Retinal Nerve Fiber Layer Analysis in RP Patients Using Fourier-Domain OCT

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PURPOSE. To determine peripapillary retinal nerve fiber layer thickness (RNFL) abnormalities in patients with retinitis pigmentosa (RP) using Fourier-domain optical coherence tomography (Fd-OCT) and to evaluate the potential effect of cystoid macular edema (CME) or axial length on RNFL measurements in such patients.

METHODS. Ninety-seven eyes of 52 patients with diagnoses of retinitis pigmentosa or Usher syndrome type II underwent complete ocular examination. Peripapillary RNFL thickness was measured using Fd-OCT in 16 segments from 4 quadrants—temporal (316°-45°), superior (46°-135°), nasal (136°-225°), and inferior (226°-315°). These measurements were compared with age- and disc size-adjusted control values. Further analyses were performed to determine the correlation of axial length or CME with RNFL thickness.

RESULTS. Thinning of the RNFL was observed in 37 eyes (38.1%) of 23 patients (44.2%). A maximum number of eyes had thinning in the nasal quadrant followed by the inferior quadrant; the superior and temporal quadrants were abnormally thin in fewer eyes. No correlation was found between axial length and RNFL thickness in the total cohort (correlation coefficient, 0.039). An abnormal increase in RNFL thickness was observed in 21.65% eyes, but no association was found between the presence of CME and increased RNFL thickness.

CONCLUSIONS. RP eyes may show abnormal thinning or increased thickness of RNFL measurements on testing with Fd-OCT. RNFL defects observed by OCT testing document the presence of anatomic defects in more anterior structures within the retina in a notable number of patients with RP. (Invest Ophthalmol Vis Sci. 2008;49:3525-3528) DOI: 10.1167/iovs.08-1842

In a previous study using time domain OCT, the authors documented thinning of the peripapillary retinal nerve fiber layer (RNFL) in patients with retinitis pigmentosa (RP).1 Transneuronal damage,2-5 vascular compromise,4 direct effect on the ganglion cells from the gene defect causing RP,5 and axonal compression leading to compromised axonal transport6 have all been postulated as various causes that potentially may lead to retinal ganglion cell loss in these patients. Further, in the study by Walia et al.,1 it was noted that some RP patients may have abnormal thickening of the RNFL, more frequently noticed in the temporal quadrant.1 Glial tissue on the surface of the optic disc may be included in the thickness measurements of the RNFL and may be the cause of an abnormally thick RNFL.

The purpose of the present study was to measure the peripapillary RNFL using Fourier domain OCT in RP patients with different degrees of disease severity. The advantages of the Fourier domain are that the thickness measurement is resampled relative to the disc center, not the scan center, so that decentering of the disc relative to the scan beam does not affect the measurement. Moreover, higher resolution and decreased scan acquisition time results in better scan quality and fewer motion artifacts.7

The authors have also now addressed some of the limitations of the previous study, including the need for correlating RNFL thickness with the size of the optic disc and the axial length of the eye. The present study also evaluates the potential impact of macular edema on the measurement of RNFL thickness.

METHODS

The study was conducted in the Department of Ophthalmology at the University of Illinois at Chicago. Fifty-two patients with RP (n = 42) or Usher syndrome type II (n = 10) were included, and informed consent was obtained. The diagnosis of RP was based on the history of night blindness, impairment in peripheral visual fields, reduction in electroretinogram rod and cone amplitudes, and presence of characteristic fundus pigmentary changes. Usher II patients had the same clinical features as RP, additional moderate congenital sensorineural hearing loss, and absence of vestibular dysfunction. This study followed the tenets of Declaration of Helsinki and was approved by an institutional review board at the University of Illinois.

All patients with a diagnosis of RP or Usher syndrome type II, who were seen by the authors during clinical examination or who returned to participate in a project relating to cystoid macular edema (CME) in RP patients, were included in the present study if they were willing to undergo OCT testing. Exclusion criteria included inability to hold steady fixation, previous history of glaucoma or raised intraocular pressure of more than 21 mm Hg, uveitis, diabetic retinopathy, optic nerve head drusen, refractive error of more than ±6 D sphere or ±2 D cylinder, dense media opacity sufficient to hinder OCT examination, and sector RP.

All patients underwent complete eye examination, including best-corrected visual acuity using an early treatment of diabetic retinopathy chart (The Lighthouse, Long Island City, NY), slit lamp examination, and intracocular pressure measurement with Goldmann applanation tonometry. Both eyes were dilated with 2.5% phenylephrine and 1% tropicamide. Fundus examination was performed using stereobiomicroscopy, and optic disc pallor was graded as normal-mild, mild-moderate, or moderate-severe. Ultrasonic axial length and pachymetry measurements were then made (Nidek Inc., Fremont, CA).

OCT was performed using Optovue technology (RTVue versions 1.2.6, 2.0.3.2, and 3.0; Optovue Inc., Fremont, CA). Although the scans were obtained using the 3 software versions, the final analysis of the RNFL thickness selectively used version 3.0. The NMH protocol was used for scan acquisition. Internal fixation was used for all patients. The total time for a single scan acquisition was 0.57 seconds. Scans

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were accepted only if they had a signal strength index greater than 35 and were free of artifacts. RNFL thickness was measured in each eye 3 times, and data were used only if they were reproducible, within normal or abnormal range, in 2 or more acquisitions. The contour of the optic nerve head was manually traced using the fundus picture generated by the OCT (Fig. 1C).

RNFL thickness was measured automatically by the existing software at a diameter of 3.45 mm around the center of the optic disc. The total number of A-scans at that circumference was 2225. RNFL thickness was measured in the temporal (316°-45°), superior (46°-135°), nasal (136°-225°), and inferior (226°-315°) quadrants. OCT software automatically measured 4 smaller segments within each quadrant. Data thus obtained were compared with the normative database provided with the software, taking the patient age and size of the optic disc into account. The RNFL was considered abnormally thin if its value was less than the 5th percentile of the age- and optic disc size-adjusted normal value and thick if it was more than the 95th percentile. Abnormal thinning or thickness in 2 of these smaller sectors within a quadrant, if reproducible, was considered significant. Data from both the eyes were averaged and used for the final analysis.

For detecting macular edema, the radial slicer protocol was used. It consisted of twelve 6-mm radial scans at 15° intervals passing through the center of the fovea. All 12 scans were acquired simultaneously, and the total time taken for their acquisition was 0.27 seconds. Each radial line consisted of 1024 A-scans.

**RESULTS**

The average age of the 52 patients in the study was 39.7 years (range, 12–78 years). Other demographic variables were as

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**Figure 1.** (A) RNFL defects in an RP patient’s eye within all segments in all quadrants. (B) RNFL defects in 2 segments within the nasal quadrant and 3 within the inferior quadrant. (C) Representative OCT-generated image of the optic disc along with manual tracing.
follows: male, 20; female, 32; Caucasian, 42 (80.7%); African descent, 8 (15.4%); Asian, 1 (1.9%); Hispanic, 1 (1.9%). Our cohort could be divided into 21 isolated cases (40.4%), 13 with autosomal dominant (25%), 6 with autosomal recessive (11.5%), and 2 with X-linked recessive inheritance (3.8%). We also included 10 patients with type II Usher syndrome (19.2%).

Average best-corrected visual acuity was 0.37 logMAR units, and the SD was 0.23 (range, 0–1, equivalent to 20/20 to 20/200 on a Snellen acuity chart). The range of intraocular pressure for all patients was 9 to 18 mm Hg (mean, 13.6 mm Hg). The average pachymetry value was 545.6 μm, with a range of 479 to 623 μm. None of the patients had a glaucomatous appearance to the optic disc.

OCT scans were obtained on 97 eyes (1 eye each was tested in 7 patients, and both eyes were tested in 45 patients). Contour lines marking the boundary of the nerve fiber layer were examined in each scan, and scans with artifacts leading to distortion of the contour lines were rejected. In 11 of these eyes, 2 of 3 scans were reproducible and used for analysis, while in 86 eyes all 3 scans were used for analysis. Values were averaged for each eye.

Thinning of the RNFL in 2 or more segments in a quadrant was seen in 37 eyes (38.14%) of 23 patients (44.23%). This finding was observed in both eyes in 14 patients and just 1 eye in 9 patients (of these, 3 had scans on only 1 eye; Figs. 1A, 1B).

We also calculated the number of patients with thinning in 3 or more segments in 1 quadrant. Twenty-six eyes (26.80%) of 16 patients (30.77%) were observed to have a thin RNFL in 3 or more segments. Among these 26, 10 patients had abnormal thinning in both eyes and 6 had abnormal thinning in 1 eye only (all 16 patients had OCT scans on both eyes).

The total number of abnormal segments from all patients was 252. The average number of abnormal segments among the 37 eyes with abnormal RNFL thinning was 6.8 segments per eye (range, 2–16).

Median visual acuity of patients with RNFL thinning was 0.36 log units (25%–75% of values were between 0.22 and 0.53). For patients with normal or thick RNFL, the median visual acuity was 0.26 (25% to 75% of values between 0.20 and 0.40). No statistical significance was observed between the 2 groups using the Mann–Whitney rank sum test (P = 0.099).

The distribution for abnormal thinning of the RNFL among the 4 quadrants is tabulated in Table 1. The nasal quadrant was found to have abnormal thinning in the maximum number of eyes, followed by the inferior quadrant.

Sixty-four eyes had normal-mild pallor of the optic disc on clinical examination, and 7 eyes had moderate to severe pallor qualitatively. Abnormal RNFL thinning was seen in 7 of 7 eyes (100%) with moderate-severe pallor and in 16 of 64 eyes (25%) with normal-mild pallor.

The value of axial length was not available in 1 patient. The average axial length in 51 patients in both eyes was 23.42 mm (±SD 0.96). No correlation was observed in the average RNFL thickness of both eyes with average axial length of both eyes in these 51 patients (correlation coefficient, −0.198; Fig. 2).

Abnormal thickening of the RNFL in 2 or more segments in a quadrant was seen in 21 eyes (21.6%) of 14 patients (26.92%). The distribution was 13 eyes (13.4%) of 10 patients (19.23%) in the temporal quadrant, 13 eyes (13.4%) of 8 patients (15.38%) in the inferior quadrant, 7 eyes (7.21%) of 5 patients (9.61%) in the nasal quadrant, and 6 eyes (6.19%) of 5 patients (9.61%) in the superior quadrant. Some eyes and patients had abnormal thickening in more than 1 quadrant, whereas 78.35% eyes did not show evidence of abnormal thickening by OCT in any segment.

All patients included in the study were also examined by OCT testing for the presence of cystoid macular edema (CME). CME was observed in 37 eyes; only 9 of these 37 eyes had abnormal RNFL thickening. Twenty-eight eyes with CME had no RNFL thickening, and 12 eyes with RNFL thickening had no CME. The distribution of abnormal thickening in eyes with CME was as follows: 7 had thickening in the temporal quadrant, 4 in the inferior quadrant, and 3 in the nasal quadrant, and 3 in the superior quadrant. The odds ratio for patients with CME and abnormal RNFL thickness and those with CME and no nerve fiber layer thickening was 1.28, with 95% confidence intervals of 0.48 to 3.43, showing no statistical difference in the 2 groups.

DISCUSSION

Peripapillary RNFL defects in 2 or more segments were observed in 38.14% of the eyes and in 3 or more segments in 26.8% eyes. These results are similar to a previous study that found that 40% of all eyes tested showed a thinning of the RNFL. However, in contrast to the previous study, which reported the superior, inferior, and nasal quadrants to be equally affected and the temporal quadrant to be least affected, abnormal thinning in the present study was seen most often in eyes in the nasal quadrant, followed by the inferior quadrant, whereas the superior and temporal quadrants showed thinning in the fewest eyes.

More extensive damage in some RP patient eyes, possibly related to enhanced light exposure, has been observed in the inferior retina. On histopathologic examination in eyes with RP, Flannery et al have also reported the greatest photoreceptor loss in the inferonasal region and the least loss in the superotemporal region. Hence, it is conceivable that maximum transneuronal damage to the ganglion cells may occur most often in the inferonasal quadrant. However, we did not analyze whether this difference between quadrants was statistically significant because a larger cohort of

**Table 1. Distribution of Abnormal Segments in Various Quadrants**

<table>
<thead>
<tr>
<th>Quadrant</th>
<th>Eyes</th>
<th>Patients</th>
<th>Eyes</th>
<th>Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nasal</td>
<td>31</td>
<td>18</td>
<td>24</td>
<td>17</td>
</tr>
<tr>
<td>Inferior</td>
<td>23</td>
<td>16</td>
<td>16</td>
<td>12</td>
</tr>
<tr>
<td>Temporal</td>
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<td>11</td>
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<td>6</td>
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<tr>
<td>Superior</td>
<td>14</td>
<td>9</td>
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**FIGURE 2.** Correlation of axial length with average RNFL thickness.
RP patients would be needed for a statistical test to be meaningful.

No significant difference was observed between the median visual acuity of patients with or without RNFL thinning. A previous study has also shown that RNFL defects develop in patients with a relatively good visual acuity.4 RNFL defects were seen in all eyes with a qualitative moderate-severe pallor of the optic disc and in only 25% of the patients with normal-mild pallor. Hence, it can be concluded that pallor of the optic nerve likely signifies ganglion cell death, not only glial tissue proliferation on the surface of the optic disc, which has been observed previously.13,14

Previous studies have documented a negative correlation between axial length and RNFL thickness.5–11 However, in the present study, no significant correlation was observed between these 2 parameters, possibly because different mutations causing RP led to different rates of photoreceptor cell loss independent of the axial length of the eye. Hence, different rates of transneuronal ganglion cell loss may be related to photoreceptor loss but independent of axial length.

Abnormal thickening of the RNFL was observed in 21.65% of eyes. In the present study, this was observed in the temporal and inferior quadrants in the greatest number of patients. However, CME was seen in fewer eyes with abnormally thick RNFL in and in more eyes with normal or thin RNFL. We did not observe that the presence of CME was one of the factors causing increased RNFL thickness. In an earlier study, the temporal quadrant was also observed to be abnormally thick in the greatest number of eyes.5 With the use of GDXVCC, another study has reported increased thickness of the average RNFL in 10 eyes of 5 RP patients with visual acuity of 20/30 or better in the superior and inferior quadrants and lesser thickness in the nasal and temporal quadrants (Nath S et al. IOVS 2007;48:ARVO E-abstract 2001). The present study had a larger cohort with a wider range of visual acuities. Differences in patient selection and in the technology used to measure RNFL thickness between our study and that of Nath et al. (IOVS 2007;48:ARVO E-abstract 2601) may account for the differences observed.

Because of the small number of patients in each genetic subtype, we did not discern whether any particular genetic subtype had a higher frequency of RNFL defects compared with the others. One limitation of the study was that the margin of the optic disc was traced manually for all patients; hence, the size of the optic disc might have been subjectively influenced by the operator.

To our knowledge, the present study is the first to use high-resolution Fourier-domain OCT to determine RNFL thickness in RP patients and to correlate the RNFL thickness with axial length and CME. RNFL thinning was observed in 38.14% of all eyes in our cohort after controlling for age and size of the optic disc. No correlation was found between axial length or CME and RNFL thickness.

For a treatment aimed at the preservation of photoreceptor cells in RP patients to result in a clinically significant improvement in visual function, ganglion cell and photoreceptor cell function will have to be preserved. Therefore, the use of OCT screening to measure RNFL thickness would seem prudent during patient selection in future treatment trials for RP patients.

References