Mediterranean Journal of Hematology and Infectious Diseases

Original Article

Growth and Endocrine Function in Tunisian Thalassemia Major Patients

Naouel Guirat Dhouib, Monia Ben Khaled, Monia Ouederni, Habib Besbes, Ridha Kouki, Fethi Mellouli and Mohamed Bejaoui.

Pediatric Immuno-Hematology Unit, Bone Marrow Transplantation Center Tunis, Tunis, Tunisia.

Competing interests: The authors have declared that no competing interests exist.

Abstract. β-thalassemia major (β–TM) is among the most common hereditary disorders imposing high expenses on health-care system worldwide. The patient's survival is dependent on lifetime blood transfusion which leads to iron overload and its toxicity in various organs including endocrine glands. This article provides an overview of endocrine disorders in beta-TM patients. This single center investigation enrolled 28 β-TM patients (16 males, 12 females) regularly transfused with packed red cell since early years of life. For each patient were determined: age, sex, number of transfusions received, history of splenectomy and anthropometric parameters. All patients underwent an evaluation of hormonal status including growth, gonadal, thyroid, adrenal cortex, and parathyroid glands. Dual-energy X-ray absorptiometry was used to diagnose low bone mass. Assessment of iron overload status was performed by measuring the serum ferritin concentration and the results of magnetic resonance imaging T_2*. Growth retardation was found in 16 of the 28 studied patients (57 %). Thirteen among them had delayed puberty. Spontaneous puberty was achieved in 16 cases. Growth hormone (GH) deficiency was found in 10 cases (35 %). Seventeen among the studied patients (60 %) developed disorders of glucose homeostasis. Subclinical hypothyroidism was found in six patients (21 %). Intensive chelation therapy had allowed the reversibility of this complication in five cases. Adrenal Insufficiency was observed in 9 cases (32%). Hypoparathyroidism has occurred in one case. Ten of the 28 studied patients had low bone mass (35%). Twenty-three of the 28 studied patients (82%) had at least one endocrine complication.

Keywords: Thalassemic syndromes, Endocrine disorders.

Citation: Dhouib N.G., Khaled M.B., Ouederni M., Besbes H., Kouki R., Mellouli F., Bejaoui M. Growth and endocrine function in Tunisian thalassemia major patients. Mediterr J Hematol Infect Dis 2018, 10(1): e2018031, DOI: http://dx.doi.org/10.4084/MJHID.2018.031

Published: May 1, 2018 **Received: March 6, 2018** **Accepted: April 16, 2018**

Correspondence to: Naouel Guirat Dhouib, Pediatric Immuno-Hematology Unit, Bone Marrow Transplantation Center Tunis, Tunis, Tunisia. Tel: + 216 98 644165. E-mail: nawel.guirat@yahoo.fr

Introduction. Long-term transfusion regimen associated with optimal chelation therapy guided by magnetic resonance imaging (MRI T_2*) technology has dramatically improved life expectancy in patients with β-TM.[1] This hemoglobinopathy, which once incompatible with prolonged survival has become a chronic disease compatible with prolonged survival. Endocrine disorders are among the most common complications in multi-transfused β-thalassemia major. They represent a leading cause of morbidity and have a significant impact on the quality of life of patients suffering from it.[2] To our knowledge; this is the first single center study reporting endocrine disorders in Tunisian β-thalassemia major patients.

Patients and Methods. Twenty-eight polytransfused thalassemia major patients older than ten years (19 ± 4,54) followed in the pediatric Immuno-hematology Department of the Bone Marrow Transplantation during a 13-year period were enrolled in the study. The diagnosis of β-TM was established by the finding of microcytic hypochromic anemia, hemoglobin analysis before blood transfusion that revealed increased amounts of hemoglobin F and genetic testing. All subjects were transfused every 3 – 4 weeks with packed red cell since early years of life in an attempt to keep their pretransfusion hemoglobin above 9.5 g/dl.

For each patient were specified demographic and clinical data (family history, age, sex, origin, consanguinity, age at diagnosis, age at the first blood transfusion, anthropometric parameters); transfusion requirements and complications related to secondary hemochromatosis; chelating therapy (date of onset, type of chelation, modalities).

The size was taken using the DETECTO metal gauge. The target size was calculated as the average of the parents' heights plus 6.5 cm for boys or minus 6.5 cm for girls. Adult height was considered to be attained when growth during the preceding year was less than 1 cm, with a bone age of over 15 years. Pubertal stages were assessed according to Tanner and Marshall.[4] Arrested puberty is characterized by a lack of pubertal progression over a year or more. Short stature is defined as height less than two standard deviations (SDs) below the mean for age and gender.[3] Body mass index (BMI) was calculated as weight (kg) divided by the square of the height (m^2) using reference charts for boys and girls. Blood glucose was determined using the glucose oxidase method on a Beckman Glucose Analyzer. Carbohydrate metabolism disorders were assessed according to the American Diabetes Association (ADA).[5]

All patients underwent hormonal evaluation testing including somatotropic, gonadotropic, corticotropic, thyrotropic and parathyroid glands. Evaluation of the GH/IGF-1 axis was performed by GH stimulation tests as well as the insulin-like growth factor (IGF-1) and insulin-like growth factor-binding-protein (IGFBP) concentrations compared to norms for age and sex.[6] The diagnosis of GH deficiency was established on an insufficient peak (less than 20 mIU /L) in response to two separate pharmacological stimuli (insulin tolerance and glucagon-propranolol tests). Subjects were arbitrarily classified according to GH peak in partial GH deficiency (GH peak between 10 and 20 mIU /L) and a total deficit if the values are less than 10 mIU/L. All subjects underwent a basal cortisolemia and after intramuscular injection of adrenocorticotropic hormone (Synacthen® test: 250 µg). An abnormal response (a serum cortisol peak below 550 nmol/L or an increment of less than 200 nmol/L from baseline or both) identifies adrenal insufficiency. Thyroid function was assessed by measuring free thyroxine (FT4) and thyrotrophic hormone (TSH). Subclinical hypothyroidism is defined as a combination of high TSH (≥ 5 mIU/L) with normal FT4 levels.

Skeletal age was evaluated according to Greulich-Pyle atlas.[7] Bone mineral density (BMD) was performed by Dual-energy x-ray absorptiometry (DXA) on L_1-L_4 lumbar spine and total hips. Low bone mass was defined as Z-score values of –2.0 SDs or lower.

Iron overload was assessed using the mean serum ferritin levels and the MRI T_2*. Cardiac and liver T_2*were assessed by a validated technique based on MRI relaxometry at 1.5 T. For the heart T_2* images, all patients underwent a single breath hold multiecho bright blood sequence with variable echo times (TEs). For the liver, a single axial slice was obtained in the center of the organ using a multiecho sequence, and a single breath hold was used to obtain images with the same parameters. Excel spreadsheet was used for image analysis and measurement of T_2*. Images were imported into a software for the region of interest (ROI) drawing. For the heart, signal intensity was obtained using an ROI drawn through the full thickness of the septum wall of the myocardial short axis image. For the liver, the signal intensity was also provided using an ROI covering the right lobe of the liver parenchyma and avoiding major vessels. The same ROI was copied across all images for each organ. Each image generated the values of both signal intensity (SI) and TEs which were manually inputted into an Excel spreadsheet. The mean signal intensity in each slice with varying TEs was used to fit the T_2* curve using the formula $SI = Ke^{-TE/T_2*}$ in the spreadsheet. A curve-fitting truncation model consisting of a monoexponential decay curve with a linear fit was applied. Excel was applied as previously described.[8] Myocardial ion concentration (MIC) was evacuated using Carpenter curves.[9] Liver iron

concentration (LIC) was calculated using Hankins curves.[10] Values of cardiac T_2* (CT_2*) <20 ms were considered to indicate cardiac siderosis which was classified as moderate (10 ms< CT_2* <20 ms) and severe (CT_2* <10 ms).[9] LIC >3 mg/g dry weight (dw) was considered to indicate liver siderosis which was classified on mild (3 < LIC <7 mg/g dw). Moderate (7< LIC <15 mg/g dw) and severe (LIC >15 mg/g dw).[11] Serum ferritin (SF) concentration was measured every 3 months using standard enzyme immunoassay. The 12-month mean SF value was considered.

The iron chelating treatments used were subcutaneous deferoxamine (Desferal®), administered in two repeated doses (40 mg/kg/day) 5-days-per-week, and oral chelators namely deferasirox in a single dose (20 - 40 mg/kg/day) and deferiprone in 3 daily taken (75 - 100 mg/kg/day). The combined treatment consisted of combining deferoxamine with oral iron chelation. A group of 13 healthy subjects was used for control.

Written informed consent was obtained from the patients or their parents.

Statistical analysis: All statistical procedures were performed using SPSS version 18.0. Results are presented in mean ± SDs. Pearson correlation analysis and unpaired T student`s test were used. P value < 0.05 was considered statistically significant.

Results. The most recurrent mutation (SNP) single-nucleotide polymorphism) found was Cd 39 (C > T) in 42 %, IVS-I-110 (G>A) in 33%, Cd 6 (A>T) in 8 %, and Cd 30 (G>C) in 4 % of patients.

Growth: sixteen (57%) of the studied patients had growth velocity standard deviation score less than -2SDs. Among them, thirteen had a pubertal delay. Bone maturation delay was present in all cases. Bone age delay and chronological age were over one year in all patients. In the absence of growth hormone deficiency, height changes with sex and age are illustrated in **figure 1**. Curves show that most children have a normal growth pattern up to the age of 9 years, and a reduced or absent height gain during puberty, which is more marked in boys than in girls. Circulating IGF-1 levels were significantly lower than controls (p = 0.00) (**Figure 2**).

Somatotropic function: GH provocation tests showed an average peak GH levels in 18 patients (40.22 ± 21.7 m IU/L; range (21-129). Ten patients (35%) had GH deficiency, among them a partial GH deficiency was found in 5 cases (16.14 mIU/L ± 2.87; range 3.7- 6.7) and a severe GH deficiency was demonstrated in 5 other cases (5.16 mIU/L ± 1.35; range 13.7- 19.7). Growth assessment for patients with complete GH deficiency showed that of the 3 patients who had attained the adult age the parental target size was reached in only one case. In patients with partial GH deficiency, statural growth was normal in 3 cases. Only one patient had a complete GH deficiency in contrast to normal IGF-1 level. Risk factors, probably related to the occurrence of GH deficiency, are: a history of splenectomy (p = 0.000), association with adrenal insufficiency (p = 0.042), low bone mass (p = 0.037). The levels of IGF-1 and IGFBP were significantly lower in patients with GH deficiency than those without (p= 0.008 and 0.037 respectively).

Puberty: spontaneous onset of puberty was obtained in 16 cases (9 boys and 7 girls) at a mean age of 15 years for boys (range 14-16 years) and 13 years for girls (range 11-15 years). Adult height was reached in 7 cases (5 boys and 2 girls) at a mean age of 20 years for boys (range 17-22 years) and 17 years for girls. Twelve of the studied

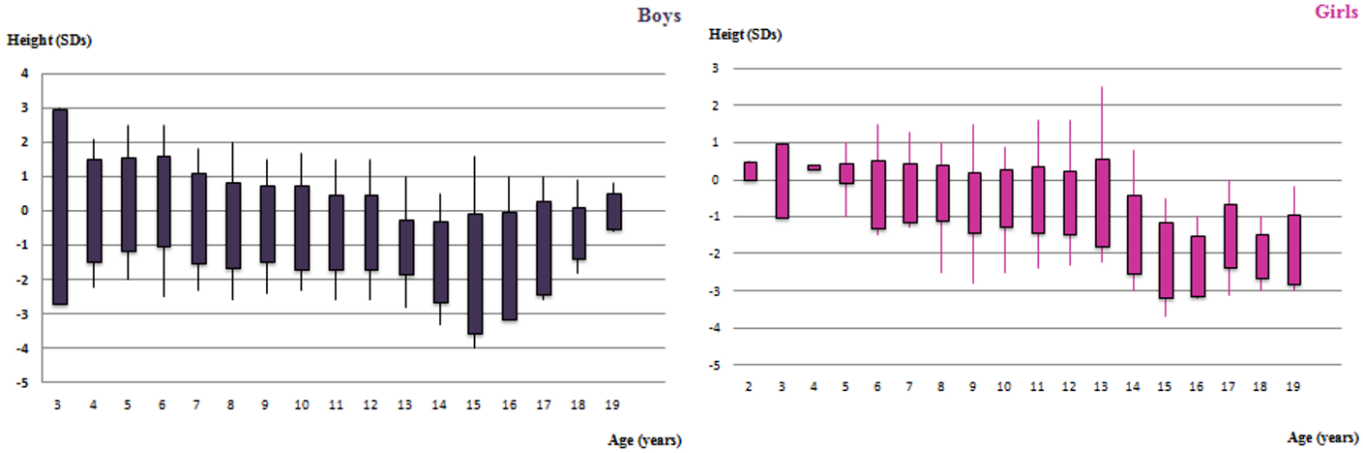

Figure 1. Mean changes in height for sex and age.

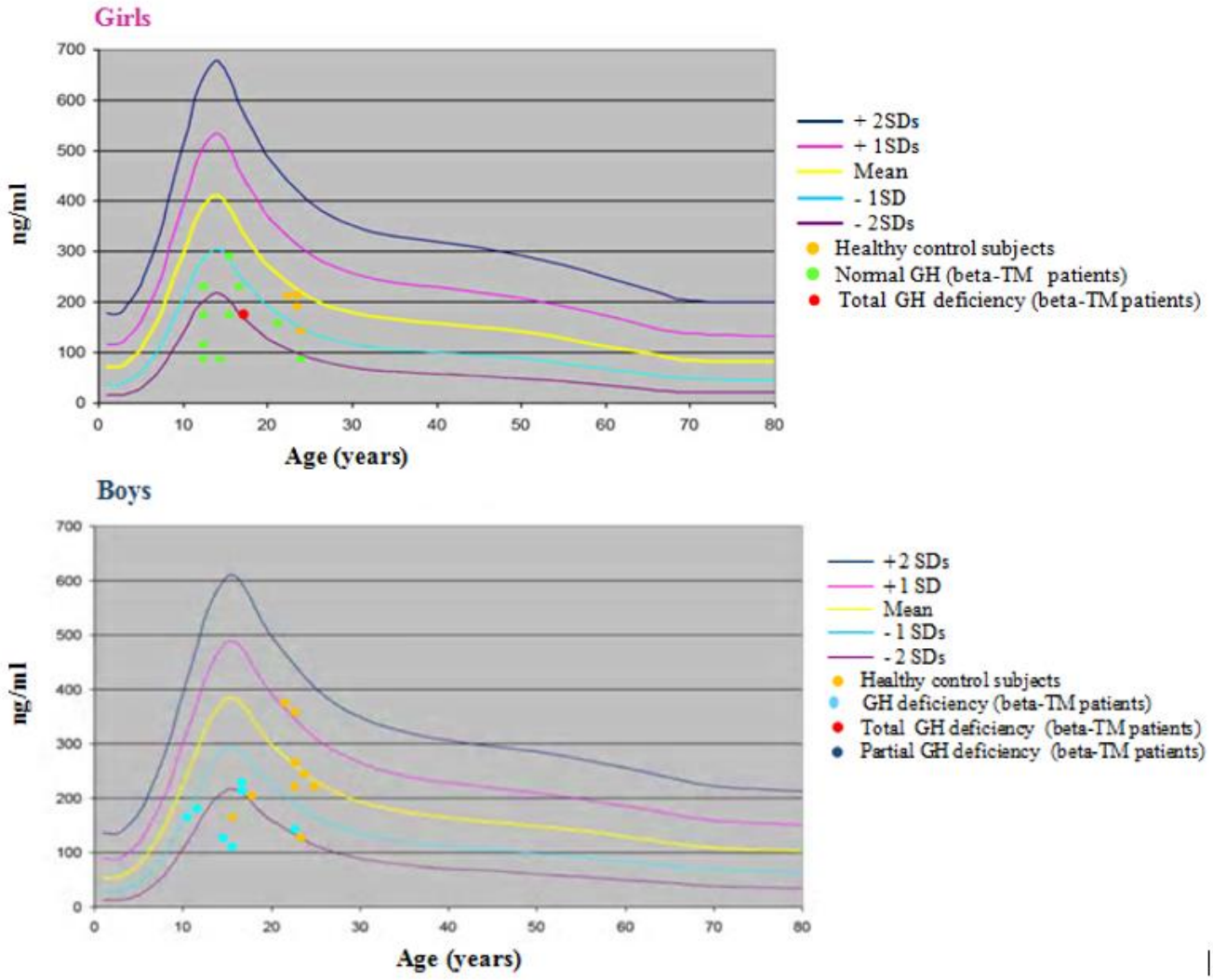

Figure 2. Distribution of IGF-1 values by age and sex.

patients (42%) had a delayed puberty and hypogonadism requiring lifelong hormone replacement therapy. Lack of pubertal progression was observed in 4 cases (2 girls and 2 boys), the absence of the onset of pubertal development in 6 cases (5 boys and one girl) and a primary amenorrhea in two other cases. Factors associated with pubertal disorders are transfusion requirements before chelating therapy ($p = 0.042$) and myocardial iron assessment by MRI T_2* ($p = 0.037$).

Carbohydrate metabolism: seventeen of the studied patients (60%) had disturbances of glucose homeostasis with an impaired fasting glycemia in four cases, impaired glucose tolerance in eight and diabetes mellitus in five. All of them except one had been diagnosed after the age of 10. Diabetes was preceded by a pre-diabetic stage in all cases with an average of 7 years (1 - 8). The mean age at the time of diagnosis was 20 ± 3.4 years (12 - 15). No significant difference was seen between males and females in the prevalence of diabetes mellitus. All diabetic patients had a family history of type I or type II diabetes in their siblings, parents or grandparents. The mean body mass index of diabetic patients was 19 ± 2.41 kg / m² (16.24-25). Overweight was noted in one patient. Two cases had first presented with diabetic ketoacidosis. Islet cell antibodies, insulin autoantibodies, and anti-glutamate decarboxylase were negative in all cases. Sixteen patients were splenectomized. Serum ferritin level in β-TM patients with diabetes and those without a history of diabetes were not significantly different. Severe cardiac loading (CT_2* ≤10ms) was present in seven patients, and 10 patients had a severe iron deposition in the liver (LIC >15 mg/g dw). All patients had metformin as an antidiabetic agent associated with combined intensive iron chelation therapy during a mean follow-up of 3 years (1-6 years). Risk factors of

carbohydrate metabolism disorders were: age at onset of chelation therapy (p = 0.025) and ferritinemia. In fact, patients with carbohydrate metabolism disorders had a higher average ferritin level than those without, and the difference was statistically significant (p = 0.03).

Thyroid function: subclinical hypothyroidism was found in six patients (mean age 17 ± 3.14 years range 14 – 16) (mean TSH levels 5.97 ± 1.31 m IU /L range 5-9). Antithyroid antibodies were negative in all cases. Mean serum ferritin level was 1405.8 ± 441.93 µg / l. Severe cardiac loading (CT_2* ≤10ms) were seen in 3 cases, and severe liver loading (LIC >15 mg/g dw) was observed in 4 cases. Only one patient required hormone replacement therapy with levothyroxine. In all other cases, combined chelation therapy allowed the normalization of thyroid hormone levels.

Adrenal function: the cortisol peak was normal in 19 patients after ACTH stimulation. Nine patients (32%) had adrenal insufficiency with a mean cortisol peak of 413.93 ± 82.76 nmol/L (range 309 -463). No patient was symptomatic. A polyendocrinopathy was found in all cases. All patients had combined chelation therapy with deferoxamine and deferasirox or deferiprone. Splenectomy was performed in all cases. None of them had received corticosteroid replacement therapy. Mean serum ferritin was 1,391.88 ± 661µg / l. Severe hepatic and cardiac loading was noticed in three patients. Factors associated with the occurrence of adrenal insufficiency were age (p = 0.033), history of splenectomy (p = 0.031), number of transfusions received (p = 0.034), and associated GH deficiency (p = 0.042).

Phosphocalcic metabolism and low bone mass: out of 28 patients, only one girl was found to have hypoparathyroidism. The mean age at diagnosis was 17 years. The patient initially complained of extremity paresthesias, mean serum calcium was 1.6 mmol/L (range 1.7 -1.9). Serum parathyroid hormone (PTH) level was low, 2 pg / ml (normal: 12-72). Among the 28 studied patients, 16 had decreased bone mineral density. Low bone mass was found in ten patients predominantly male (7 patients). All patients had one or more associated endocrinopathy. Factors related to the development of low bone mass include: hypothyroidism (p = 0.007), GH deficiency (p = 0.037), decreased IGF-1 levels (p = 0.002), and iron overload on cardiac (p = 0.021) and liver (p = 0.002) T_2* MRI.

Associated endocrinopathies: five among the studied patients had no endocrine disorder, and 23 (82%) had at least one endocrinopathy with one endocrinopathy in six cases, two in eight cases, three in four cases and four in five cases. The mean age of these patients was 18.4 ± 1.3 years. The prevalence rates of endocrine disorders are shown in **Table 1**. There was a significant difference between mean serum ferritin in thalassemic patients with endocrine complications (1660 ± 1208 µg /l) and those without endocrinopathies (1166 ± 823 µg/l): p = 0.01.

Table 1. Endocrine disorders according to their number

Associated endocrinopathies	1	2	3	4
Delayed puberty	2	3	4	3
Disturbances of glucose homeostasis	3	6	3	5
Adrenal insufficiency	1	3	1	4
Hypothyroïdism	0	1	2	3
GH deficiency	0	3	2	4
Hypoparathyroïdism	0	0	0	1

Discussion. Endocrine abnormalities are widespread among multi-transfused β -TM patients. The most frequent endocrine complications reported are growth retardation, delayed puberty, hypogonadism, carbohydrate metabolism disorders, impaired thyroid, parathyroid and adrenal functions. These complications are largely explained by the toxic effect of iron overload secondary to chronic blood transfusions because the human body lacks a mechanism to excrete excess iron.[12] The pathogenesis of the statural delay in β-TM patients is multifactorial.[13] In our study, in agreement with the findings of Skordis et al.,[14] growth disorders varied depending on age at presentation. In early childhood, growth retardation is mainly due to hypoxia, anemia, ineffective erythropoiesis and nutritional factors. During late childhood, iron overload affecting GH-IGF-1 axis and other potential endocrine complications are the main factors affecting growth. After 11 years of age, delayed or arrested puberty is an important contributing factor to growth failure. In accordance with other studies, maintaining a pretransfusion hemoglobin level above 9–10.5 g/ dl promotes normal growth during the first years of life for the majority of patients in our study. Total growth hormone deficiency is an associated factor that may contribute to the growth delay in

patients with β-thalassemia major. In our study, we found that five out of 28 patients had complete growth hormone deficiency. Four of them had a height below the average of 2 standard deviations. On the other hand, among the five patients with partial growth hormone deficiency, four had normal growth rate suggesting the likely secondary origin of growth hormone deficiency in β-thalassemia major patients. Several studies assessed the GHRH-GH-IGF-1 axis function pointing out its impairment in a large number of short patients. Neurosecretory GH disorders with different prevalence are reported in thalassemia patients with short stature while contradictory data are available on GH reserve. It was reported normal or reduced with a wide variability (8-80%) in short patients, due to defects in the pituitary gland and/or in the hypothalamus[15]. Other authors have reported normal GH and GHBP levels but low levels of IGF-1 and IGFBP which did not properly increase with IGF-1 generation test, suggesting that insensitivity to GH action may be the cause of abnormal growth.[16] The reduction of serum IGF-1 levels in β-TM with no growth hormone deficiency in the different stimulation tests supports these findings.[17,18] However, a lack of correlation between IGF-1, IGFBP, and height SDS in β-TM children with growth failure may indicate that growth failure is not specifically related to GH-IGF- 1 axis. In accordance with what has been described, we have found IGF-1 levels to be significantly lower compared to controls without any growth hormone deficiency. Also, patients with growth hormone deficiency had lower levels of IGF-1 than non-deficient patients. Hypogonadotropic hypogonadism, is the most frequent endocrinopathy in patients with transfusion-dependent thalassemia.[19,20] In male patients, clinical presentations of hypogonadotropic hypogonadism include lack, delay, and/or block of pubertal sexual maturation and, in adult life, decreased libido, erectile dysfunction, worsened sense of well-being, and lower quality of life. Spermatogenesis is impaired, and the volume of ejaculate is decreased. In female patients, hypogonadism is clinically diagnosed by the absence of pubertal development or discontinuation or regression of the maturation of secondary sex characteristics.[20] Low serum concentrations of sex hormones and gonadotropins confirm the diagnosis. Several questions were asked about their diagnosis, their central or peripheral origin and their reversibility to the intensification of the iron chelating treatment. The GnRH (gonadotrophin-releasing hormone) test is unhelpful in the clinical assessment of the hypothalamic-pituitary axis in β-TM patients especially when the chronological and bone ages have not reached pubertal levels. The ability of the testes to produce testosterone under the stimulatory effect of chorionic gonadotropins reflecting the hypophyseal origin of hypogonadism. Among girls, pubertal anomalies are mainly represented by menstrual cycle disorders. According to a study by Borgna-Pignatti et al.,[21] studying 118 patients with β-TM, only 23 (19.4%) had spontaneous menarche. Our study has shown that nearly two-thirds of patients have carbohydrate metabolism disorders. According to the ADA criteria, 12 patients (42%) had a pre-diabetic state with impaired glucose tolerance and impaired fasting glucose, while five patients had developed diabetes. However, none of them required insulin therapy. The incidence of pre-diabetic state is considerably higher than the rate of patients developing IDDM which is 4.2% according to a recent study by Bejaoui et al. in a Tunisian multicenter study.[22] The higher prevalence in our study could be related to the age of patients being studied, with lower rates in younger patients. Indeed, according to the French national register, among 215 β-TM, diabetes was found in 13 patients (6%).[23] According to the same register, it goes from 1.3%, for patients less than 15 years of age, to 4.1% for those between 15 and 24, reaching more than 11% for those above 25 years. The pathogenesis of diabetes in β-TM patients is not fully understood. Studies report the early occurrence of insulin resistance and hyperinsulinemia leading to IGT.[24-27] With age, the persistence of insulin resistance exhausts β-cells and reduces insulin secretion leading to DM. However, other studies report that a defective insulin secretion resulting from toxic effects of iron deposition in the pancreas may precede the development of glucose intolerance. As described before, myocardial and hepatic T_2* values were significantly higher among patients with carbohydrate metabolism disorders. Diabetes is often associated with other endocrinopathies. Six (21%) among the studied patients had primary hypothyroidism (3 girls and 3 boys) with a mean age of 17 ± 3.14 years. Thyroid dysfunction has been reported in 13–60% of patients with

thalassemia, but its severity is variable in different series. These discrepancies cannot be attributed to differences in patients' ages, but rather to difference treatment protocols, including differing transfusion rates and chelation therapies.[28] Milder forms of thyroid dysfunction are much more common.[29-31] Subclinical hypothyroidism was found in all cases with thyroid levels mean of 5.97 ± 1.31 m IU / L. According to others,[32] antithyroid antibodies were negative in all cases. Mean serum ferritin level was 1405.8 ± 441.93 ng / ml. Severe overload was shown in 3 cases on Cardiac T_2* analysis, and liver overload in 4 cases. Only one patient required hormone replacement therapy with levothyroxine. In all other cases, combined chelation treatment allowed the normalization of thyroid hormone levels. In a large prospective study, hypogonadism was diagnosed in 86% of patients, hypoparathyroidism in 23% of patients, and hypothyroidism in 18% of cases. Among 5 diabetic studied patients, pubertal development delay was found in 4 cases, GH deficiency in two cases and subclinical hypothyroidism in 2 cases. Adrenal insufficiency is not a rare complication in β-TM. However, it is of little or no clinical impact under basal conditions. Accordingly, glucocorticoid replacement therapy might be advised only for stressful conditions.[33] HPT has been considered as a typical complication of the second decade of life in transfusion-dependent patients with TM. It is thought to be mainly the consequence of iron deposition in the parathyroid glands. In most patients the onset of HPT was preceded or followed by other endocrine complications.[34] The patient with hypo-parathyroidism had a total absence of pubertal development, diabetes and low bone mass. Low bone mass represents a prominent cause of morbidity among young adults of both genders with β-TM and the incidence of low bone mass in well treated β-TM patients has been found to be approximately 40–50%. In addition to genetic factors, bone marrow expansion, direct iron toxicity to osteoblasts, deferoxamine iron chelation, and endocrine gland involvement appear to play a major role. According to others, bone mineral density was significantly lower in β-TM patients with endocrinopathy. Subclinical impairment of adrenocortical function in patients with β-TM is not uncommon, however, it is of little or no clinical impact under basal conditions but may have a potential relevance during stressful events. Accordingly, glucocorticoid treatment coverage might be advised only for stressful conditions. Clinical adrenal insufficiency and adrenal crisis are very rare. Parathyroid insufficiency is a rare and late complication of iron overload.[35] Most patients are asymptomatic or has moderate impairment, requiring thus a periodic follow-up of the phosphocalcic balance in β-TM patients. This complication is often associated with other endocrinopathies that they always precede. Our patient with hypoparathyroidism had a total absence of pubertal development, diabetes and low bone mass. With longer life expectancy, low bone mass becomes increasingly marked. Our study revealed that 35% of the patients had low bone mass. According to others, we found the bone mineral density is significantly lower in patients with associated endocrinopathy. Our study showed higher prevalence of multiple endocrine complications than that recently reported by De Sanctus et al.[32] However, the percentage of patients without multiple endocrine complications is similar in the two study groups.

Conclusions. Endocrine and metabolic disorders are very common among multi-transfused β-TM patients. Ferritin concentrations are not a reliable predictor of these complications. Patients with evidence of cardiac iron overload have more frequently endocrinopathies. This suggests that tissue iron loading is a crucial factor in leading to these disorders. Early detection of these abnormalities as well as multidisciplinary management with standardized protocols are the best means to ensure a better quality of life for patients. Moreover, the promise of new chelators in development can be viewed with an intelligible optimism for a new age of iron chelation therapy.

References:

1. Telfer PT, Warburton F, Christou S, Hadjigavriel M, Sitarou M, Kolnagou A, Angastiniotis M. Improved survival in thalassemia major patients on switching from desferrioxamine to combined chelation therapy with desferrioxamine and deferiprone. Haematologica 2009; 94 (12):1777-1778. http://dx.doi.org/10.3324/haematol.2009.009118 PMid: 19815834
2. De Sanctis V, Roos M, Gasser T, Fortini M, Raiola G, Galati MC. Italian Working Group on Endocrine Complications in Non-Endocrine Diseases. Impact of long-term iron chelation therapy on growth and endocrine functions in thalassaemia. J Pediatr Endocrinol Metab

2006 ; 19(4):471-480. PMID:16759032
3. Sempé. Sempé M, Pédron G, Roy-Pernot MP .Auxologie méthode et séquences. Théraplix. Paris; 1979.
4. Tanner J.M. Growth at adolescence. 2nd Edition Blackwell Scientific Publications; 1962.
5. Diagnosis and classification of diabetes mellitus. American diabetes association. Diabetes Care 2010; 33: 62-69. http://dx.doi.org/10.2337/dc10-S062 PMid: 20042775
6. Brabant G, von zur Mühlen A, Wüster C, Ranke MB, Kratzsch J, Kiess W, Ketelslegers JM, Wilhelmsen L, Hulthén L, Saller B, Mattsson A, Wilde J, Schemer R, Kann P. German KIMS Board. Serum insulin-like growth factor I reference values for an automated chemiluminescence immunoassay system: results from a multicenter study. Horm Res 2003; 60:53-60. http://dx.doi.org/10.1159/000071871 PMid: 12876414
7. Greulich WW, Pyle SI. Radiographic atlas of skeletal development of the hand and wrist. 2nd Ed. Stanford University Press; 1959.
8. Fernandes JL, Sampaio EF, Verissimo M, Pereira FB, da Silva JA, de Figueiredo GS, Kalaf JM, Coelho OR . Heart and liver T2 assessment for iron overload using different software programs. Eur Radiol 2011; 21(12):2503–2510. http://dx.doi.org/10.1007/s00330-011-2208-1 PMid:21842212
9. Carpenter JP, He T, Kirk P, Roughton M, Anderson LJ, de Noronha SV, Sheppard MN, Porter JB, Walker JM, Wood JC, Galanello R, Forni G, Catani G, Matta G, Fucharoen S, Fleming A, House MJ, Black G, Firmin DN, St Pierre TG, Pennell DJ. On T2* magnetic resonance and cardiac iron. Circulation 2011; 123(14):1519–1528. http://dx.doi.org/10.1161/CIRCULATIONAHA.110.007641 PMid:21444881
10. Hankins JS, Mccarville MB, Loeffler RB et al. R2* magnetic resonance imaging of the liver in patients with iron overload. Blood 200; 113(20):4853–4855. http://dx.doi.org/10.1182/blood-2008-12-191643 PMid:19264677
11. Di Tucci AA, Matta G, Deplano S, Gabbas A, Depau C, Derudas D, Caocci G, Agus A, Angelucci E. Myocardial iron overload assessment by T2* magnetic resonance imaging in adult transfusion dependent patients with acquired anemias. Haematologica 200 ; 93(9):1385–1388. http://dx.doi.org/10.3324/haematol.12759 PMid:18603557
12. Ouederni M , Ben Khaled M , Mellouli F , Ben Fraj E , Dhouib N , Yakoub IB , Abbes S , Mnif N , Bejaoui M .Myocardial and liver iron overload, assessed using T2* magnetic resonance imaging with an excel spreadsheet for post processing in Tunisian thalassemia major patients. Ann Hematol 2017 ; 96(1):133-139. http://dx.doi.org/10.1007/s 00277-016-2841-5 PMid:27730342
13. Soliman A T, Khalafallah H, Ashour R. Growth and factors affecting it in thalassemia major. Hemoglobin 2009; 33(S1):S116–S126. http://dx.doi.org/10.3109/03630260903347781 PMid:20001614
14. Skordis N, Kyriakou A. The multifactorial origin of growth failure in thalassaemia. Pediatr Endocrinol Rev 2011 ; 8 Suppl 2:271-277. PMid:21705977
15. Galanello R, Origa R. Beta-thalassemia. Orphanet J Rare Dis 2010;5:11. http://dx.doi/10.1186/1750-1172-5-11 PMID: 20492708
16. Kyriakou A, Skordis N.Thalassaemia and Aberrations of Growth and Puberty. Mediterr J Hematol Infect Dis. 2009; 1(1): e2009003. PMID: 21415985
17. De Sanctis V, Skordis N, Galati MC, Raiola G, Giovannini M, Candini G, Kaffe K, Savvides I, Christou S . Growth hormone and adrenal response to intramuscular glucagon test and its relationship to IGF-1 production and left ventricular ejection fraction in adult B-thalassemia major patients. PediatrEndocrinol Rev 2011 ; 8 Suppl 2:290-294. PMid:21705980
18. Pincelli AI, Masera N, Tavecchia L, Perotti M, Perra S, Mariani R, Piperno A, Mancia G, Grassi G, Masera G. GH deficiency in adult B-thalassemia major patients and its relationship with IGF-1 production. Pediatr Endocrinol Rev 2011 ; 8 Suppl 2:284-289. PMid:21705979
19. De Sanctis V, Elsedfy H, Soliman AT, Elhakim IZ, Pepe A, Kattamis C, Soliman NA, Elalaily R, El Kholy M, Yassin M. Acquired hypogonadotropic hypogonadism (AHH) in thalassaemia major patients: An underdiagnosed condition? Mediterr J Hematol Infect Dis 2016, 8(1): e2016001. http://dx.doi.org/10.4084/MJHID.2016.001
20. De Sanctis V, Soliman AT, Elsedfy H, Albu A, Al Jaouni S, Anastasi S, Bisconte MG, Canatan D, Christou S, Daar S, Di Maio S, El Kholy M, Khater D, Elshinawy M, Kilinc Y, Mattei R, Mosli HH, Quota A, Roberti MG, Sobti P, AL Yaarubi S, Canpisi S, Kattamis C. Review and recommendations on management of adult female thalassemia patients with hypogonadism based on literature review and experience of ICET-A network specialists. Mediterr J Hematol Infect Dis 2017, 9(1): e2017001. http://dx.doi.org/10.4084/MJHID.2017.001
21. Borgna-Pignatti C, De Stefano P, Zonta M, Vullo C, De Sanctis V, Melevendi C, Naselle A, Masera G, Terzoli S, Gabitti V, Piga A.Growth and sexual maturation in thalassemia major. J Pediatr 1985 ; 106: 150-156. PMid:3965675
22. Bejaoui M, Guirat N. Beta thalassemia major in a developing country: epidemiological, clinical and evolutionary aspects. Mediterr J Hematol Infect Dis 2013;5(1):e2013002. http://dx.doi.org/10.4084/MJHID.2013.002 PMid:23350015
23. Thuret I, Pondarré C, Loundou A, Steschenko D, Girot R, Bachir D, Rose C, Barlogis V, Donadieu J, De Montalembert M, Hagege I, Pegourie B, Berger C, Micheau M, Bernaudin F, Leblanc T, Lutz L, Galactéros F, Siméoni MC, Badens C. Complications and treatment of patients with β-thalassemia in France: results of the National Registry. Haematologica 2010 ; 95(5):724-729. http://dx.doi.org/10.3324/haematol.2009.018051 PMid: 20007138
24. Toumba M, Sergis A, Kanaris C, Skordis N . Endocrine complications in patients with thalassaemia major.Pediatr Endocrinol Rev 2007; 5: 642-648. PMId:18084158
25. Gamberini MR, Fortini M, De Sanctis V, Gilli G, Testa MR.. Diabetes mellitus and impaired glucose tolerance in thalassaemia major: incidence, prevalence, risk factors and survival in patients followed in the Ferrara Center.Pediatr Endocrinol Rev 2004 ; 2 (Suppl 2): 285-291. PMid:16462713
26. Au WY, Lam WW, Chu W, Tam S, Wong WK, Liang R, Ha SY . A T2* magnetic resonance imaging study of pancreatic iron overload in thalassemia major. Haematologica 2008 ; 93:116-119. http://dx.doi.org/10.3324/haematol.11768 PMid:18166794
27. Bas M, Gumruk F, Gonc N, et al. Biochemical markers of glucose metabolism may be used to estimate the degree and progression of iron overload in the liver and pancreas of patients with β-thalassemia major. Ann Hematol 2015 ;94:1099-2104. http://dx.doi.org/10.1007/s00277-015-2342-y PMid:25740381
28. Phenekos C, Karamerou A, Pipis P, Constantoulakis M, Lasaridis J, Detsi S, Politou K. Thyroid function in patients with homozygous β-thalassemia. Clin Endocrinol (Oxf) 1984 ; 20:445–450. PMid:6424976
29. Magro S, Puzzanio P, Consarino C, Galati MC, Morgione S, Porcelli D, Grimaldi S, Tancre D, Arcuri V, De Santis V. Hypothyroidism in patients with thalassemia syndromes. Acta Haematol (Basel) 1990 ; 84:72–76. http://dx.doi.org/10.1159/000205032 PMid:2120889
30. Depaz G, Deville A, Coussement N, Manassero J, Mariani R. Thyroid function in thalassemia major. Ann Pediatr (Paris) 1985 ; 32:809–811. PMid:4091451
31. Landau H, Matoth I, Landau-Cordova Z, Goldfarb A, Rachmilewitz EA, Glaser B. Cross-sectional and longitudinal study of the pituitary-thyroid axis in patients with thalassaemia major. Clin Endocrinol (Oxf) 1993 ; 38:55–61.PMid:8435886
32. De Sanctis V, Elsedfy H , Soliman AT., Elhakim IZ , Kattamis C, Soliman NA, Elalaily R. Clinical and Biochemical Data of Adult Thalassemia Major patients (TM) with Multiple Endocrine Complications (MEC) versus TM Patients with Normal Endocrine Functions: A long-term Retrospective Study (40 years) in a Tertiary Care Center in Italy. Mediterr J Hematol Infect Dis 2016; 8(1): e2016022. http://dx.doi.org/10.4084/MJHID.2016.022 PMid: 27158435
33. Huang KE , Mittelman SD, Coates TD, Geffner ME, Wood JC. A significant proportion of thalassemia major patients have adrenal insufficiencydetectable on provocative testing. J Pediatr Hematol Oncol. 2015; 37(1):54-59. http://dx.doi.org/10.1097/MPH.0000000000000199 PMid:24942024
34. De Sanctis V , Soliman AT, Canatan D , Elsedfy H, Karimi M , Daar S , Rimawi H , Christou S, Skordis N , Tzoulis P, Sobti P, Kakkar S, KilincY, Khater D, Alyaarubi SA, Kaleva V, Lum SH, Yassin MA, Saki F, Obiedat M, Anastasi S, Galati MC, Raiola G, Campisi S , Soliman N, Elshinawy M, Al Jaouni S, Di Maio S, Wali Y, Elhakim IZ, Kattamis C. An ICET- A survey on Hypoparathyroidism in Patients with Thalassaemia Majorand Intermedia: A preliminary report. Acta Biomed 2018; 16;88(4):435-444. http://dx.doi.org/10.23750/abm.v88i4.6837 PMid:29350657
35. Casale M, Citarella S, Filosa A, De Michele E, Palmieri F, Ragozzino A, Amendola G, Pugliese U, Tartaglione I, Della Rocca F, Cinque P, Nobili B, Perrotta S. Endocrine function and bone disease during

long-term chelation therapy with deferasirox in patients with β-thalassemia major. Am J Hematol 2014; 89(12):1102-1106. http://dx.doi.org/10.1002/ajh.23844 PMid:25197009