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Retinal Nerve Fiber Thickness Measurements in Choroideremia Patients with Spectral-Domain Optical Coherence Tomography

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Abstract

Aims—To evaluate the presence of peripapillary retinal nerve fiber layer (RNFL) defects in patients with choroideremia by using spectral-domain OCT (SD-OCT).

Methods—Twenty-nine eyes of 16 patients with choroideremia underwent peripapillary RNFL thickness measurements by using SD-OCT.

Results—The mean ($\pm$ SD) age of the study population was 44.0 ± 16.0 years (range, 13–63 years). Thirteen eyes (45%) showed a thinning of the peripapillary RNFL in at least one quadrant in either one or both eyes. Thinning was most commonly found in the superior (13 eyes) and inferior (10 eyes) quadrants. Twenty-one eyes (72%) showed a thickening of the peripapillary RNFL in at least one quadrant in either one or both eyes. Of these 21 eyes, all had thickening in the temporal quadrant. Additionally, two eyes in each of the other three quadrants were found to be abnormally thick.

Conclusion—Our study demonstrated the presence of defects in the peripapillary RNFL thickness in patients with choroideremia by using SD-OCT. It would be clinically prudent that choroideremia patients considered for various treatment options be considered for RNFL thickness measurements.

Keywords

Retinal nerve fiber layer; Choroideremia; Spectral-domain OCT

Introduction

Choroideremia (CHM) is a generalized progressive chorioretinal degeneration inherited in an X-linked recessive manner.¹ The onset of symptoms including night vision complaints and peripheral visual field loss usually occurs in the first or second decade of life. The fundus changes in the affected individual undergo a typical progression. The initial appearance is a “salt and pepper” pigment mottling at the equator and posterior pole. At this stage, the electroretinogram is often abnormal, showing a reduced or absent scotopic component, and final dark-adapted perimetric thresholds are elevated.² There are focal disturbances in the retinal pigment epithelium (RPE), consisting of pigmentary loss or a metallic sheen. However, the underlying choroid may appear normal. Atrophy of the choroid follows with eventual loss of the entire layer and exposure of bare sclera. These changes begin in the mid-periphery and progress centrally, the macula being less affected with

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central vision preserved until late in the disease. In the final stage, the entire fundus shows a diffuse yellowish-white reflex of the sclera.³

CHM is caused by mutations in the *CHM* gene, which encodes Rab escort protein-1 (REP-1), an escort protein involved in the prenylation of Rabs. Under-prenylation of certain Rabs, as a result of loss of function mutations in REP-1, could affect vesicular trafficking, exocytosis, and secretion in peripheral cells of CHM patients.^{4,5,6} Histopathologic studies show that there is a loss of the outer and midretinal layers and RPE. Bruch's membrane may remain, as well as remnants of the inner retinal layers.^{7,8,9} In CHM, patients usually have peripapillary chorioretinal tissue preservation until later stages. To our knowledge, only one case report,¹⁰ which used scanning laser polarimetry with serial analysis, has documented a retinal nerve fiber layer (RNFL) "retardation" (thinning) in a choroideremia patient.

Spectral-domain (SD) optical coherence tomography (OCT) technology provides better scan resolution and a greater number of scans acquired at a faster rate than time-domain (TD) OCT technology.^{11,12} These improvements have the potential to provide clinicians with enhanced tools for the diagnosis and follow-up of patients with retinal and optic nerve pathology. The purpose of the current study was to use high-speed, high-resolution SD-OCT to evaluate the presence of any RNFL defects in patients with CHM.

Patients and Methods

Participants

Twenty-nine eyes of 16 patients with a diagnosis of CHM were included in this prospective investigative study. The participants were recruited for examination during the period from February 1st, 2010, through April 31st, 2010 at the Department of Ophthalmology at the University of Illinois at Chicago. The study was conducted in accordance with the ethical standards stated in the 1964 Declaration of Helsinki and was approved by an institutional review board at the University of Illinois. Informed written consent was obtained from all participants.

The diagnosis of CHM was based on the patients' history of night blindness, variable degrees of peripheral field restriction, the characteristic fundus findings (including choroidal and RPE degenerative changes throughout the posterior pole and mid-peripheral retina), and an abnormal full-field electroretinogram was obtained on 13 of 16 patients.

Patients with a diagnosis of CHM who were seen by two of the authors (MAG & GAF) during their routine clinical follow-up examinations were included in the present study if they were willing to undergo SD-OCT testing. Additional patients previously seen by one of the authors (GAF) were contacted by telephone and asked to participate in the study based on their prior diagnosis of CHM.

Inclusion and Exclusion Criteria

Participants had to meet the following inclusion criteria: refractive error not exceeding ± 6.00 diopter (D) spherical equivalent and ± 2.00 D astigmatism and transparent ocular media (nuclear color/opalescence, cortical or posterior subcapsular lens opacity < 1) according to the Lens Opacities Classification System III system.¹³ Subjects with history of optic neuropathy, any other retinal diseases other than CHM, past history of glaucoma and/or increased intraocular pressure (IOP) of ≥ 21 mmHg, prior history of intraocular surgery except for uncomplicated cataract extraction, and poor OCT image quality were excluded from the study.

Ocular Examination

All participants underwent a complete ocular examination, including best-corrected visual acuity (BCVA) using an early treatment diabetic retinopathy study screening chart (ETDRS) in most of the patients [except 1 patient where a Snellen chart was used and converted to log MAR visual acuity during the data analysis], slit-lamp biomicroscopic examination, and Goldmann applanation IOP measurement. Dilated fundus examination was done by using both direct and indirect ophthalmoscopy. Any optic disc pallor was recorded and graded as (mild, moderate, and severe) by two of the authors (MAG & GAF).

SD-OCT Examinations

All subjects included in the current study underwent RNFL measurements using an OPKO spectral-domain OCT/SLO instrument (OPKO Instrumentations, Miami, FL), which utilizes SD technology to acquire high resolution OCT data. It has 5 μm axial resolution, 15 μm transverse resolution, and scans at 27 000 A-scans per second. The instrument is a combination OCT and confocal scanning laser ophthalmoscope (SLO) designed to image vitreous, retinal, and choroidal structures. The system has point-to-point registration with eye tracking features.

For the peripapillary RNFL measurements, the RNFL exam protocol was used for scan acquisition, which was measured automatically by the existing software at a diameter of 3.45 mm around the center of the optic nerve. The RNFL scan acquisition consists of three single circle scans acquired consecutively and subsequently averaged.

The peripapillary RNFL thickness parameters calculated by OPKO software and evaluated in this study were: average thickness (360 degrees), temporal quadrant thickness (316–45 degrees), superior quadrant thickness (46–135 degrees), nasal quadrant thickness (136–225 degrees), and inferior quadrant thickness (226–315 degrees). The OCT software automatically measured 3 smaller segments within each quadrant (total of 12 segments and 4 quadrants).

The peripapillary RNFL thickness was measured at least 3 times in each eye, and data were used only if the scans were reproducible, within either a normal or abnormal range, in at least 2/3 of the acquisitions. Also, eyes with scans that were inconsistent on the first set of 3 OCT RNFL scans, were rescanned with another set of 3 scans. We then used the criteria that 2/3 of these scans should be consistent in order to be included in the analysis.

Internal fixation was used for all scans. Scans with signal strength <6 were excluded, as per manufacturer's instructions. In addition, criteria for determining scan quality as reported in previous studies were followed.^{14,15} The optic disc and scan circle were visible on the fundus image, color saturation was even and dense throughout the entire scan with red color visible in the RPE and the RNFL was visible with no missing or blank areas within the scan pattern.

Data Analysis

The total peripapillary RNFL thickness was considered abnormally thin if its value was less than the 5th percentile of the age-corrected normal value provided by the manufacturer and thicker if it was more than the 95th percentile. Abnormal thinning or thickening of the RNFL in at least one quadrant, consistently in at least 2/3 of the RNFL OCT scans was considered significant and therefore abnormal.

We measured the average of RNFL thickness $\pm$ standard deviation (SD) in all 4 individual quadrants and compared these values with age-corrected normative data provided by the

manufacturer that were comprised of 245 eyes of 123 visually normal subjects aged 19 to 85 years (mean [SD] age was 43.1[14.9] years). Statistically significant differences in the mean peripapillary total circumferential RNFL thickness between our cohort and that from normative control data were analyzed using the two-tailed *t*-test. Additionally, linear regression was used to evaluate the effect of age on the overall peripapillary RNFL thickness measurements.

Results

Our study cohort included 29 eyes of 16 patients with choroideremia with 3 eyes excluded due to the presence of an old traumatic retinal detachment in one eye and poor ocular fixation that made the acquisition of a reliable scan difficult in two eyes. The average age of the patients was 44.0 ± 16.0 years (range, 13–63 years). All patients were males with the exception of 1 female patient, who was diagnosed with choroideremia based on characteristic clinical fundus appearance, ERG changes, and a genetic molecular mutation. The study cohort was comprised of 15 Caucasian patients and 1 Hispanic patient. In 3 patients (18.8%), disease causing mutations in the CHM gene were previously identified. A missense mutation, C to T change at codon 787, exon 6 was identified in a 44-year-old Caucasian male and a missense mutation, C to A change at codon 907, exon 7 creating a stop codon in a 31-year-old Caucasian female. A hemizygous splice site IVS3-2 A to G, intron 3 mutation was identified in a 55-year-old Caucasian male.

The average best-corrected visual acuity for all patients was 0.54 ± 0.72 log MAR (range, 0.04–2.80), which is equivalent to 20/20⁻² on a Snellen acuity chart to light perception. The range of IOP for all patients was from 8 to 17 mmHg (mean ($\pm$ SD), 12.6 ($\pm$ 2.6) mmHg). Only one patient (6.3%) had mild disc pallor clinically in both eyes.

The peripapillary RNFL was found to be thinner than normal (below the 5th percentile of the age-corrected normal value) in at least one quadrant for 13 of the 29 eyes studied (45%). In eyes in which thinning was observed, the RNFL thickness was an average of 39.9 ± 9.0 μ m thinner than normal (average and SD among all eyes). Of these 13 eyes, 2 eyes showed thinning in three quadrants, 8 eyes in two quadrants, and 3 eyes in one quadrant (there was a total of 25 abnormally thin quadrants). Figure 1 provides an example of a patient with significant thinning in two quadrants. Thinning was most commonly found in the superior (13 eyes) and inferior (10 eyes) quadrants. Thinning was only observed in two eyes in the nasal quadrant, and was never observed in the temporal quadrant.

Thickening of the peripapillary RNFL was also found in our cohort of patients. The peripapillary RNFL was found to be thicker than normal (above the 95th percentile of the age-corrected normal value) in at least one quadrant for 21 of the 29 eyes studied (72%). In eyes in which thickening was observed, the RNFL thickness was an average of 38.8 ± 11.7 μ m thicker than normal (average and SD among all eyes). Of the 21 eyes that showed thickening, 3 eyes showed thickening in three quadrants, no eyes showed thickening in two quadrants, and 17 eyes showed thickening in one quadrant only (there was a total of 26 abnormally thick quadrants). Figure 2 provides an example of a patient with significant thickening in three quadrants. Of the 21 eyes with thickening, all had an abnormally thick temporal quadrant. Additionally, two eyes in each of the other three quadrants were found to be abnormally thick.

Further analysis was performed on individual eyes that showed either thickening only or thinning only in at least one quadrant. The average RNFL thickness of the 7 eyes that showed only thinning in at least one quadrant was 83.4 μ m (averaged across all four quadrants in these eyes). For the 14 eyes that showed only thickening in at least one

quadrant, the average RNFL thickness was 118.9 μm (averaged across all four quadrants in these eyes). The values of the eyes with thinning only and the eyes with thickening only were significantly different from normal ($t=3.68$, $p<0.01$ for thinning; $t=3.17$, $p<0.01$ for thickening).

A quantitative comparison of RNFL thickness of the patients and the normative database was performed using t-tests. Since there was no statistically significant difference between the RNFL thickness of the left and right eyes of the patients ($t = -0.25$, $p = 0.80$), the data for the two eyes were averaged and the analyses were based on the mean data. There was no statistically significant difference between the overall average RNFL thickness of the patients and normal controls ($t=0.78$, $p=0.43$). However, the average RNFL thickness in the temporal quadrants showed a statistically significant thickening when compared to normal, whereas the average RNFL thickness in both the superior and inferior quadrants showed a statistically significant thinning when compared to normal. Table 1 provides the mean thickness measurements for the patients and controls, as well as the results of the statistical analysis.

Previous studies have shown that the overall RNFL thickness can decrease with age.^{14–16} The effect of age on the overall RNFL thickness for the choroideremia patients is shown in Figure 3. In this plot, overall RNFL thickness (averaged across all four quadrants) is plotted as a function of age for each patient. The data are fit with a linear regression line that has a slope of -0.70 , which is significantly different from zero ($t=-2.20$, $p<0.05$). This finding indicates that there is an approximate 7.0 μm loss of RNFL thickness per decade of life.

Discussion

Recently, SD-OCT technology has been introduced that provides faster scanning (up to 100x) and finer axial resolution (up to 2x) compared with TD-OCT.¹² This evolving technology may serve as a useful adjunct method to provide complementary information that may facilitate the detection of disease progression over time. To our knowledge, our current study is the first to demonstrate the presence of peripapillary defects in RNFL thickness by using SD-OCT in patients with choroideremia. Among our study cohort, our data showed that 13 out of 29 eyes (45%) had thinning of the RNFL in one or more quadrants in at least one eye. This finding was similar to a previous report that showed the presence of retinal nerve fiber layer thinning by using GDx laser polarimetry.¹⁰ Our study also showed that 21 eyes (72%) had thickening of the RNFL in at least one quadrant. Abnormal thinning was more commonly found in the superior and inferior quadrants, while the abnormal thickening was found in the temporal quadrants. Of note, the results highlight the importance of examining the RNFL quadrants individually, as the overall RNFL thickness can be normal, with individual quadrants showing abnormalities. The functional significance of the RNFL defects, if any, is unclear.

Our findings in regard to RNFL thickness changes in choroideremia (a primary outer retinal layer degenerative disease) raises questions regarding the role of transsynaptic retinal degeneration or direct causal effects from the consequences of outer chorioretinal layer degeneration with possible secondary anatomical and physiological inner retinal layer alternations. A knowledgeable explanation for these changes in the RNFL thickness awaits further future investigations. Previous studies^{17,18} that used TD-OCT testing on a single choroideremia patient and carriers of choroideremia ($N=21$) noted the presence of retinal thickening, which was similar to a previous study conducted by our group (under peer-review) that showed the presence of retinal thickening and macular edema in patients with choroideremia. Also, previous reports^{8,18} observed that retinal gliosis and choroidal fibrosis may occur in choroideremia patients, which might partially explain the high percentage of

RNFL thickening in our study population. Our findings add to the list of inherited retinal dystrophies in which abnormalities in RNFL thickness may be found.^{14,15,20}

The data also showed that there was an approximate 7.0 μm loss of RNFL thickness per decade of life in our cohort of patients with choroideremia. The loss of RNFL thickness with age in patients with choroideremia is consistent with findings in patients with XLRS and cone-rod dystrophy.^{14,15}

A potential limitation of the current study is that the axial lengths of the studied eyes were not determined, which could influence the RNFL thickness measurements.^{21,22} However, differences in axial length among the subjects is not likely to be significant, given that only patients with relatively small refractive errors were recruited for the study.

In conclusion, the present study demonstrated the presence of defects in the peripapillary RNFL thickness in patients with choroideremia using SD-OCT. Measurement of RNFL thickness may be useful for patient selection in future treatment trials, as patients who have preservation of the inner retina may benefit most from gene-directed therapies or pharmaceutical interventions while those with a notable degree of inner retinal changes on SD-OCT testing might not achieve a clinically significant improvement in visual function based on treatment strategies directed towards the preservation of photoreceptors and/or retinal pigment epithelial cells.

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Figure 1. Abnormal thinning of the peripapillary RNFL in 3 out of 4 quadrants (superior, nasal, and inferior) and abnormal thick temporal quadrant in the right eye of an affected male (42-year-old) with choroideremia. The Green color means normal RNFL thickness, yellow means thin RNFL (outside 95% confidence limit of normal), red means thin RNFL (outside 99% confidence limit of normal), and white means thick RNFL. The figure also shows a cross-sectional retinal infrared (IR) image sampled along a 3.4 mm circle centered to optic disc, an OCT scan, and RNFL thickness graph.

Figure 2.

Abnormal thickening of the peripapillary RNFL in 3 out of 4 quadrants (superior, temporal, and inferior) in the left eye of an affected female (31-year-old) with choroideremia. The Green color means normal RNFL thickness, yellow means thin RNFL (outside 95% confidence limit of normal), red means thin RNFL (outside 99% confidence limit of normal), and white means thick RNFL. Also shown in the figure, an OCT scan, infrared image of the optic disc, and a graph represents the RNFL thickness in different quadrants.

Figure 3. RNFL thickness as a function of age. The solid line is a linear regression line fit to the data, as described in the text.

Table

shows the mean thickness of the peripapillary RNFL in each quadrant and in overall circumferential thickness in our study cohort vs. controls.

	Control thickness (μm)	Patient thickness (μm)	p-value
Superior	129.18 $\pm$ 15.92	112.03 $\pm$ 25.79	<0.01
Inferior	132.08 $\pm$ 16.82	115.25 $\pm$ 26.82	<0.01
Nasal	87.50 $\pm$ 16.12	82.72 $\pm$ 16.21	0.25
Temporal	71.30 $\pm$ 12.36	97.50 $\pm$ 19.71	<0.01
Overall	105.02 $\pm$ 15.31	101.88 $\pm$ 18.98	0.43