Glucose metabolism in conventionally treated patients with β-thalassaemia major assessed with oral glucose tolerance test

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Abstract

Introduction: The aim of the study was to assess prevalence and risk factors of glucose intolerance in thalassaemic patients, optimally treated since early in life. In addition, the evolution of glycaemic indices (β-cell function, insulin resistance and insulin sensitivity) with aging and their correlation with several parameters were investigated.
Material and methods: Seventy-four patients (35 M and 39 F) with β-thalassaemia major (mean age: 20.52±5.12), treated with regular blood transfusions and adequate chelation treatment, were enrolled in the study. They were all assessed with oral glucose tolerance test, whereas data regarding serum ferritin concentrations, hepatitis C status, splenectomy, and body mass index (BMI) were available.
Results: Results showed a prevalence of 12.2 and 5.4% for impaired glucose tolerance (IGT) and diabetes mellitus, respectively. Ferritin concentrations were significantly increased in patients with IGT, whereas there was no clear association between hepatitis C virus infection, splenectomy or BMI and the development of glucose intolerance. A significant decrease in β-cell function index was noted in diabetic patients in comparison to patients with both IGT and normal glucose metabolism. A deterioration of β-cell function was observed with ageing, although it was not statistically significant. Insulin sensitivity index was significantly decreased in thalassaemic patients with IGT.
Conclusions: The results indicate that glucose intolerance is still frequent among thalassaemic patients, especially in their third decade of life. Moreover, our data suggest that glucose intolerance in β-thalassaemia major is characterized by progressive deterioration of insulin secretory capacity with a concomitant reduction in insulin sensitivity.

Key words: β-thalassaemia major, glucose intolerance, OGTT, diabetes, prevalence.

Introduction

β-thalassaemia major is characterized by a hereditary defect in synthesis of the beta chain of adult haemoglobin, resulting in ineffective erythropoiesis. Conventional management, consisting of regular blood transfusions and adequate chelation therapy, has led to life prolongation and improvement of quality of life in patients with β-thalassaemia major. Complications of the disease and its treatment are still reported; however,

they appear later in life and usually in a milder degree. Iron-induced heart failure still remains the commonest cause of death among thalassaemic patients, being responsible for more than half of them [1]. Disturbances of glucose metabolism are frequently reported in patients with β-thalassaemia major, but the prevalence of diabetes mellitus (DM) and impaired glucose tolerance (IGT) varies in different studies [2-9]. This fluctuating prevalence is mainly attributed to the heterogeneity of the studied population regarding age, management and compliance with treatment.

The exact pathogenetic mechanism for the development of glucose intolerance in β-thalassaemia major is not clearly elucidated. Severe insulin deficiency due to haemochromatosis-induced pancreatic β-cell defect seems to be present only in advanced stages [10]. Glucose metabolism disturbances in early stages are characterized by elevated insulin levels in order to compensate for reduced insulin sensitivity [5]. In addition, hepatic dysfunction, commonly seen in the thalassaemic population, contributes to decreased hepatic insulin extraction, resulting in hyperinsulinaemia [11]. Recently, a triggered autoimmune response has been postulated to participate in the pathogenesis of diabetes associated with β-thalassaemia major [12].

The objective of this study was to determine prevalence and risk factors of glucose metabolism disturbances in patients with β-thalassaemia major treated optimally with conventional management since early life. Moreover, our aim was to correlate glycaemic indices (β-cell function, insulin resistance and insulin sensitivity), as these were determined by the oral glucose tolerance test (OGTT), with demographic and clinical parameters.

Material and methods

In line with the routine clinical management in our centre, every patient with β-thalassaemia major aged >10 years is assessed with an OGTT every 2 years. Between October 2005 and May 2006, we performed an OGTT in every thalassaemic patient aged 10-30 years followed in our centre. All were treated conventionally with regular blood transfusions, in order to maintain pre-transfusion Hb levels ≥9.5 g/dl and adequate chelation treatment with deferoxamine (DFO), deferiprone or a combination of these two chelation agents. Seventy-four patients (35 M and 39 F) with a mean age of 20.52±5.12 were recruited. OGTT was performed after a 12 hours fasting period. Glucose was ingested at a dose of 1.75 g/kg body weight (max =75 g) and venous blood samples were collected at 0, 30, 60, 90 and 120 min for the measurement of plasma glucose and insulin. Glucose was measured in serum after immediate centrifugation using the glucose-oxidase method with a coefficient of variation of ±1.6%. Insulin was determined by an immuno-radiometric assay technique with a detection limit of 2 mU/l and a coefficient of variation of 6% within the assay and 9% between assays.

According to the World Health Organization's definition [13], DM is confirmed when fasting glucose is ≥126 mg/dl or 2 hours post glucose load ≥200 mg/dl, whereas IGT is diagnosed when fasting glucose is <126 mg/dl and 2 hours post glucose load =140–200 mg/dl. Insulin resistance and β-cell function were derived from the HOmeostasis Model Assessment (HOMA) [14], according to the following formulae:

$$\text{insulin resistance index (IRI)} = I_0 \times (G_0/18)/22.5$$
$$\beta\text{-cell function index } (\beta\%) = 20 \times I_0/[(G_0/18) - 3.5].$$

Insulin sensitivity was evaluated by an index proposed by Matsuda and DeFronzo [15], according to the following formula:

$$\text{insulin sensitivity index (ISI)} = 10000/[\text{square root}(G_0 \times I_0 \times G_{mean} \times I_{mean})],$$

where G_0, G_{mean} – glucose at 0 and mean glucose concentration (mg/dl), I_0, I_{mean} – insulin at 0 and mean insulin concentration (mU/l).

Data regarding body mass index (BMI), liver function, serum ferritin concentrations, hepatitis C viral (HCV) status and history of splenectomy were extracted from medical files for every patient. BMI standard deviation scores (SDS) were calculated according to an age- and sex-matched normal Greek population.

Statistical analysis and figures were created using the Office Excel® 2003 software program, Microsoft®, Redmond, WA, USA. Results are expressed as mean values ± SD. χ^2 test and unpaired Student t-test were employed to compare individual studied groups. A P value less than 0.05 was considered to be statistically significant.

Results

Four out of the 74 studied patients (5.4%) had DM. Three of them were >20 years old, while one was aged less than 20 years. The overall prevalence of IGT was 12.2% (5.8 and 17.9% for the groups of <20 and >20 years, respectively). All the data of the patients, including HOMA indices and ISI, categorized in groups regarding age, as well as for the totality of the studied population, are shown in Table I and Figure 1.

When patients' data were analyzed in relation to the results of the OGTT, no statistical significance was noted regarding sex, HCV status, or BMI in the three formed groups (Normal, IGT and DM). Ferritin levels were significantly increased in the IGT group,

Table I. Data expressed as mean ± SD or n (%) in thalassaemic patients categorized regarding age

Parameters	10≥ Age <20	20≥ Age ≥30	P	Total
N	35	39		74
Age [mean ±SD, years]	15.99±2.87	24.58±2.66		20.52±5.12
Sex [M/F]	17/18	18/21		35/39
BMI SDS [mean ±SD]	0.39±0.89	–0.04±1.19	NS	0.17±1.08
Ferritin [mean ±SD, μg/l]	1575±1126	1981±1382	NS	1789±1275
HCV+ [%]	1/35 (2.9)	9/39 (23.1)	**<0.001**	10/74 (13.5)
Splenectomy [%]	3/35 (8.6)	10/39 (25.6)	**<0.001**	13/74 (17.6)
IGT [%]	2/35 (5.8)	7/39 (17.9)	**0.001**	9/74 (12.2)
DM [%]	1/35 (2.9)	3/39 (7.7)	0.07	4/74 (5.4)
IRI [mean]	1.21±0.6	1.09±0.7	NS	1.15±0.65
β-cell function [mean]	62.51±41.5	53.23±30.5	NS	57.62±36.2
ISI_{0-120} [mean]	15.41±8.6	18.06±11.6	NS	16.81±10.3

Table II. Data expressed as mean ± SD or n (%) in patients categorized regarding the result of the OGTT

Parameters	Normal	IGT	DM
N	61	9	4
Age [mean ±SD, years]	20.03±5.26	22.83±4.32	22.75±2.41
Sex [M/F]	27/34	5/4	3/1
BMI SDS [mean ±SD]	0.19±1.04	–0.04±1.38	0.34±1.16
Ferritin [mean ±SD, μg/l]	1696±1225	2439±1244*	1736±1984
HCV+ [%]	8/61 (13.1)	1/9 (11.1)	1/4 (25)
Splenectomy	11/61 (18)	1/9 (11.1)	1/4 (25)
IRI [mean]	1.13±0.55	1.26±1.01	1.12±1.09
β-cell function index [mean]	62.01±37.3	42.56±23*	24.54±11.8*,§
ISI_{0-120} [mean]	17.3±10.3	12.28±5.2*	14.59±11.7

**P<0.05 compared to normal group, §P<0.05 compared to IGT group*

compared to Normal (2439±1244 vs. 1696±1225 μg/l, P=0.047). However, patients with DM did not demonstrate a significant increase in ferritin levels (1736±1984 μg/l). There was a significant deterioration of β-cell function index in DM patients compared to IGT (24.54 vs. 42.56, P=0.045) as well as compared to patients with normal glucose metabolism (24.54 vs. 62.01, P=0.0005). ISI was significantly decreased in patients with IGT in comparison to patients with normal glucose metabolism (12.28 vs. 17.3, P=0.016). All these data are shown in Table II and Figure 2.

Discussion

The prevalence of DM and IGT among thalassaemic patients has been gradually reduced over the years. Indeed, epidemiological studies [2-4] performed before the systematic use of DFO, the first chelation agent, reported a prevalence of 19 and 31% for DM and IGT, respectively. In subsequent studies, the prevalence of diabetes associated with β-thalassaemia major varied from 4.9 [6] to 22.6% [12]. These discrepancies could be partly attributed to differences in age distribution of the studied population, treatment modalities followed in individual centres worldwide and criteria for the diagnosis of glucose intolerance that have been employed. However, it has been widely appreciated that thalassaemic patients born after 1970 treated with regular blood transfusions and adequately chelated show a more advanced age of onset and a consequently lower incidence of diabetes. In our study, the prevalence of DM among thalassaemic patients aged <30 years is 5.4%, and this is in agreement with other studies performed in Europe [6, 16]. The prevalence of IGT in our study is lower than in other recent studies, with the exception of a Taiwanese study [9] which reports a low prevalence

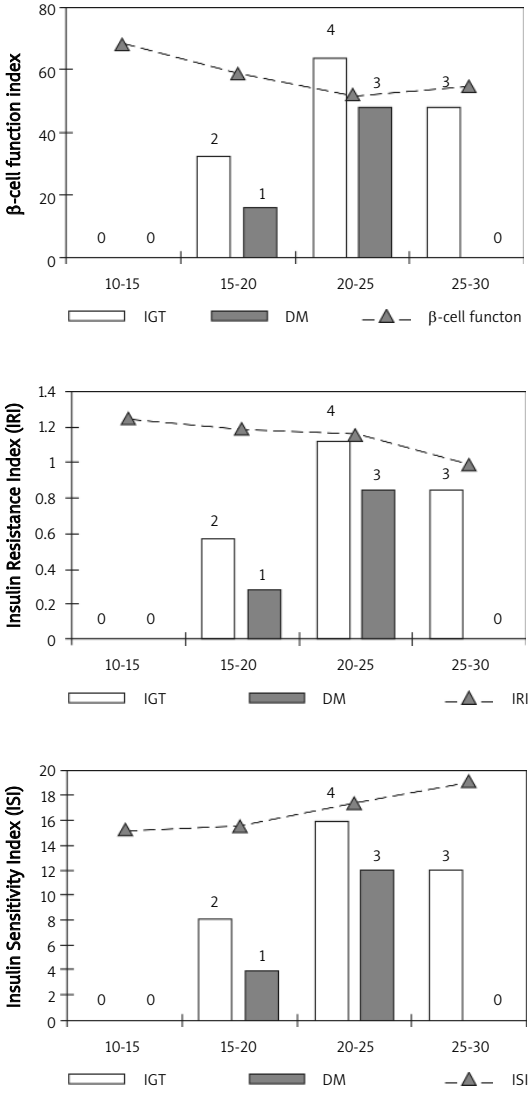

Figure 1. Schematic appearance of the results of OGTT [impaired glucose tolerance (IGT) and diabetes mellitus (DM)] as well as β-cell function index, insulin resistance index (IRI) and insulin sensitivity index (ISI) in thalassaemic patients in relation to age

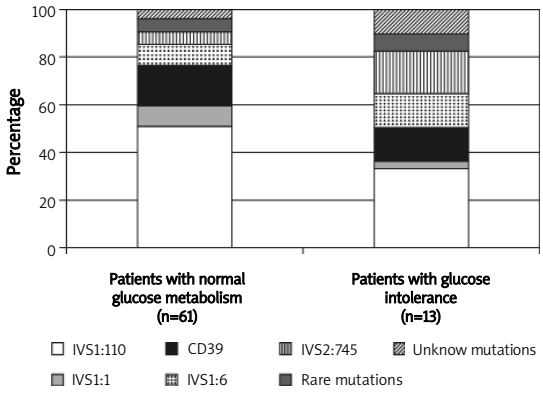

Figure 3. β-globin gene mutation distribution between thalassaemic patients with normal (n=61) and patients with abnormal (n=13) glucose metabolism

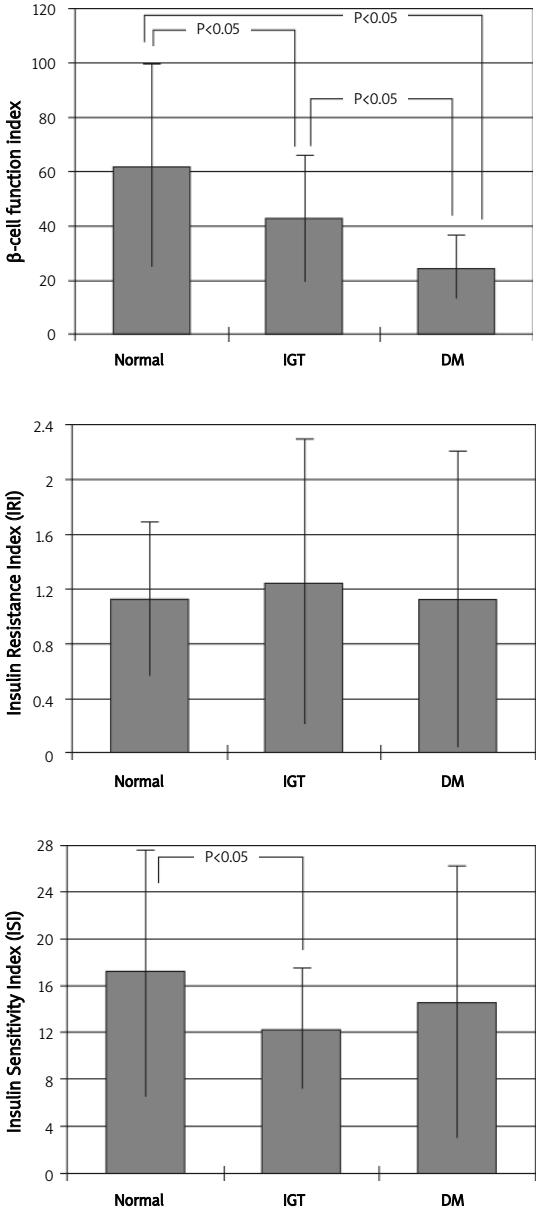

Figure 2. Schematic appearance of β-cell function index, insulin resistance index (IRI) and insulin sensitivity index (ISI) in thalassaemic patients with normal glucose metabolism, impaired glucose tolerance (IGT) and diabetes mellitus (DM)

of IGT (8.5%) but a very high prevalence of DM (19.5%) in patients with β-thalassaemia major.

Elevated ferritin levels, hepatitis C infection, age, familial history of diabetes and splenectomy have been proposed as risk factors for the development of glucose intolerance in patients with β-thalassaemia major. In our study, serum ferritin levels were significantly elevated in patients with IGT but not in diabetic patients. Moreover, a recent study [17] indicates that IVS II nt 745 genotype is positively correlated with the development of glucose intolerance in thalassaemic patients. Although this

particular genotype is rare in the Greek population and with respect to the limited number of patients with abnormal glucose metabolism in our study, our mutation distribution analysis did not reveal any association between glucose intolerance and any specific genotype (Figure 3).

For the assessment of insulin secretory capacity, insulin sensitivity and insulin resistance we used indices that have been previously shown to closely correlate with hyperglycaemic clamp and to the frequently sampled, intravenous glucose tolerance test (FSIGTT) in non-thalassaemic patients. The latter methods are technically difficult to perform, especially in multi-transfusion subjects such as our patients. Previous studies based on hyperglycaemic clamp or FSIGTT on thalassaemic patients did not show a deterioration in insulin secretory capacity [5, 11, 18], with the exception of one study [19] which showed a reduction in thalassaemic patients with IGT. In our study, β-cell function index was statistically reduced as we moved from normal to IGT and to DM. This result is in agreement with a previous study [10], using HOMA assessment in a thalassaemic population. In addition, our study showed a progressive reduction in β-cell function index with ageing, although not statistically significant. In a study by Messina et al. [20], β-cell function index was significantly decreased in patients with β-thalassaemia over a 3-year period.

Regarding insulin sensitivity, our results showed that there was a statistically significant reduction of ISI in thalassaemic patients with IGT compared to thalassaemic patients with normal glucose tolerance. However, thalassaemic patients with DM showed only a moderate reduction of ISI, something which can be attributed to the additional insulin secretion deficiency. This reduction of insulin sensitivity in thalassaemic patients with glucose metabolism disturbances has been shown by previous studies [5, 10, 11, 18, 19], indicating the major role of insulin resistance for the development of glucose intolerance in β-thalassaemia major. When correlated with age, ISI showed, surprisingly, a positive correlation. This can be partly attributed to the fact that our younger patients were peri-pubertal and had increased BMI, factors that both affect insulin sensitivity. In contrast, in the study by Messina et al. [20], ISI was significantly reduced over the 3-year study period.

In conclusion, disturbances of glucose metabolism are still reported among thalassaemic patients, despite advances in conventional treatment. With respect to the limitations of our study regarding the use of OGTT instead of a more accurate method and the cross-sectional design of this study, our results indicate that glucose intolerance in β-thalassaemia major is characterized by a progressive deterioration of insulin secretory capacity with a concomitant reduction in insulin sensitivity. Recent studies [21, 22] suggest that combined chelation treatment is favourable in terms of preserving normal glucose metabolism and even reversing glucose intolerance in thalassaemic patients. Close surveillance with regular screening, preventive intervention and early intensification of chelation therapy are of great importance as achieved life prolongation in thalassaemic patients requires an improvement in the quality of their life.

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