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Original Article

Assessment of glucose homeostasis in young adult female β-thalassemia major patients (β-TM) with acquired hypogonadotropic hypogonadism (AHH) never treated with sex steroids compared to eugonadal β-TM patients with spontaneous menstrual cycles

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Abstract. *Background*: Acquired ypogonadotropic hypogonadism (AHH) is the most prevalent endocrine complication in thalassemia major (TM). *Study design:* Considering the detrimental effect of estrogen deficiency on glucose metabolism, the ICET-A Network promoted a retrospective study on the long-term effects of estrogen deficiency on glucose homeostasis in female β-TM patients with HH without hormonal replacement therapy (HRT). *Patients and Methods:* Seventeen β-TM patients with AHH (4 had arrested puberty; Tanners' breast stage 2-3), never treated with sex steroids, and 11 eugonadal β-TM patients with spontaneous menstrual cycles at the time of referral were studied. A standard 3-h OGTT was performed in the morning, after an overnight fast. Six-point plasma glucose and insulin level determinations, indices of insulin secretion and sensitivity, early-phase insulin insulinogenic index (IGI), HOMA-IR and β-cell function (HOMA-β), oral disposition index (oDI), glucose and insulin areas under the OGTT curves were evaluated. *Results:* Abnormal glucose tolerance (AGT) or diabetes was observed in 15 (88.2%) of 17 patients with AHH and 6 (54.5%) of 11 patients with eumenorrhea. The difference between the two groups was statistically significant (P: 0.048). However, the group of eugonadal patients was younger compared to AHH patients (26.5 ± 4.8 years vs. 32.6 ± 6.2 years ; P: 0.010). Advanced age, severity of iron overload, splenectomy, increased ALT levels and reduced IGF-1 levels were the main clinical and laboratory risk factors for glucose dysregulation observed in β-TM with AHH compared to eugonadal β-TM patients with spontaneous menstrual cycles. *Conclusion:* These data further support the indication for an annual assessment of OGTT in patients with β-TM. We believe that a registry of subjects with hypogonadism is necessary for a better understanding of the long-term consequences of this condition and refining treatment options. (www.actabiomedica.it)

Key words: β-thalassemia major, glucose tolerance, insulin sensitivity, β-cell secretion, acquired hypogonadotropic hypogonadism.

Introduction

Subjects with β-thalassemia major (β-TM) or transfusion-dependent thalassemia (TDT) have various medical needs to meet the complications of thalassemia throughout their lives (1,2). Most of the major disease-related complications are commonly found beyond the second and third decades of life (1).

Regular blood transfusions which are the major part of the supportive care regimen to correct anemia, represent the main source of iron overload in β-TM (420ml/unit of donor blood contains 200 mg of iron).

Iron accumulation induces progressive failure of the liver, heart, and endocrine glands. Increased systemic iron levels lead to the saturation of the physiological systemic iron carrier transferrin and the excess free iron produce the non-transferrin bound iron (NTBI) and its reactive fraction, labile plasma iron (LPI) (3). LPI enters cells through calcium channels that are not regulated by intracellular iron concentration and may cause oxidative damage to cellular membranes, proteins and DNA. NTBI/LPI-mediated toxicity and tissue iron overload may also exert multiple detrimental effects that contribute to the pathogenesis of complications in β-TM (4).

Serum ferritin (SF), a relatively inexpensive and widely available measurement, is useful to assess iron overload and to monitor chelation therapy. The LPI assay by fluorescence can serve as an alternative method to SF in cases where SF could be fallacious. However, it requires standardization and clinical validation, and risky levels of LPI have yet to be identified (4). Magnetic resonance imaging (MRI) offers the most accurate and widely available non-invasive method for assessing liver and cardiac iron concentration (5).

Acquired hypogonadotropic hypogonadism (AHH) is the most prevalent reported endocrine complication, affecting a large number of β-TM patients (1,6,7). The prevalence and severity of hypogonadism vary between studies, depending on the age, genotype, number of transfusions, age at the start of chelation therapy and the type of iron chelation therapy in the study cohort of patients (3,7).

In general, the early forms of hypogonadotropic hypogonadism (HH) in females have a clinical impact starting from the age of 10–11 years and are manifested by decreased of growth velocity and, around the age of 13 years, by delay of pubertal development. In pubertal patients, the appearance of hypogonadism results in the arrest of puberty in the early stages of breast development and primary amenorrhea or, later, secondary amenorrhea (1,6,7).

In one study, on multivariate logistic regression analysis, patients with a SF level >2,500 ng/mL were 3.53 times (95% CI 1.09–11.40) more likely to have diabetes mellitus, 3.25 times (95% CI 1.07–10.90) more likely to have hypothyroidism, 3.27 times (95%, CI 1.27–8.39) more likely to have hypoparathyroidism, and 2.75 times (95%, CI 1.38–5.49) more likely to have hypogonadism compared to patients with SF ≤ 1,000 ng/mL. However, splenectomized patients who had SF levels less than 2,500 ng/mL also had high rates of endocrine disorders (8).

Endocrinologists taking care of such patients often see subjects with HH in late adolescence or early adulthood, in whom the main complaint is the lack of pubertal development (primary amenorrhea) or secondary amenorrhea.

Considering the well-recognized possible detrimental effect of estrogen deficiency on glucose metabolism (9,10), the ICET-A Network (International Network of Clinicians for Endocrinopathies in Thalassemia and Adolescence Medicine) promoted a retrospective study to investigate the long-term effects of estrogen deficiency on glucose homeostasis in female β-TM patients with HH who were considered to be at increased risk for HRT-related adverse events or who refused HRT. In addition, we reviewed their medical records, collecting data on disease evolution and related data on treatment and complications.

Patients and Methods

A. Study population and design

All β-TM patients, over the age of 17 years, who were consecutively referred for consultation or

second opinion for endocrinological and metabolic problems to a single Italian centre (Pediatric and Adolescent Outpatient Clinic, Private Accredited

Quisisana Hospital, Ferrara, Italy) from October 2010 to July 2022, were reviewed.

Seventeen β-TM patients with AHH (4 had arrested puberty; Tanners' breast stage 2-3), never treated with sex steroids, and 11 eugonadal β-TM patients with spontaneous menstrual cycles (from 28 to 45 days) at the time of referral were selected for the study, and in addition 8 adult healthy females. The exclusion criteria included: 1) non-transfusion-dependent thalassemias; 2) mental illness (depression, anxiety disorders, eating disorders); 3) renal insufficiency; 4) history of severe head trauma and brain injury; 5) alterations in nutritional status; 6) bone marrow transplantation; 7) HIV positivity; and 8) patients taking contraceptives.

The following data were collected: age, gender, ethnicity, anthropometric indices, pubertal status, age at first transfusion, interval between transfusions, mean annual pre-transfusion hemoglobin (Hb), annual blood consumption, peak of SF (defined as the highest level registered from the beginning of chelation therapy to the consultation), splenectomy, liver iron concentration (LIC) and cardiac T2* assessed by magnetic resonance imaging (MRI), bone mineral densitometry (BMD), and associated endocrine complications.

B. Auxological measurements, classifications and definitions

Height and weight were measured according to international recommendations (11). Short stature was defined as height below the third percentile using the growth charts of Cacciari et al. for the Italian population (12,13). Body mass index (BMI) was calculated (weight in kg/height in m^2). A subject was considered overweight when the BMI was between 25 and 30 and obese above 30 (13). Sexual maturation was determined by physical examination. Pubertal arrest was defined as the lack of pubertal progression for more than 3 years after spontaneous breast bud onset. Primary amenorrhoea (PA) was defined as the absence of menarche at the age of 16 years (14).

The presence of associated endocrine complications was defined according to the I-CET guidelines, published in 2013 (14). Ranges of normal insulin-growth factor-1 (IGF-1) values set at the 2.5th–97.5th percentile in the three age ranges were 95.6–366.7 ng/ml between 25 and 39 yrs and 60.8–297.7 ng/ml between 40 and 59 yrs (15). Serum FSH, LH and E_2 were measured by chemiluminescent assay using a commercial kit.

Iron overload was assessed by measuring SF levels. Iron overload 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).

C. Oral glucose tolerance test (OGTT)

A standard OGTT (max 75 g of glucose in 250–300 mL water) was performed in the morning, after

an overnight fast, in subjects who were clinically stable and without a history of acute infection in the previous 3 weeks. Venous blood samples were collected at 0, 30, 60, 90, 120 and 180 min after glucose load for plasma glucose (PG) and insulin assay. Glucose tolerance was classified in accordance with the American Diabetes Association criteria (16).

Serum glucose was measured by the glucose oxidase method in a glucose analyzer. The percent coefficient of variation of the glucose oxidase method is 1.4–1.5%. Insulin was measured using the Immulite immunoassay (Diagnostic Products Corporation, Los Angeles, CA, USA). The analytical sensitivity was 2 μIU/mL.

D. Calculations of insulin secretion and sensitivity indices derived from OGTT

Indices of insulin secretion and sensitivity [Early-phase insulin secretion (insulinogenic index:IGI) and β-cell function (HOMA-β)], HOMA-IR quantitative insulin sensitivity check index (QUICKI), Matsuda index (MI 0-120), oral disposition index (oDI), glucose and insulin areas under the OGTT curves) were calculated as previously described (17,18). Patients were considered insulin resistant (IR) when the HOMA-IR index was > 2.5 (19).

The quantitative insulin sensitivity check index (QUICKI) was calculated using the following formula: 1/[log fasting insulin (mIU/mL) + log fasting glucose (mg/dL)]. IR was defined as a QUICKI value < 0.357 (19).

The presence of indeterminate glucose tolerance (INDET) was defined as a normal fasting PG and normal 2- h post challenge glucose with any intermediate OGTT plasma glucose level ≥ 200 mg/dL (20). The delayed timing of post-load glucose peak (>30 minutes) associated with lower oDI was considered an index supporting a decline of β-cell function and was an additional tool to enhance the prediabetes risk stratification (21).

Statistical analysis

All numeric variables were expressed as mean ± standard deviation (SD). For the statistical analysis, a software program was used and validated, according to Alder and Roesser (22). Comparison of variables in the two groups of patients were made using an independent sample *t* test and variables with non-normal distribution were compared using non-parametric Wilcoxon's signed
rank test. Pearson's correlation tests (2-tailed) were used to study correlations between variables with parametric and non- parametric distributions. Differences in proportions between the 2 groups were evaluated using the Chi-Square test and Fisher's exact test. A P value < 0.05 was considered statistically significant.

Ethics

All procedures were in accordance with the 1964 Helsinki declaration and its later amendments.
According to the Italian regulations, ethics approval by the local Ethics Committee was not required for the following reasons: no identifiable private information was collected; patients underwent only routine diagnostic and therapeutic procedures according to current guidelines (23,24) and an anonymized dataset was analyzed. Informed consent was obtained from all patients after detailed explanation of the nature and purpose of the study and the likely risks and benefits associated with study participation (25).

Results

A. Patients' characteristics

The demographic, anthropometric, and clinical data of enrolled patients are summarized in Table 1.

Two β-TM patients with AHH were classified overweight and one obese. In the group of eugonadal β-TM patients, 3 were overweight and no one was obese. Patients with AHH were older compared to eugonadal β-TM patients (P: 0.010).

Patients were regularly transfused with genotyped, white cell depleted, washed packed red blood cells (PRBC) (Table 1). Ten out of 17 patients with AHH had undergone splenectomy because of increased transfusion requirements and/or the presence of signs of hypersplenism. Splenectomy was done at the mean age of 14.9 years. None of 11 eugonadal patients or those with spontaneous menstruation had been splenectomized.

Menarche was reported at the mean age of 13.5± 0.9 years. Two eugonadal patients had spontaneous pregnancy.

Mean alanine aminotransferase (ALT) level was consistently higher in patients with HH compared to eugonadal β-TM patients (P: 0.0045).

At consultation, in β-TM patients with HH, a SF level < 1,000 ng/mL was present in 1 patient, between > 1,000 and < 2,000 ng/mL in 6 patients, and > 2,000 ng/mL in 10 patients. In eugonadal β-TM patients with spontaneous menstruation, a SF level < 1,000 ng/mL was present in 3 patients, between > 1,000 and < 2,000 ng/mL in 5 patients, and > 2,000 ng/mL in 3 patients.

The commonest associated endocrine complication in 6/17 patients with AHH (35.2%) was subclinical hypothyroidism (Table1). The results of glucose abnormalities are reported in the next section. Multiple endocrine complications (> 2), in addition to AHH, were present in 8 patients. All patients received hormonal replacement therapy in accordance with the ICET-A guidelines (14).

At consultation, 12 β-TM patients with AHH and 7 eugonadal patients were taking oral bisphosphonate (alendronate 70 mg/week). Spine and femur osteoporosis (defined as a T score of –2.5 or lower)

Table 1. Comparison of clinical characteristics, laboratory and diagnostic results between 17 Ŧthalassemia major (β-TM) patients with primary amenorrhea and 11 β-TM eugonadal patients with spontaneous menstruation. Data are expressed as mean ± SD.

Variables	β-TM patients with AHH	Eugonadal β-TM patients	P value
Number of β-TM patients	17	11	-
Age at consultation (yrs)	32.6 ± 6.2	26.5 ± 4.8	**0.010**
Family history of diabetes:	-	-	-
None	14	11	-
Yes (n. and %)	3 (17.6%)	2 (18.1%)	0.95
Age at first blood transfusion (months)	12.5 ± 15.1	14.0 ± 11.6	0.78
Mean annual pre-transfusional Hb level (g/dL)	8.79 ± 0.27	8.80 ± 0.35	0.93
Annual consumption of PBRC in mL/kg BW	124.3 ± 22.1	130.5 ± 15.0	0.42
Number of splenectomized patients	10/17	0/17	-
Age at splenectomy (yrs)	14.9 ± 10.8	-	-
Body Mass Index (kg/m^2) at consultation	23.0 ± 3.1	23.1 ± 3.2	0.93
Final standing height (cm)	157.8 ± 7.4	152.5 ± 8.0	0.084
Iron chelation therapy at consultation:	-	-	-
Desferrioxamine (DFO) (n)	11/17	6/11	-
Deferiprone (DFP) (n)	5/17	3/11	-
Deferasirox (DFX) (n)	1/17	2/11	-
SF at consultation (ng/mL)	2601.9 ± 1652.7	1336.0 ± 615.3	**0.022**
SF peak (ng/dL)	4371.0 ± 2919.1	2344.2 ± 1488.3	**0.043**
ALT (normal values: < 40 IU/L) at consultation	44.4 ± 23.8	21.3 ± 7.0	**0.0045**
IGF-1 (ng/mL) at last consultation	62.1 ± 37.2	94.0 ± 36.3	**0.032**
MRI LIC (mg/g d.w.) assessed 9 -18 months before consultation (n)	4.6 ± 3.6 (13)	5.2 ± 4.5 (10)	0.71
MRI global myocardial T2* (ms) assessed 9-18 months before consultation (n)	16.0 ± 13.1 (13)	29.9 ± 9.3 (10)	**0.010**
Number of patients with T2* < 20 ms (n and %)	5/13 (38.4%)	1/10 (10.0%)	0.18
Final height and endocrine complications registered at consultation	-	-	-
1. Short final stature (≤ 3rd centile) (n)	4/17	5/11	0.23
2. β-TM- RD (n)	2/17	0/11	0.24
3. Subclinical hypothyroidism (n)	6/17	0/11	**0.048**
4. Central HT (n)	1/17	0/11	0.42
5. Hypoparathyroidism (n)	2/17	0/11	0.24
6. Hypocortisolism (n)	0/17	0/11	-

Legend = PBRC: packed blood red cells; BW: body weight; AHH:acquired hypogonadotropic hypogonadism; n: number; SF: serum ferritin, ALT: alanine aminotransferase; IGF-1: Insulin Growth Factor-1; MRI: Magnetic Resonance Imaging; LIC: liver iron concentration; Central HT: Central hypothyroidism.

assessed by DEXA, one year before starting alendronate, was present in 83.3% and 57.1, respectively (P: 0.22).

B. Glucose homeostasis at consultation

At first consultation, in β-TM patients with AHH (mean age of 32.6 years), an abnormal glucose tolerance (AGT: including isolated IFG, EGI, IGT and IFG+IGT) and diabetes classified during OGTT, was found in 12/14 patients (85.7%) (Figure 1). Three patients had been previously diagnosed with TM-related diabetes (Th-RD) during adolescence. A normal glucose tolerance was present in only 2 patients (14.2%).

When the total group of 17 β-TM patients with AHH and diabetes or AGT (88.2%) were compared to 11 eugonadal β-TM patients with spontaneous menstrual cycles and ATG (54.5%) (Figure 2), the difference was statistically significant (P: 0.048). However, the latter group of eugonadal patients was younger compared to AHH patients (26.5 ± 4.8 years vs. 32.6 ± 6.2 years; P: 0.010).

The curve profiles of PG and insulin, during OGTT, in the 2 groups of patients are illustrated in figures 3

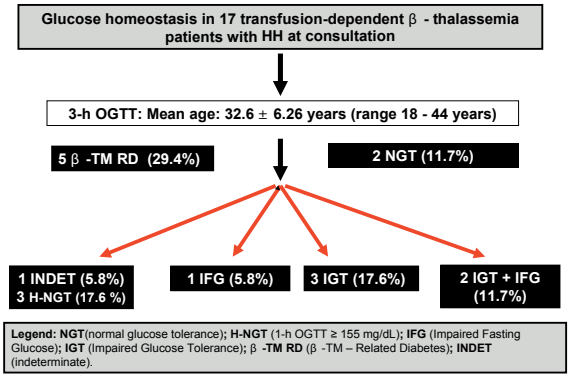

Figure 1. Number of β-TM patients with abnormal glucose homeostasis and β-TM related diabetes during 3-h OGTT, at consultation (mean age 32.6 years). Three patients had been previously diagnosed with TM-related diabetes (Th-RD) during adolescence.
Indeterminate glucose tolerance (INDET) was defined as a normal fasting PG and normal 2- h post challenge glucose with any intermediate OGTT plasma glucose level ≥ 200 mg/dL.

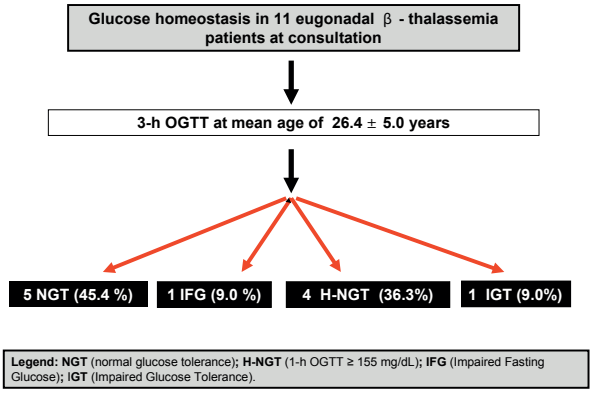

Figure 2. Number of eugonadal β-TM patients with spontaneous menstruation who had abnormal glucose homeostasis during 3-h OGTT (mean age 26.4 years).

and 4, and the detailed mean levels at 0',30',60,120 and 180' minutes are reported in table 2. A statistical difference of PG level was observed at baseline and 60' minutes during OGTT in patients with AHH compared to eugonadal patients, but no significant differences were observed between the two groups of patients regarding plasma insulin level (Figure 4).
Higher insulin levels at 120 min vs. 30 min were found

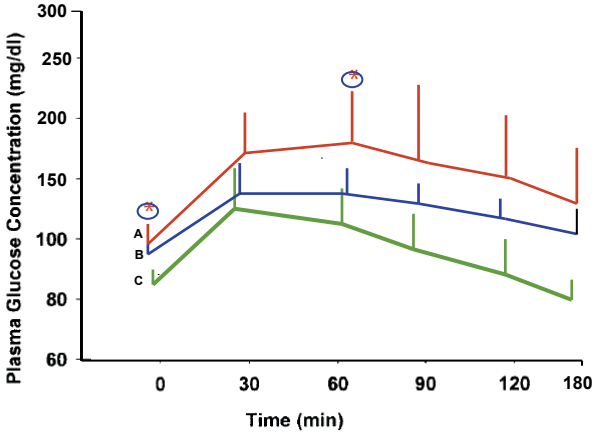

Figure 3. Profiles of plasma glucose during oral glucose tolerance test in 11 eugonadal Tβ-TM patients (A) versus 14 Tβ-TM patients with AHH (B) and 8 healthy adult females (C). (*) = P: 0.042 and 0.035, respectively.

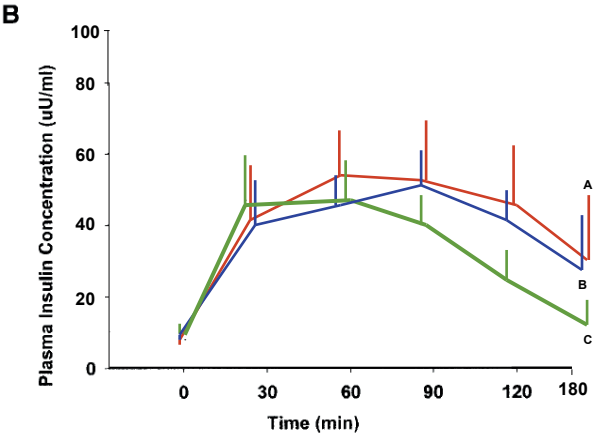

Figure 4. Profiles of plasma insulin during oral glucose tolerance test in 11 eugonadal Tβ-TM patients (A) versus 14 Tβ-TM patients with AHH (B) and 8 healthy adult females (C).

in 4 patients with AHH (65.75 ± 21.8 vs. 35.75 ± 21.7 μU/mL) and in 3 eugonadal β-TM patients (45.1± 22.5 vs. 21.0 ± 10.6 μU/mL).

C. Insulin secretion and sensitivity indices

Substantially, the indices of insulin secretion and sensitivity were not different between the two

groups of patients but all were statistically different when compared to 8 healthy adult females subjects (Table 3).

Table 2. Comparison of plasma glucose (PG) and plasma insulin levels during OGTT in 14 β-TM patients with AHH versus 11 eugonadal β-TM patients. Data are expressed as mean ± SD.

Variables	β-TM patients with AHH (14 patients)	Eugonadal β-TM patients (11 patients)	P value
Fasting plasma glucose (PG) (mg/dL)	96.5 ± 9.3	89.7± 5.4	**0.042**
PG 30 min. after OGTT (mg/dL)	164.6 ± 39.7	141.0 ± 23.8	0.095
PG 1-h after OGTT (mg/dL)	176.7 ± 40.6	146.5 ± 21.2	**0.035**
PG 2- h after OGTT (mg/dL)	154.4 ± 46.1	128.1 ± 13.4	0.081
PG 3- h after OGTT (mg/dL)	126.5 ± 40.3	112.2 ± 17.7	0.28
$AUC_{GLU\ 0\text{-}120}$ (mg/dL)	334.1± 83.6	282.1±26.1	0.059
Fasting insulin (μU/mL)	6.8 ± 4.2	6.9 ± 3.0	0.98
Insulin 30 min after OGTT (μU/mL)	42.5 ± 22.0	38.1 ± 17.0	0.58
Insulin 1-h after OGTT (μU/mL)	49.0 ± 22.0	41.0 ± 16.9	0.32
Insulin 2-h after OGTT (μU/mL)	43.3 ± 23.5	39.7 ± 14.3	0.65
Insulin 3-h after OGTT (μU/mL)	31.2 ± 21.0	32.1 ± 17.2	0.90
$AUC_{INS\ 0\text{-}120}$ (μU/mL)	86.01 ± 27.9	72.7 ± 26.2	0.23

Legend: OGTT, oral glucose tolerance test; $AUC_{GLU\ 0\text{-}120}$, area under curve; $AUC_{INS\ 0\text{-}120}$, insulin area under the curve;

Table 3. Comparison of insulin secretion and sensitivity indices during OGTT in 14 β-TM patients with AHH versus 11 eugonadal β-TM patients with spontaneous menstruation, and 8 healthy adult female patients. Data are expressed as mean ± SD.

Variables	β-TM patients with AHH (14 patients)	Eugonadal β-TM patients (11 patients)	Normal values
HOMA-IR (*)	1.69 ± 1.24 (**§**)	1.40 ± 0.78 (§)	See legend
HOMA-β(*)	72.8 ± 33.5 **(^)**	92.64± 38.9 (§)	See legend
MATSUDA INDEX (MI 0-120) (*)	6.54 ± 3.66 (**§**)	6.94 ± 2.47 (§)	See legend
QUICKI (*)	0.36 ± 0.03 **(^)**	0.36± 0.02 (^^)	See legend
Insulinogenic Index (IGI) (*)	0.59 ± 0.34 **(^)**	0.64 ± 0.21 (^^)	See legend
Oral disposition Index (oDI) (*)	3.89 ± 3.0 **(^)**	4.42 ± 1.97 (^^)	See legend

Legend: HOMA-IR, homeostasis model of assessment; HOMA- β homeostasis model assessment for estimation of index β-cell secretion; QUICKI, quantitative insulin sensitivity check index; AUC, area under the curve; Note: Three β-TM patients have been not included because developed diabetes before consultation; Comparison between the two groups of β-thalassemia major patients = (*): NS; Comparison between β-TM patients and 8 adult healthy females = HOMA-IR: n.v. 1.2 ± 0.7(§): NS; HOMA-β n.v. 123.0 ± 48; (^) P:< 0.01; MATSUDA INDEX : n.v. 8.7 ± 3.4 (§): NS; QUICKI: n.v. 0.39 ± 0.03; (^) P: 0.035; (^^) P: 0.017; Insulinogenic Index (IGI): n.v. 1.7 ± 1.3; (^) P: 0.006; (^^) P: 0.015; Oral disposition Index: n.v. 13.6 ± 9.9; (^) P: 0.0001; (^^) P: 0.0076; (extrapolated from: Ref. 26).

c. Significant correlations

Correlation analysis between the collected variables including age, SF level, ALT and IGF- 1 values was performed in the two groups of patients. Interestingly, the strongest correlations were found between $AUC_{GLU\ 0\text{-}120}$ and HOMA-IR, oral disposition index, IGI and PG at 2-h during OGTT, and QUICKI vs. HOMA-IR and MATSUDA index in patients with HH, and between $AUC_{INS\ 0\text{-}120}$ and QUICKI, IGF-1 vs. oral disposition Index, and QUICKI vs. HOMA-IR and MATSUDA index in eugonadal β-TM patients. The significant variables are reported in table 4.

Discussion

This retrospective study was carried out on a unique cohort of 17 adult female β-TM patients with AHH never treated with sex steroids. To the best of our knowledge, this is the first study in the literature, analyzing the prevalence of glucose dysregulation in

Table 4. Significant correlations between the two groups of β-TM patients with primary amenorrhea (14 patients) and eugonadal and spontaneous menstruated (11 patients).

Correlations	β-TM patients with primary amenorrhea (14 patients)	Eugonadal Tβ-TM patients (11 patients)
$AUC_{GLU\ 0\text{-}120}$ (mg/dL) vs. MATSUDA INDEX	**r:** -0.551, **P: 0.041**	**r:** -0.2666, P: 0.055
$AUC_{GLU\ 0\text{-}120}$ (mg/dL) vs. HOMA-IR	**r:** 0.6622, **P: 0.009**	**r: :** -0.0262, P: 0.93
$AUC_{GLU\ 0\text{-}120}$ (mg/dL) vs. Insulinogenic Index (IGI)	**r:** -0.6751, **P: 0.008**	**r:** -0.5061, P: 0.11
$AUC_{GLU\ 0\text{-}120}$ (mg/dL) vs. Oral disposition Index	**r.** -0.838, **P: 0.0001**	**r:** -0.5288, P: 0.09
$AUC_{GLU\ 0\text{-}120}$ (mg/dL) vs. PG at 1-h during OGTT	**r:** -0.1497, P: 0.61	**r:** 0.7104, **P: 0.014**
$AUC_{GLU\ 0\text{-}120}$ (mg/dL) vs. PG at 2-h during OGTT	**r.** 0.7544, **P: 0.001**	**r:** 0.1462, P: 0.66
$AUC_{INS\ 0\text{-}120}$ (μU/mL) vs. QUICKI	**r**: -0.0534, P: 0.86	**r:** : -0.7885, **P:0.003**
IGF-1 vs. Oral disposition Index	**r:** : 0.2436, P: 0.61	**r:** -0.6259, **P: 0.041**
QUICKI vs. MATSUDA INDEX	**r:** 0.8404, **P: 0.0001**	**r. :** 0.8573, **P: 0.0007**
QUICKI vs. HOMA-IR	**r:** -0.8929, **P: 0.00001**	**r:** 0.8491, **P: 0.0009.**

young adult female patients with a long clinical history of untreated hypogonadism.

The prevalence of abnormal glucose handling was significantly higher in adult female β-TM patients with AHH compared to eugonadal ones, as evidenced by statistically significant differences in glucose levels at baseline as well as one hour after a 75-gram oral glucose load. In addition, as many as 29% of hypogonadal females had diabetes mellitus versus no diabetes in eugonadal patients.

Advanced age, severity of iron overload, splenectomy, increased ALT levels and reduced IGF-1 levels were the main clinical and laboratory risk factors for glucose dysregulation observed in β-TM with HH compared to eugonadal β-TM patients with spontaneous menstrual cycles. The emerging, yet unanswered question is whether estrogen deficiency is an "innocent bystander", reflecting the severity of iron overload, or does it *per se* contribute to abnormal glucose regulation, potentially through reducing insulin sensitivity ?

Iron loading of the pancreas starts early in transfused patients receiving poor or suboptimal iron chelation therapy (27-29) because pancreatic β- cells are vulnerable to oxidative stress. Iron overload disrupts insulin signalling in liver and skeletal muscle tissue, causing insulin resistance (IR), and at the same time, due to to minimal endogenous antioxidant defence, induces apoptosis of pancreatic β-islet cells with consequent insulin deficiency (29).

Matter et al. (30) evaluated pancreatic iron overload by T2*-weighted gradient-echo MRI in young β-TM patients, correlating it with glucose disturbances, LIC, SF and splenectomy. β-TM patients with diabetes and abnormal glucose tolerance showed a higher degree of pancreatic and hepatic siderosis compared to thalassemics with normal glucose tolerance and controls (P < 0.001, P < 0.0001). Moreover, splenectomized thalassemic patients had significantly lower signal intensity (SIR) of the pancreas compared to non-splenectomized patients (P: < 0.05), suggesting that, after splenectomy, iron deposition in the pancreas may be accelerated.

Fifty percent of HH patients had ALT levels above the normal limit of 40 IU/L and 6 were HCV-RNA positive. The liver is the primary organ for glucose metabolism because it plays a major role in blood glucose homeostasis by maintaining a balance between the uptake and storage of glucose via glycogenolysis and gluconeogenesis. Therefore, the liver may, in part, be responsible for the detection of glucose abnormalities during OGTT.

In the present study, the role of IGF-1 in β-TM patients with AHH on glucose homeostasis was less consistent compared to a previous observation (31), because no correlation was observed between IGF-1 and $AUC_{GLU\ 0\text{-}120}$, $AUC_{INS\ 0\text{-}120}$ indices of insulin secretion and sensitivity, and ALT. However, in the 11 eugonadal patients with spontaneous menstruation a significant correlation was found between IGF-1 and oral disposition Index (r: -0.6259, P: 0.041). IGF-1 has been shown to lower blood glucose through stimulating glucose transport to fat and muscle and inhib-

iting hepatic glucose output (32,33). In conclusion, further studies are needed to assess the IGF-1 level in patients with NGT or AGT (isolated IFG, EGI, IGT, and IFG+IGT) before defining an obvious association.

We cannot exclude the potential role of estrogen deficiency in modulating insulin sensitivity and glucose tolerance in patients with HH. The role of estrogens in the regulation of glucose homeostasis has been extensively investigated in animal and clinical studies (34). There are three major forms of physiological estrogens in females: estrone (E1), estradiol (E2, or 17β-estradiol), and estriol (E3). Their action has been attributed to the activation of the nuclear receptors (ERTand ERβ) and the novel cell surface membrane receptors (GPR30 and ER-X) (35,36). Ovarian hormones, especially E_2, participate in the regulation of the pancreatic secretion of insulin, insulin sensitivity, and carbohydrate metabolism as observed in ovariectomy and menopause (37).

Although the mean (SD) indices of insulin secretion and sensitivity were not different between the two groups of β-TM patients, the potential risk factor for developing insulin resistance, that is, the reduction in circulating estrogens, was also observed in our AHH patients. The $AUC_{GLUC\ 0\text{-}120}$ during OGTT correlated significantly with PG level at 120 min, IGI, HOMA-IR, Matsuda index (MI) and oral disposition index. The latter index indicates that these patients have inadequate β-cell compensation for the degree of insulin resistance present.

Several interesting additional aspects emerged from this retrospective study. First, 6 out of 11 (54.5%) eugonadal β-TM patients had an abnormal glucose tolerance in the absence of other associated endocrine complications. Their SF peak was identified as severe (3.046 ± 550.5 ng/mL). This observation supports the importance of frequent assessment of SF, and evaluation of the peak SF levels during the long-term evolution of iron overload and not to restrict to the last assessment of SF levels.

Second, the insulin secretion and sensitivity indices (QUICKI, IGI and oDI) of the two groups were statistically different compared to controls. These findings indicate that measurement of oDI may be clinically useful in patients with β-TM to detect subtle defects in β-cell function and identify progression of the disease in patients with β-TM. QUICKI and IGI seem to be better indices than HOMA-IR and HOMA-β to identify insulin sensitivity and insulin secretion and could be suitable as surrogate indices for estimating patients at risk of AGT. Nevertheless, a longitudinal analysis will be necessary to confirm the predictive value of these markers.

Third, the delayed timing of post-load glucose peak (>30 minutes) associated with lower oDI observed in 7/11 eugonadal β-TM indicates a decline of β-cell function and could be an additional tool to enhance the prediabetes risk stratification (21,38).

Finally, based on our results and in the context of the existing literature, we recommend the evaluation of the surrogate indices for the β-cell function and insulin sensitivity in addition to PG and insulin levels during OGTT in β-TM patients. Insulin sensitivity is the ability of insulin to lower FPG by suppressing hepatic glucose production and promoting glucose uptake in peripheral tissues while insulin resistance is defined as an impaired biological response to insulin. We are aware of the wide range of publications discussing the pros and cons of surrogate measures from various perspectives. Therefore, we advise to be cautious in the interpretation of OGTT-derived insulin sensitivity values as differences in OGTT may reflect variations in β-cell function, and/or glucose absorption rather than variations in insulin sensitivity (39,40).

In summary, we acknowledge that the current study is based on limited data and the retrospective characteristics of the study represent only one time point of laboratory data collection and thus lack the strength of a prospective or a longitudinal study. Moreover, we did not perform the measurement of insulin clearance that may be an important aspect of glucose metabolism in subjects with estrogen deficiency. Nevertheless, this study provides, for the first time, a framework of clinical and laboratory evidence that mirrors core aspects of β-TM patients with HH never treated with sex steroids. We hope that our study will be helpful and applicable for counseling patients in daily practice. Finally, we believe that a registry of subjects with hypogonadism is necessary to provide a better understanding of the long-term consequences of this condition and to refine treatment options.

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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