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ORIGINAL PAPER

Growth hormone reserve in adult beta thalassemia patients

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Abstract Reduced serum insulin-like growth factor-1 (IGF-1) and hypogonadotrophic hypogonadism are common features of adult β-thalassemia, and warrant evaluation of the growth hormone (GH)-IGF-1 axis. The aim of this study was to determine GH reserve in β-thalassemia patients (9 females, 7 males, 15 major, 1 intermedia), age 29.3 ± 6.9 years, BMI 21.3 ± 1.9 kg/m^2, and in 20 age, sex and BMI-matched healthy controls, using the GH-releasing hormone (GHRH)–arginine test. The associations between peak GH response and hormonal and biochemical indices were evaluated. Using BMI-related cut-off limits for peak GH response in the GHRH–arginine test, 4/16 β-thalassemia patients had peak GH lower than 11.5 μg/l, the cut-off limit suggested for lean subjects, and were diagnosed as GH deficient (GHD). Using 9 μg/l as the cut-off limit 2/16 patients were GHD. Reduced serum IGF-1 and IGFBP-3 were present in 69% and 19% of the patients, respectively. Peak GH did not correlate with serum IGF-1, TSH, and fT_4 levels or gonadal status. Neither peak GH nor IGF-1 correlated with serum ferritin. Our findings suggest that GHD is present in up to a quarter of adult β-thalassemia patients. The clinical benefits of GH therapy need to be determined. GHD alone does not account for the high prevalence of reduced IGF-1 in adult β-thalassemia.

Keywords Adult beta thalassemia · Growth hormone reserve · GHRH–arginine · IGF-1

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Introduction

Thalassemia is a hereditary anemia resulting from defects in hemoglobin production. β-Thalassemia (β-thal) is caused by any of more than 200 point mutations in the β-globin gene, resulting in decreased production of β-globin chains [1]. Transfusion therapy is the mainstay of β-thal, but may lead to iron overload and hemosiderosis. Despite chelation therapy, iron deposition in the heart, liver and endocrine glands is still an important cause of variable organ damage in β-thal patients. Impairment of growth, hypogonadotrophic hypogonadism, delayed puberty, hypothyroidism, hypoparathyroidism, diabetes mellitus, and bone disease are common endocrine complications in β-thal major and intermedia [2]. Osteoporosis represents an important cause of morbidity in adult β-thal patients, and is often accompanied by disabling pain and fractures. The pathogenesis of osteoporosis in β-thal is multifactorial, and includes bone marrow expansion, hypogonadism, vitamin D deficiency, and deferoxamine-induced bone dysplasia [3].

Others and we have previously reported a high prevalence of reduced serum insulin-like growth factor-1 (IGF-1) level in adult β-thal patients, which correlates with their decreased bone mineral density (BMD), and contributes to the development of osteoporosis [4, 5]. Indeed, low serum IGF-1 level has been identified as an independent risk factor for osteoporosis in women and men [6]. The etiology

of reduced serum IGF-1 in adult β-thal patients is not completely understood. Studies of the GH-IGF-1 axis in pediatric β-thal patients were equivocal, with some demonstrating GH deficiency (GHD) due to a pituitary secretory defect, while others reported GH resistance [7–10]. Moreover, GH therapy was attempted in pediatric β-thal patients, and has had variable success [11–16]. Data in adult β-thal patients is sparse. In a recent study, GH reserve was assessed in a group of young adult β-thal patients who had been diagnosed with GHD during childhood [17]. A quarter of these patients were found to be GHD at adulthood, similar to the prevalence reported in idiopathic childhood GHD. To our knowledge, the GH-IGF-1 axis has not been studied in unselected adult β-thal patients. As GHD might be an additional contributing factor to congestive heart failure and osteoporosis, the evaluation of GH reserve in these patients is warranted. In this study, we assessed GH response to standardized GHRH–Arginine stimulation testing in adult β-thal major and intermedia patients.

Results

Clinical characteristics

The clinical and biochemical characteristics of the study population are presented in Tables 1 and 2. There were no significant differences in BMI or age among the three groups. The female β-thal patients were shorter than the female controls (Table 1), but none were below the 3rd percentile for height. Fifty percent of β-thal patients had hypogonadotrophic hypogonadism (total testosterone <9.5 nmol/l and low gondadotropins in males, low estradiol and gonadotropins in females), and were treated with sex hormone replacement therapy. Four β-thal patients had hypothyroidism and were treated with L-thyroxine. Six β-thal patients had evidence of chronic liver disease (hemosiderosis and/or chronic hepatitis C).

Peak GH response during the GHRH–arginine test

A negative correlation between peak GH in response to GHRH–arginine and BMI was observed ($r = –0.53$, $p = 0.02$, $r = –0.48$, $p = 0.06$ in the control subjects and β-thal patients, respectively).

The individual peak serum GH concentrations are shown in Fig. 1. None of the β-thal patients or the control subjects had a peak GH concentration lower than 4.1 μg/l, while all three patients with MPHD had a peak GH level of less than 3 μg/l. Four lean β-thal patients (BMI 20.3–22.8 kg/m^2) had a peak GH level lower than 11.5 μg/l (range 7.9–11.27 μg/l). One overweight control subject (BMI 28.7 kg/m^2) had a peak GH response of 8.8 μg/l. Thus, 4/16 β-thal patients and none of the control subjects were found to be GHD.

Mean peak GH concentrations following GHRH–arginine stimulation were significantly higher in the control female subjects compared to β-thal female patients (Table 1). In β-thal patients, peak GH was not significantly different in hypogonadal and eugonadal patients (23.2 ± 11.1 μg/l vs. 23.5 ± 13.1 μg/l $p = 0.95$, respectively). There was no correlation between peak GH and serum IGF-1 ($r = 0.25$, $p = 0.42$). Peak GH level did not correlate with either TSH or FT_4 levels. There was no association between peak GH and serum ferritin ($r = 0.07$, $p = 0.8$).

Serum IGF-1 and IGFBP-3 in β-thal patients

Eleven patients (69%) had IGF-1 levels below the 95% confidence interval for age (Table 2). Serum IGF-1 level did not correlate with serum ferritin ($r = 0.24$, $p = 0.4$) or liver enzymes. Three patients (19%) had IGFBP-3 levels below the 95% confidence interval for age (Table 2). There was no difference in serum IGFBP-3 level between hypogonadal and eugonadal patients (2.43 ± 0.47 vs. 2.48 ± 0.68, $p = 0.89$).

Table 1 Clinical characteristics of the study population

	β-Thal patients	Healthy controls	MPHD patients	*p*-Value
N	16	20	3	
Age (yrs)	29.3 ± 6.9	31.7 ± 7.6	36.3 ± 15.9	0.35
Female/male	9/7	13/7	2/1	
Height (m) (female/male)	1.60 ± 0.07/1.69 ± 0.09	1.66 ± 0.05/1.77 ± 0.04	1.60/1.75	0.08[a]/0.03[b]
BMI (kg/m^2)	21.3 ± 1.9	22.6 ± 3.2	23 ± 3.8	0.17
Peak GH (μg/l) (female/male)	16.4 ± 7.3/32.2 ± 10.4	72 ± 58/35.1 ± 27	1.22/0.68	0.005[a]/0.8[b]

Data are mean ± SD. Group means of β-thal patients and healthy controls were compared using the non-paired *t*-test

[a] *p*-Value in females

[b] *p*-Value in males. β-thal—beta thalassemia major and/or intermedia, MPHD—multiple pituitary hormone deficiency, BMI—Body Mass Index

Table 2 Hormonal and biochemical indices in β-thal patients

TSH (0.15–4.0 mIU/l)	2.23 ± 0.6
Free T_4 (10.3–24.4 pmol/l)	14.2 ± 2.4
% Patients with hypogonadotrophic hypogonadism	50
% Patients with reduced serum IGF-1	69
% Patients with below-normal serum IGFBP-3	19
Ferritin (11–290 ng/ml)	1932 ± 1892
Serum AST (2–60 IU/l)	58 ± 22.1
ALT (6–53 IU/l)	36 ± 22.1

Data for continuous variables are mean ± SD

TSH—thyroid stimulating hormone, AST—Aspartate aminotransferase, ALT—Alanine aminotransferase, IGF-1—Insulin-like Growth Factor 1, IGFBP-3—IGF-binding protein 3

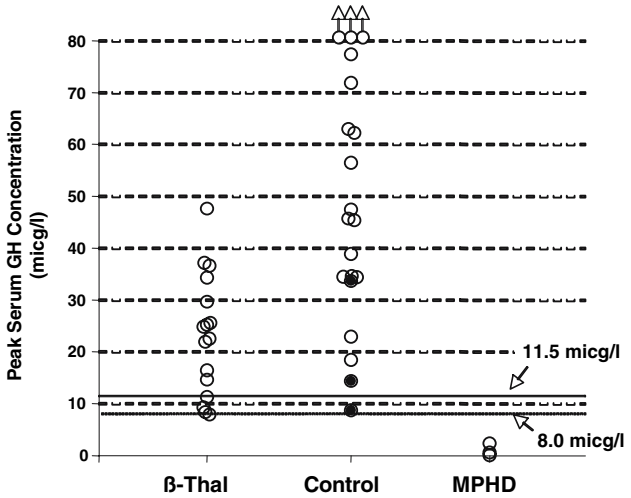

Fig. 1 Individual peak serum GH concentrations in response to stimulation with GHRH–arginine in β-thalassemia (β-Thal) patients, control subjects, and in patients with multiple pituitary hormone deficiency (MPHD). Three control subjects (filled circles) had a BMI >25 and <30 kg/m^2, whereas all of the patients with β-thalassemia and MPHD had BMIs <25 kg/m^2. The solid horizontal line indicates the lower limit of normal for subjects with BMI <25 kg/m^2 (11.5 micg/l), while the dotted line indicates the lower limit of normal for subjects with BMI >25 and <30 kg/m^2 (8.0 micg/l)

Discussion

Previous reports of childhood GH deficiency in β-thal, as well as reduced IGF-1 and hypogonadotrophic hypogonadism in adult β-thal patients warranted evaluation of the GH-IGF-1 axis in this age group. Moreover, a pituitary secretory defect in β-thal was previously suggested by demonstrating iron deposition in the anterior pituitary in correlation with gonadal function [18]. Our findings suggest that up to one quarter of adult β-thal patients are GH deficient. These findings are compatible with the only published study, which examined GH reserve in adult β-thal patients with childhood-onset GHD [17]. To our knowledge this is the first study to test GH reserve in unselected adult β-thal patients. As GHD is a possible contributing factor to osteoporosis and cardiovascular morbidity in thalassemia, the clinical efficacy of GH replacement in these patients needs to be evaluated. However, over two thirds of the β-thal patients in this study had reduced serum IGF-1, which cannot be accounted for by GHD alone.

To determine GH reserve we used the GHRH–arginine stimulation testing. While the Insulin Tolerance Test (ITT) is considered the gold standard for the evaluation of GHD, it can be associated with severe side effects such as myocardial ischemia and seizure disorder, and is therefore often avoided in chronic patients such as β-thal patients [19]. The GHRH–arginine test was suggested as a safe and reproducible alternative to ITT [19–21]. A sensitivity of 95% and a specificity of 91% of the GHRH–arginine test using ITT as the gold standard was shown in patients suspected of having a pituitary GH secretory defect in the context of a pituitary mass [19]. The GHRH–arginine stimulation test was also attempted in conditions with no structural hypothalamic pituitary disease such as in patients with human immunodeficiency virus lipodystrophy [22]. As the ITT was not performed in this study, it is unknown how well the GHRH–arginine test compares with the ITT in β-thal patients. In addition, the overnight GH secretion and pulse GH amplitude were not determined.

The cut-off limit for the diagnosis of adult GHD using the GHRH–arginine stimulation test is not well established, and is influenced mainly by BMI. Increased failure rates to respond to GHRH–arginine were reported in patients with obesity and increased visceral adiposity [23]. Our findings of an inverse relationship between peak GH and BMI in the control subjects and in the β-thal patients are consistent with these reports. Biller et al. found that in patients with adult-onset hypothalamic pituitary disease and MPHD, using a cut-off of 4.1 μg/l has 95% sensitivity and 91% specificity for the diagnosis of GHD using ITT as the gold standard [19]. However, the mean BMI in the control subjects in that study was 30.3 ± 5.8 kg/m^2. A recent large study evaluated specific BMI-related cut-off limits in 322 patients with organic hypothalamic–pituitary disease and in 318 control subjects [24]. Applying these cut-off limits to our study population resulted in a diagnosis of GHD in 4/16 thalassemia patients and in none of the controls. Using a lower cut-off level of 9 μg/l as suggested by others [17] would have resulted in diagnosing 2/16 β-thal patients as GHD.

Over two thirds of the β-thal patients (69%) had serum IGF-1 below the 95% confidence interval for age, which cannot be explained by GHD alone. A plausible mechanism is GH resistance. Primary and secondary GH

resistance has been reported in chronic liver disease, and was attributed to GH receptor loss [25]. Reduced IGF-1 mRNA and serum levels along with decreased acid labile subunit (ALS) gene expression and serum levels have been demonstrated in cirrhotic patients [25]. Furthermore, worsening of hepatocellular function parallels the decrease in serum IGF-1 levels [26]. Despite treatment with chelating agents, liver iron accumulation is still an important complication of β-thal, and cirrhosis is not uncommon in these patients [1]. The lack of association between serum ferritin and IGF-1 levels in this study is not surprising, as serum ferritin is a highly unreliable measure of iron stores, particularly when liver disease is present [27]. Measurement of hepatic iron by liver biopsy, or non-invasively by magnetic susceptometry is a more accurate methodology to determine liver iron stores [28]. Finally, sex hormones and thyroxine deficiencies which can result in a reduction of plasma IGFBP3, thus affecting IGF-1 concentrations, are unlikely, as β-thal patients in this study were hormonally replete.

Our findings suggest that up to a quarter of adult β-thalassemia patients are GHD, thus justifying the assessment of GH reserve in these patients. The clinical benefits of GH replacement therapy remain to be determined. GHD cannot account however for the high prevalence of reduced IGF-1 in β-thalassemia patients. Since IGF-1 deficiency is a contributing factor to osteoporosis, an important cause of morbidity and disability in β-thalassemia patients, additional mechanisms and therapies for this condition should be further explored.

Methods

Subjects

Adult homozygous β-thal patients were randomly recruited from the thalassemia clinic at Hadassah-Hebrew University Medical Center, Jerusalem, Israel, as previously described by us [5]. Fifty adult β-thal patients are followed in this clinic, and 16 consecutive patients (7 males and 9 females) with β-thal major ($N = 15$) and intermedia ($N = 1$), who gave consent to participate in the study were recruited. Twenty healthy, age, sex, and BMI-matched volunteers comprised the control group. Three patients with known pituitary disease and MPHD (≥3 pituitary hormone deficiency, excluding prolactin) were also studied. Two patients had panhypopituitarism after pituitary surgery for a pituitary adenoma, and one patient underwent resection of a craniopharyngioma. This small group of patients with MPHD was included to demonstrate the validity of the GHRH–arginine test. None of the β-thal patients were evaluated for GHD during their childhood, and none received prior GH therapy. Data collection from medical charts included use of chelating agents and hormone replacement therapy, average serum ferritin level over the past year, TSH and free T_4 levels and a history of any endocrinopathy.

Biochemical and endocrine measurements

GH reserve assessment

All subjects underwent GHRH and arginine stimulation testing in the morning after a 12-h fast. Standing height was measured using Harpenden stadiometer and body mass index (BMI) was determined in the same visit. GHRH, (Geref diagnostic, Serono, Inc., Norwell, MA), 1 μg/kg was administered by IV bolus along with a 30-min IV infusion of arginine hydrochloride (0.5 g/kg, maximum dose 30 g). Blood samples for GH level were collected at –15, 0, 30, 60, 90, and 120 min after GHRH administration.

Blood samples were also obtained for serum IGF-1, IGFBP3, aspartate aminotransferase (AST), alanine aminotransferase (ALT), and ferritin. Written informed consent was obtained from all subjects in accordance with the ethics committee on Human Studies at the Hadassah-Hebrew University Medical Center. GH was measured by a two-site chemiluminescent immunometric assay with an intra-assay coefficient of variation (CV) of 4.6% (Euro/DPC, Gwynedd, UK). The inter-assay CV was 6.6%. The sensitivity of the assay was 0.01 μg/l. IGF-1 was measured by an enzyme-labeled chemiluminescent immunometric assay with an intra-assay CV of 3.9% (Euro/DPC, Gwynedd, UK). The inter-assay CV was 7.7%. The sensitivity of the assay was 2.6 nmol/l. IGFBP-3 was measured by an enzyme-labeled chemiluminescent immunometric assay with an intra-assay CV of 4.8% (Euro/DPC, Gwynedd, UK). The inter-assay CV was 7.2%. The sensitivity of the assay was 0.02 mg/l.

Three BMI-related cut-off levels for GH peak response to GHRH–arginine were evaluated, as previously suggested [24]: 4.1 μg/l in obese subjects (BMI ≥30 kg/m^2), 8.0 μg/l in overweight subjects (BMI ≥25 kg/m^2 and <30 kg/m^2), and 11.5 μg/l in lean subjects (BMI <25 kg/m^2).

IGF-1 and IGFBP-3 were considered reduced if below the lower value of the 95% confidence interval for age and sex.

Statistical analysis

Descriptive statistics are presented as mean ± standard deviation (SD) unless otherwise specified. Differences in age, BMI, peak GH during the GHRH–arginine test among the three groups were tested using Kruskal–Wallis analysis of variance (ANOVA). For comparisons between β-thal

patients and healthy controls the unpaired Student's *t*-test was used. The correlation coefficients were calculated using Pearson coefficients. Statistical analysis was performed using Statistix 8.0 (Analytical Software, Tallahassee, FL). Statistical significance was defined as $p < 0.05$.

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