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Abnormal vascular and neural retinal morphology in congenital lifetime isolated growth hormone deficiency

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ABSTRACT

Objective: Experimental models demonstrate an important role of GH in retinal development. However, the interactions between GH and the neuro-vascularization of the human retina are still not clear. A model of untreated congenital isolated GH deficiency (IGHD) may clarify the actions of GH on the retina. The purpose of this work was to assess the retinal neuro-vascularization in untreated congenital IGHD (cIGHD).

Design: In a cross sectional study, we performed an endocrine and ophthalmological assessment of 25 adult cIGHD subjects, homozygous for a *null* mutation (c.57 + 1G > A) in the GHRH receptor gene and 28 matched controls. Intraocular pressure measurement, retinography (to assess the number of retinal vascular branching points and the optic disc and cup size), and optical coherence tomography (to assess the thickness of macula) were performed.

Results: cIGHD subjects presented a more significant reduction of vascular branching points in comparison to controls (91% *vs.* 53% [$p = 0.049$]). The percentage of moderate reduction was higher in cIGHD than in controls ($p = 0.01$). The percentage of individuals with increased optic disc was higher in cIGHD subjects in comparison to controls (92.9% *vs.* 57.1%). The same occurred for cup size (92.9% *vs.* 66.7%), $p < 0.0001$ in both cases. There was no difference in macula thickness.

Conclusions: Most cIGHD individuals present moderate reduction of vascular branching points, increase of optic disc and cup size, but have similar thickness of the macula.

1. Introduction

The visual system is fundamental for the neuro-motor development, environmental adaptation and survival capacity. Visual acuity depends on a well-developed eye that is capable to form the image on the retina and process it in the central nervous system. Body size is heavily influenced by the effect of circulating GH and its main effector IGF-I on bone and cartilage tissues. It has been proposed that retinal development may reflect autocrine or paracrine ocular production of GH, IGF-I, IGF type II (IGF-II) and other peptides like fibroblast growth factor (FGF), vascular endothelial growth factor (VEGF) and platelet-derived growth factor (PDGF) [1–5]. Accordingly, in mice, rats and chickens GH and GH receptor gene expression was documented in developing neural retina [3].

More than a half century has passed since the first description of regression of neovascularization in diabetic retinopathy after pituitary infarction and resultant GH deficiency, which led to the use of hypophysectomy as a therapy for proliferative diabetic retinopathy (GHD) [6]. However, the interaction between GH/IGFs and vascularization of the human retina is still not completely understood [5]. More recently, reduced retinal vascularization was shown in GHD children [7] and in GH insensitivity syndrome (Laron syndrome) [8], suggesting that the GH-IGF-I axis is critical for normal vascularization of the human retina. Nevertheless, in both studies there was some overlap between patients and controls, suggesting that other factors may affect the pattern of vascularization. The consequences of GHD or Laron syndrome in human retina may not be identical. While both models cause very low serum IGF-I levels, in the former there is often some GH secretion, albeit low, whereas the action of GH is completely impaired in Laron

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syndrome. Furthermore, congenital GHD can be isolated or associated to other pituitary deficits, and caused by several genetics or embryological insults, with direct consequences to the visual system [9,10]. Therefore, it is important to assess if individuals with inherited (therefore not caused by intrauterine insults) isolated GHD (IGHD) exhibit abnormalities in retinal neuro-vascularization. However, IGHD is a rare disease, occurring in 1:3480 to 1:10,000 live births [11], and most cases are treated with GH replacement during childhood. We have described in rural Northeastern Brazil a cohort of congenital IGHD (cIGHD) individuals due to a homozygous mutation (c.57 + 1 G > A) in the GHRHR gene (*GHRHR*) [12]. Despite severe short stature with adult height ranging from 1.07 to 1.36 m in pooled genders [13], these individuals cope well with daily challenges, do not exhibit neurodevelopmental problems, and have normal life expectancy [14]. Therefore, we hypothesized that these individuals present satisfactory retinal health, contributing to their normal survival. The objective of this study is to assess retinal neuro-vascularization in these untreated IGD individuals.

2. Subjects and methods

2.1. Subjects

In a cross sectional study, adult treatment-naïve cIGHD subjects, and age and sex-matched controls were recruited by advertising in the local Dwarfs Association, and by word of mouth among inhabitants of Itabaianinha County. Inclusion criterion for cIGHD was genotype-proven homozygosis for the c.57 + 1 G > A *GHRHR* mutation, whereas for controls was proven homozigosity for the wild-type allele. Genotyping method was described before [12]. Exclusion criteria for both groups were previous GH replacement, the presence of other genetic or acquired diseases that could alter the eye fundus appearance, hypertension requiring more than four drugs, diabetic retinopathy and glaucoma. Twenty-five cIGHD and 28 controls volunteered. The Federal University of Sergipe Institutional Review Board approved this study, and all subjects gave written informed consent.

2.2. Endocrine assessment

Before the eye protocol, all the subjects fulfilled a clinical questionnaire and collected blood after an overnight fast for total cholesterol, triglycerides, glucose and IGF-I. Total cholesterol, triglycerides, and glucose were measured by standard techniques. IGF-I was measured by a solid-phase, enzyme-labeled chemiluminescent immunometric assay (IMMULITE 2000, Siemens Healthcare Diagnostics Products Ltd., Malvern, PA, USA), with a with intra- and inter-assay variabilities of 4.2 and 5.1%.

2.3. Study protocol

All individuals underwent a complete eye examination, including applanation tonometry for intraocular pressure (Kowa Applanation Tonometer HA-2, Kowa, Japan), retinography (fundus photography) (Visucam 500, Carl Zeiss Meditec AG, Jena, Germany) and optical coherence tomography (OCT), (Stratus 3000, Carl Zeiss Meditec Inc. Dublin, CA, USA), under drug induced mydriasis. All fundus photographs were digitally recorded, sent to Göteborg, Sweden and analyzed by a unique examiner with clinical long-term experience (A. H.) blinded to the GH status.

2.4. Retinography

Branching point reduction was assessed in all the visualized area in only well-focused photographs where all vessels were clearly visualized (11 cIGHD individuals and 17 controls). Both eyes were examined and there was no discrepancy between the two eyes of each patient regarding vascular branching points. The examiner classified in a blind fashion the branching point reduction in the initial categories: no reduction, mild reduction, moderate reduction and severe reduction. As no cIGHD had no reduction, and no control had mild reduction, we pooled no reduction and mild reduction in the final classification as minimal reduction. The same examiner evaluated the optic disc size (decreased, normal and increased) and cup size (normal or increased) in 14 cIGHD and 28 controls (one control had only the optic disc assessed).

2.5. OCT

Twenty-five cIGHD subjects and 22 controls were analyzed and the average of each right and left macular area were calculated before comparison. Three patients had only one eye examined: two in cIGHD group (one due to toxoplasmosis and another because retinal detachment) and one in control group (severe cataract). We measured the thickness of the fovea and eight more macular areas: inner temporal, inner superior, inner nasal, inner inferior, outer temporal, outer superior, outer nasal, and outer inferior.

2.6. Statistical analysis

Continuous variables were expressed as mean and standard deviation. Categorical variables were expressed in absolute number and percentage. The Student's *t*-test was used to compare continuous variables. Fisher's exact test was used to compare the categorical variables. Statistical analysis was performed using the statistical software IBM®SPSS® Version 20. Probability values <0.05 on a two tailed test were considered statistically significant.

3. Results

Table 1 shows the clinical, biochemical and intraocular pressure in the cIGHD and controls subjects. Only the height, weight and IGF-I levels exhibited severe reduction in cIGHD group in comparison to control subjects. Table 2 shows the vascular branching point reduction in cIGHD subjects and controls in absolute number (n) and percentage (%) in the initial categories. Fig. 1 shows the final classification of the rate of vascular branching point reduction. Fisher's exact test revealed that cIGHD subjects presented more reduction of vascular branching points in comparison to controls ($p = 0.049$). The percentage of moderate reduction in cIGHD was higher than in control (91% *vs.* 53%, $p = 0.01$). Figs. 2 and 3 show that the rates of individuals with increased optic disc and cup size were increased in cIGHD in comparison to controls ($p < 0.0001$ in both cases). The percentage of individuals with increased optic and cup was significantly higher in cIGHD than in controls ($p = 0.005$, and $p = 0.028$, respectively). Fig. 4 shows the vascular branching point reduction and the optical disc and cup increase in an cIGHD individual and in a control. Table 3 shows that there was no difference in the thickness of the macula between the groups (fovea and eight more areas).

Table 1
Clinical, biochemical and intraocular pressure (IOP) in the IGHD and control subjects.

	IGHD	Control	p
Age (years)	50.1 (15.9)	51.1 (14.0)	0.799
Sex (M)	13	15	0.764
Smoking	1	2	1
Height (m)	1.2 (0.18)	1.6 (0.1)	<0.0001
Weight (kg)	39.3 (8.7)	71.8 (14.4)	<0.0001
Systolic BP (mm Hg)	122.1 (18.9)	124.4 (15.0)	0.622
Diastolic BP (mm Hg)	78.8 (9.3)	80.4 (6.5)	0.486
IGF-I (ng/ml)	1.9 (0)	132 (55)	<0.0001
Glucose (mg/dl)	105.5 (22.7)	105.9 (70.5)	0.975
Cholesterol (mg/dl)	227.0 (65.1)	210.7 (28.2)	0.257
Tryglicerides (mg/dl)	142.0 (95.5)	135.3 (51.3)	0.762
IOP (mm Hg)	15.2 (3.1)	14.6 (1.9)	0.510