Screening for glucose dysregulation in β-thalassemia major (β-TM): An update of current evidences and personal experience

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Abstract. Glucose dysregulation (GD) in patients with β-thalassemia major (β-TM) usually develops gradually. Prediabetes consists of two abnormalities, impaired fasting glucose (IFG) and impaired glucose tolerance (IGT), the latter detected by a standardized oral glucose tolerance test (OGTT). Diagnosis of prediabetes is essential for an early identification of high-risk individuals who will benefit from intensive iron chelation therapy and lifestyle modification. Therefore, patients with β-TM should undergo annual screening for glucose abnormalities, according to international recommendations, starting from the age of 10 years. OGTT remains the preferred screening method as it is more sensitive for GD than fasting plasma glucose (FPG), although it is poorly reproducible. The use of HbA_{1c} measurement has limited use as it is generally considered unreliable in patients with thalassemia. Continuous glucose monitoring system (CGMS) is an accurate method to detect the variability of glucose fluctuations and offers the opportunity for better assessment of glucose homeostasis in a selected group of β-TM patients. Pancreatic Magnetic Resonance Imaging (MRI) associated with insulin secretion-sensitivity index-2 (ISSI-2) could be a complementary test, minimizing the necessity for OGTT and identifying high-risk patients before irreversible pancreatic damage occurs. The aims of this short report are to give practical guidance for an early identification of GD in β-TM patients, to summarise our experience, and to offer an impetus for further research in the field. (www.actabiomedica.it)

Key words: β-thalassemia major, glucose dysregulation, prediabetes, screening

Background

Multiple studies have shown that glucose related disturbances in patients with β-thalassemia major (β-TM) are common complications particularly in inadequately iron chelated patients, and less frequently in well transfused and efficiently chelated patients (1-5).

Glucose dysregulation (GD) occurs at an earlier stage during adolescence and the incidence increases with age (6,7), while diabetes occurs at later stages due a progressive and early loss of pancreatic β-cell mass, along with persistent insulin resistance (IR). Insulin deficiency may be caused by iron deposition in the islet β-cells of pancreas that begins after the first decade of

life (8), exhaustion of β-cells or a combination of both. The occurrence of IR may be due to: (a) direct effect of iron overload and/or (b) hepatic dysfunction leading to reduced hepatic extraction of insulin and excess release of glucose into the bloodstream (6,7).

Chronic hepatitis C, common in older β-TM patients, may also play a role in the pathogenesis of abnormal glucose tolerance (6). Moreover, zinc (Zn) deficiency, encountered frequently in β-TM patients (9), might lead to an exacerbation of the inability of the pancreas to secrete sufficient amounts of insulin in response to glucose stimulation (10). Zn is a potent physiological regulator of insulin signal transduction through its inhibitory effect on protein tyrosine phosphatase 1b, the key phosphatase that dephosphorylates the insulin receptor (11).

The high prevalence of diabetes mellitus (DM) in subjects with β-TM warrants targeted screening for detection of GD as early as possible. Several studies have analyzed strategies based on clinical and diagnostic data aimed at identifying individuals at high risk of GD. However, screening practices vary, with many centers not yet following the recommendation of periodic OGTT in all β-TM patients starting from the age of 10 years (12).

This review updates and summarizes the recent advances and personal experience in the field of GD in β-TM patients. By outlining current modalities and utility of screening tests, the authors hope to help physicians and researchers to improve clinical care and encourage further research.

Bloody glucose homeostasis in healthy humans

In healthy humans, circulating glucose concentration is maintained within a narrow range, in part by the ability of glucose itself to regulate endogenous glucose production in both prandial and fasting states. Blood glucose metabolism has a strong diurnal variation with seasonal and age variations. In normal healthy individuals glucose is normally maintained at ~90 mg/dl. This is achieved through an intricate balance between glucose uptake and endogenous glucose production to maintain constant glucose concentration (13). Among the tissues contributing to the maintenance of normal range of blood glucose are the liver, skeletal and cardiac muscles, fat and brain. After a carbohydrate meal, ~33% of the glucose is taken up by the liver, another ~33% by muscles and adipose tissues, and the remainder by the brain, kidney and red blood cells (14). The liver plays a major role in maintaining normal whole body glucose levels by regulating the processes of de novo glucose production (gluconeogenesis) and glycogen breakdown (glycogenolysis), thus controlling the levels of hepatic glucose release (13). Insulin and glucagon are two central glucose-dependent counter regulatory hormones that orchestrate the peripheral tissues' responses to control rates of utilization and production of glucose in order to maintain glycemia within narrow range (15).

Etiological classification of DM and peculiarities of GD in patients with β-TM

DM represents a group of diseases of heterogeneous etiology, characterized by chronic hyperglycemia and other metabolic abnormalities. Depending on the severity of the metabolic abnormality, diabetes may be asymptomatic, or may be associated with symptoms (thirst, polyuria, and weight loss), or may progress to ketoacidosis and coma.

The etiological classification of diabetes and related disorders includes: (a) type 1 diabetes (T1DM), (b) type 2 diabetes (T2DM), (c) those due to specific mechanisms and diseases, and (d) gestational diabetes mellitus. T1DM is characterized by destructive lesions of pancreatic β-cells either by an autoimmune mechanism or an unknown cause. T2DM is characterized by a combination of decreased insulin secretion and decreased insulin sensitivity (IS). Category (c) includes two subgroups: subgroup A is a diabetes in which specific mutations have been identified as cause of genetic susceptibility, while subgroup B is diabetes associated with other pathological conditions or diseases (16-18).

A typical example of subgroup B is the diabetes of β-TM patients, that we have recently proposed would be better defined as thalassemia-related diabetes (Th-RD) (19), as it has not yet been clearly classified. It differs from T1DM and T2DM, although

it has similarities with both (insulin insufficiency and variable insulin resistance) (6,7,20). Moreover, in contrast to T1DM, microvascular complications are not as frequent or as severe (20-23). Lastly, macrovascular complications (cerebrovascular disease and peripheral vascular disease) are rare (22).

Current criteria for the diagnosis of prediabetes and DM

Prediabetes is an intermediate state of hyperglycemia with glycemic parameters above normal but below the diabetes threshold. While the diagnostic criteria of prediabetes are not uniform across various international professional organizations, it remains a state of high risk for developing diabetes with an annual conversion rate of 5% -10% (24).

Currently, different definitions of prediabetes have been issued and identify phenotypes on the basis of the tests for hyperglycemia: fasting plasma glucose (FBG), 2-h plasma glucose (2-h PG), and hemoglobin A_{1c} (HbA_{1C}).

The World Health Organization (WHO) has defined prediabetes as a state of intermediate hyperglycemia using two specific parameters: impaired fasting glucose (IFG) defined as fasting plasma glucose (FPG) of 110 to 125 mg/dL (6.1-6.9 mmol/L) and impaired glucose tolerance (IGT) defined as 2- h plasma glucose of 140-199 mg/dL (7.8-11.0 mmol/L) or a combined IFG/IGT after oral glucose tolerance test (OGTT) (16). The test should not be performed in patients who fulfil the criteria for diabetes and in patients who are under physical stress, e.g. post-surgery, trauma, infection or extreme psychological stress, as these may give misleading results.

The American Diabetes Association (ADA), on the other hand, has the same cut-off value for IGT (140-199 mg/dL), but has a lower cut-off value for IFG (100-125 mg/dL; 5.6 -6.9 mmol/L) and, in addition, has an HbA_{1c} level of 39-47 mmol/mol (5.7-6.4%) for the definition of prediabetes (17).

While the National Institute for Health and Care Excellence (NICE) and the International Expert Committee (25) suggest a higher cut-off point of 6.0-6.4% (42-47 mmol/mol) for prediabetes.

Because HbA_{1C} is a measure of chronic hyperglycemia, it may reflect impairment in both fasting and 2-h glucose.

Both organizations define diabetes mellitus (DM) as: (a) a fasting glucose of ≥126 mg/dL (≥ 7.0 mmol/L), or (b) a 2-hour glucose on an oral glucose tolerance test (OGTT) of ≥ 200 mg/dL (≥ 11.1 mmol/L) (16,17). In the absence of unequivocal hyperglycemia, the ADA recommends that the result should be confirmed with repeat testing, or (c) a random glucose of ≥ 200 mg/dL (11.1 mmol/L) with classic diabetes symptoms (17).
In the case of symptomatic hyperglycemia, the diagnosis is obvious and a confirmatory test is not required before treatment is initiated. However, when the results of more than one test are available and the results are discordant, the test(s) should be repeated (26).

Screening of GD in patients with β-TM: where do we stand ?

In the light of evidence showing that: (a) pancreatic iron loading starts in early childhood, especially in patients receiving suboptimal iron chelation (8), (b) an insidious onset of glucose abnormalities (6,12), and (c) improvement of insulin secretion and glucose metabolism in patients with β-TM intensively treated with iron chelators in the early stages of dysglycemia (27), the International Network of Clinicians for Endocrinopathies in Thalassemia and Adolescent Medicine (ICET-A) recommend two different screening parameters for abnormalities of glucose homeostasis (12):
(a) a periodic assessment of fasting plasma glucose (FPG) from the age of 5 years;
(b) a 2-h OGTT, preferably combined with determination of insulin secretion at 10, 12, 14, and 16 years and annually thereafter.

At present, the criteria for the interpretation of OGTT do not support the use of HbA_{1C} for defining prediabetes (28). The credibility of HbA_{1c} has been questioned because the hemoglobin composition in thalassemia patients is considerably modified due to regular and frequent transfusions. The results may be falsely increased or decreased depending on the proximity to transfusion, shortened erythrocyte lifespan and the assay used (29,30). However HbA_{1c}, in effi-

ciently transfused β-TM patients seems valuable in diagnosis and monitoring treatment of DM (31).

Other glycemic markers such as glycated albumin and fructosamine have the potential for identifying prediabetes. However, these biomarkers have not been incorporated into guidelines, and there is currently no consensus on their use in clinical practice for defining glycemic status (12).

ADA versus WHO criteria: The personal experience in patients with β-TM

It is still unclear whether the ADA diagnostic criteria (17) or higher thresholds, as suggested by WHO (16), should be used in β-TM patients for the prompt identification of high-risk patients who would benefit from intensive iron chelation therapy and lifestyle modification. Therefore, to avoid inadequacies of threshold levels for diagnosing prediabetes and an underestimation of this disorder, we studied the prevalence of IFG in different age groups of patients, using different FPG cut points.

In the course of an ongoing ICET-A retrospective study on GD in β-TM patients, we collected data on 397 patients (aged 5-40 years; 56.3% males) followed from January 1988 to June 2021 by the same endocrinologist (VDS) at Pediatric Endocrinology and Adolescent Medicine Outpatient Clinic of St. Anna Hospital and Pediatric Endocrinology and Adolescent Medicine Outpatient Clinic of Quisisana Hospital of Ferrara. Exclusion criteria for the study were bone-marrow transplanted patients, subjects who were overweight/obese, or those on treatment with steroids.

All OGTTs were performed following WHO recommendations (16). Briefly, a first blood sample was drawn in the morning after an overnight fast to determine the FPG, then a standardized oral challenge was performed with 1.75 g glucose per kg body weight (maximum 75 g). Subsequent blood samples were collected from a venous catheter at 30, 60, 90, 120 and 180 minutes to measure plasma glucose and insulin. OGTT results were categorized following ADA and WHO criteria using the FPG and the PG level registered 2-h after OGTT. Variables are expressed as numbers and percentages (%), and comparison of data were analyzed by Chi-square (χ^2) test.

a. At baseline

Based on FPG levels, the 4 groups of β- TM patients (Table 1) were assigned to one of the following categories of glucose tolerance: normal FPG, isolated IFG $_{low}$ (based on ADA criteria), isolated IFG $_{high}$ (using

Table 1. Different fasting plasma glucose (FPG) cut-off points in 397 β- thalassemia major patients aged 5-40 years with normal FPG, impaired fasting glucose (IFG $_{low- high}$) and diabetes at baseline, using ADA and WHO criteria.

Fasting Plasma Glucose (FPG)	**5-10 years Mean age 7.7 ± 2.0 (R: 5.5 - 10.9) SF:1,781 ± 886**	**11-20 years Mean age 17.2 ± 1.7 (R: 16 - 20) SF:2,432 ± 1,240**	**21-30 years Mean age 22.6 ± 2.4 (R: 21 - 26.7) SF:1,615 ± 1,410**	**31-40 years Mean age 35.2 ± 4.1 (R: 31.2- 39.11) SF:1,430 ± 1,260**
	N. patients: 78	**N. patients: 156**	**N. patients: 100**	**N. patients: 63**
FPG: <100 mg/dL (< 5.6 mmol/L)	**63 (80.7%)**	**78 (50.0%)**	**53 (53.0%)**	**40 (61.5%)**
IFG $_{low}$: 100-109 mg/dL (5.6 - 6.0 mmol/L)	**11 (14.1%)**	**47 (30.1%)**	**19 (19.0%)**	**14 (22.2%)**
IFG$_{high}$: 110-125 mg/dL (6.1- 6.9 mmol/L)	**4 (5.1%)**	**25 (16.0%)**	**23 (23.0%)**	**7 (11.1%)**
Total cases of IFG: 100-125 mg/dL (5.6 - 6.9 mmol/L)	**15 (19.2%)**	**102 (36.1%)**	**42 (42%)**	**21 (33.3%)**
Diabetes labelled on the basis of confirmed FPG levels: ≥ 126 mg/dL (≥ 7.0 mmol/L)	**0 (0%)**	**6 (3.8%)**	**5 (5.0%)**	**2 (3.1%)**

Legend = SF: serum ferritin; R: age range; IFG: impaired fasting glucose; ADA: American Diabetes Association; WHO: World Health Organization. The criteria of WHO and ADA for the definitions of DM are the same.

WHO criteria), and diabetes without fasting hyperglycemia (FPG levels: ≥ 126 mg/dL; ≥ 7.0 mmol/L).

Using the ADA criteria, the prevalence of isolated IFG was 23.6 %, while increasing the threshold value of FPG to 110 mg/dL (6.1 mmol/L) according to WHO criteria, decreased the prevalence to 15.3%. Thirteen patients presented with a confirmed FPG level compatible with newly diagnosed diabetes (Table 1).

The total prevalence of IFG $_{low+ high}$ (19.2%) documented in the youngest group of patients (78 patients: mean age: 7.7 ± 2 years; 66 % males), chelated with monotherapy (DFO, DFP or DFX; SF:1,781 ± 886 ng/ml), was significantly lower compared to that reported by Liang et al. (32) (30 % in 81 patients).

The analysis of FPG in the 4 age groups of β-TM patients showed a higher prevalence of GD mainly in the second to third decade of life with a slow but not statistically significant decline (χ^2: 0.889; P= 0.34) in the older group of patients (age group: 31-40 years). This effect might be due to mild clinical phenotypes of patients as well as to differences in transfusions rate, annual consumption of blood and a better compliance to chelation therapy as patients get older.

b. After OGTT

With the aim of increasing the sensitivity of IFG cut-off to earlier identify β-TM patients at risk of disturbances of glucose homeostasis, we retrospectively reviewed the OGTTs in 4 groups of 384 patients followed in the same Center with FPG: <100 mg/dL (234 patients), IFG $_{low}$:100-109 mg/dL (91 patients) and IFG $_{high}$:110-125 mg/dL (59 patients).

Based on the OGTT, 44 of 234 β-TM patients presented with NFG (18.8%), 3 (1.2%) with IGT and 1 with a new diagnosis of Th-RD. In the group of patients with IFG the probability of diagnosing IGT was higher (46.1%) in subjects with FPG between 100 and 109 mg/dL (5.6 - 6.0 mmol/L) compared to subjects with FPG between 110 and 125 mg/dL (6.1- 6.9 mmol/L= 26.3% mg/dL; χ^2: 7.258 , P = 0.0071) (Figure 1). IGT was diagnosed even in the 5-10 years age group. The detailed number of patients with IGT and diabetes, after OGTT, are shown in tables 2 and 3.

Both ADA and WHO criteria for IFG missed the diagnosis of Th-RD in 4 of 91 patients (4.3%) and 11 of 59 patients (18.6%), respectively. The num-

Table 2. Diagnostic value of different fasting glucose cut-off points in the detection of impaired glucose tolerance (IGT: N. and % of diagnostic positivity) two hours after oral glucose tolerance test in 384 β- thalassemia major patients aged 5-40 years.

Fasting Plasma Glucose (FPG)	**5-10 years N. (%)**	**11-20 years N. (%)**	**21-30 years N. (%)**	**31-40 years N. (%)**
FPG :<100 mg/dL (< 5.6 mmol/L)	0/63 (0%)	14/78 (17.9 %)	19/53 (35.8 %)	11/40 (27.5 %)
IFG $_{low}$: 100-109 mg/dL (5.6 - 6.0 mmol/L)	2/11 (18.1%)	21/47 (44.6%)	10/19 (52.6%)	9/14 (64.2%)
IFG $_{high}$: 110-125 mg/dL (6.1- 6.9 mmol/L)	2/4 (50%)	8/25 (32%)	12/23 (52.1%)	3/7 (42.8%)
Total cases of impaired fasting glucose: 100-125 (mg/dL) (5.6 - 6.9 mmol/L)	4/78 (5.1 %)	29/150 (19.3 %)	22/95 (23.1 %)	12/61 (19.6 %)

Table 3. Diagnostic value of different fasting glucose cut points in the detection of β- thalassemia major related diabetes (Th-RD: N. and % of diagnostic positivity) two hours after oral glucose tolerance test in 384 patients aged 5-40 years.

Fasting Plasma Glucose (FPG)	**5-10 years N. (%)**	**11-20 years N. (%)**	**21-30 years N. (%)**	**31-40 years N. (%)**
FPG < 100 (mg/dL) (< 5.6 mmol/L)	0/63	0/78	0/53	3/40
Total number of patients: 40	(0 %)	(0 %)	(0 %)	(7.5%)
IFG $_{low}$: 100-109 mg/dL (5.6 - 6.0 mmol/L)	0/11	0/47	2/19	2/14
Total number of patients: 33	(0 %)	(0 %)	(10.5%)	(14.2%)
IFG $_{high}$: 110-125 mg/dL (6.1- 6.9 mmol/L)	0/4	1/25	6/23	4/7
Total number of patients: 55	(0 %)	(4%)	(26.0%)	(57.1%)
Total cases of newly diagnosis of diabetes	0/78	1/150	8/95	9/61
PG after OGTT: ≥ 200 mg/dL (≥ 11.1 mmol/L)	(0 %)	(0.6 %)	(8.4 %)	(14.7 %)

ber of patients with a new diagnosis of diabetes, after OGTT, increased progressively starting from the age of 11 years (Table 3).

In conclusion, our clinical experience has shown that: (a) many β-TM patients who have a normal FPG may present with GD after OGTT, (b) glucose homeostasis disturbances may be present also in very young patients, (c) ADA criteria used for the diagnosis of IFG identified an additional group of patients with IGT, and (d) OGTT screening seems to be cost-effective. However further studies are needed to evaluate if it is also applicable to very young β-TM patients.

We acknowledge that this preliminary study is subject to several limitations. First, the number of patients is relatively small and data were collected retrospectively from a single unit, so the applicability of this study to the general population of β-TM in real time may be limited. Second, we defined and validated cases with GD, except diabetes, on the basis of a single measurement rather than using repeated measurements to confirm the diagnosis. Third, the retrospective study considered patients who received, over time, different protocols for the treatment of iron overload. Nevertheless, to the best of our knowledge, our data are the first comparing different FPG cut-off levels in relation to the PG level at 2-h after OGTT in a cohort with satisfactory chelation regimes.

Can we identify β-TM patients at high-risk for GD earlier and with more sensitive biomarkers?

In the general population, hyperglycemia can be determined at least in three ways by measuring

fasting glucose, post-challenge (or postprandial) glucose, and HbA_{1c}. The first is, by definition, the lowest glucose level during of the day. Post-challenge glucose levels show the magnitude of glucose elevation (peak) after a glucose load, lasting 1–3 hours. However, it is noteworthy that OGTT results are not always reproducible and can vary over time. Continuous glucose monitoring (CGM) has modernized the landscape of diabetes screening and management mainly in subjects with diabetes type 1 (T1DM) and diabetes type 2 (T2DM); however, its application in β-TM patients is increasing as it can detect early stages of glycemic abnormalities compared to other screening tests (33-35). Nevertheless, it is not currently approved as a diagnostic tool for β-TM patients and more information is needed to establish a consensus on screening parameters/ thresholds, e.g. the number of glucose elevations required to indicate prediabetes or diabetes and its correlation with clinical outcomes.

Other measures of ß-cell function depend on the pattern of insulin response during OGTT (36), and 1-hour PG ≥ 155 mg/dL after OGTT (37), as well as pancreas R2* MRI combined with insulin secretion-sensitivity index-2 (ISSI-2) (38). However, the reproducibility and predictivity of these tests in thalassemia are not fully known and should be further investigated to confirm their clinical significance.

Conclusion and recommendations for clinicians caring for patients with β-TM

Although thalassemia-related diabetes shares certain characteristics with both T1DM and T2DM, it appears to be a separate entity with a unique pathophysiology. The high prevalence of Th-RD in subjects with β-TM requires targeted screening for detection of GD as early as possible.

In general, OGTT is the accepted gold standard for screening, and its use is widely advocated with the recommendation that the test should be carried out, preferably combined with determination of insulin secretion, at 10, 12, 14, and 16 years and annually thereafter. However, the clinical utility of the OGTT in the general population has been questioned (39), due to test variability, inconvenience related to oral glucose load, time spent in the laboratory, and cost. Moreover, even when the fasting and 2-h OGTT glucose levels are normal, variable and intermittent post-prandial hyperglycemia can be detected by CGMS in children and adults affected with β-TM (34,35).

Although the determination of the optimal method for identifying patients at risk for deterioration of glucose homeostasis is still challenging, the recent retrospective personal observations confirm the utility of OGTT screening, using ADA criteria, for the detection of IGT and Th-RD in β-TM patients with normal fasting plasma glucose (FPG). Nevertheless, we

look forward to prospective studies in larger groups of patients with β-TM.

In low- and middle-income countries, screening for GD in β-TM patients poses particular challenges, in particular, where healthcare centers are overcrowded, understaffed, and insufficiently resourced. However, the major concern is with those who would be deemed normal by current FPG criteria. Therefore, a selective approach should be considered and discussed as reasonable alternative with acceptable sensitivity for case detection.

Practically, we can consider two approaches to address this problem. The first, to lower the threshold for FPG, as suggested by Noetzli et al. (40) and Pepe et al. (41) for increasing the sensitivity for early detection of GD. Pepe et al. (41) found that FPG > 98 mg/dL identified an abnormal OGTT result. This cut-off is similar to that previously identified (97 mg/dL) by Noetzli et al. (40) in a small cohort of patients and suggests the need to use lower cut-offs than population norms to increase specificity of the diagnosis in β-TM patients.

The 50th percentile of FPG in children (from 5 to 11 years) varies from 82 to 90 mg/dL (4.6 - 5.0 mmol/L) and between 20 and 44 years is 94 mg/dL (5.2 mmol/L) (42). The second approach is to keep the FPG criteria as currently defined and use clinical and laboratory information (severity of iron overload, poor compliance to iron chelation therapy, presence of chronic liver disease, other complications of iron overload, overweight/obesity) to identify those β-TM patients who should have annual OGTT screening. If the implementation of annual GD screening with the OGTT is problematic for some individuals and/or centers, monitoring of fasting and 2-h post-prandial glucose (PPG) is recommended.

In clinical practice, it is difficult to give a definitive cut-off level of SF to define patients at risk of GD. Chelation therapy is tailored to maintain the liver and cardiac iron in an acceptable levels and to match ongoing transfusional iron loading. Multiple methods of assessing the degree of iron overload exist, and each method has benefits and limitations. SF is the least expensive. Typically, the goal is to keep the liver iron concentration between 2 and 7 mg/g dry weight, corresponding to a SF level between 500 and 1,500 ng/mL in patients with β-TM (43).

The suggested guidance requires further investigation of the natural history of the disease. This will permit harmonization of the results and will provide a better patient outcome. The information gleaned from this process must be communicated to patients and/or parents for a better adherence to the diagnostic process and for the general treatment of disease.

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

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