between variables were assessed using Pearson's linear correlation for normally distributed variables and Spearman's Rank Correlation for non-normally distributed quantitative variables. According to Swinscow, the correlation coefficient was considered as follows:  $<0.4$  as weak, from 0.4 to 0.59 as moderate, from 0.6 to 0.79 as strong, and  $\geq 0.8$  as very strong.<sup>6</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. Multiple linear regression analyses were employed to evaluate the relationship between the dependent variable, 2-h PG after OGTT, and potential associated risk factors: age, gender, BMI, pre-transfusion hemoglobin level, oral iron chelating agents, SF, history of splenectomy, and positive family history for T1 DM and T2 DM. Other variables were not assessed because the major risk factors for dysglycemia currently reported in the literature include older age, high BMI, elevated SF levels, chronic liver disease, and history of splenectomy.<sup>1,2,7</sup> A p-value  $<0.05$  was considered statistically significant.

**Ethics.** All patients provided informed consent in accordance with principles of the Declaration of Helsinki. Patients underwent only routine diagnostic procedures in accordance with international recommendations for the diagnosis and management of dysglycemia in  $\beta$ -TDT patients.<sup>2</sup> The study was approved by the local institutional review boards or was waived in accordance with the local legislation and institutional requirements.

**Results.** The cohort included 71  $\beta$ -TDT patients (34 males and 37 females), with a mean age of  $25.6 \pm 10.5$  years (range: 13–59.7 years). Of these, 19/71 patients (26.7%) were under 18 years of age. 60/71 patients were of normal weight (84.5%), 3/71 (4.2%) were underweight, and 8/71 (11.2%) were overweight or obese.

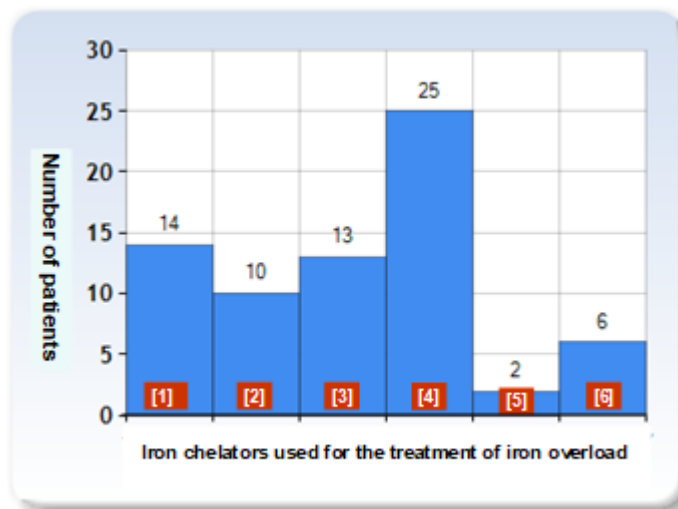
Genotypic data were available for 49 patients, with the majority (39/49; 79.5%) being homozygous for  $\beta^0$ -thalassemia and 10 patients (20.4%) having a compound heterozygous  $\beta^0/\beta^+$  genotype. The mean annual pre-transfusion Hb level was  $9.2 \pm 0.38$  (range: 8–10.2 g/dL; median: 9.1 g/dL).

A positive family history for type 1 or 2 DM was reported in 26/66 (39.3%) patients. A past history of splenectomy was reported in 46/71 (56.3%) patients with  $\beta$ -TDT. Mean and median ALT in 49/71 patients were  $59.8 \pm 44.3$  and 49 IU/L, respectively. A positive HCV RNA history was reported in 19/49 (38.7%).

For IOL treatment, various iron chelators have been used over the last 3 decades. Over the years, different combinations have been developed to allow individualized therapy in  $\beta$ -TDT patients with poor adherence to iron chelation therapy, persistent IOL, and/or organ damage. The most common treatment was the combination of desferrioxamine (DFO) plus deferiprone (DFP) (25/70 patients; 35.7%), followed by subcutaneous desferrioxamine (DFO: 14/70; 20.0%). One adolescent patient refused any iron chelation therapy (**Figure 1**).

The mean value of SF was  $3,492 \pm 3,063$   $\mu$ g/L (95% CI 2,780–4,204). In 9/71 (12.6%) patients, the SF levels were  $< 800$   $\mu$ g/L, and in 37/71 (52.1%) patients, they were  $> 2,500$   $\mu$ g/L. A 14-year-old patient with Th-RDM refused ICT.

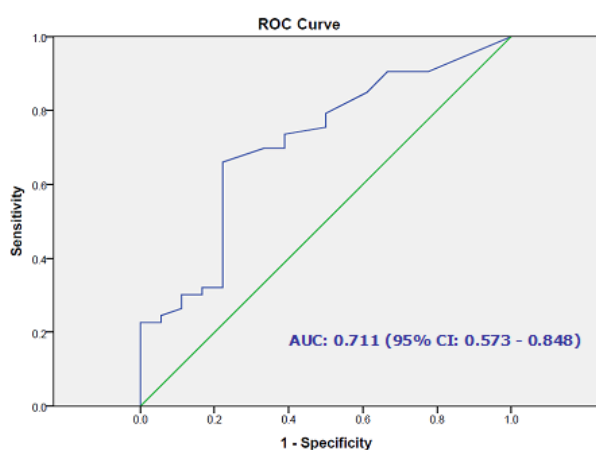
The mean FPG was  $141.6 \pm 16.5$  mg/dL (95% CI 137.7–145.4). The mean 1-h PG, after glucose load, was  $253.4 \pm 73.1$  (95% CI 236.3–270.4) and the mean



**Figure 1.** 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.

2-h PG, after glucose load, was  $233.8 \pm 67.0$  mg/dL (95% 218.215–249.385). Among 71  $\beta$ -TDT patients with FPG  $\geq 126$  mg/dL, 2-h PG  $\geq 200$  mg/dL was documented in 53 patients (74.6%), while PG 2-h after OGTT was 140-199 mg/dl in 12 patients (16.9 %) and  $< 140$  mg/dl in 6 patients (8.5%).

The ROC curve analysis showed that the optimal cutoff value for FPG  $\geq 126$  mg/dL to detect 2-h PG  $\geq 200$  mg/dL after OGTT was 133.6 mg/dL, with a sensitivity of 66% and specificity of 77.8% (Figure 2).



**Figure 2.** Receiver operating characteristic (ROC) curve using the performance of FPG measurements ( $\geq 126$  mg/dL) as outcomes for 2-h PG  $\geq 200$  mg/dL, after OGTT. The best cutoff value was 133.6 mg/dL, with a sensitivity of 66% and a specificity of 77.8%, yielding an AUC (area under the ROC curve) of 0.711.

Data on insulin secretion, sensitivity, and resistance were available for 27 patients. The surrogate indices of insulin secretion and

sensitivity/resistance in 17  $\beta$ -TDT patients with FPG  $\geq 126$  mg/dL and 2-h PG after OGTT  $\geq 200$  mg/dL were compared to those in 16 young adult healthy controls (Table 1). The increased HOMA-IR index was associated with a significant defect in  $\beta$ -cell secretion (IGI) and a reduction in oDI, indicating insufficient compensation for IR and reflecting a decline in pancreatic  $\beta$ -cell function. In the remaining 10 patients, 6 had FPG  $\geq 126$  mg/dL and PG 2-h after OGTT between 140 -199 mg/dL, and 4 patients had FPG  $\geq 126$  mg/dL with PG 2-h after OGTT  $< 140$  mg/dL; comparison with healthy controls was not performed due to the small numbers.

A linear, significant but weak correlation was found between SF and 2-h PG after OGTT ( $r$ : 0.3909,  $P$ : 0.00089). A moderate correlation was documented between FPG and 2-h PG after OGTT ( $r$ : 0.4669;  $P$ : 0.000055), and between 30-min vs. 2-h PG after OGTT (47 patients;  $r$ : 0.429,  $P$ : 0.0036).

A strong correlation was observed between 1-h PG and 2-h PG after OGTT (48 patients;  $r$  = 0.7325,  $P$   $<$  0.00001). Moreover, the combination of FPG  $\geq 126$  mg/dL and 1-h PG 253.5 mg/dL (1st quartile) was associated with 2-h PG  $> 200$  mg/dL in 44/48 (91,6%)  $\beta$ -TDT patients.

The multivariate linear regression model confirmed an association between 2-h PG after OGTT and SF level (t-stat: 2.4798;  $P$ : 0.015), but not with the other investigated variables.

**Conclusions.** Pancreatic IOL in  $\beta$ -TDT patients generally begins after the first decade of life and increases with age. The incidence of dysglycemia is primarily driven by chronic IOL from frequent blood transfusions, through reactive oxygen species (ROS)- mediated impairment of insulin synthesis, secretion, and apoptosis, contributing to the development of Th-RDM.<sup>8</sup>

A significant correlation was found between SF and 2-h PG after OGTT ( $r$ : 0.3909,  $P$ : 0.00089). In 9/71 (12.6%) patients, the SF levels were  $< 800$   $\mu$ g/L, and in 37/71 (52.1%) patients,  $> 2,500$   $\mu$ g/L (95% CI: 2,780–4,204). A high risk of Th-RDM has been reported in the UK, using a univariate analysis, in  $\beta$ -TDT patients with persistently elevated SF levels (an average 10-year SF level  $> 1,500$   $\mu$ g/L) compared to those with lower average SF levels.<sup>9</sup> In addition to IOL, other risk factors for developing dysglycemia in  $\beta$ -TDT patients include: severity of genotype and clinical phenotype, advanced age at onset of chelation therapy, poor

**Table 1.** Derived indices of insulin sensitivity/resistance, secretion, and  $\beta$ -cell function in 17  $\beta$ -TDT patients with FPG  $\geq$  126 mg/dL and 2-h PG after OGTT  $\geq$  200 mg/dL (**Group A**) versus 16 young adult healthy controls (**Group B; from Ref 1**).

Variables	Group A $\beta$ -TDT patients (n:17)	GROUP B Healthy controls (n: 16)	P-value
Mean age (years)at OGTT	20.8 $\pm$ 6.6	23.6 $\pm$ 3.5	0.14
Gender (Males/Females)	9/8 47	8/8 50	0.81
Body mass index (BMI: Kg/m <sup>2</sup> )	22.5 $\pm$ 4.6	21.5 $\pm$ 2.0	0.30
Family history of T1DM/T2 DM (N.and %)	5/17 (29.4%)	4/16 (25%)	0.78
HOMA-IR and 95% CI	4.63 $\pm$ 3.33 [3.0–6.2]	1.2 $\pm$ 0.8 [0.80–1.59]	0.0004
Insulinogenic Index (IGI) and 95% CI	0.36 $\pm$ 0.55 [0.098–0.62]	1.7 $\pm$ 1.5 [0.96–2.43]	0.0016
MATSUDA Index (MI 0-120) and 95% CI	4.25 $\pm$ 3.29 [2.68–5.81]	8.6 $\pm$ 3.5 [6.8–10.3]	0.0009
Oral disposition index (oDI30) and 95% CI	1.19 $\pm$ 1.83 [0.32–2.06]	13.8 $\pm$ 10.1 [8.85–18.74]	< 0.0001

compliance with chelation therapy, chronic liver disease, overweight/obesity, and history of splenectomy. Additional risk factors include associated endocrine complications, zinc deficiency, pancreatic fatty replacement, low insulin-like growth factor-1 (IGF-1), and reduced physical activity.<sup>1,2,6,8</sup>

The present study has important implications for clinical practice: the use of high FPG as a single screening test is not sufficiently discriminative for diagnosing diabetes in patients with  $\beta$ -TDT. The ROC analysis showed that the best cutoff value of FPG for detecting 2-h PG  $\geq$  200 mg/dL after OGTT was 133.6 mg/dL, with a sensitivity of 66% and specificity of 77.8%. Nonetheless, even with this cutoff, 15/29  $\beta$ -TDT patients (51.7%) had 2-h PG < 200 mg/dL.

The reported sensitivity of FPG  $\geq$ 126 mg/dL to detect 2-h OGTT diagnosed diabetes was 44.7% in Japanese subjects,<sup>10</sup> 70.1% in UK<sup>11</sup>, and 41% in US subjects.<sup>12</sup> Basically, the strength of association between FPG and 2-h PG is highly variable and depends on various factors.<sup>10-12</sup> Reproducibility and accuracy of PG levels may be influenced by pre-analytical, analytical, and post-analytical issues. The pre-analytical phase is the most important and requires standardized procedures to minimize variability and bias, both in the analytical methods used and in biological variability.<sup>13,14</sup>

For the foregoing reasons, clinicians should be prudent when two different tests are used, and the results of FPG  $\geq$  126 mg/dL versus 2-h PG post-glucose load are discordant. In practice, healthcare professionals might decide to closely follow patients at risk and consider a second OGTT, as

recommended by the ADA.<sup>3</sup> The long-term follow-up in 10  $\beta$ -TDT patients with discordance between FPG and 2-PG levels documented the development of diabetes in 7/10 patients, after 4.5  $\pm$  2.9 years (range: 1-10 years), and an isolated impaired fasting glucose in 3 patients, after 1, 4, and 7 years.

Several studies across different ethnic groups have shown that intermediate glucose values at 1 h post-glucose load predict an increased risk of incident T2DM.<sup>15</sup> In the present study, the combination of FPG  $\geq$  126 mg/dL and 1-h PG 253.5 mg/dL (1st quartile) was strongly associated with 2-h PG  $\geq$  200 mg/dL. Additional studies are needed to expand our understanding of the role of elevated 1-h PG in patients with dysglycemia and to evaluate whether shortening the OGTT from 2-h to 1-h would be more practical, acceptable, and effective in clinical practice.<sup>15-18</sup>

The major limitations in this study were evaluated. First, the small number of patients included in the retrospective survey. Second, there is a need to obtain further evidence on the diagnostic accuracy of FPG and to establish the optimal cutoff for maximum diagnostic accuracy, the concentration in large numbers of patients with  $\beta$ -TDT in different countries. Third, although a single FPG measurement is acceptable for epidemiological studies, its day-to-day variability can lead to misclassification of glucose tolerance.<sup>19</sup> The day-to-day intraindividual coefficients of variation range from 6.4 to 11.4% for FPG and 14.3 to 16.7% for 2-h PG during OGTT.<sup>13,14</sup> Finally, our study did not perform direct clamp measurements of insulin sensitivity and secretion; these were assessed using surrogate indices: HOMA-IR, IGI,

MI (0-120), and oDI.

Despite these limitations, this study provides, for the first time, valuable insights into the diagnostic accuracy of elevated FPG for screening dysglycemia in  $\beta$ -TDT patients. The concordance rate between elevated FPG and 2-h PG was high at 74.6%, but it also highlighted disagreement between the two values in 25% of our patients with  $\beta$ -TDT. Substantially, it indicates that the risk of diabetes increases with the degree of fasting hyperglycemia, underlining the importance of long-term follow-up in patients with discordant levels between FPG and 2-h PG post glucose load, and suggests that 1-h PG, like in the general population,<sup>20</sup> is a sensitive marker for detecting  $\beta$ -TDT patients at high risk for Th-RDM.

**Author contributions.** VDS conceived and designed the retrospective observational study, wrote the original draft. VDS, MF, and SD are the guarantors of the data integrity and take full responsibility for its content. VDS and IE performed formal analysis and data interpretation. SD and PT played a pivotal role in shaping the final paper version. MF, ATS, FS, and MK contributed to the intellectual content. All authors have accepted responsibility for the entire content of this scientific letter and approved its submission.

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**Competing interests:** The authors declare no conflict of Interest.

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