Quantification of Retinal Nerve Fiber Layer Thickness in Normal Eyes, Eyes With Ocular Hypertension, and Glaucomatous Eyes With SD-OCT

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BACKGROUND AND OBJECTIVE: To quantitatively evaluate and compare retinal nerve fiber layer (RNFL) thickness between normal eyes, eyes with ocular hypertension (OHT), and glaucomatous eyes in an Indian population using spectral domain optical coherence tomography (SD-OCT).

PATIENTS AND METHODS: Average and quadrant RNFL values were compared among three groups (66 normal eyes, 55 OHT eyes, and 51 glaucomatous eyes) and the discriminating power of each parameter was evaluated by calculating areas under receiver operator curves (AROCs).

RESULTS: The mean RNFL thickness was 93.45 ± 16.9 µm in glaucomatous eyes, significantly less than in normal (112.48 ± 6.8 µm) and OHT (110.09 ± 10.9 µm) eyes. OHT eyes had significantly thinner RNFL thickness in the temporal quadrant (P = .006) than normal eyes. RNFL thickness in glaucomatous eyes differed significantly from normal eyes in most parameters except nasal quadrant (P = .1) and from OHT eyes in most parameters except temporal (P = .4) and nasal quadrants (P = .3). The parameter with the largest AROC to discriminate between OHT and normal eyes was temporal quadrant (0.65). Inferior quadrant RNFL thickness had the largest AROC (0.93) to distinguish between OHT and glaucomatous eyes and normal and glaucomatous eyes.

CONCLUSION: SD-OCT identified differences in most parameters between eyes with glaucoma and normal eyes and also between eyes with glaucoma and OHT. Overlap of RNFL thickness between normal and OHT eyes limits the ability of this instrument to differentiate between normal and OHT subjects.

INTRODUCTION

Glaucoma is an optic neuropathy characterized by progressive degeneration of retinal ganglion cells and their axons that leads to optic disc cupping and retinal nerve fiber layer (RNFL) loss. Significant axonal loss may precede the development of visual field defect and identifiable optic disc cupping.¹ Improving dif-

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Differentiation between healthy eyes and eyes with ocular hypertension and glaucoma by RNFL thickness measurements may aid in detection of eyes suspected to have early glaucoma or likely to progress to glaucoma. Hence, earlier detection of RNFL thinning using imaging devices has become an active area of research interest.

Optical coherence tomography (OCT) is a non-contact, non-invasive imaging modality that provides high-resolution, quantitative assessment of RNFL thickness and optic disc parameters. It is a sensitive method for the detection of early structural glaucomatous nerve alterations that precede optic disc and visual field damage.

It measures echo time delay and intensity of back-scattered light from various retinal layers using an optical correlation technique known as Michelson low coherence interferometry. Recently, a new method of acquiring OCT scans was developed called spectral domain OCT (SD-OCT) that uses an interferometer with a high-speed spectrometer and the reflectance interference between the reference arm and retina at each A-scan location, which is Fourier transformed to simultaneously (rather than sequentially, as in time domain OCT) to acquire all points along the depth of A-scan without reference arm movement. The advantage of the Fourier domain OCT is improved speed and resolution. Because each A-scan is acquired at one time in SD-OCT, the image acquisition rate is much higher at 16,000 to 40,000 A-scans per second as opposed to the time domain OCT rate of 400 A-scans per second. This acquisition rate allows for much faster scanning time, reducing motion artifacts, and enabling denser and other novel patterns. SD-OCT is also able to improve axial resolution from 8 to 10 µm to 3 to 6 µm, which improves the ability to visualize intraretinal structures. The faster scanning speed allows for acquisition of denser patterns across the optic nerve head.

We undertook this study to quantitatively measure and compare RNFL thickness in normal eyes, eyes with ocular hypertension, and glaucomatous eyes and assess the clinical importance of the data obtained using SD-OCT in Indian eyes.

**Patients and Methods**

This was a prospective study that included normal volunteers from staff and patients from a glaucoma clinic at the Institute. The study followed the guidelines contained in the Declaration of Helsinki and was approved by the ethics committee of the Institute. All participants were Asian Indians.

All subjects underwent complete ophthalmic evaluation, including best-corrected visual acuity; refraction; slit-lamp biomicroscopy; intraocular pressure (IOP) measurement by Goldmann applanation tonometer (three readings on three occasions spaced 2 weeks apart at approximately the same time of the day to avoid diurnal fluctuation of IOP); gonioscopy by Volk four-mirror Sussman Goniolens; optic disc and stereoscopic fundus evaluation with +78-diopter lens; central corneal thickness measurement with Ultrasonic Pachymeter (DGH 550, Pachette 2; DGH Technology, Inc., Exton, PA); achromatic automated perimetry using Swedish Interactive Threshold Algorithm (SITA), Standard 24-2 program with Humphrey visual field analyzer (Carl Zeiss Meditec, Dublin, CA); and RNFL thickness measurement with SD-OCT (SLO V2.26 version; OPKO/OTI Spectral OCT/SLO, a division of OPKO Health, Inc., Miami, FL).

Informed consent was obtained from each subject. Only one randomly selected eye per subject was included in the study. For inclusion, subjects had to have best-corrected visual acuity of 20/30 or better, refractive error within ±3 diopters sphere and ±1.5 diopters cylinder, clear ocular media (nuclear opalescence, nuclear color, and cortical changes up to grade 3 [NO1-3, NC1-3, C1-3] on Lens Opacities Classification System III), and open angles on gonioscopy.

Subjects with intraocular surgery, ocular trauma, neurological disease, corneal pathology, uveitis, retinal or macular pathology, abnormal discs (eg, tilted discs), and peripapillary atrophy were excluded.

Normal subjects had normal ocular examination, IOP less than 21 mm Hg, no history of raised IOP, normal optic disc appearance (ie, absence of glaucomatous optic neuropathy [defined as no vertical cup–disc asymmetry between 2 eyes and intact neuroretinal rim]), and normal and reliable achromatic automated visual field. Normal visual field indices were defined as mean deviation (MD) and pattern standard deviation (PSD) within 95% confidence limits and glaucoma hemifield test within normal limits. Reliability criteria for automated perimetry were fixation losses of 20% or less and false-positive and false-negative results of 33% or less.
Inclusion criteria for eyes with ocular hypertension was the same as that for normal subjects with the exception that their IOP was between 24 and 32 mm Hg after diurnal day tension recording from 8:00 a.m. to 7:00 p.m.

Patients with glaucoma had IOP greater than 22 mm Hg on more than three occasions, characteristic glaucomatous optic nerve damage (defined as vertical cup–disc asymmetry > 0.2 between 2 eyes, neuroretinal rim thinning, notching or excavation, localized pallor, or RNFL defect), and associated achromatic visual field loss in a corresponding location (which satisfied ≥ 2 of Anderson and Parella’s criteria³). Visual field results needed to be reproducible on at least two consecutive examinations. Thirty patients had early glaucoma (MD > -6 dB) and 21 patients had moderate glaucoma (MD between -6 and -12 dB). Patients with advanced glaucoma (MD worse than -12 decibels) were not included in the study because severe disease is associated with increased sensitivity of any imaging modality.

OCT Technique

After dilation with 1% tropicamide eye drops, all images were acquired by a single operator (TM) using SD-OCT. During the scanning mode, the Spectral OCT/SLO produces and displays the cross-sectional OCT image and confocal fundus image through the same optics at the same time. These images are displayed simultaneously on the computer screen and correspond pixel to pixel.

The RNFL thickness was measured with RNFL scanning mode. The subjects were asked to look at the internal fixation target and the circular scan with a diameter of 3.4 mm was centered around the optic disc while the location was observed on the scanning laser ophthalmoscope image to ensure proper positioning of the scan in relation to the optic nerve head. Once the system captures three sequential OCT images where the RNFL thickness is within 5% variation, the scanning automatically stops. The software tracks the position of RNFL OCT scan and its position in relation to the optic nerve.

The RNFL analysis uses an automated OCT software algorithm to identify anterior and posterior margins of RNFL and averages the measurements around the circular scan to obtain average RNFL thickness globally and for four quadrants (superior, nasal, inferior, and temporal). The sectors were defined in clockwise order for the right eye and counter-clockwise order for the left eye.

To be acceptable for inclusion, scans must have been properly centered on the optic disc. Scans with bad centering, artifacts, RNFL discontinuity, or misalignment were excluded. Quality of the scan image was subjectively assessed by the experienced operator (TM) so that there must have been red color visible in the retinal pigment epithelium and RNFL, dense and even color saturation across the entire scan, and a signal-to-noise ratio of greater than 7.

Statistical Methods

A previous study⁶ with Stratus OCT reported a standard deviation ranging between 10 and 15 µm and an approximately 20-µm difference in RNFL thickness measurement between glaucomatous and normal eyes. The minimum sample size required with a significance level of 5% and a power of 90% was calculated to be: N = [2 × (1.96 + 1.28 )² × 15²]²0² = 11.8 in each group.

Statistical analysis was performed using SPSS for Windows software, version 15 (SPSS Inc., Chicago, IL). Descriptive analysis including mean and standard deviation values with 95% confidence intervals was calculated for RNFL parameters in the three groups. Analysis of variance with post hoc Bonferroni comparisons was used to compare the difference between various RNFL parameters among the three groups. A P value of .05 or less was considered statistically significant.

The discriminating power of each OCT parameter was evaluated by calculating areas under receiver operating characteristic (AROC) curves. To determine effect of age differences on mean and quadrant RNFL thickness between the groups, a linear regression model was applied.

RESULTS

One hundred seventy-two patients were enrolled in the study. This included one randomly selected eye of 66 normal patients, 55 patients with ocular hypertension, and 51 patients with glaucoma. Baseline characteristics of the three groups are listed in Table 1.

The mean age ± standard deviation was 51.1 ± 12.9 years (range: 33 to 79 years) in normal eyes, 51.6 ± 10.5 years (range: 31 to 65 years) in eyes with ocu-
Lar hypertension, and 57.4 ± 10.4 years (range: 31 to 69 years) in glaucomatous eyes, and was significantly higher (analysis of variance; \( P = .007 \)) in patients with glaucoma compared with normal patients and patients with ocular hypertension. However, linear regression model revealed that age was not correlated with any RNFL thickness parameter. Hence, age was not considered a significant factor in subsequent analyses.

There was no significant difference between groups regarding gender, refractive error, and central corneal thickness (Table 1).

With reference to global indices, patients with glaucoma had MD and PSD significantly different from those of normal eyes and eyes with ocular hypertension (\( P = .001 \)). There was no difference in MD (\( P = .9 \)) and PSD (\( P = 0.7 \)) between normal eyes and eyes with ocular hypertension (Table 1).

Mean RNFL thickness was 112.5 ± 6.8 µm in normal eyes (range: 92 to 128 µm), 110.1 ± 10.9 µm (range: 88 to 138 µm) in eyes with ocular hypertension, and 93.4 ± 16.9 µm (range: 73 to 128 µm) in glaucomatous eyes. Mean RNFL thickness between normal eyes and eyes with ocular hypertension did not show any significant difference (\( P = .3 \)). However, mean RNFL thickness showed a significant difference between glaucomatous eyes, normal eyes, and eyes with ocular hypertension (\( P < .001 \)) (Table 2).

Temporal quadrant RNFL thickness measurements

| TABLE 1 | Baseline Characteristics of Subjects in Three Subgroups |
| --- | --- | --- | --- |
| Characteristic | Normal \((n = 66)\) | Ocular Hypertension \((n = 55)\) | Glaucoma \((n = 51)\) |
| No. of eyes | 66 | 55 | 51 |
| Age ± SD (y) | 51.06 ± 12.9 | 51.64 ± 10.5 | 57.45 ± 10.4 |
| Gender | | | |
| Males | 28 (42.4%) | 28 (50.9%) | 23 (45.1%) |
| Females | 38 (57.6%) | 27 (49.1%) | 28 (54.9%) |
| Mean deviation ± SD (dB) | -1.27 ± 0.82 | -1.52 ± 0.60 | -8.20 ± 3.75 |
| Pattern standard deviation ± SD (dB) | 1.29 ± 0.24 | 1.71 ± 0.17 | 6.42 ± 3.03 |
| Refractive error ± SD (D) | -0.49 ± 2.07 | -0.55 ± 0.28 | -0.44 ± 1.27 |
| Central corneal thickness ± SD (µm) | 540 ± 30.18 | 535 ± 20.09 | 538 ± 32.32 |

SD = standard deviation; dB = decibels; D = diopters.

| TABLE 2 | Mean RNFL Thickness Measurement in Three Subgroups |
| --- | --- | --- | --- |
| RNFL Parameters | Normal \((n = 66)\) | OHT \((n = 55)\) | Glaucoma \((n = 51)\) |
| Average | 112.48 ± 6.8 (110.82 to 114.15) | 110.09 ± 10.9 (107.14 to 113.04) | 93.45 ± 16.9 (88.70 to 98.21) |
| Superior quadrant | 136.86 ± 14.3 (133.35 to 140.38) | 136.76 ± 14.7 (132.79 to 140.74) | 105.88 ± 26.6 (98.41 to 113.35) |
| Temporal quadrant | 71.32 ± 11.6 (68.47 to 74.16) | 65.31 ± 12.0 (62.06 to 68.56) | 67.04 ± 12.1 (63.63 to 70.45) |
| Inferior quadrant | 140.88 ± 12.9 (137.70 to 144.06) | 139.91 ± 11.7 (136.73 to 143.09) | 105.31 ± 16.8 (100.60 to 110.03) |
| Nasal quadrant | 100.02 ± 12.1 (97.04 to 102.99) | 98.51 ± 13.0 (94.99 to 102.03) | 95.61 ± 18.9 (90.28 to 100.93) |

RNFL = retinal nerve fiber layer; OHT = ocular hypertension.

\( ^a \) ± standard deviation (95% confidence interval).

\( ^b \) P value.
were significantly thinner in eyes with ocular hypertension compared with normal eyes \((P = .006)\). RNFL thickness in glaucomatous eyes differed significantly from normal eyes in most parameters except the nasal quadrant \((P = .1)\). The majority of RNFL parameters were significantly thinner in eyes with glaucoma than in eyes with ocular hypertension except the temporal \((P = .4)\) and nasal \((P = .3)\) quadrants. There was overlap in RNFL thickness between eyes with ocular hypertension and normal eyes (Table 2).

AROC curves to discriminate normal eyes from eyes with ocular hypertension and eyes with glaucoma are shown in Table 3. An AROC of 1 represents perfect discrimination, whereas an area of 0.5 represents chance discrepancy. Temporal quadrant RNFL measurements had the largest AROC curve to discriminate eyes with ocular hypertension from normal eyes \((0.65)\) (Fig. 1). The inferior quadrant \((0.93)\) had the largest AROC curve to distinguish between normal and glaucomatous eyes (Fig. 2). The inferior quadrant \((0.93)\) had the largest AROC curve among all parameters to distinguish between eyes with ocular hypertension and eyes with glaucoma (Fig. 3).

### DISCUSSION

Glucomatous optic nerve damage results in RNFL thinning that has been known to develop earlier than the morphological change in the optic disc.\(^1\) Hence, assessment of such alteration is of help in early diagnosis of glaucoma.

Long-term follow-up studies of subjects with ocular hypertension report a conversion to glaucoma in a range of 0% to 52%.\(^7\) Quigley et al.\(^8\) showed preexisting RNFL defects in 57% of eyes with ocular hypertension that converted from normal to defective visual fields.

### TABLE 3

<table>
<thead>
<tr>
<th>RNFL Parameters</th>
<th>Normal vs OHT AROC and (Standard Error)</th>
<th>OHT vs Glaucoma AROC and (Standard Error)</th>
<th>Normal vs Glaucoma AROC and (Standard Error)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average</td>
<td>0.616 (.05)</td>
<td>0.763 (.05)</td>
<td>0.778 (.05)</td>
</tr>
<tr>
<td>Superior quadrant</td>
<td>0.515 (.05)</td>
<td>0.816 (.05)</td>
<td>0.819 (.05)</td>
</tr>
<tr>
<td>Temporal quadrant</td>
<td>0.651 (.05)</td>
<td>0.470 (.06)</td>
<td>0.601 (.06)</td>
</tr>
<tr>
<td>Inferior quadrant</td>
<td>0.554 (.05)</td>
<td>0.932 (.02)</td>
<td>0.934 (.02)</td>
</tr>
<tr>
<td>Nasal quadrant</td>
<td>0.530 (.05)</td>
<td>0.593 (.06)</td>
<td>0.611 (.06)</td>
</tr>
</tbody>
</table>

\(AROC = \) area under receiver operating characteristic; RNFL = retinal nerve fiber layer; OHT = ocular hypertension.
Lalezary et al.\textsuperscript{2} reported that thinner OCT RNFL measurements at baseline were associated with development of glaucomatous change in eyes with suspect glaucoma. The clinical significance of determining RNFL thickness with OCT in normal eyes, eyes with ocular hypertension, and glaucomatous eyes is to know whether the lower measurements obtained for eyes with ocular hypertension are really predictors of those who will develop glaucoma.

Although some studies have failed to find significant differences in RNFL thickness between eyes with ocular hypertension and normal eyes,\textsuperscript{9-11} others have found significantly thinner RNFL thickness in eyes with ocular hypertension.\textsuperscript{12-14} This may be due to racial differences, differences in sample size, imaging procedures, population age studied, criteria used to select the study population, and the interindividual peripapillary RNFL thickness variability.

Anton et al.\textsuperscript{9} reported that mean RNFL thickness around the disc and superior and inferior RNFL thickness were significantly thinner in glaucomatous eyes than in eyes with ocular hypertension or normal eyes ($P < .001$). They found no significant difference in RNFL parameters between eyes with ocular hypertension and normal eyes. Using Stratus OCT, Mistlberger et al.\textsuperscript{10} reported that although the mean RNFL thickness tended to be thinner in eyes with ocular hypertension than in normal eyes, this difference was not statistically significant (83.7 ± 16.6 vs 90.9 ± 14.2 µm; $P = .1$). RNFL thickness was less in glaucomatous eyes (56.9 ± 21.5 µm; $P < .001$) than in normal eyes and eyes with ocular hypertension.

Hoh et al.\textsuperscript{11} reported that the mean RNFL thickness measured with OCT was significantly less in glaucomatous eyes (56.9 ± 21.5 µm) than in eyes with ocular hypertension (83.7 ± 16.6 µm) and normal eyes (90.9 ± 14.2 µm). Although RNFL thickness tended to be greater in normal eyes than in eyes with ocular hypertension, this difference was not statistically significant.

Tjon-Fo-Sang et al.\textsuperscript{12} reported significantly decreased mean superior and inferior RNFL thickness in patients with ocular hypertension compared with those of normal subjects as measured by scanning laser polarimetry. They found that a mean RNFL thickness in patients with ocular hypertension was 30% less than that in age-matched control subjects.

Bowd et al.\textsuperscript{13} used OCT 2000 and found that the mean RNFL was significantly thinner in eyes with ocular hypertension than in normal eyes, 72.8 µm (range: 66.4 to 78.1 µm) and 85.8 µm (80.2 to 91.7 µm), respectively. The mean RNFL was thinned by an average of 15% in eyes with ocular hypertension. Inferior quadrant RNFL thickness was 84.8 µm (range: 75.6 to 94 µm) versus 107.6 µm (range: 99.3 to 115.9 µm) in normal eyes and nasal quadrant RNFL thickness was 44.1 µm (range: 37.5 to 51.7 µm) versus 61.8 µm (range: 53 to 65.6 µm) in normal eyes. Mean and quadrant RNFL thickness was significantly thinner in glaucomatous eyes than in eyes with ocular hypertension and normal eyes.

Gyatsho et al.\textsuperscript{14} used Stratus OCT to compare 23 eyes with glaucoma, 24 eyes with ocular hypertension and 48 normal Indian eyes and found that superior, inferior, and global RNFL measurements were significantly thinner in eyes with ocular hypertension compared with normal eyes ($P = .031, .019, \text{ and } .022$, respectively). Inferior and superior RNFL measurements had the largest AROCs (0.717 and 0.700, respectively) to distinguish eyes with ocular hypertension from normal eyes. All five RNFL parameters were significantly thinner in the glaucoma group compared with the ocular hypertension group ($P < .001$). Parameters with the largest AROCs to distinguish these two groups were average and inferior average RNFL measurements ($0.989$ and $0.979$, respectively).

Unlike previous studies, we measured and compared RNFL thickness in eyes with ocular hypertension, normal eyes, and glaucomatous eyes using SD-OCT. Gurses-Ozden et al.\textsuperscript{15} used second-generation
OCT and showed that increasing the sampling density or the number of A-scans can increase the reproducibility of measurements. The SD-OCT enables RNFL thickness measurements with increased resolution, sampling rate, and density. Improved resolution of SD-OCT may increase the likelihood of OCT becoming a useful clinical tool in the management of glaucoma.

In our study, except for temporal quadrant RNFL thickness, none of the parameters showed a significant difference between normal eyes and eyes with ocular hypertension. We found that SD-OCT identified differences in most parameters between patients with glaucoma and normal individuals and also between patients with glaucoma and patients with ocular hypertension, which confirms previous reports. Most studies using Stratus OCT have identified the average and inferior average RNFL thickness as the best discriminators between normal and glaucomatous eyes. We found inferior quadrant RNFL thickness to be the best parameter to discriminate between normal and glaucomatous eyes and eyes with ocular hypertension and glaucoma. The finding that initial RNFL thinning occurs at the inferior quadrant in eyes with ocular hypertension is of interest because visual field defects associated with glaucoma usually occur initially in the superior visual field corresponding to defect in the inferior pole of the optic disc. With these quantitative measures, it may be possible to detect RNFL change to monitor glaucomatous progression in eyes with ocular hypertension.

RNFL thickness is known to have inter-individual and inter-racial variation. The mean peripapillary thickness measured in different studies has ranged from a mean of 80 to 140 µm, which may be due to population age, racial difference, imaging procedures, or the generation of OCT being used. Gyatsho et al. suggested that the slight difference in RNFL thickness may have a bearing on the normative database of the machine and it may be better to have race-specific data.

A limitation of the study is that our patients with ocular hypertension and glaucoma were recruited from an Institute-based glaucoma practice and although most patients with glaucoma had early to moderate glaucoma (indicated by MD), they might not reflect the distribution of RNFL thickness in the general population of patients with ocular hypertension and glaucoma. Although the eyes with ocular hypertension in our study had normal visual fields on achromatic perimetry, other visual function testing by short-wave-length automated perimetry or frequency-doubling perimetry was not done. There is evidence that a subset of patients with ocular hypertension may have preclinical optic nerve damage that is not detected by conventional achromatic perimetry. However, we included only patients with ocular hypertension who had an IOP of more than 24 mm Hg recorded on more than three occasions, and thus normal patients and patients with ocular hypertension were distinguished by a minimum IOP difference of 3 mm Hg, decreasing the likelihood of a patient with ocular hypertension having IOP that overlaps with normal subjects.

In our study, influence of optic disc area on RNFL thickness between the three groups was not assessed. It has been reported that RNFL thickness measurements obtained by Stratus OCT may be influenced by the optic disc size, whereas other studies have failed to find significant correlation.

SD-OCT identified differences in most parameters between patients with glaucoma and normal individuals and also between patients with glaucoma and ocular hypertension. Although there was significant difference in RNFL thickness between eyes with ocular hypertension and normal eyes in the temporal quadrant, other parameters failed to show a statistically significant difference. Overlap in RNFL thickness between normal eyes and eyes with ocular hypertension limits the ability of this instrument to differentiate between normal eyes and eyes with ocular hypertension. Patients at risk of developing glaucoma would be well served if SD-OCT proves to be more sensitive to differentiate RNFL thickness in normal eyes and eyes with ocular hypertension and in eyes with ocular hypertension and glaucoma. Its role as a screening instrument requires longitudinal studies to show whether OCT can detect glaucomatous progression in eyes with ocular hypertension.

REFERENCES