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Assessment of retinal thickness as a marker of brain masculinization in children with congenital adrenal hyperplasia: a pilot study

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Abstract

Objective: To investigate the relationship between brain masculinization and retinal thickness in children with congenital adrenal hyperplasia (CAH).

Methods: Forty-five patients with CAH aged between 4 and 18 years and 30 age-matched healthy controls were included in this prospective study. Macular area was examined with optical coherence tomography (OCT); central subfield thickness (CST), cube volume (CV) and macular retinal thickness (MT) were measured in each subject. A gender identity questionnaire (GIQ) was used for the evaluation of gender happiness index.

Results: Girls with CAH had a higher CV ($p = 0.002$) and MT ($p = 0.003$) than healthy girls. No significant difference was found between boys with CAH and healthy boys regarding the retinal thickness measurements. Mean CST, CV and MT were significantly higher in boys than in girls in the control group ($p = 0.013$, $p < 0.001$, respectively), but there was no significant difference in those parameters between girls and boys with CAH. The gender happiness index was not different between healthy boys and boys with CAH, but was significantly lower in girls with CAH than healthy girls ($p = 0.01$).

Conclusions: As retina is part of the brain, our finding appears to be a morphological evidence of the excess androgen exposure on brain structures in girls with CAH. In addition, we suggest using retinal thickness measurements as a marker of prenatal excess androgen exposure in future studies.

Keywords: congenital adrenal hyperplasia; disorders of sex development; gender dysphoria; retinal thickness.

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Introduction

Disorders of sex development (DSD) are defined as congenital conditions in which development of chromosomal, gonadal or anatomical sex is atypical [1]. Congenital adrenal hyperplasia (CAH) constitutes the majority of all DSD patients presenting with genital ambiguity [2]. 21-Hydroxylase deficiency (21-OHD), the most common form of CAH, disrupts glucocorticoid and mineralocorticoid synthesis and leads to glucocorticoid deficiency, as well as clinically apparent mineralocorticoid deficiency in two-thirds of patients. Reduced glucocorticoid feedback to the hypothalamic-pituitary axis results in increased adrenal androgen production, subsequent systemic androgen excess and ambiguous genitalia [3].

The process of sexual differentiation of the human brain has not been completely elucidated yet, but experimental studies revealed that prenatal cerebral exposure to critical sex hormones is crucial for the development of sexual behavior and sexually dimorphic brain anatomy [4]. Rat studies showed an association of brain masculinization with the development of retina [5–9]. Salyer et al. [10] showed that retinal thickness was influenced by perinatal hormone manipulation via aromatizable androgens in rats. Furthermore, clinical and population-based

studies have documented differences between the sexes in macular retinal thickness (MT) with boys having thicker retinas than girls [11].

In this study, we investigated the relationship between brain masculinization and retinal thickness in children with 21-OHD and age-matched healthy controls. To this end, we examined the macular area with laser ophthalmoscope-optical coherence tomography (OCT) and used a gender identity questionnaire (GIQ) for the evaluation of gender happiness index.

Materials and methods

Subjects

Forty-five patients with classical 21-OHD (40 salt losing, five simple virilizing), aged between 4 and 18 years (mean age, 11.0 ± 3.5 years) were included in this prospective study. Eighteen of them (40%) had 46,XY karyotype and 27 (60%) had 46,XX karyotype. Assigned genders of all cases were compatible with their karyotype. The control group consisted of 30 age-matched healthy children (15 males, 15 females and mean age, 11.0 ± 4.4 years). The participants who had congenital ocular abnormalities, low OCT signals, alignment problems, and intraocular pressure more than 21 mmHg were excluded. The study (No.: 5-2013) was approved by the Ethics and Research Committee of Kanuni Sultan Suleyman Training and Research Hospital and the procedures followed were in accordance with the ethical standards of the institution on human experimentation. A written informed consent was obtained from the parents of every participant.

Retinal thickness

One ophthalmologist, who was blind to the clinical status of the subjects, examined the macular area with laser ophthalmoscope-OCT (OPKO/OTI Spectral OCT/SLO, OPKO Health, Inc., Miami, FL, USA). OCT was shown to provide reliable, accurate, and repeatable measures in children [12–15]. MT scans were performed through dilated pupils with three cycles of tropicamide 0.5% (one drop), administered 5 min apart. The three-dimensional volumetric macular scan protocol covered a 6 × 6 mm^2 area centered on the fovea with 512 A-scans × 256 B-scans in 0.8 s. Choroidal thickness was mapped using the Early Treatment Diabetic Retinopathy Study grid, which comprised inner and outer rings (diameters, 1–3 mm and 3–6 mm, respectively) divided into four quadrants: superior, inferior, temporal, and nasal. The grid was divided into nine subfields: inner and outer temporal, inner and outer superior, inner and outer inferior, inner and outer nasal, and the central area (Figure 1). We measured mean central subfield thickness (CST), cube volume (CV) and MT in each subject.

Gender identity questionnaire

A 16-item parent-report GIQ was administrated by the same pediatric psychiatrist. This GIQ was originally developed by Elizabeth and Green [16] and validated by Johnson et al. [17] with a specificity of 95% and a sensitivity of 86.8%.

Statistical analysis

Statistical analysis was done using SPSS 19.0 software (IBM, Armonk, NY, USA). Independent t-test was used for comparing means. Data were presented as mean ± standard deviation (SD) of the mean and a p-value of <0.05 was considered significant.

Results

Girls with CAH had a higher CV and MT than healthy girls (Table 1), but no significant difference was found between boys with CAH and healthy boys regarding the retinal thickness measurements (Table 1). Mean CST, CV and MT were significantly higher in boys than in girls in the control group (Table 2), but there was no significant difference in those parameters between girls and boys with CAH (Table 2).

The gender happiness index was lower in girls with CAH than healthy girls (Table 3), but it was not statistically different between boys with CAH and healthy controls (Table 3). All children in the control group and all boys with CAH were satisfied with their gender, but two girls with CAH had gender dysphoria and five girls had tomboy identity. There was no significant correlation between retinal thickness measurements and gender happiness index scores in both genders (data are not shown).

Discussion

We observed that girls with CAH had a thicker retina than age-matched healthy controls, nearly as thick as boys with CAH and healthy boys. In contrast, retinal thickness was not significantly different in boys with CAH than healthy boys. To the best of our knowledge, this is the first study showing increased retinal thickness in girls with CAH. In previous studies, retinal thickness was reported to be significantly greater in male compared to female subjects [11]. In addition, sex steroid receptors and enzymes metabolizing steroids have been identified on the retina. Although the role of these steroids in humans has not been fully identified yet, sexual differentiation of the brain was shown to have an association with biomorphic differentiation and development

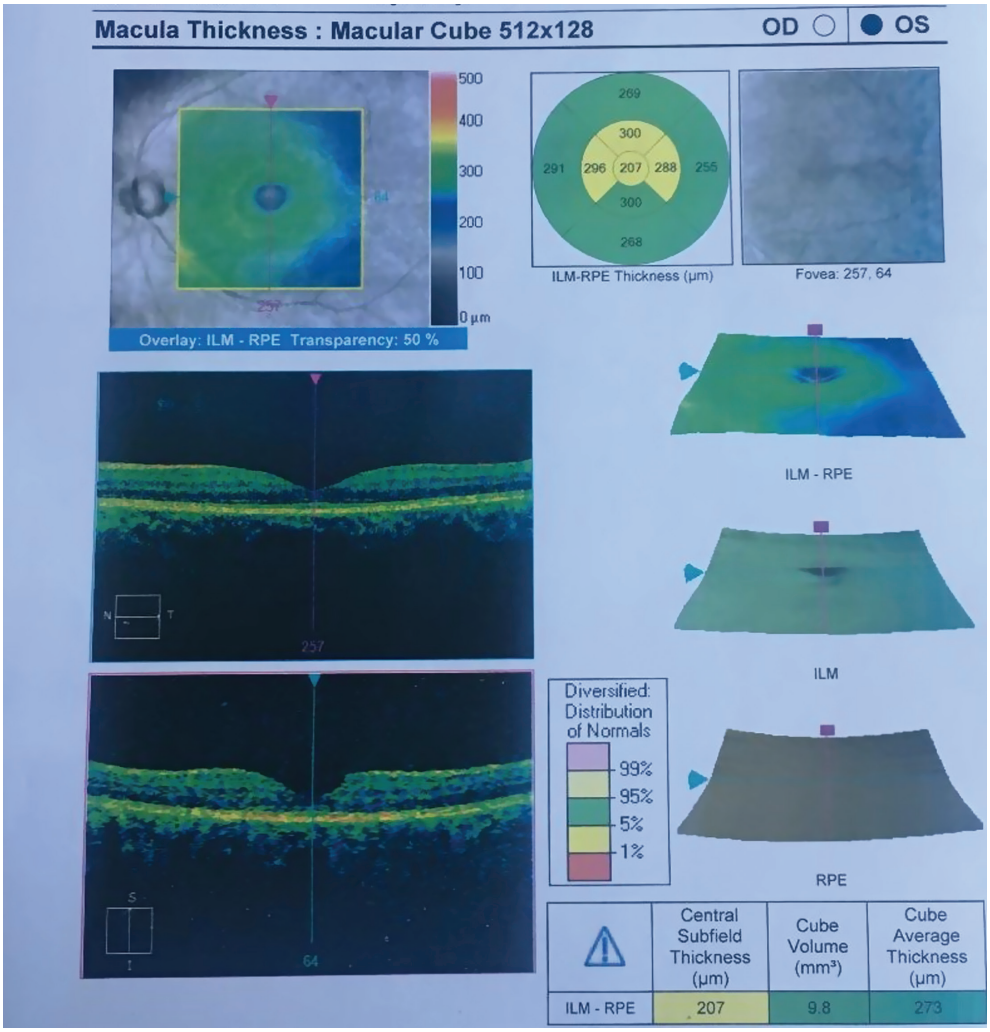

Figure 1: The macular area measurement using laser ophthalmoscope-optical coherence tomography.

of retina in rat studies [5–9]. As retina is part of the brain, our finding appears to be a morphological evidence of the excess androgen exposure on brain structures in patients with CAH.

Exposure of the developing brain to sex steroids during fetal life influences postnatal gender and sexual development, including sexual orientation [16]. Gender dissatisfaction or gender dysphoria denotes unhappiness with assigned sex, and occurs more frequently in DSD patients. The biological mechanisms of this condition have not been fully discovered yet and it is not always predictable from the degree of genital virilization [18, 19]. Gender dysphoria is more often observed in subjects assigned to the female sex with considerable prenatal or postnatal androgen exposure [20]. It was reported that the rates of bisexual and homosexual orientation were increased in women with CAH correlated with the degree of prenatal androgenization [21]. Dessens et al. [22] reported that the majority of the patients with CAH raised female later developed a gender identity as women, but 5% had serious problems with their gender identity. Pasterski et al. [23] suggested that early androgens have a masculinizing effect on both aggressive behavior and activity level in girls with CAH rather than boys. Additionally, boys with CAH do not show increased masculinization of toy, playmate or activity preferences as well as alterations in gender identity [24]. The absence of increased male-typical behavior in boys with CAH was suggested to occur because androgen concentrations in boys with CAH are largely similar to those in boys without CAH prenatally [24]. In accordance with previous studies, the gender happiness index was lower in girls with CAH than their age-matched healthy peers in our study group. In addition, two girls with CAH had gender dysphoria and five had tomboy identity. However, all children in the control group and all boys with CAH were satisfied with their gender.

Table 1: Comparison of retinal measurements between patients with congenital adrenal hyperplasia and healthy controls.

Gender	Group	n	Mean	Standard deviation	p-Value
Male					
Central subfield thickness, mmol	Patient	18	252.9444	25.73273	0.95
	Control	15	252.5333	16.37449	
Cube volume, mm^3	Patient	18	10.2444	0.45532	0.43
	Control	15	10.3600	0.36606	
Macular retinal thickness, mmol	Patient	18	286.2778	12.94243	0.33
	Control	15	290.2667	9.72821	
Female					
Central subfield thickness, mmol	Patient	27	244.0000	20.38287	0.17
	Control	15	234.9333	19.60855	
Cube volume, mm^3	Patient	27	10.2000	0.46904	**0.002**
	Control	15	9.74	0.38	
Macular retinal thickness, mmol	Patient	27	285.5556	13.18605	**0.003**
	Control	15	272.8000	10.81797	

A p-value <0.05 is accepted as significant and indicated with bold letters.

Table 2: Comparison of retinal measurements of boys and girls in patients with CAH and healthy controls.

Group	Referred gender	n	Mean	Standard deviation	p-Value
Patient					
Central subfield thickness, mmol	Female	27	244.0000	20.38287	0.20
	Male	18	252.9444	25.73273	
Cube volume, mm^3	Female	27	10.2000	0.46904	0.75
	Male	18	10.2444	0.45532	
Macular retinal thickness, mmol	Female	27	285.5556	13.18605	0.85
	Male	18	286.2778	12.94243	
Control					
Central subfield thickness, mmol	Female	15	234.9333	19.60855	0.013
	Male	15	252.5333	16.37449	
Cube volume, mm^3	Female	15	9.74	0.38	<0.001
	Male	15	10.3600	0.36606	
Macular retinal thickness, mmol	Female	15	272.8000	10.81797	<0.001
	Male	15	290.2667	9.72821	

Table 3: Comparison of gender identity questionnaire scores between patients with congenital adrenal hyperplasia and healthy controls.

	Group	n	Mean	Standard deviation	p-Value
Male					
Gender identity questionnaire scores	Patient	18	3.94	0.175	0.20
	Control	15	4.00	0.00	
Female					
Gender identity questionnaire scores	Patient	27	3.69	0.437	0.01
	Control	15	4.00	0.00	

Study limitations

This is a pilot study with small numbers of children; this would be the reason for lack of correlations between retinal thickness measurements and gender happiness index scores in both genders.

Conclusions

We showed that girls with CAH had a higher macular retinal thickness than age-matched healthy controls. As retina is part of the brain, our finding appears to be a morphological evidence of the excess androgen exposure on brain structures in girls with CAH. New OCT techniques are good tools for noninvasive, *in vivo*, high-resolution visualization of the retina. We suggest using retinal thickness measurements as a marker of prenatal excess androgen exposure in future studies.

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