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Original article

Insulin-like growth factor -1 (IGF-1) and glucose dysregulation in young adult patients with β-thalassemia major: causality or potential link?

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Abstract. *Background:* Insulin-like growth factor-1 (IGF-1) has been shown to lower blood glucose through stimulating glucose transport to fat and muscle and inhibiting hepatic glucose output. Although previous cross-sectional reports reported an association between low circulating concentrations of IGF-1 and glucose dysregulation (GD), its role is still debated. *Aims of study:* The present retrospective study was designed to assess the circulating IGF-1 levels in β-thalassemia major (β-TM) patients with normal oral glucose tolerance test (NGT-OGTT) and GD referred for an endocrine evaluation to explore the potential link between low IGF-1 and GD. *Study design and methods:* Our study included 34 young adult patients with β-TM; 12 patients with NGT after OGTT, 7 with impaired glucose tolerance (IGT), 9 with impaired fasting glucose (IFG) plus IGT, and 6 patients with β-TM-related diabetes mellitus (β-TM- DM). *Results:* Twenty-two β-TM patients with GD or β-TM- DM and 1 patient with NGT had IGF-1 levels below the 2.5th percentile. Correlation of IGF-1 with fasting plasma glucose, HOMA-IR (homeostatic model assessment for insulin resistance) and OGIS (oral glucose insulin sensitivity) was found. Moreover, a negative correlation was documented between ALT and the Insulinogenic Index (IGI) and a positive correlation between serum ferritin and PG 2-h after OGTT. *Conclusion:* This study reports for the first time an association between low levels of IGF-1 and GD in β-TM patients. Despite some limitations, our study can serve to generate proposals for more convenient and efficient methods to identify and treat early GD in patients with β-TM, and to conduct more extensive studies. (www.actabiomedica.it)

Key words: β-thalassemia major, Insulin-like growth factor-1, glucose homeostasis, oral glucose tolerance test

Introduction

To overcome the effects of severe anemia, β-thalassemia major (β-TM) patients require regular blood transfusions (Transfusion Dependent Thalassemia -TDT). Since humans have no mechanism for elimination of excess iron accumulated by multiple transfusions of red blood cells, they inevitably become progressively iron overloaded. Cumulative iron overload, in turn, leads to iron toxicity with organ

dysfunction and damage, particularly of the liver and heart (1). After the iron storage ability of the reticuloendothelial cells is saturated, the iron spills into blood where it normally binds to transferrin. Once transferrin is saturated, the level of non-transferrin bound iron steadily increases (1). The unbound iron begins to accumulate in various organs, mainly heart, liver, pancreas and endocrine glands, inducing increased production of reactive oxygen species (ROS), especially hydroxyl radicals by the Fenton and Haber Weiss reaction (2,3). The formed ROS cause lipid peroxidation of the cell membrane; one of the products is malondialdehyde (MDA), which is toxic and mutagenic (4). Proper management and efficient, regular and accurate monitoring of total body iron with timely administration of iron chelators is essential to prevent and reduce iron burden (1).

The different mechanisms of iron uptake and accumulation in different organs may be responsible for the variation in the risk of complications. The liver is one of the first organs to accumulate iron and has the greatest iron content, followed by the pancreas and heart (5). Pancreatic β-cells are rich in mitochondria and are highly sensitive to oxidant generating substances (6). Oxidative stress is believed to modify a number of the signaling pathways within a cell that can ultimately lead to insulin resistance and hyperglycemia can lead to increased production of ROS in endothelial cells, liver and pancreatic β-cells (6). It has also been shown that in adult β-TM patients, iron accumulation is heterogeneous in the pancreatic head, body and tail regions, and fatty replacement of the pancreas is commonly seen in patients with overt DM (7-9).

In the literature, the estimated prevalence of diabetes mellitus (DM) in β -TM patients ranges from 9.7% to 29% (10-13). The duration of progression to overt hyperglycemia is highly variable, ranging from a few to several years (12,13). Risk factors for developing glucose dysregulation (GD) in thalassemia include severity of genotype and clinical phenotype, advanced age at onset of chelation therapy, poor compliance with chelation therapy, chronic liver diseases (particularly chronic active hepatitis C) and zinc deficiency (10-14). We suggested that diabetes in thalassemia should be defined as "β-TM- related diabetes (β-TM -RD)" (10) because it differs in pathogenetic mechanisms from type 1 diabetes (T1DM) and type 2 diabetes (T2DM) although it has similarities with both.

The oral glucose tolerance test (OGTT) is probably the best and simplest method to assess glucose homeostasis because it provides information about both insulin resistance and glucose intolerance. A 2-hour OGTT, preferably combined with assessment of insulin secretion, at 10, 12, 14, and 16 years and annually thereafter has been recommended for TDT patients (12,13).

Insulin-like growth factor-1 (IGF-1) is a polypeptide hormone produced mainly by the liver in response to the endocrine GH stimulus, but it is also secreted by other tissues for autocrine/ paracrine purposes. Insulin-like growth factors (IGFs), specifically IGF-1 and IGF-2, promote glucose metabolism, with their activity regulated by IGF-binding proteins (15).

Cross-sectional population studies have shown that both low and high IGF-1 are associated with increased insulin resistance, highlighting the complexity of the IGF-1 system (16,17). Moreover, low IGF-1 levels predicted an increased risk for development of impaired glucose tolerance (IGT) and T2DM in healthy people in 2 large prospective cohorts (18,19).

The biological action of IGF-1 is thought to be mediated via its type 1 receptors and/or hybrid insulin/IGF-1 receptors. It has been proposed that an increased proportion of hybrid insulin/IGF-1 receptors would reduce insulin sensitivity in target tissues of insulin action, contributing to cellular insulin resistance. Hybrid insulin/IGF-1 receptors are widely distributed in human tissues, including skeletal muscle and adipose tissue (17). However, it is not clear whether these associations are related to underlying impairment in β-cell function because portal insulin is an important promoter of hepatic IGF-1 synthesis (20).

In a previous study, we found that IGF-1 levels were below 2SDs in 50% of β-TM patients compared to healthy subjects. In males, the IGF-1 concentrations ranged from 18.3 to 147.7 ng/mL (mean 68.29 ± 33.5 ng/mL; median 62.5 ng/mL), whereas in females the range was from 19.5 and 195.5 ng/ml (mean 76.46 ± 41.84 ng/mL (21). The median IGF-1 levels in healthy Italian subjects of both sexes are 206 ng/ml in the 25-39 yr range and 147 ng/ml in the 40-59 yr range (22).

In multivariate regression analysis, height, weight, body mass index (BMI), serum ferritin (SF), alanine aminotransferase (ALT), hepatitis C virus (HCV) serology and left ventricular ejection fraction (LVEF) were not significantly related to IGF-1, but a significant correlation was found in females between HCV-RNA positivity and IGF-1, ALT and SF (21).

Thus, the present retrospective study was designed to assess the circulating IGF-1 levels in β -TM patients with normal OGTT and GD, and to explore the potential link between low IGF-1 and GD.

Subjects and methods

a. Design

Young adults with β -TM, consecutively referred to Pediatric and Adolescent and Thalassemia Endocrinology Outpatients Quisisana Clinic for an endocrine assessment and/or for a second opinion between September 2010 and March 2022, were selected for this observational retrospective study.

The inclusion criteria included Transfusion Dependent (TD) β -TM patients who: (a) were older than 21 years; (b) provided full information of clinical history and laboratory assessment and complete 2-h OGTT, including plasma glucose and serum insulin at baseline, 30, 60, 90, and 120 minutes. Exclusion criteria were: (a) patients already diagnosed with β-TM -RD (23) or were on treatment with anti-diabetic agents; (b) non-transfusion dependent β-thalassemia patients; (c) bone-marrow transplanted patients; (d) subjects using drugs affecting glucose metabolism, and (e) subjects with other major chronic illnesses besides β -TM. In total, 34 anonymized subjects' data sets met our inclusion criteria.

Patients had been diagnosed with β -TM on the basis of clinical and laboratory data (24). All patients were on regular blood transfusions at 2 -3 week intervals and were on iron chelation therapy.

For the diagnosis of associated endocrine complications we used the criteria previously described by the international network on endocrine complications in thalassemia (ICET) (25).

b. Study measurements

Height, body weight and BMI were determined following the standard procedures. A patient was considered obese when BMI exceeded 30 Kg/m^2, overweight when BMI was 25 - 30 kg/m^2. Glucose metabolism status was assessed by 2-h OGTT (75 g). Glucose tolerance status was classified according to the ADA criteria by a single OGTT (26). Patients with fasting plasma glucose (FPG) between 100 to 125 mg/dL (5.6-6.9 mmol/L) were classified as impaired fasting glucose (IFG) and those with 2-h-PG values after OGTT between 140–199 mg/dL (7.8–11.0 mmol/L) as IGT. Patients with diabetes (β-TM- RD) were those with FPG ≥ 126 mg/dL or 2 h-PG ≥ 200 mg/dL (FPG: ≥7.0 mmol/L or 2-h- PG: ≥11.1 mmol/L).

Plasma glucose was measured by the glucose oxidase method and plasma insulin was analyzed by commercial chemiluminescent immunoassays (Diagnostic Products Corporation, Los Angeles, CA or Roche Diagnostics, Germany).

Plasma total IGF-1 was measured in a single laboratory by a chemiluminescent immunometric assay method (IMMULITE 2000, Siemens Health care Diagnostics, USA). Intra-assay and inter-assay coefficient of variation declared by the manufacturer was 2.5–7.6 %.

The following data were also collected from the provided data: concentration of ALT, positivity for hepatitis C infection (HCVAb) and for active hepatitis C (HCV-RNA), SF, global cardiac iron and liver iron content (LIC), assessed by magnetic resonance imaging (MRI) T2* performed 15 -21 months before the endocrine consultation.

Iron overload was arbitrarily classified as mild with SF < 1.000 ng/mL, moderate SF >1.000 ng/mL and < 2.000 ng/mL and severe SF >2.000 ng/mL. Cardiac MRI T2* values were expressed in msec (T2* normal values: > 20 msec) and LIC in mg/g dry weight (d.w.). Normal LIC levels range between 0.17 and 1.8 mg/g of liver dry weight tissue (mg/g d.w.) (27). A LIC value above 3 mg/g d.w. is considered indicative of liver siderosis, which is classified as mild if the value is >3 and < 7 mg/g d.w., moderate <15 mg/g d.w. and severe if >15 mg/g d.w. (9).

c. Surrogate measures of insulin secretion and insulin sensitivity

Early-phase insulin secretion (I/GI) and glycemic metabolism indices, including quantitative insulin sensitivity check index (QUICKI), homeostatic model assessment for insulin resistance (HOMA-IR) and insulin sensitivity (OGIS) were calculated from the OGTT (Table 1) (28,29). Oral disposition index (oDI) estimates were calculated according to the following formula: IGI x 1/Fasting Insulin (30).

Ethics

This retrospective observational study was developed in accordance with the Helsinki declaration (www.wma.net). Anonymised data sets were analyzed, no identifiable private information was collected and patients underwent only routine diagnostic procedures according to the national Italian protocols and following International Guidelines (34). All patients provided informed consent.

Statistical analysis

All numeric variables were expressed as mean ± standard deviation (SD), range, median and quartile. Comparison of different variables in the two groups was made using unpaired student t-test and Mann-Whitney test for normal and non-parametric variables, respectively. Chi-square (χ^2) test was used to compare the frequency of qualitative variables among the different groups. The data obtained were analyzed using Pearson's correlation test for those with normal distribution or Spearman correlation test for the ones with an abnormal (non- parametric) distribution. A p-value < 0.05 was considered statistically significant. For the statistical analysis, a software program was used and validated, according to Alder and Roesser (35).

Results

This retrospective study included 34 patients with β-TM (mean age 38.8 ± 5.6 yrs, range 30-47.4 yrs; 15 males, 44.1%) at the time of recent evaluation. The mean age at the first blood transfusion was 0.9 ± 0.6 months; 16/34 patients (47.0 %) had undergone splenectomy. The median transfusion interval was 21.5 days (range 14–23 days). The mean SF level was 930,7 ± 850,0 ng/mL (range: 317–3,665 ng/ml). Their mean BMI was 23.21 ± 4.0 Kg/m^2 (range: 18-40.1 Kg/m^2). Seven females (36.8%) and 5 males (33.3%) presented a standing height < 3rd percentile for the Italian population (149.5 ± 3.2 cm, in females and 156.2 ± 2.3 cm, in males).

β-TM patients were categorized in 3 groups in accordance to disturbances of glucose metabolism

Table 1. Insulin sensitivity and β-cell function indices derived from fasting and OGTT measurements of glucose and insulin.

Index	Formula/equation and interpretation
HOMA-IR	Fasting glucose (mg/dL) x fasting insulin (μIU/mL)/405. It can be used as an index representing insulin resistance and β-cell function. The mean 80th percentile in Italian population, considered as cut-off value for insulin resistance, is 2.77 (31).
QUICKI	1/log insulin + log glycemia in mg/dL. The quantitative insulin sensitivity check index (QUICKI) is an empirically derived mathematical transformation of fasting blood glucose and plasma insulin concentrations that provides a reliable, reproducible, and accurate index of insulin sensitivity. The 75th percentile in non-Italian population 0.35 (range 0.34–0.37) (32).
OGIS (0-120 min.)	This method is expressed in ml per minute per square meter of body surface area (ml.min $^{-1}$. m $^{-2}$). Oral Glucose Insulin sensitivity (OGIS) is comparable to the calculation of glucose clearance obtained in the clamp (32). The mean normal value in non-Italian population is 424. In subjects with GD vary from 323 to 375 (33).
IGI	[Δ insulin (30–0 minutes) (μIU/mL)/Δ glucose (30–0 minutes) (mg/dL)] . Insulinogenic Index (IGI) is used to estimate insulin secretion and is calculated as the change in insulin divided by the change in glucose from 0 to 30 minutes (28,29).
oDI	IGI x 1/Fasting Insulin. Oral Disposition index (oDI) is expressed by calculating the product of insulin secretory capacity and insulin sensitivity. In healthy subjects, the product of insulin sensitivity and insulin secretion is constant (29).

Abbreviations: OGTT, Oral Glucose Tolerance Test; **GD**, Glucose Dysregulation.

based on ADA criteria and after OGTT: Group A (12 patients with normal glucose tolerance, NGT), Group B (7 patients with IGT and 9 with IFG plus IGT) and Group C (6 patients with β-TM-RD).

In patients of group A with normal OGTT, the annual evaluation of glucose tolerance in the previous 8.6 ± 4.2 years (range 2-17 years) was reported as normal. In patients of group B, the first documentation of GD was observed 5.1 ± 2.9 years earlier (range: 1-9 years), and in 3 patients of group C a diabetic curve after OGTT had been reported in the previous 1, 2 and 7 years, respectively.

The demographic data and laboratory findings of the 3 groups of patients are shown in Table 2.

Statistical analysis of the relevant demographic and laboratory variables among the three groups (A with NGT; B with IFG and IFG plus IGT; and C with β-TM-RD) revealed no statistical significance in the majority of variables studied. However, statistically significant differences were identified only in: (a) IGF-1 between A vs. B and A vs. C (P= 0.0001), but not between B vs. C as in both groups IGF-1 was extremely reduced; (b) age between A vs. B (P= 0.0002); (c) LIC between A vs. C (P= 0.0073); (d) plasma glucose 1-h OGTT A vs. B (P= 0.0005); (e) plasma glucose 2-h after OGTT A vs. B (P= 0.0017), and (f) delayed insulin peak A vs. B (P=0.011).

An abnormal ALT value (> 80 U/L) was observed in 2 male β-TM patients of group B and 1 female of group C, and none of group A. Their IGF-1 levels were: 43.9, 18.1 and 31.7 (ng/mL), respectively.

SF level >2,000 ng/mL (severe iron overload) was present in 2 female patients of group A (16.6%), 1 male patient of group B (6.2%) and 3 patients (2 females) of group C (50%). The mean and SD of ALT and SF between the 3 group of patients was not statistically significant (Table 2).

A liver iron concentration > 7 mg/g dry weight was reported in 1 female of group C, and global cardiac T2* < 20 msec in 2 patients (1 male and 1 female) of group A (18.1%), in 3 patients (2 males) of group B (25%) and 1 female of group C (33.3%). Lower cardiac T2* values were found in 3 patients of group C (29.6 ± 6.1) (Table 2). Their mean IGF-1 was 57.9 ± 32.7 ng/mL (range: 19.5-113 ng/mL).

Twenty-three β-TM patients (1 male patient in group A and all patients in groups B and C), had IGF-1 levels below the 2.5th percentile of the normal values for the Italian population (< 95.6 ng/mL for ages 25-39 yrs and <60.8 ng/mL aged 40-59 yrs). The mean serum IGF-1 concentrations were significantly lower in patients in groups B and C versus those in group A (P = < 0.0001;Table 2). Growth hormone status, after conventional stimulation tests, was previously assessed in 6 patients of group B and was reported as normal. No data were available on vitamin D and serum zinc levels.

A significant percentage of β-TM patients presented with associated endocrinopathies [9/12 patient (75%) in group A and in all patients of group B and C]. Hypogonadotropic hypogonadism (HH), arrested puberty (AP), acquired hypogonadotropic hypogonadism (A-HH: in males) and secondary amenorrhea (SA) in females were the commonest endocrine complications (75% in group A and 100% in group B and C) followed by primary or central hypothyroidism [1female patient of group A (8.3%) and 6 in group B and C (27.2%)]. One case of hypoparathyroidism in a 37.8 yrs old female patient of group B (6.25%) was reported. Six patients out of 34 (3 males) had more than 1 endocrine complication (17.6%). Five males (2 with HH and 3 with A-HH) and 9 females (4 with HH, 3 with AP and 2 with SA) patients were not receiving hormone replacement therapy through personal choice, medication cost or reported "medical contraindications". All hypothyroid and hypoparathyroid β-TM patients were taking thyroxine or calcitriol, respectively. No adrenal insufficiency was reported. No patient was on treatment with recombinant growth hormone.

The mean and SD of surrogate indices of insulin secretion and insulin sensitivity in the 3 groups of β-TM patients are reported in table 3 and the significant correlations of IGF-1, BMI, ALT and SF with different clinical and laboratory parameter are presented in table 4. A linear correlation was present between BMI and glucose level at 2-h after OGTT but no correlations were observed between age and other clinical or laboratory parameters.

Discussion

In β -TM patients, glycemic disturbances often deteriorate over time before eventually progressing to iabetes.

Table 2. Epidemiological, clinical and laboratory findings in 34 β-thalassemia major (β-TM) patients at final evaluation, classified in three groups based on OGTT: A with NGT; B with IGT and IFG plus IGT and C with β-TM-RD.

Variables	Group A (NGT) (n.12)	Group B (IGT and IFG+ IGT) (n.16)	Group C (β-TM-RD) (n.6)
Chronological age (yrs)	34.6 ± 5.3	42.0 ± 3.8	38.6 ± 4.7
Gender (Males/Females)	3/9	10/6	2/4
BMI (Kg/m^2) **Mean and SD** **Range**	 22.1 ± 2.8 18 -23.8	 22.8 ± 2.3 19.9-27.7	 26.2 ± 7.1 20.5- 40.1
Family history of diabetes: **Type 1** **Type 2**	1 1	4 3	- 1
Serum ferritin (ng/mL) - Mean and SD **< 500 ng/mL (n. and %)**	862.8 ± 668.7 5 (41.6%)	661.8 ± 484.9 5 (31.2%)	1730.0 ± 1326.5 1 (16.6%)
LIC (mg/g dry weight) **Cardiac T2* (msec)**	1.87 ± 0.93 (11 pts) 35.0 ± 14.5 (11 pts)	2.10 ± 1.8 (13 pts) 25.8 ± 9.6 (12 pts)	7.0 ± 5.5 (4 pts) 20.9 ± 6.1 (3 pts)
Chelation therapy: **Desferrioxamine (DFO)** **Deferiprone (DFP)** **Deferasirox (DFX)** **DFO plus DFP**	 7 1 2 2	 13 2 1 -	 2 2 - 2
ALT (U/L) - **Mean and SD**	27.3 ± 7.7	43.8 ± 31.3	58.6 ± 31.5
HCV-ab positive (%) **HCV –RNA positive** (%)	91.6 41.6	100 62.5	100 66.6
IGF-1 (ng/mL) - **Mean and SD** **Range** **Median** **Quartile: Q_{1-3}**	**126.2 ± 34.1** **50.7- 197.3** **123.6** **109.7-143.8**	**56.0 ± 38.3** **27.3 - 150.5** **40** **30.2-71.3**	**31.2 ± 6.0** **19.1-37** **31.9** **29.4 -37.0**
Plasma glucose (mg/dL) at baseline - Mean and SD	92.8 ± 4.0	98.1 ± 9.3	138.8 ± 26.9
Plasma glucose (mg/dL) after 1 h OGTT- Mean and SD	128.1 ± 18.7	168.4 ± 36.0	254.0 ± 40.4
Plasma glucose (mg/dL) after 2 h OGTT- Mean and SD	122.5 ± 12.2	161.1 ± 16.5	251.5 ± 35.6
Fasting insulin (μU/mL)	5.7 ± 2.5	5.6 ± 2.8	9.6 ± 4.0
Insulin peak (μU/mL) during OGTT- Mean and SD	49.2 ± 18.5	59.1 ± 30.6	43.0 ± 30.6
Delayed insulin peak (90'-120' minutes) after OGTT	33.3 %	81.2%	83.3%
Plasma insulin (μU/mL) after 2 h OGTT - Mean and SD	35.0 ± 19.6	56.3 ± 33.7	42.0 ± 31.2

In 34 β -TM patients referred for endocrine evaluation and/or for a second opinion, an increased IR, assessed by HOMA-IR, and a reduced insulin sensitivity, assessed by OGIS, were observed. Moreover, in patients with later stages of GD, the capacity to compensate for insulin resistance was decreased, resulting in lower oDI (Group B) compared to patients with NGT (Group A), and an additional acute β-cell impairment (IGI) occurred in β -TM-RD.

Table 3. Surrogate indices of insulin secretion and insulin sensitivity in the 3 groups of β-TM patients (Mean, SD and P values).

Group	IGI	HOMA	QUICKI	OGIS	oDI
Group A: NGT (n.12)	13.72 ± 9.0	1.31 ± 0.59	0.71 ± 1.14	439.75 ± 21.96	2.55 ± 1.87
Group B: IGT or IFG+ IGT (n.16)	9.0 ± 9.11	1.54 ± 0.89	0.38 ± 0.05	391.18 ± 45.94	1.88 ± 1.0
Group C: β-TM- RD (n.6)	5.23 ± 3.68	3.34 ± 1.71	0.32 ± 0.02	315.33 ± 36.52	3.29 ± 1.81
P value: A vs. B	0.18	0.44	0.25	**0.002**	0.24
P value: B vs. C	0.17	**0.003**	0.01	**0.001**	**0.032**
P value: A vs. C	**0.043**	**0.0016**	0.42	**< 0.0001**	0.43

Table 4. Correlations between different clinical and laboratory parameters.

	IGF-1	BMI	ALT	Serum ferritin
Age (yrs)	**r:-0.4513; P= .007**	N.S.	=	=
BMI (Kg/m 2)	N.S.	=	=	=
ALT (U/L)	**r:--0.534; P= .001**	=	=	N.S.
Serum ferritin (ng/ml)	N.S.	=	N.S.	=
LIC (mg/g dry weight)	N.S.	=	=	**r: 0.472; P= .00003**
Cardiac T2* (msec)	**r: 0.4147; P: .035**	=	=	N.S.
FPG	**r: 0.4174; P= .014**	N.S.	**N.S.**	**r: 0.6105; P= .0001**
PG 2-h after OGTT	**N.S.**	**r: 0.3889; P= .023**	**N.S.**	r: **0.3814 P=.026**
Basal Insulin	N.S.	N.S.	**N.S.**	**r: 0.3458; P= .045**
Insulin peak after OGTT	N.S.	N.S.	N.S.	N.S.
HOMA-IR	**r: -0.2651; P= N.S.**	N.S.	**r: 0.3559; P=.038**	**r: 0.6201; P=.000092**
QUICKI	N.S.	N.S.	N.S.	N.S.
OGIS	**r:0.4912. P=.0031**	N.S.	**N.S.**	**r:- 0.4154; P= .014**
IGI	N.S.	N.S.	**r: -0.410; P=.016**	N.S.
oDI	N.S	N.S.	N.S.	N.S.

Twenty-two β-TM patients with GD or β-TM-RD and 1 patient with NGT had IGF-1 levels below the 2.5th percentile of the normal values for the Italian population. The low IGF-1 levels were correlated with age, BMI, FPG, HOMA-IR and OGIS. In addition, a strong correlation was also observed between metabolic parameters and BMI, liver enzyme (ALT), and SF (Table 4). Interestingly, a linear correlation was observed between IGF-1 and cardiac T2* (P=0.035) in 26 patients (Table 4).

These observations are supportive of the notion that low circulating IGF-1 levels can lead to impaired insulin action in thalassemic subjects with different degrees of glucose tolerance. Moreover, the findings are consistent with animal studies demonstrating reduced insulin sensitivity in mice with liver-specific deletion of the IGF-1 gene that is reversed by treatment with recombinant human IGF-1 (36,37). However, we urge caution in interpreting the results because the present study was limited by the fact that the analyses

were based on total IGF-1 rather than the biologically active free IGF-1. In addition, we did not measure IGF-binding proteins (IGFBPs), which play a key role in regulating the bioavailability of circulating IGF-1. IGFBP-1 synthesis is suppressed by hepatic portal insulin, and low levels of this binding protein have been associated with insulin resistance, IGT, and the metabolic syndrome (38,39).

Recent data from our group showed that IGF-1 concentrations were significantly correlated with serum ALT ($r = -0.26$, $p = 0.05$) and SF ($r = -0.29$, $p = 0.02$) concentrations. These data indicate that liver dysfunction, secondary to iron overload, hepatitis or both, may negatively affect the hepatic IGF-1 synthesis (40). Moreover, the magnitude of of IGF-1 increase in response to exogenous growth hormone (GH) stimulation was lower in thalassemic patients versus those with growth hormone deficiency (GHD) (41,42). These reports support the hypothesis that the main pathogenetic mechanism of IGF-1 deficiency seems to be related to impairment of liver function that is due to iron toxicity (increased LIC) and chronic liver inflammation (high ALT and serum ferritin) basically due to chronic active hepatitis C and B. In addition, the production and secretion of IGF-1 is also influenced by GHD in adolescent and young adults with β -TM (43-45), sex hormones (46,47), as well as age, sex, quantity and quality of nutrition (46,47).

Current screening guidelines in TDT patients include a 2-h OGTT, starting from the age of 10 years (25,34). However, in our retrospective study, we noted that only 65.3% of patients with β -TM were compliant with this recommendation, probably due to the inconvenience of frequent patients' visits and multiple investigations, intolerance of glucose load, and hospitalization. Therefore, many patients with GD could remain undiagnosed for a long time. The use of HbA_{1c} measurement has limited use as it is generally considered unreliable in patients with thalassemia. Considering the limited evidence of other markers (e.g. fructosamine; 1,5-AG; and glycated albumin) on the diagnostic performance, further research is necessary to define the precise role in the diagnosis and management of GD in patients with β -TM (12). Pancreatic MRI could minimize the necessity for OGTT and may identify high-risk patients before irreversible pancreatic damage occurs, but at present it is available in relatively few centers (12).

Our study has some limitations due to: (a) the retrospective design; (b) the relative low number of patients; (c) the small number of patients with β -TM RD, and (d) failure to assess serum free-IGF-1 and IGFBP-3.

In conclusion, it is evident that low IGF-1 serum levels are frequently found in TDT patients and are associated with the development of disorders in glucose homeostasis. The question which remains unanswered is whether this is just an association or causation. On the one hand, low IGF-1 levels may just be surrogate markers of higher iron overload. On the other hand, IGFs are known to support endocrine and exocrine pancreatic development and a reduction of them might contribute to the profound deficiency of total pancreas mass in subjects with Type 1 DM (15) and low IGF-1 may lead to worsening insulin resistance and glucose dysregulation (18,19).

It remains to be proved whether low serum IGF-1 by itself could contribute to dysglycemia in adult patients with β-TM and GD and also whether growth hormone replacement could improve glucose homeostasis. Prospective studies to monitor potential benefits versus possible side-effects will enable endocrinologists to define recommendations on dosage and the long term effects (48).

Despite some limitations, our study can serve to generate a reflection for more convenient and efficient methods to identify and treat early GD in patients with β-TM. If our data can be replicated in more extensive studies, IGF-1 could be identified as a potential marker for the detection of β-TM patients at-risk for GD.

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.

Author Contributions: VDS conceived the study, have the responsibility for the integrity and accuracy of the data analysis, and edited the first draft. SDM advised on statistical analysis. ATS, SD, PT, SDM and CK reviewed and edited the manuscript. MAY contributed to discussion. All the authors approved the final version of submitted manuscript.

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