Assessment of Retinal Nerve Fiber Layer Internal Reflectivity in Eyes With and Without Glaucoma Using Optical Coherence Tomography

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Objective: To assess the internal reflectivity of the retinal nerve fiber layer in normal, ocular hypertensive, and glaucomatous eyes using optical coherence tomography.

Methods: All patients underwent complete ophthalmic examination and achromatic automated perimetry. Intraocular pressure was 21 mm Hg or less for low-tension glaucoma and normal eyes and at least 25 mm Hg on 2 separate occasions in ocular hypertensive and high-tension glaucoma eyes. All glaucomatous eyes had characteristic glaucomatous optic neuropathy and associated achromatic automated perimetry defect. Relative retinal nerve fiber layer internal reflectivity was measured on optical coherence tomography images using a software program of our own design.

Results: We enrolled 98 eyes (19 normal, 34 ocular hypertensive, 28 low-tension glaucoma, and 17 high-tension glaucoma). Relative internal reflectivity was less in eyes with glaucoma than in normal (P<.001, t test) and ocular hypertensive eyes (P<.001, t test). There was no difference in relative internal reflectivity between normal and ocular hypertensive eyes (P=.32) and between eyes with high-tension glaucoma and low-tension glaucoma (P=.43). Internal reflectivity correlated with mean deviation on achromatic automated perimetry ($r^2=0.49$, P<.001, quadratic regression analysis).

Conclusion: Relative retinal nerve fiber layer internal reflectivity may provide useful information about the extent of retinal nerve fiber layer injury in glaucoma.


Retinal nerve fiber layer (RNFL) damage may precede the development of glaucomatous optic neuropathy or visual field loss. Retinal nerve fiber layer photography may identify RNFL injury years before visual field damage becomes apparent, suggesting that patients with progressive glaucomatous neuropathy might be detected by newer methods of RNFL assessment, before they develop reproducible damage detectable by perimetry.

Optical coherence tomography (OCT) is a noninvasive, noncontact transpupilary imaging technique for obtaining cross-sectional images of the retina in vivo. Optical coherence tomography provides qualitative information about retinal disorders as well as quantitative measurements of retinal anatomy. Schuman et al reported the ability of OCT to detect differences in RNFL thickness between normal and glaucomatous eyes, with a high correlation between RNFL thickness and visual field deficit.

The RNFL thickness measurement, however, is only one way to assess RNFL structure. The optical characteristics of the RNFL, which correspond to its internal reflectivity, may provide additional information regarding RNFL structure and damage in glaucoma. The RNFL thickness measurements have not yet been compared with internal reflectivity measurements to determine which method is more accurate and sensitive in the detection of early RNFL damage.

The goal of this study was to establish a method to assess the internal reflectivity of the RNFL in OCT images and to evaluate its usefulness in the detection of RNFL damage.

RESULTS

Ninety-eight patients were enrolled from September 1997 to April 1999. There were 19 normal eyes, 34 OHT eyes, 28 LTG eyes, and 17 HTG eyes. There was no difference among the groups with respect to sex, race, and refraction. The LTG and HTG patients tended to be older than both...
PATIENTS AND METHODS

Normal ocular hypertensive (OHT) and glaucomatous individuals meeting the entry criteria were enrolled in this prospective study. Informed consent was obtained from all patients using a consent form approved by the Institutional Review Board for Human Research of the New York Eye and Ear Infirmary, New York. All patients were between 20 and 75 years of age and had visual acuity of 20/40 or better, refractive error not exceeding 5.00 diopters sphere and/or 2.00 diopters cylinder, and no prior incisional surgery. All subjects underwent complete ophthalmic examination, including slitlamp biomicroscopy, stereophotography of the optic nerve head, achromatic automated perimetry, confocal scanning laser ophthalmoscopy, and OCT. Achromatic automated perimetry was performed with the Humphrey Field Analyzer (Humphrey Systems Inc, Dublin, Calif) using program numbers 24-2 or 30-2. Visual field reliability criteria included less than 23% fixation losses and false-negative and false-positive rates. When both eyes met the enrollment criteria, only the right eye was enrolled.

Normal subjects had no history of ocular disease or family history of glaucoma. All had intraocular pressures (IOPs) of 21 mm Hg or less by Goldmann applanation tonometry, normal optic disc appearance based on clinical examination and review of stereoscopic photography, and normal automated achromatic perimetry. Absence of glaucomatous optic neuropathy was defined as a vertical asymmetry of 0.2 or less, a cup-disc ratio of 0.6 or less, and intact neuroretinal rim without peripapillary hemorrhages, notches, localized pallor, or RNFL defect. Visual field indices showed a deviation (CPSD) within 95% confidence limits and a glaucomatous hemifield test result within normal limits.

Ocular hypertension was defined as an IOP of 25 mm Hg or more (with or without antiglaucoma medication) on at least 2 occasions, whereas low-tension glaucoma (LTG) patients had IOPs of 25 mm Hg or more (with or without antiglaucoma medication) on at least 2 occasions, whereas low-tension glaucoma (LTG) eyes had no measured IOP of 22 mm Hg or more. Each eye was dilated with 1% tropicamide and 2.5% phenylephrine hydrochloride. Optical coherence tomography (software version 4.1, Humphrey Systems Inc) was performed using near-infrared, low-coherence illumination (840 nm) with a tissue resolution of approximately 10 to 17 µm. After pupilary dilation, three 360° circular scans with a diameter of 3.4 mm centered on the optic disc were performed. Scan acquisition time was 1.0 second. Each scan consisted of 100 individual A-scan samples evenly distributed along the circle circumference. All studies were completed within a 3-month period.

The OCT raw data were exported to an IBM-compatible personal computer and analyzed using a software program of our own design running on Microsoft Windows 95. The software automatically detects the inner and outer borders of the RNFL using a relative threshold technique, which is similar to the algorithm used in the commercial version of OCT software (Figure 1). The thickness of the RNFL is then determined. The reflectivity of pixels within these borders is collected by analysis of the raw data array, and the mean reflectivity is calculated automatically. The relative internal reflectivity is computed by taking a ratio of measured reflectivity against the saturation reflectivity of each image, which represents "pure white" colors in the false-color scheme.

Statistical analyses were performed using JMP software (SAS Institute Inc, Cary, NC). Analysis of variance (ANOVA) was used to compare the different parameters among the groups. P<.05 was considered statistically significant.

![Figure 1. Optical coherence tomography raw data circular scan of a normal subject. Note the high reflectivity of the retinal nerve fiber layer (red color). Limits are detected automatically by the software.](http://archophth.jamanetwork.com/pdfaccess.ashx?url=/data/journals/ophth/9877/ on 06/19/2017)
internal reflectivity between normal and OHT eyes \((P = .32)\) or between eyes with HTG and LTG \((P = .43)\) (Figure 2). Internal reflectivity correlated with MD \((r^2 = 0.49, P < .001)\), quadratic regression analysis) and with CPSD on achromatic automated perimetry \((r^2 = 0.34, P < .001)\), quadratic regression analysis (Figure 3). The correlation was improved when the mean relative internal reflectivity of 3 scans was used rather than a single scan. Relative internal reflectivity was strongly correlated to RNFL thickness \((r^2 = 0.61, P < .001)\) (Figure 4). There were no statistically significant differences in relative internal reflectivity between eyes of women and men \((P = .2)\). Accurate assessment of RNFL damage is important in the early detection and longitudinal evaluation of glaucomatous optic neuropathy. Optical coherence tomography has been reported to be able to differentiate normal from glaucomatous eyes measuring the RNFL thickness.\(^7\) Although the internal limiting membrane and RNFL posterior limits are defined by a change in the reflectivity, the current OCT software does not provide any information regarding the internal reflectivity status of the tissue.

In this prospective study, we measured the internal reflectivity of the RNFL. Since the absolute reflectivity can vary according to a wide variety of factors, such as media opacity or scan technique, relative numbers were used. Each value was a percentage of the local maximum, allowing comparison of different scans in the same patient or among different patients.

Our data are the first to report the use of RNFL internal reflectivity in the detection of glaucoma and sug-

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**Patient Demographics and Visual Field Parameters**

<table>
<thead>
<tr>
<th></th>
<th>Low-Tension Glaucoma</th>
<th>High-Tension Glaucoma</th>
<th>Normal</th>
<th>Ocular Hypertension</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, mean ± SD, y (range)</td>
<td>59.7 ± 9.1 (39-71)</td>
<td>56.6 ± 12.5 (36-72)</td>
<td>53.7 ± 11.5 (33-72)</td>
<td>54.1 ± 12.3 (25-72)</td>
<td>.20*</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Male</td>
<td>10</td>
<td>10</td>
<td>6</td>
<td>17</td>
<td>.26†</td>
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<tr>
<td>Female</td>
<td>18</td>
<td>7</td>
<td>13</td>
<td>17</td>
<td></td>
</tr>
<tr>
<td>Mean deviation, dB</td>
<td>−9.4 ± 7.0</td>
<td>−7.0 ± 7.3</td>
<td>−0.4 ± 1.3</td>
<td>−0.7 ± 1.4</td>
<td>&lt; .001*</td>
</tr>
<tr>
<td>Corrected pattern standard deviation, dB</td>
<td>9.2 ± 4.3</td>
<td>5.6 ± 2.7</td>
<td>1.0 ± 0.6</td>
<td>1.2 ± 0.7</td>
<td>&lt; .001*</td>
</tr>
</tbody>
</table>

*Analysis of variance.
†x² Test.
suggest that the intensity of the RNFL relative internal reflectivity correlates with its thickness. It also correlates with functional visual loss as measured by MD and CPSPD during achromatic automated perimetry. Optical coherence tomography differs from scanning laser polarimetry (GDx; Laser Diagnostic Technologies, Inc, San Diego, Calif), which provides an estimate of the peripapillary RNFL thickness using the polarization properties of the axon bundles. Both OCT and RNFL relative internal reflectivity are not affected by the polarization properties of the cornea or crystalline lens.

Layers in the retina can be differentiated by OCT because these tissues have different optical reflection or scattering properties and are displayed in the false-color image of the OCT. The posterior limit of the RNFL is detected by the OCT, searching from the photoreceptor image of the OCT. The posterior limit of the RNFL is because these tissues have different optical reflection or scattering properties.

In summary, RNFL relative internal reflectivity is a good predictor of glaucomatous damage. Optical coherence tomography RNFL thickness and its internal reflectivity are able to separate normal and OHT from glaucomatous eyes. Expansion and longitudinal evaluation of the present data set and refinement of the OCT scan technique will aid in our understanding of this optic neuropathy. Early diagnosis and treatment of glaucoma may be possible with a better understanding of the RNFL and its changes consequent to glaucomatous injury.

Accepted for publication January 27, 2000.

This study was supported in part by the Joel and Elaine Levitt Research Fund of the New York Glaucoma Research Institute, New York, NY, The New York Eye and Ear Infirmary Department of Ophthalmology Research Fund, New York (Dr Dou), and an unrestricted grant from Allergan, Inc, Irvine, Calif (Dr Pons).


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REFERENCES