Respiratory Medicine 137 (2018) 61–69

Contents lists available at ScienceDirect

Respiratory Medicine

journal homepage: www.elsevier.com/locate/rmed

Direct and indirect effects of Growth Hormone Deficiency (GHD) on lung function in children: A mediation analysis

Giovanna Cilluffo[a,b,*,1], Giuliana Ferrante[c,1], Salvatore Fasola[a,b], Alessandro Ciresi[d], Irene Cardillo[d], Giancarlo Tancredi[e], Giovanni Viegi[a,f], Carla Giordano[d], Nicola Scichilone[d], Stefania La Grutta[a,c]

[a] National Research Council of Italy, Institute of Biomedicine and Molecular Immunology, Palermo, Italy
[b] Department of Economics, Business and Statistical Science, University of Palermo, Italy
[c] Department of Science for Heath Promotion and Mother and Child Care, University of Palermo, Italy
[d] Biomedical Department of Internal and Specialist Medicine (DIBIMIS), University of Palermo, Palermo, Italy
[e] Department of Pediatrics, Sapienza University, Rome, Italy
[f] National Research Council of Italy, Institute of Clinical Physiology, Pulmonary Environmental Epidemiology Unit, Pisa, Italy

ARTICLE INFO

Keywords:
Children
Growth-hormone-deficiency
Lung function tests
Mediation analysis

ABSTRACT

Background: Studies on pulmonary function tests (PFTs) in Growth Hormone Deficiency (GHD) children are lacking. The aims of this study were: (i) to investigate PFTs in GHD pre-pubertal children with respect to Controls, before starting Growth Hormone Therapy (GHT) (T0); (ii) to evaluate changes of PFTs in GHD vs Controls, after 1-year GHT (T1). For both aims the mediation analysis (MA) was applied to evaluate the extent to which the relationship between GHD and PFTs could be ascribed to a height-mediated (indirect) or a GH direct effect.

Methods: 47 pre-pubertal GHD children (aged 5–14 years) underwent PFTs at T0 and T1. At T0, 47 healthy children matched for age and sex were enrolled as Controls. A MA was performed to assess the relationship between GHD and PFTs and height. Statistical analyses were performed using the statistical software R (https://cran.r-project.org/mirrors.html). A p-value < 0.05 was considered significant.

Measurements and main results: At T0, PFTs indices were significantly lower in GHD than in Controls. From T0 to T1 a significant improvement was found in PFTs. The percentages of the mediated effect on FVC, FEV_1, $FEF_{25–75\%}$ and TLC were $< 50\%$ at T0, suggesting that the direct effect was prevalent. At T1, the percentages of the mediated effect for spirometry indices were $\geq 50\%$, indicating that the indirect (height-mediated) effect was the most relevant.

Conclusions: The study shows that pre-pubertal children with GHD have an impairment of lung function not exclusively attributable to the indirect (height-mediated) effect, but also to the direct GH action which is mitigated after 1-year of GHT.

1. Introduction

Isolated Growth Hormone Deficiency (GHD) is a rare cause of children short stature with an estimated prevalence of 1–5:10000 (www.orpha.net), ranging from 1:1800 to 1:30000 [1]. Recombinant human GH is currently the only treatment option for growth failure in children affected by GHD [2,3].

GH induces the physiological and anatomical changes detected in the lung of adult subjects after both GH excess [4] and deficiency [5,6]. GH Receptor (GHR) gene expression has been demonstrated in pulmonary tissues of animal models [7,8]. In addition, GH mRNA has been found in human alveolar macrophages [9], and there is evidence of GH immunoreactivity in fetal and adult human lungs [10,11]. Therefore, GH may act as a local growth factor in the mammalian lung, since the prenatal life, being an endocrine regulation of lung growth and function throughout development.

Pulmonary Function Tests (PFTs) in ten GHD adult patients was investigated by Merola et al., showing that vital capacity (VC), functional residual capacity (FRC) and total lung capacity (TLC) were significantly reduced compared to healthy subjects, whereas no significant

* Corresponding author. National Research Council of Italy, Institute of Biomedicine and Molecular Immunology "A. Monroy", Via Ugo La Malfa 153, 90146 Palermo, Italy.
E-mail address: giovanna.cilluffo@ibim.cnr.it (G. Cilluffo).
[1] Co-primary authors.

https://doi.org/10.1016/j.rmed.2018.02.019
Received 21 December 2017; Received in revised form 21 February 2018; Accepted 25 February 2018
Available online 26 February 2018
0954-6111/

changes were observed for forced expiratory volume in one second/forced vital capacity ratio (FEV_1/FVC), residual volume (RV) and carbon monoxide diffusing lung capacity (DLco) [5,6]. Subsequently, the same Authors investigated lung volume before and after twelve-month GH replacement therapy (GHT) in twenty adult patient diagnosed as GHD, showing significant improvement of lung volume only in those with childhood-onset GHD, in comparison with whose with adult-onset GHD [5,6].

To date, no study investigated PFTs in GHD children before starting the replacement therapy as well as no study assessed changes in PFTs after a long-course of GHT in order to elucidate the relationship among PFTs, height and GHD.

The aims of this study were: (i) to investigate PFTs in GHD prepubertal children with respect to Controls, before starting GHT (T0); (ii) to evaluate changes in PFTs in GHD with respect to Controls, after 1-year GHT (T1). For both aims the mediation analysis (MA) was applied in order to evaluate the extent to which the relationship between GHD and PFTs could be ascribed to a height-mediated (indirect) or to a GH direct effect.

2. Methods

2.1. Study population

47 newly diagnosed GHD children attending the Section of Endocrine-Metabolic Diseases of the Biomedical Department of Internal and Specialist Medicine (DIBIMIS), University of Palermo, Italy, were recruited since January 2014 to July 2015. In all patients, height, body mass index (BMI) and insulin-like growth factor-I (IGF-I) were measured at baseline (T0) and twelve-month GHT (T1). Patients underwent PFTs both at T0 and at T1. At T0, 47 healthy children matched for age and sex were also studied as Controls.

GHD patients received GHT once a day at bedtime with a pen injection system. Following a published protocol [12], each child, regardless of GH peak, received an initial daily dose of 0.025 mg/kg of GH with a gradual increase to 0.030 mg/kg after 6 months in order to keep the IGF-I levels (μg/l) in the normal range: 85–230 (6–9 years); 98–404 (9–12 years); 142–525 (12–15 years).

The local ethics committee approved the study (*AOUP Paolo Giaccone, Palermo, Italy, N.4/2013*), and a written informed consent was obtained prior to testing from parents or legal guardians. The approved study was inserted into the central registration system ClinicalTrials.gov (identifier: NCT02507245).

2.2. Inclusion and exclusion criteria

The inclusion criteria were: (i) age between 6 and 14 years; (ii) first stage of sexual development (in order to avoid any interference of puberty onset with auxological and biochemical parameters) [13]; (iii) diagnosis of isolated idiopathic GHD established by auxological (height and growth velocity throughout 1 year before the diagnosis), radiological (a bone age delay of at least one year with respect to the chronological age, estimated by an X-ray of the left wrist and hand, evaluated according to the methods of Greulich and Pyle [14]), and biochemical criteria (failure of GH to respond to two stimuli tests (arginine and glucagon) yielding GH peaks below 8 μg/L) of the GH Research Society [15].

The exclusion criteria were: (i) multiple pituitary hormone deficiency; (ii) any other hormonal replacement therapy; (iii) any reported respiratory tract infection within 4-weeks prior to enrollment; (iv) anatomical chest deformities causing airway restriction, e.g. pectus excavatum [16,17]; (v) active smoking.

3. Procedures

3.1. Hormone and biochemical assays

All biochemical data were collected after overnight fasting. Serum GH levels were measured by immunoradiometric assay using commercially available kits (Radim, Italy). The sensitivity of the assay was 0.04 μg/l. The intra- and inter-assay coefficients of variation (CVs) were 2.5–3.9 and 3.8–5.0%, respectively. GH concentrations were reported in μg/l of IS 98/574.

Serum IGF-I levels were measured by a chemiluminescent immunometric assay (Immulite 2000; Diagnostic Products Corp., Los Angeles, CA) using murine monoclonal *anti*-IGF-I antibodies. The standards were calibrated against the World Health Organization second IS 87/518. The assay had an analytical sensitivity of 1.9 μg/l. The intra and inter-assay CVs were 2.3–3.9% and 3.7–8.1%, respectively.

3.2. Pulmonary function tests (PFTs)

PFTs were performed according to the European Respiratory Society and American Thoracic Society (ERS/ATS) standardization document using an appropriate device (Cosmed Quark PFT Rome, Italy) [18]. Spirometry was carried out in the morning under standard environmental conditions. Height (in cm) and weight (in kg) were measured in standing position without shoes, using a stadiometer (Wunder HR1, Italy) and an electronic weighing scale (Seca, Hamburg, Germany). Out of three acceptable tests, the best forced vital capacity (FVC) and flow expiratory volume in the first second (FEV_1) were retained, from which FEV_1/FVC ratio was also computed.

Moreover, all patients underwent multiple-breath N2 washout (MBW), which consists in washing out the N2 from the lungs, while the patient breathes 100% oxygen. The N2 washout ends when the end-tidal gas concentration reaches values below 1/40th of its starting concentration. The following static lung volumes were obtained: functional residual capacity (FRC), residual volume (RV) and total lung capacity (TLC). The volume of air that remains in the lungs after quiet expiration is FRC, while the one remaining after maximal expiration is RV. TLC is the total volume of air present in the lungs after maximal inspiration. Carbon monoxide diffusing capacity (DL_{CO}) was measured by the single breath method. The test begins with a rapid inhalation of a mixture of gas containing helium (He) and a small concentration of carbon monoxide (CO) starting from residual volume and reaching TLC. After a short period of apnea (10 ± 2 s), the subject exhales air: a sample is collected and analyzed for the He and CO concentrations. Alveolar Volume (VA) was obtained by multiplying the inspired volume for the He dilution fraction. DLco/VA, i.e. the transfer coefficient for the diffusion of CO into the blood, was also computed.

PFTs were transformed in percentages of the predicted values using: GLI 2012 reference equations [19] for spirometry (FVC, FEV_1, FEF_{25-75} and FEV_1/FVC), Polgar reference equations [20] for lung volumes (VC, FRC, RV and TLC) and Cotes reference equations [21] for diffusion capacity indices (DLco and DLco/VA).

4. Statistical analysis

Mean values were compared between GHD and Control subjects using *t*-test. Differences of categorical variables were evaluated using Chi-squared test. Differences in PFTs between GHD children at T0 and T1 were assessed through Student's t-test for paired data.

The extent to which the relationship between GHD and PFTs could be ascribed to a height-mediated (indirect) or a GH direct effect was explored by means of Mediation Analysis (MA). It allows to assess whether the relationship between a predictor and an outcome variable can be explained by their relationship with a third, mediator variable. MA was carried out both at T0 and T1, separately by means of