

**Retinal neural and vascular structure in isolated growth hormone deficiency children and evaluation of growth hormone treatment effect on retina**

**Short Running Title:** Retinal morphology in growth hormone deficiency

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**What is already known on this topic**

Reduced retinal vasculature has been shown in patients with GH deficiency and insensitivity. our results provide additional support for this hypothesis. however, retinal neural morphology in these patients have been evaluated in different studies, which reported different results. Unlike retinal vascularization, data regarding the retinal neural structure are discrepant between different studies, which reported either increased or decreased retinal nerve fibre layer thickness or macular thickness. Additionally there are a limited number studies about the effect of GH treatment on ocular tissues, which evaluated different parameters.

**What this study adds**

Our findings suggest that GH deficiency may lead to decreased retinal vascularization; however, retinal neural growth and differentiation were not affected by GH deficiency. We also evaluated the GH treatment effect on retina. we observed that the GH treatment did not cause any retinal changes.

**Abstract**

**Objective:** Our aim was to evaluate neural and vascular retinal morphology of children with isolated growth hormone deficiency (GHD) and to determine any retinal changes of GH treatment.

**Methods:** Twenty-eight children with isolated GHD and 53 age-, gender- and body mass index-matched healthy volunteers were enrolled in this prospective study. The retinal nerve fibre layer(RNFL), macular thickness(MT) were measured, as well as intraocular pressure(IOP) . The

number of retinal vascular branching points were calculated. GH treatment efficiency on retina and IOP was evaluated in the 1-year of treatment. Measurements were also made in the control group at baseline and following the initial examination. Pre- and post-treatment changes were compared with each other and with the controls. A correlation between ocular dimensions and IGF-I levels were also analysed.

**Results:** The number of branching points was significantly lower in GHD patients compared with control subjects ( $15.11 \pm 2.67$  and  $19.70 \pm 3.37$ , respectively,  $p < 0.05$  for all comparisons). The mean RNFL, MT and IOP in GHD patients were found to be no statistically significant difference than in control subjects. GH treatment did not create any significant changes in the retinal vascularization or other retinal neural parameters and IOP either within the patient group or when compared with the control group. No correlations were observed between ocular dimensions and IGF-I levels.

**Conclusion:** Our findings suggest that isolated GHD may lead to decreased retinal vascularization. However, retinal neural growth and differentiation were not affected by GHD. These findings may be related to fetal development process of pituitary somatotrophic cells and retina. Additionally, GH treatment did not cause any changes on retinal neural and vascular tissues.

**Keywords:** Growth hormone deficiency, retinal neural development, retinal vascularization, growth hormone treatment.

## INTRODUCTION

Growth hormone (GH) is generally regarded as an endocrine hormone that is released from the pituitary somatotrophs into the circulation and is essentially required for postnatal growth and development. However, this belief has been challenged by the experimental models who have demonstrated the presence and effect of pituitary GH in many extrapituitary sites, including the nervous, reproductive, immune and vascular systems (1,2). Ocular neural and vascular system is one of them.

The possible role and effect of pituitary GH and IGF-I, which mediates many aspects of GH, on retinal development is controversial. Recently, a limited number of human studies has also been emphasized its functional role. Although GH and various growth factors (i.e., IGFs, VEGF, FGF and TGF $\beta$ ) are often thought to be produced locally and act in autocrine/paracrine ways to promote the maintenance, survival and differentiation of retinal tissues, this is partially true for the vascularization and neurogenesis of the retina before the functional differentiation of pituitary somatotrophs (3).

Abnormal ocular findings such as optic nerve hypoplasia, disc dysfunction, increased corneal thickness, reduced retinal vascularization and short axial length in GHD patients exemplify GH's effects on the developing ocular tissues (4-8). Similarly, pituitary GH in the human retina and vitreous fluid can also provide further evidence for a possible role of GH in the ocular development (9,10). Consequently, ocular tissues seem to represent a target site for pituitary GH action as suggested by several human and animal studies. Based on these studies, we wanted to evaluate retinal neural and vascular structure in isolated GHD patients. The another objective of this study is to assess any retinal changes under the GH treatment.

## METHODS

This prospective study consisted of 28 patients with severe short stature (height SDS less than -3) at diagnosis and whose growth velocity was lower than 4cm/year or below expectations for the pubertal stage. Patients were excluded if they had a history of being a preterm or were small for their gestational age at birth; a diagnosis of cardiovascular, thyroid, hepatic or renal disease, obesity, current hypertension, chromosomal abnormalities in addition to their already known ocular disease, severe refractive errors, or a family history of ocular hypertension/glaucoma.

Pubertal staging was assessed by the Tanner stage according to breast development in girls and genital development in boys (11). Routine biochemical tests, complete blood counts, thyroid

function tests and serum tissue transglutaminase antibodies were obtained in all patients. Bone age was evaluated by using the Greulich and Pyle atlas. Pituitary MRI was performed in all to exclude the presence of structural anomaly. After then, all were underwent GH stimulation test. A GH stimulation test was performed with L-Dopa and Clonidine. IGF-I and IGFBP-3 levels were also measured. Peak GH responses after two stimulation tests below 10 µg/l were regarded as GH deficiency. Thereafter, the diagnosis of GHD was established by the clinical, auxological and biochemical criteria of the GH Research Society (12). In diagnosed patients, recombinant human GH treatment was started at the initial daily dose 0.025 mg/kg. During the study, IGF-I and IGFBP-3 levels were measured intervals of 3-6 months and GH dose was adjusted to maintain serum IGF-1 levels above + 2SD, but to not exceed + 3 SD levels.

Fifty-three healthy children who were carefully matched for age, gender, body mass index (BMI) and pubertal staging were recruited as the control group from the siblings of patients and children who had presented to the health care unit for a routine examination. Healthy volunteers were recalled in the first year following initial examination.

The study protocol was approved by the ethics committee of our hospital. Parents of the patients and controls were informed about the study and informed consent was obtained.

### **Assessment of Retinal Vascularization and Retinal Thicknesses**

All patients and controls underwent a complete ophthalmologic examination, including an auto-refractometer (RM8900; Topcon), best-corrected visual acuity (BCVA) measurements with a 6-m Snellen eye chart, slit-lamp biomicroscopy, fundus examination and intraocular pressure (IOP) measurement. The subjects also underwent an examination with the Heidelberg Spectralis-OCT (Spectralis; Heidelberg Engineering, Heidelberg, Germany). Central subfield macular thickness (MT) and peripapillary retinal nerve fibre layer (RNFL) thickness were assessed to evaluate retinal neurogenesis. The RNFL measurements were determined globally and for six regions including temporal, superotemporal, superonasal, nasal, inferotemporal and inferonasal. The number of retinal vascular branching points was obtained from infrared images. Optic nerve head (ONH) centered Near-infrared reflectance (IR) pictures were obtained with a 30° field of view. A frame to calculate retinal vascular branching points was set for each eye to minimize individual disparity. Temporal border of the fovea was defined as the temporal border of the frame. The other borders was calculated as 4000 µm away from the center of ONH.

RNFL thickness, MT and retinal vascularity were evaluated again after 12 months of treatment and determined any retinal changes, as well as IOP. Additionally, the correlation of changes with IGF-I levels were calculated. The controls were also called back one year following their initial examination to observe the retinal changes associated with age and puberty.

### **Statistical Analysis**

All statistical calculations were performed using SPSS version 20 (SPSS, Inc., Chicago). Subjects' right eyes were selected for statistical analysis. Descriptive statistics were computed as means ± standard deviations (SD). Parameters with normal distribution were analysed with t test and parameters with non-normal distribution were analysed with Mann-Whitney U test. Differences between values before and after GH treatment were evaluated using paired samples t-tests. The linear relationships between variables were evaluated using Spearman's correlation tests. A p value < 0.05 was considered statistically significant.

### **RESULTS**

A total of 28 (female:male = 14:14) isolated GHD patients and 53 (female:male = 33:20) age- and gender-matched healthy children aged 12.46± 2.41 and 11.32 ± 3.1 years, respectively, were included in the study. No statistical difference was observed among the groups in terms of age, gender, BMI (p>0.05). Puberty was compatible with Tanner stage 1 in 9 patients, and in 19 control subjects (p>0.05). Others had advanced pubertal development. Routine laboratory tests, thyroid function tests and serum tissue transglutaminase antibodies were within normal limits. Pituitary MRI was normal in all patients.

The best corrected visual acuities of all subjects were 6/6. None of the differences in spherical equivalent, MT and four quadrant RNFL thickness, IOP among patients and control subjects were significant ( $p>0.05$  for all). The mean number of vascular branching points was  $15.11 \pm 2.67$  in the study group and  $19.70 \pm 3.37$  in the control group ( $p<0.01$ , figure-1). All ocular parameters of both groups are compared in Table-1.

At the end of the first year, eye parameters of all 28 patients were checked. According to this any significant changes were not observed in the MT, RNFL thickness, IOP and the number of vascular branching points with treatment ( $p>0.05$ ; Table-2). In one GHD patient, optic disc drusen were detected and the patient was followed.

Additionally, the retinal vascularization, IOP, MT and RNFL thickness did not disclose any significant correlation with an increase in IGF-I levels ( $r=0.003$ ;  $r=0.12$ ;  $r=-0.06$ ;  $r=0.16$ , respectively,  $p>0.05$  for all).

The first year evaluation of the control group could be performed in only 17 subjects. No significant differences in the secondary ocular parameters regarding the pubertal development and age were observed in these subjects. The statistical result of the initial comparison was not different from the statistical result of the secondary comparison in which we compared the retinal measurements of the patients that were under treatment.

## DISCUSSION

In this prospective study, we have essentially examined the retinal neural and vascular structures in patients with isolated GHD and whether GH treatment will cause any retinal change. We observed that the MT, RNFL thickness of patients was not different from the healthy controls, while retinal vascularization decreased. On the other hand, GH treatment did not cause any retinal neurovascular changes.

Firstly, Hellström et al. reported reduced retinal vasculature among isolated GHD patients and later suggested the importance of GH and IGF-I for normal retinal vascularization in another one of their studies (7). More recently, Pereira-Gurgel et al reported moderate reduction of retinal vascular branching points in isolated GHD patients (13). Another recent study also evaluated the effects of the GH/IGF-I axis on retinal vascular branching and characteristics in patients with GH insensitivity and shown that reduced retinal vasculature and tortuosity of the retinal vessels (14). Similarly, our patients were shown reduced retinal vasculature. Based on these data that have parallel results, we can suggest that the GH/IGF-I axis has an effect on the retinal vasculature. However, we cannot suggest that the pituitary GH has a similar effect on retinal neural development. In fact, GH and GH mRNA proteins have also been identified in retinal ganglion cells and can be traced within their axons in the retina within the optic fibre layer and outside the retina within the optic nerve, optic chiasm and optic tract (10,15,16). In this case, we expected that the GHD would lead to greater thinning of the MT and RNFL thickness, but it did not, although the decreased peripapillary nerve fiber thickness and decreased optic disc size were previously described in some children with congenital GHD (17,18).

The reasons for these differences are probably caused by the variety of causes of GHD, and different methods used.

Why retinal neural structure was shown normal development while retinal vascularization decreased in GH deficiency could be partially explained through embryonic development process of these structures and somatotrop cells. GH producing cells can be identified at 9 weeks of gestation (19), whereas embryonic neural development occurs in an earlier embryonic period. Therefore, the nervous system, showing normal development despite the lack of pituitary GH, should be considered that as an extrapituitary GH production site. Our findings also suggested that locally produced growth hormone and factors are more effective in neural retinal development. This possibility is supported by the presence of GH immunoreactivity in the brain prior to the ontogeny of the pituitary gland and somatotroph differentiation, as demonstrated in the human and chicken brain (20,21). Whereas vascularization of the retina normally starts at approximately 12 weeks of gestational age, while the pituitary somatotroph GH production has already begun and continues during fetal development, with little or no vascularization after birth (22,23). Normally, it is expected to affect more by IGF-II when

fetal somatic and ocular development considered (24). Although, angiogenic GH and IGF-I effect on retinal vessels cannot be ignored. this hypothesis was already supported by several studies, showing decrease in retinal vascularization on patients with GHD and GH insensitivity.

The another finding of this study is that the GH treatment did not create any significant retinal changes, as well as IOP. The effect of a lack of GH treatment on retina could be explained by various proposals. One proposal suggests that the GH and IGF-I cannot pass from the inner retinal barrier. Other proposals suggest that normalized GH and IGF-I levels might not enough to any retinal changes or the follow-up time may be inadequate to evaluate retinal changes.

Previous data suggesting induction of neovascularization by IGF-I refer to clinical conditions where an excess of IGF-I is present in the serum (25-28). Treatment with GH, on the other hand, aims to normalize IGF-I levels as much as possible, and is not expected to induce a sustained excess of IGF-I as in acromegaly or patient with diabetic retinopathy. Another explanation may also be related to the impaired of retinal blood barrier permeability and/or integrity in diabetic retinopathy.

Our study has some limitations, just as in other similar studies, the lack of genetic diagnosis was the most significant limitation. the other one was the use of a semi-quantitative system to assess the retinal vascularization. The long-term treatment outcomes should be evaluated to determine correct and more information about the effect of treatment on retina. this was the potential limitation of our study.

#### **CONCLUSION:**

The current study, to the best of our knowledge, is the first comprehensive, prospective study to evaluate retinal structure (neural and vascular) in isolated GHD children. A selective reduction of retinal vascularization and normal retinal neural architecture may suggest that the GH/IGF-I axis regulates retinal vascular development, but not the neural retina. Besides, our findings suggested that GH treatment is not associated with retinal changes. However, monitoring time, treatment dose, genetic cause of GHD are the important points to be taken into consideration while saying that the treatment is ineffective. We recommend, therefore, that ophthalmologic evaluations should be performed in all GHD patients before institution of GH treatment and repeated every year. Also, further studies with larger groups are required to clarify the functional role of GH on ocular growth and differentiation by using advanced measurement techniques, as well GH treatment.

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**Table 1: Ocular parameters of patient and control groups at baseline**

Variables	Groups		
	Patient(n=28)	Control (n=53)	p value
IOP	16.18 ± 2.79	15.47 ± 2.55	0,27
CSFMT	268.07 ± 18.7	266.38 ± 20.94	0,711
N	70.21 ± 13.44	70.58 ± 10.39	0,899
I	243.18 ± 42.16	255.19 ± 26.15	0,177
T	73.32 ± 8.87	74.53 ± 10.79	0,592
S	240.75 ± 41.64	250.09 ± 28.35	0,293
G	96.39 ± 12.28	99.72 ± 8.3	0,206

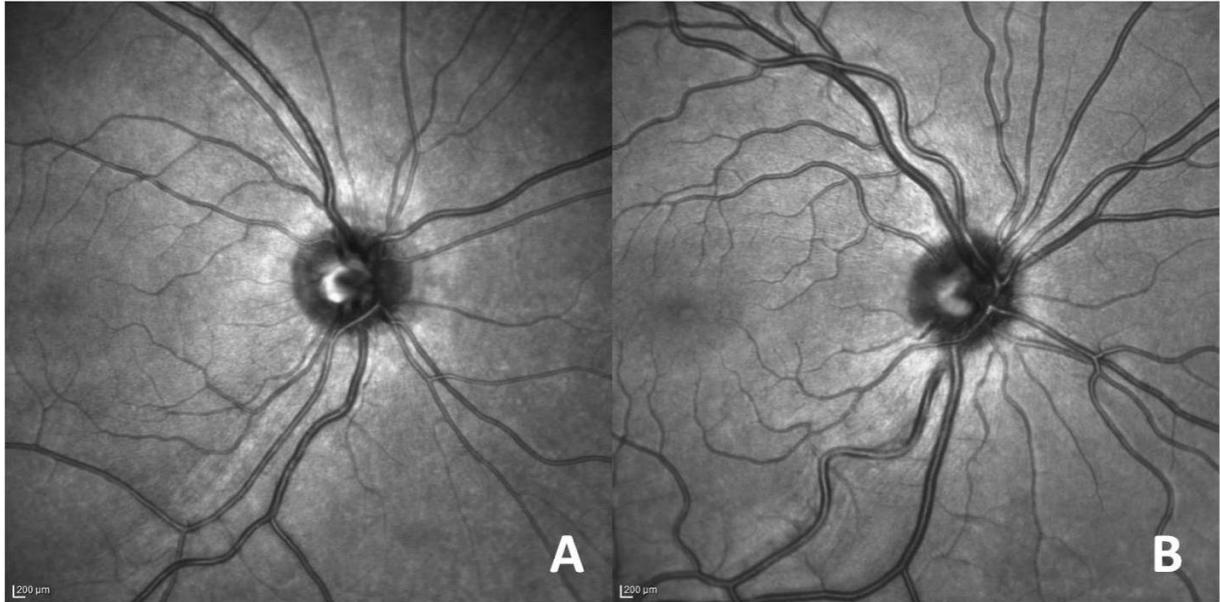
<b>Number of branching points</b>	<b>15,11 ± 2,67</b>	<b>19,70 ± 3,37</b>	<b>&lt;0,001</b>
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*n*:number IOP:Intraocular pressure, CSFMT: Central subfield macular thickness Peripapillary retinal nerve fiber layer; N:nasal, I:inferior,T:temporal, S:superior, G:global

**Table 2: Ocular parameters of patients before and after 1-year of GH treatment**

<b>Variables</b>	<b>Patients(n:28)</b>		<b>p value</b>
	<b>Before treatment</b>	<b>After treatment</b>	
<b>IOP</b>	<b>17.28± 1.90</b>	<b>16.46±1.68</b>	<b>0,093</b>
<b>CSFMT</b>	<b>260.14± 20.15</b>	<b>260.79± 21.86</b>	<b>0,661</b>
<b>N</b>	<b>69.71 ± 12.04</b>	<b>72.93± 14</b>	<b>0,08</b>
<b>I</b>	<b>253.64 ± 35.5</b>	<b>241.36± 44.5</b>	<b>0,157</b>
<b>T</b>	<b>71.36 ± 7.38</b>	<b>71.21± 9</b>	<b>0,889</b>
<b>S</b>	<b>238.71± 48.53</b>	<b>238.57 ± 47</b>	<b>0,976</b>
<b>G</b>	<b>94.57± 10.17</b>	<b>95.93 ± 13.22</b>	<b>0,254</b>
<b>Number of branching points</b>	<b>14.43 ± 2.1</b>	<b>13.14 ± 1.9</b>	<b>0,079</b>

*n*:number, IOP:Intraocular pressure, CSFMT: Central subfield macular thickness, Peripapillary retinal nerve fiber layer; N:nasal, I:inferior,T:temporal, S:superior, G:global



**Figure 1:** In infrared images; A: Reduced retinal vascularization in 10 year old GHD patient, B: Normal retinal vascularization in 10 year old healthy subject

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