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ORIGINAL ARTICLE

Deregulation of the growth hormone/insulin-like growth factor-1 axis in adults with cystic fibrosis

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Abstract
Purpose Patients with cystic fibrosis (CF) present with signs and symptoms that overlap with those of adult growth hormone deficiency (GHD) syndrome: loss of muscle mass, bone fragility and lower stress tolerance. In literature, the prevalence of GHD in pediatric CF patients ishigher than general population, but these studies have been performed on children with growth delay. To our knowledge, there are no studies on adult patients. The aim of this paper is to evaluate GH–IGF1 axis in an adult CF population.
Methods Fifty clinically stable adult patients, 30 males; age 36 ± 2 years; BMI 21.39 ± 0.22 kg/m^2 and FEV_1 67 ± 4% were studied. Data regarding glycometabolic status and results of pituitary, thyroid, parathyroid, gonadal and adrenal function tests were recorded. All patients underwent a GH releasing hormone (GHRH) + Arginine stimulation test to confirm a GHD.
Results GHRH + Arginine test revealed the presence of GHD in 16 patients (32%); specifically 7 patients had a severe deficiency and 9 a partial deficiency.
Conclusions Adult patients with CF may show GHD. These patients should be followed over time to assess if the GHD could impact the clinical progression of CF.

Keywords Growth hormone deficiency · Insulin-like growth factor-1 · Cystic fibrosis · Adult patients

Introduction

Recent advances in the management of cystic fibrosis (CF) have significantly improved the life expectancy of affected patients. This is achieved by the use of therapies that include nebulised mucolytics, bronchodilators, antibiotics also used for prophylaxis, respiratory physiotherapy and new drugs aimed to modulate the cystic fibrosis transmembrane conductance regulator (CFTR) protein function [1]. The annual 2015 data report of the Cystic Fibrosis Foundation Patient Registry estimated an average life expectancy of 45.2 years for children born with CF in 2015 [2]. The more chance of survival for these patients has focused attention on new complications of disease, like as endocrine disfunctions, which impact on quality of life. Some endocrine complications are well known and documented, such as diabetes, affecting 50–75% [3] of adolescents and adults with CF. Early cystic fibrosis involves the exocrine pancreas but may progress to involve the endocrine portion. Infertility in males also occurs due to obstruction/atresia of the vas deferens [4]. CF patients have bone damage caused by malabsorption of vitamin D and the frequent use of corticosteroids. Corticosteroid therapy inhibits the achievement of peak bone mass, makes their bones fragile and prone to fractures during adolescence [5]. These fractures, if they occur in the ribs can cause severe pain and compromise respiratory mechanics [6]. There are other endocrine complications that are still little investigated to date, such as growth hormone deficiency (GHD). Adult patients with GHD can have a variety of signs and symptoms, most of which are unspecific [7]. Often the symptoms are understood only after the start of replacement therapy

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with recombinant human growth hormone (rhGH), which induces changes in body composition and a feeling of wellness [8–10]. Previous studies on GHD in CF were mainly performed on children and adolescents with short stature. Prevalence of patients with GHD in the general population is consistently reported to be in the 0.02–0.03% range while prevalence in CF patient population was 4.21% [11]. In these studies, children and adolescents with CF have been found to have a normal spontaneous GH secretion, with low levels of effector proteins such IGF-1, and insulin-like growth factor binding protein 3 (IGFBP-3) [12], which correlated with height and body mass index (BMI) [13]. The chronic inflammation present in patients with CF causes the production of proinflammatory cytokines such as interleukin-1β (IL-1β), interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α), which reduce IGF-1 levels [14]. Thus, the short stature in patients with CF also seems to be due to a relative insensitivity to GH [15, 16]. We hypothesize that the etiopathogenic mechanism of a possible late-onset GHD in adults may be due to chronic hypoxia of the pituitary gland, as is documented occur in other chronic diseases [17, 18]. Chronic hypoxia, due to the CF, would cause ischaemic damage in pituitary cells, particularly somatotrope cells, which are more prone to ischaemia due to their external position within the anterior pituitary gland. In addition, the state of severe and chronic malnutrition and hepatic impairment contribute in a multifactorial way to reduce the IGF-1 production. The low levels of IGF-1 induce lean muscle mass loss and hypotrophy of respiratory muscles that contribute to respiratory impairment and poor lung function [19]. Therefore, we thought it useful to investigate the GH/IGF-1 axis in adult CF patients, regarding the impact of GH on the bone mineral matrix [20–22], muscle mass, physical endurance and lipid profile.

Materials and methods

We studied 50 adult patients with CF (30 males) with a mean age of 36 ± 2 years (range 18–60 years). All patients were regularly followed up at the time of diagnosis to date, at the Cystic Fibrosis Centre of Lazio Region. The study was approved by the Ethics Committee of the Umberto I Polyclinic University of Rome with protocol number 818/13. All patients signed an informed consent and received all the information about the study to which they adhered. The exclusion criteria of the study were: patients with an age less than 18 years, patients with a pulmonary exacerbation in the 4 weeks prior to the study, patients on the waiting list for lung transplantation and those who had undergone lung or liver transplantation, patients with forced expiratory volume in one second (FEV_1) < 30% of the predicted value and on oxygen therapy. All patients in the study were evaluated for anthropometric parameters and had all achieved the genetically determined target height. None of the patients were evaluated for GHD during their childhood, and none of them had previously received rhGH. For all subjects a careful history and clinical assessment both by electronic database and clinical documents were conducted. The blood levels of the following hormones were measured: IGF-1, free T_3 (FT3), free T_4 (FT4), thyroid-stimulating hormone (TSH), luteinizing hormone (LH), follicle-stimulating hormone (FSH), testosterone (Estradiol in females), prolactin (PRL), adrenocorticotropic hormone (ACTH), cortisol, sex hormone-binding globulin (SHBG) and parathyroid hormone (PTH). In women with regular menstrual cycles, samples for hormone assays were taken in the follicular phase of the menstrual cycle (between the third and the eighth day). The hormonal determinations of TSH, FT3, FT4, estradiol or testosterone, FSH, LH, SHBG and PRL were performed with commercially available automated Chemiluminescence immunoassay systems. The reference ranges for these hormones were as follows: TSH (0.35–4.94 mlU/L), FT4 (0.7–1.48 ng/dL), FT3 (1.71–3.71 pg/mL), Testosterone (10.4–38.2 nmol/L), estradiol (35–169 pg/mL), LH (1.8–8.16 mIU/mL in males and 2.89–21.72 mIU/mL in females), FSH (1.38–9.58 mIU/mL in males and 3.35–21.63 mIU/mL in females), PRL (2.64–13.13 ng/mL in males and 3.34–26.72 ng/mL in females), and SHBG (11.7–137.2 nmol/L in females and 11.2–78.1 nmol/L in males). The hormonal determinations of ACTH, PTH and GH were performed with immunoradiometric assay (IRMA). The reference ranges for the other assays were as follows: ACTH (10–90 pg/mL), PTH (11–62 pg/mL) and baseline GH (0–10 ng/mL).The hormonal determinations of cortisol and IGF-1 were performed with radioimmunoassay (RIA). The reference ranges for the other assays were as follows: cortisol (266–720 nmol/L) and IGF-1 (70–250 ng/mL in males and 100–415 ng/mL in females). At the same time fasting glucose and other biochemical and hematological tests were performed. All subjects were tested for stimulation with GHRH and Arginine in the morning after a 12-h fast. A 1 mg/kg dose of GHRH (Geref Diagnostic®, Serono) was administered by intravenous bolus, followed by arginine hydrochloride by intravenous infusion over 30 min (0.5 g/kg, with a maximum dose of 30 g). Blood samples for GH level were collected at time 0, 30, 45, and 60 min after stimulation [23]. The values of peak GH after stimulation were compared to the body mass index (BMI). Severe GHD was defined as a peak GH lower than 11.5 ng/mL for patients with a BMI < 25 kg/m^2, and lower than 8.0 ng/mL for those with a BMI > 25 kg/m^2. No patient in the sample had a BMI > 30 kg/m^2 [24]. A partial GHD was defined until 16 ng/mL. We chose the GHRH + arginine stimulation test not only because it is the gold standard for the diagnosis of GHD in adults, but also because many of the patients had CF related diabetes

(CFRD) which made it inappropriate to use the insulin tolerance test (ITT) or the glucagon stimulating test [25]. For 41 of the 50 patients in the study, we measured the levels of IGF-1, IGF-2, IGF-BP3, IGF-BP4, IGF-BP5, IGF-BP6 and IGF-BP7 with Bio-Plex Pro™ RBM (BIORAD) using two plates support and cryopreserved the blood samples at − 80 °C. In each patient we also evaluated the lumbar (L1–L4) and proximal femur bone mineral density (BMD) using densitometry with dual-energy X-ray absorptiometry (DXA).We then asked our patients to complete a questionnaire (HQL-S) [26] as a self-assessment of their quality of life to objectify their perception of well-being with a specific test for adult patients with GH deficiency.

Statistical analysis

The data are presented as mean and standard deviations. The association between binary variables (such as sex and therapies) and groups was investigated through Pearson's Chi-squared test. A binary logistic regression was performed to quantify the association between groups and independent variables through odds ratio (OR) with corresponding 95% confidence intervals. Kruskal–Wallis nonparametric one-way analysis measures the statistical significance of the difference between groups. For the statistical analysis, we used IBM SPSS Statistics version 21.0. A value of $p < 0.05$ was considered to be significant.

Results

The general characteristics of the study population are shown in Table 1. Only 6 out of the 50 patients had normal pancreas function and so did not require enzyme replacement therapy. Of these, five were heterozygous for mutation F508del and mild mutation (IV or V class) of the CFTR gene and all had normal function of GH–IGF-1 axis except one patient with GH partial deficiency. Fourteen patients (28%) were suffering from CFRD and were on insulin therapy with NovoRapid® (NovoNordisk) and Lantus® (Insulin Aspart). Two patients were already receiving levothyroxine Eutirox® (Bracco) for hypothyroidism; the study did not reveal any other hormonal deficiencies in the patients enrolled. Periodically all patients in the study received inhaled corticosteroids cycles. However, GHRH plus Arginine test were performed at least 60 days after the last inhaled corticosteroid therapy. The assessment of the secretory patterns of GH and IGF-1 by the GHRH + Arginine test, in accordance with data in the literature for the normalization of GH values adjusted for BMI, showed the presence of GHD in 16 of the 50 patients in the study (32%). Of these 44% (7/16) of the patients had severe deficiency and 56% (9/16) had partial deficiency. The results are shown in Fig. 1. We divided the patients into two groups according to the presence (group A) or absence (group B) of GHD. Group A includes patients with a severe and partial deficiency of GH. All results of the two groups with and without GHD are reported in Table 2. Comparing groups A and B, statistically significant differences were not detected for the respiratory function tests: FEV_1: p value = 0.541, forced vital capacity (FVC): p value = 0.479, and forced expiratory flow (FEF) 25–75%: p value = 0.307. The p value of the mean score of lumbar DXA in A group compared to B group is 0.939. The p value of the mean score of the femoral DXA in A group compared to B group was 0.775. Comparing the two groups (A vs B) for total body DXA did not reveal any statistically significant differences:

Table 1 The general characteristics of the study population

Sex	30 males, 20 females
Age (years)	36.3 ± 2.12
BMI (kg/m^2)	21.39 ± 0.22
WC (cm)	92.35 ± 11.55
Pancreatic insufficiency (%)	88 (44/50 pts)
CFRD (%)	28 (14/50 pts)
FEV_1 (%)	67
FVC (%)	74
Lumbar DXA *T* score	− 1.1 ± 1.3
Femoral DXA *T* score	− 1.3 ± 0.7
Total body DXA *T* score	0.4 ± 1
Total body DXA fat%	17.3 ± 0.14
Questionnaire HQL-S average value	38.5 ± 9.1
Questionnaire HQL-S *Z* score	0.7 ± 0.2

Data are expressed as mean ± SD

BMI body mass index, *WC* waist circumference, *FEV_1* forced expiratory volume in one second, *FVC* forced vital capacity, *CFRD* Cystic Fibrosis Related diabetes

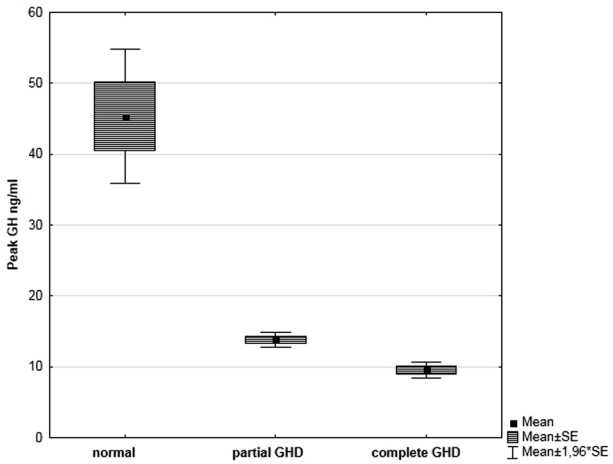

Fig. 1 Results of GHRH stimulation test

Table 2 A comparison of the two groups with and without GHD

Variables	Group A	Group B	*p* value
Sex (M vs F) pts	F:2 M:14	F:18 M:16	0.006*
BMI (kg/m^2)	23.10 ± 2.39	20.94 ± 2.06	0.001*
WC (cm)	102.25 ± 5.67	87.69 ± 10.66	0.000*
FVC (%)	78 ± 16	73.2 ± 20.8	0.479
FEV-1 (%)	63.3 ± 17.7	59.3 ± 20.6	0.541
FEF 25–75 (%)	47.2 ± 30	37.5 ± 20.4	0.307
TSH (μIU/mL)	2.1 ± 1.06	2.04 ± 0.87	0.02*
CFRD (% no. pts)	50% (8/16 pt)	17.6% (6/34 pts)	0.02*
Lumbar DXA (*T* score)	− 1.1 ± 1.4	− 1.1 ± 1.1	0.939
Femoral DXA (*T* score)	− 0.7 ± 0.9	− 0.8 ± 0.9	0.775
Fasting glucose (mg/dl)	115.9 ± 29.1	87.7 ± 13.6	0.04*
IGF-1 SDS	− 1.09 ± 1.72	− 0.92 ± 1.06	0.71

BMI body mass index, *WC* waist circumference, *FVC* forced vital capacity, *FEV-1* forced expiratory volume in 1 s, *FEF 25–75%* forced inspiratory flow 25–75%, *TSH* thyroid-stimulating hormone, *CFRD* Cystic Fibrosis Related diabetes, *DXA* Dual-energy X-ray absorptiometry, *IGF-1 SDS* insulin-like growth factor-1 standard deviation scores, *GHD* growth hormone deficiency

* Statistically significant values

Table 3 Comparison of patients with and without GHD in terms of the average values of the effector proteins of GH

Variables	Group A	Group B	*p value*
IGF-1	205.4 ± 78.9	204.4 ± 54.5	0.967
IGF-2	212.8 ± 72	213.3 ± 90.7	0.985
IGFBP-1	27.1 ± 26.2	15.5 ± 12	0.172
IGFBP-2	86.8 ± 52.8	67 ± 52	0.308
IGFBP-3	2009.4 ± 743.6	1816.9 ± 540.3	0.446
IGFBP-4	211.1 ± 51.6	225.6 ± 57	0.458
IGFBP-6	229.8 ± 58.1	238.2 ± 44.1	0.671
IGFBP-7	29.3 ± 5.6	32.1 ± 7.3	0.212

GHD growth hormone deficiency, *IGF-1* insulin-like growth factor-1, *IGF-2* insulin-like growth factor-2, *IGFBP-1* insulin-like growth factor binding protein 1, *IGFBP-2* insulin-like growth factor binding protein 2, *IGFBP-3* insulin-like growth factor binding protein 3, *IGFBP-4* insulin-like growth factor binding protein 4, *IGFBP-6* insulin-like growth factor binding protein 6, *IGFBP-7* insulin-like growth factor binding protein 7

% fat *p* value = 0.76. The *p* value of the absolute value of HQL-s in group A compared to group B was 0.11. The *p* value of the HQL-s *Z* SCORE in A group compared to B group was 0.29. We noticed that among patients with GHD (both partial and severe), 50% were homozygous for mutation F508del, while among those without GH deficiency, 39% were homozygous for the mutation F508del of the CFTR gene ($p = 0.707$). Comparing the group with normal GH and the group with GH deficiency one, we found no statistical differences in the mean absolute values of IGF-1 and in mean IGF-1 standard deviation score (SDS) between A group (− 0.92 ± 1.06) and B group (− 1.09 ± 1.72) *p* value = 0.71 [27]. Taking note of this result, at a later time, on blood samples, stored at − 80 °C, we performed assays of IGF-BP3 [28], protein IGF-2 [29], the other related binding proteins GH/IGF-1 axis [30, 31], using Bio-Plex Pro RBM—BIORAD technology. The assay was performed on only 41 of the 50 patients in the study, out of which 34.2% (14/41) had GH deficiency, severe in 12.2% (5/41) and partial in 22% (9/41). Again, we compared the 41 patients dividing them into two groups: those with GH deficiency (partial and severe) and those with no deficiency. There was no statistically significant difference between the two groups. The results are shown in Table 3.

Discussion

Our study shows that in a population of 50 adult patients with cystic fibrosis who had normal growth and have never been treated with rhGH, 32% of patients (16/50) had GHD. The 37.5% of the patients with GHD showed impaired fasting glucose and a greater prevalence of cystic fibrosis related diabetes (CFRD). The BMI was normal in all patients but was significantly higher in those with deficit. The waist circumference was significantly higher in those with GHD. The data on thyroid function can be considered as a baseline for a follow up over time. The self-perception of quality of life was similar in the two groups. This result is understandable considering the heavy impact of the underlying disease on quality of life; we expect much improvement after treatment with rhGH. Pulmonary function values are not significantly different between patients with and without GH deficiency. Unlike the pediatric populations who show deficiency of IGF-1 and IGFBP-3, in the adults studied, the values of all the effector and related transport proteins within the GH/IGF-1 axis were normal and there was no difference between the groups with and without GHD. In pediatric patients, IGF-1 is the most reliable parameter for measuring the state of GH secretion. Unfortunately, especially in adult patients, for diagnostic purposes, IGF-1 is much less trusted. In fact, in adults with overt GH deficiency, the values of IGF-1 are frequently normal. The importance of the blood levels of IGF-1, from a diagnostic point of view, can only be considered in an appropriate clinical context. In fact, in adults IGF-1 is more often used in patients already diagnosed with GH deficiency to measure their response to replacement therapy with rhGH. To evaluate the response of treatment with rhGH in these CF patients with GH deficiency, we should follow the clinical response because IGF-1 is not useful. This study has some limitations, as the number of the patients studied and the lack of measures of the proinflammatory cytokines.

In conclusion, in this pilot study, we showed a significant prevalence of GH deficiency in adult CF patients. Then in these patients, the pituitary GH secretory capacity is not related to the IGF-1 levels. Finally, we found an association between serious genetic mutations and GHD. So, further studies should evaluate the patient's genotype as factor causing GHD rather than the hypothesis of hypoxia. To validate this hypothesis, it is necessary to consider a large CF population especially for the high genetic variability in the Italian CF patients.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflicts of interest in this work.

Ethical approval The present study was approved by the ethics committee of the University and adhered to the tenets of the Declaration of Helsinki.

Informed consent Additionally, the written informed consents were signed by all participants.

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