The Retinal Nerve Fiber Layer Thickness in Ocular Hypertensive, Normal, and Glaucomatous Eyes With Optical Coherence Tomography

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Objective: To quantitatively assess and compare the thickness of the retinal nerve fiber layer (RNFL) in ocular hypertensive eyes with normal and glaucomatous eyes using the Optical Coherence Tomograph (OCT 2000, software version A4X1; Humphrey Instruments, San Leandro, Calif).

Methods: The mean RNFL thickness of ocular hypertensive (n = 28) eyes was compared with age-matched normal (n = 30) and glaucomatous (n = 29) eyes. Subject eyes were classified into diagnostic groups based on intraocular pressure, stereoscopic disc photographs, and standard automated perimetry. Three circular scans were obtained for each eye at a diameter of 3.4 mm around the optic disc. In each eye, average RNFL thickness measurements were obtained in temporal, superior, nasal, and inferior quadrants. A single index of average RNFL thickness throughout 360° also was obtained.

Results: Mean (95% confidence interval) RNFL was significantly thinner in ocular hypertensive eyes than in normal eyes, 72.8 µm (66.4-78.1 µm) and 85.8 µm (80.2-91.7 µm), respectively. More specifically, RNFL was significantly thinner in ocular hypertensive eyes than in normal eyes in the inferior quadrant, 84.8 µm (75.6-94.0 µm) vs 107.6 µm (99.3-115.9 µm); and in the nasal quadrant, 44.1 µm (37.5-51.7 µm) vs 61.8 µm (53.0-65.6 µm). Retinal nerve fiber layer was significantly thinner in glaucomatous eyes than in ocular hypertensive and normal eyes throughout 360° and in all quadrants.

Conclusion: These findings suggest that quantitative differences in RNFL thickness exist between age-matched ocular hypertensive, normal, and glaucomatous eyes.

Arch Ophthalmol. 2000;118:22-26

Optical coherence tomography (OCT) is one promising technology that has been developed to assess tissue thickness in vivo, such as that of the retinal nerve fiber layer (RNFL). This technology was initially designed for fiberoptic use. Early biological uses of this technology involved imaging of the human anterior chamber, peripapillary retina, and coronary artery. Optical coherence tomography has been demonstrated to detect changes in tissue thickness with micrometer-scale sensitivity. With a prototype instrument, OCT data were reported to correlate with the known topography of human retinas. This instrument also performed well in detecting induced RNFL lesions in monkeys. Reproducibility studies using an OCT prototype have shown SDs of measurement of RNFL and retinal thicknesses of approximately 10 to 20 µm (10%-20%) in normal and glaucomatous eyes. Finally, visual field loss correlated with RNFL thickness as determined by this OCT prototype in glaucomatous eyes.

As there is considerable evidence that RNFL loss precedes visual field loss in patients with glaucoma, it is of interest to quantify this loss in patients with ocular hypertension who are at risk for developing glaucoma. In this study, we assessed RNFL thickness in ocular hypertensive eyes using the commercially available Optical Coherence Tomograph (OCT 2000, software version A4X1; Humphrey Instruments, San Leandro, Calif).

RESULTS

Retinal nerve fiber layer thickness measurements for the 3 diagnostic groups overlapped considerably (Figure 1). Mean RNFL thickness (360° measurement) ranged from 40 to 105 µm in OHT eyes, from 46 to 106 µm in normal eyes, and from 4 to 85 µm in glaucomatous eyes. Finally, visual field loss correlated with RNFL thickness as determined by this OCT prototype in glaucomatous eyes.

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A 1-way analysis of variance on mean RNFL thickness (360° measurement) data showed a significant main effect of diagnostic group, F(2,84) = 40.23, P ≤ .001. Mean (95% confidence interval) RNFL thickness was

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SUBJECTS AND METHODS

SUBJECTS

Three age-matched groups, classified as ocular hypertensive (OHT) (n = 28), normal (n = 30), and glaucomatous (n = 29), were recruited from the Glaucoma Center of the University of California, San Diego. Mean (± SD) age of the OHT, normal, and glaucoma groups was 65.9 (±10.5), 63.4 (±10.0), and 66.7 (±13.9) years, respectively. Subjects were age-matched because preliminary analysis of OCT data from 61 normal eyes (from patients aged 23-80 years; mean age, 53.4 ± 14.6 years) revealed a significant correlation between age and RNFL thickness in the temporal quadrant ($r = -0.32, P = .01$), and a trend toward a significant correlation between age and RNFL thickness in the superior quadrant ($r = -0.23, P = .08$). Correlations were weak between age and RNFL thickness in the nasal and inferior quadrants ($r = -0.02, P = .91$ and $r = -0.03, P = .81$, respectively).

All subjects had a complete ophthalmologic examination including direct and indirect ophthalmoscopy, intraocular pressure (IOP) measurement, stereoscopic photographs of the optic disc, and visual field testing prior to OCT image acquisition. One eye of each subject was randomly selected for study. All test eyes had a best-corrected visual acuity of at least 20/40. Informed consent was obtained from all participants and the methods were approved by the University of California San Diego Human Subjects Committee.

Test eyes were classified as glaucomatous, OHT, or normal based on the following criteria. Glaucomatous (open angle) eyes had a measured IOP of 24 mm Hg or more on at least 2 occasions. These eyes had a glaucomatous appearance of the optic disc defined as thinning of the neuroretinal rim, hemorrhage, notch, excavation, an RNFL defect, or asymmetry of vertical cup or disc of greater than 0.2 based on masked standardized grading of stereoscopic photographs. Glaucomatous visual field damage on the Humphrey Field Analyzer program 24-2 (or 30-2) required a corrected pattern SD outside of the 95% normal limits or glaucoma hemifield test outside of the 99% normal limits. Mean deviation (95% confidence interval) in these glaucomatous eyes was $-5.71$ (−4.16, −7.26) dB, indicating early to moderate visual field damage.

Ocular hypertensive eyes had a measured IOP of 24 mm Hg or more on at least 2 occasions. These eyes had intact rims, no evidence of hemorrhage, notch, excavation, or RNFL defect, and had symmetrical optic discs (asymmetry of vertical cup or disc < 0.2) based on masked analysis of stereoscopic photographs. Visual field results on the Humphrey Field Analyzer program 24-2 (or 30-2) showed a corrected pattern SD within the 95% normal limits and glaucoma hemifield tests within normal limits.

Normal eyes had a highest measured IOP of 22 mm Hg or lower with no history of elevated IOP. Stereoscopic photography and visual field analysis criteria were the same as those for OHT eyes.

INSTRUMENTATION

Optical coherence tomography (OCT) assesses RNFL thickness by analyzing the temporal delay of backscattered light from tissue structures. The echo delay time of the backscattered light from different layers in the retina is determined using low-coherence interferometry. First, low-coherence light is directed onto a beam-splitter, resulting in 2 beams, one directed at the tissue of interest (signal beam) and the other at a reference mirror (reference beam). The amplitude and delay of tissue reflection is determined by an interferometer that adds the electromagnetic waves in the 2 reflected light beams. Because of the low coherence of the light source, interference of light reflected from the signal and reference beams only occurs when the delay of the reflections is almost matched, resulting in high resolution.

In the commercially available OCT 2000, software version A4X1, RNFL is differentiated from other retinal layers using a thresholding algorithm that detects the anterior edge of retinal pigment epithelium and determines the photoreceptor layer position. For each scan, the posterior edge is determined to be the first occurrence of signal above threshold anterior to the photoreceptor layer of the RNFL. Nerve fiber layer thickness is defined as the number of pixels between the anterior and posterior edges of the RNFL.

In circle scan mode, this instrument generates RNFL thickness measurements (in micrometers) at 100 points along a 360° circular path (1 thickness value per 3.6°). This information is presented graphically on a continuous x and y plot, where x is the retinal position (eg, temporal, superior, nasal, inferior) and y is RNFL thickness in micrometers. In addition, RNFL thickness is presented on 2 circular charts, 1 with 12 equal sectors each representing 1 hour around the clock face, and the other with 4 equal 90° hour glass sectors each representing 1 quadrant. These charts display RNFL thickness (micrometers) within each sector. A single mean RNFL thickness for the full 360° scan is also displayed.

PROCEDURE

Three 3.4-mm-diameter circular scans centered on the optic disc judged to be of acceptable quality were obtained for each test eye, the mean of which was used in the analysis. This approximate scan diameter was found to be optimal for RNFL analysis in a prototype instrument. For each subject, RNFL thickness was assessed in 4 retinal regions: temporal (316° to 45° on unit circle), superior (46° to 135° on unit circle), nasal (136° to 225° on unit circle), and inferior (226° to 315° on unit circle). Average RNFL thickness was also assessed (0° to 359° on unit circle).

Within-subject reproducibility was examined by determining the mean and SD of RNFL thickness between the 3 images obtained for each study eye in each quadrant.

We also used a confocal scanning laser ophthalmoscope (Heidelberg Retina Tomograph [HRT]; Heidelberg Engineering, Heidelberg, Germany) to assess the optic disc area (see “Results” section). Three circular scans centered on the optic disc judged to be of acceptable quality were obtained for each test eye, the mean topography image of which was used in the analysis. Details of this instrument have been described elsewhere.

STATISTICAL ANALYSIS

Multiple comparisons between groups were conducted using analysis of variance. Pairwise comparisons for all significant main effects were conducted using the Tukey-Kramer Honestly Significant Difference test (all $\alpha = .05$).
RNFL was significantly thinner in glaucomatous eyes, 56.5 µm (45.7-67.4 µm) than in OHT eyes, 101.4 µm (93.6-109.2 µm) and normal eyes, 105.7 µm (98.0-112.6 µm). Retinal nerve fiber layer thickness in the superior quadrant was similar in OHT and normal eyes. In the nasal quadrant, the RNFL was thinnest in glaucomatous eyes, 29.2 µm (21.2-37.1 µm) and increased significantly in OHT eyes, 44.1 µm (37.5-51.7 µm) and normal eyes, 61.8 µm (53.0-65.6 µm). Finally, in the inferior quadrant, the RNFL was thinnest in glaucomatous eyes, 49.2 µm (39.2-59.2 µm) and increased significantly in OHT eyes, 84.8 µm (75.6-94.0 µm) and normal eyes, 107.6 µm (99.3-115.9 µm) (Figure 2).

The influence of optic disc area on RNFL thickness measurements between the 3 subject subpopulations was also assessed. It is known that RNFL is thickest near the optic disc, primarily owing to lateral fusion of nerve fiber bundles.19 Further, there may be more nerve fibers in eyes with larger optic discs.20,21 Because OCT scans were centered on the optic disc, we suspected that measurements taken from subjects with large discs might be thicker. This is because measurements in these subjects would be taken closer to the disc margin than in subjects with smaller discs, owing to the fixed diameter of the circular scan. To test this possibility, we first investigated the correlation (r) between optic disc area and RNFL thickness for normal eyes. We then compared optic disc area between each diagnostic group. Disc area was not significantly correlated with RNFL thickness in normal eyes in any quadrant (all P>.05). Further, there was no significant difference in disc area between diagnostic groups, F(2,54) = 2.18, P = .12.

This study quantified and compared RNFL thickness in OHT eyes with that in normal eyes and glaucomatous eyes by using OCT. There was considerable within-group variability in measured RNFL thickness. Average RNFL was significantly thinner in OHT eyes than in normal eyes. Specifically, RNFL was significantly thin-
ner in OHT eyes than in normal eyes in the inferior and nasal quadrants. Finally, RNFL was significantly thinner in glaucomatous eyes than in OHT and normal eyes in each quadrant and throughout 360° measurement.

It is not surprising that we found significant thinning of RNFL in glaucomatous eyes compared with OHT and normal eyes because glaucomatous appearance of the optic disc and/or RNFL was an inclusion criterion for glaucoma diagnosis in our study. Differences in RNFL thickness between glaucomatous and normal eyes are well documented.22-28 The results of this investigation, showing significant differences in RNFL between OHT and normal eyes, are less established. Our results suggest that thinner RNFL may be characteristic of some OHT as well as glaucomatous eyes.

In this study, we found an average decrease in RNFL thickness of about 15% in OHT eyes compared with normal eyes. Similar findings have been reported elsewhere using different evaluative techniques. Using stereophotogrammetric measures, Schwartz and Takamoto29 showed a significant decrease in RNFL thickness of about 18% in OHT eyes compared with normal eyes. Using scanning laser polarimetry, Anton et al30 found a significant decrease in retardation measurements (an indirect measure of RNFL thickness) of about 7% in OHT eyes compared with normal eyes. Similar results were obtained by Tjon-Fo-Sang31 also using scanning laser polarimetry. Finally, us-

### Table 2. RNFL Thickness in OHT, Normal, and Glaucuomatous Eyes by Quadrant

<table>
<thead>
<tr>
<th>Quadrant</th>
<th>OHT (n = 28)</th>
<th>Normal (n = 30)</th>
<th>Glaucuomatous (n = 29)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temporal</td>
<td>58.8 (50.6-67.0)</td>
<td>66.2 (61.3-71.2)</td>
<td>41.6 (33.7-49.3)</td>
<td>&lt;.001†</td>
</tr>
<tr>
<td>Superior</td>
<td>101.4 (93.6-109.2)</td>
<td>105.7 (98.0-112.6)</td>
<td>56.5 (45.7-67.4)</td>
<td>&lt;.001†</td>
</tr>
<tr>
<td>Nasal</td>
<td>44.1 (37.5-51.7)</td>
<td>61.8 (53.0-65.6)</td>
<td>29.2 (21.1-37.1)</td>
<td>&lt;.001†</td>
</tr>
<tr>
<td>Inferior</td>
<td>84.8 (75.6-94.0)</td>
<td>107.6 (99.3-115.9)</td>
<td>49.2 (39.3-59.2)</td>
<td>&lt;.001†</td>
</tr>
<tr>
<td>Mean§</td>
<td>72.8 (66.4-78.1)</td>
<td>85.8 (80.2-91.7)</td>
<td>44.4 (36.4-52.6)</td>
<td>&lt;.001†</td>
</tr>
</tbody>
</table>

*Values are expressed in micrometers as mean SDs (95% confidence intervals). RNFL indicates retinal nerve fiber layer; OHT, ocular hypertensive.
†Glaucuoma group significantly different from OHT and normal groups (Tukey-Kramer Honestly Significant Difference test, P < .05).
‡All diagnostic groups significantly different from each other (Tukey-Kramer Honestly Significant Difference test, P < .05).
§Average RNFL thickness for 360° measurement. This value is slightly different than the arithmetic mean of the quadrant values due to sampling area difference.

It is possible that thinner RNFL in the inferior quadrant, making these eyes particularly susceptible to the effects of increased IOP.

Several studies have failed to find significant differences in RNFL thickness between OHT and normal eyes.30-41 It is possible that these normal results are related to sensitivity of the measurement instruments, sample size, or difference in study population. For instance, Lester et al40 used confocal scanning laser ophthalmoscopy (HRT) to measure retinal height among other parameters. These authors found no significant retinal height difference between OHT and normal eyes. These findings might be due to a difference in sensitivity between the HRT and the OCT for measuring RNFL thickness. The HRT was not designed to measure the RNFL directly. The thickness measurements provided by the HRT software are indirect measurements that rely on a reference plane.

The negative results of Lachkar and Cohn30 might be due to the relatively small sample size used in their study (17 OHT eyes). In this study we found considerable RNFL thickness overlap between the 3 diagnostic groups. This result indicates a large within-group variance in each diagnostic group that might mask between-group variances in a smaller sample population.

Differences in RNFL thickness between OHT and normal eyes in this study may be evidence of early damage to the optic nerve. It is possible that patients with OHT eyes with RNFL thickness measurements more similar to those of patients with glaucoma are at a greater risk of developing glaucomatous visual field defects. Similarly, it is possible that the OHT eyes in our study and those in other studies are early-stage glaucomatous eyes.

Although the OHT eyes in our study had normal visual fields and showed no evidence of optic disc or RNFL defects based on stereoscopic photograph assessment, it is possible that visual field defects might be detected in these eyes using other visual function techniques such as short wavelength automated perimetry or frequency doubling perimetry.

While not directly addressed by our study, our results provide some insight into the ability of OCT to cat-
ogerizes as hypertensive, glaucomatous, or normal. We found considerable overlap in RNFL thickness among study groups. This suggests that a single OCT RNFL thickness measurement would not be sufficient for diagnosis. However, this is most likely not a limitation of OCT technology itself, but rather a by-product of large individual differences in RNFL thickness. Recent data presented by Zangwill et al. and Nakla et al. showed that OCT is as good as scanning laser polarimetry and HRT in differentiating glaucomatous eyes from normal eyes despite the fact that OCT image analysis is based on 100 data points rather than several thousand (e.g., scanning laser polarimetry and HRT).

Results from this study may be somewhat limited in their generalizability. Patients with OHT and glaucomatous eyes in our study were recruited from a university-based glaucoma practice. These patients may not reflect the RNFL characteristics of the population as a whole. Further, our patients were predominantly white (88%). Several studies have shown racial differences in measurements of optic disc parameters, including differences in RNFL thickness. 

In summary, OCT revealed statistically significant quantitative differences in RNFL thickness between OHT and normal eyes within our study population. Longitudinal studies should be conducted to determine if these differences result in an increased likelihood of conversion from normal to glaucomatous visual fields for patients with OHT eyes.

Accepted for publication August 16, 1999.