Analysis of Retinal Nerve Fiber Layer and Optic Nerve Head in Glaucoma with Different Reference Plane Offsets, Using Optical Coherence Tomography

Christopher Kai-shun Leung, 1 Wai-man Chan, 2 Yung-lam Hui, 1 Wing-bo Yung, 3 Jackson Woo, 1 Moon-kwong Tsang, 1 and Kwok-kay Tse 1

PURPOSE. To evaluate the performance of retinal nerve fiber layer (RNFL) thickness and optic nerve head (ONH) parameters analyzed with different offsets of reference plane in detecting early glaucomatous changes and in correlation with visual function using optical coherence tomography (OCT).

METHODS. This was a cross-sectional study consisting of 41 normal subjects and 30 with early and 40 with advanced glaucoma. RNFL thickness and ONH parameters were measured with reference planes positioned at 95, 150, and 205 μm above the level of retinal pigment epithelium (RPE). Discriminating power for early glaucoma detection and correlation with visual field MD for each parameter at different levels of reference plane were compared by using the analyses of area under the receiver operating characteristic curves (AUCs) and linear regression, respectively.

RESULTS. All ONH measurements were significantly different between normal and glaucoma groups, irrespective of the level of reference plane. In normal eyes, changing the reference plane position resulted in significant differences in ONH measurements. Among all the parameters examined, integrated rim volume and RNFL thickness measured at 150 μm above the RPE showed the largest AUC (0.966) for early glaucoma detection, and the strongest correlation with visual function (r = 0.793), respectively.

CONCLUSIONS. OCT analysis of the ONH and RNFL is useful for early glaucoma detection. Among the three reference planes examined in this study, measurements analyzed at 150 μm above the RPE demonstrated the best performance for glaucoma detection and correlation with visual function. Compared with ONH measurements, RNFL thickness may be a better indicator, reflecting retinal ganglion cell function and monitoring disease progression. (Invest Ophthalmol Vis Sci. 2005;46:891–899) DOI: 10.1167/iovs.04-1107

Optic nerve head (ONH) assessment is one of the most crucial elements in diagnosing and monitoring glaucoma. Although subject to interobserver variability, qualitative evaluations of the ONH neuroretinal rim, cup-to-disc ratio, and nerve fiber layer (NFL) defect have been found useful for evaluation of different degrees of glaucomatous damage. As structural changes in the ONH and NFL are usually evident before functional visual field loss, quantifying ONH topology may provide a more reliable and reproducible assessment in glaucoma. The introduction of modern imaging devices such as the confocal scanning laser ophthalmoscope, scanning laser polarimetry, and optical coherence tomography have offered objective and reproducible measurements of the topographic parameters of the ONH and NFL. Although the scanning laser polarimeter (GDX Nerve Fiber Analyzer; Laser Diagnostics Technologies, San Diego, CA) was designed primarily to measure the NFL thickness around the optic disc, confocal scanning laser ophthalmoscopy (Heidelberg Retinal Topography [HRT]; Heidelberg Engineering, Heidelberg, Germany) and optical coherence tomography (OCT; Carl Zeiss Meditec Inc., Dublin, CA) provide quantitative data of both NFL thickness and topographic parameters of the ONH, such as neuroretinal rim area and volume, cup-to-disc area ratio, and cup volume. In several studies, it has been shown that the OCT evaluation of NFL thickness can differentiate between normal and glaucomatous eyes, and yet, it remains uncertain whether NFL thickness or ONH topographic measurement is more closely related to visual field changes and which provides a more sensitive marker for detection of glaucoma.

In contrast, most published studies on ONH topology analysis are oriented around HRT, less attention has been focused on assessing the capability of analyzing the optic disc changes in glaucoma using OCT. Theoretically, OCT is more advantageous over HRT in offering a higher axial resolution, automated outlining of optic disc margin, and a consistent and stable reference plane for delineation of the neuroretinal rim boundary. The determination of the position of the reference plane is the most essential element in ONH topographic analysis, because accurate calculations of ONH parameters depend heavily on an appropriate choosing and marking of a reference plane. OCT determines the level of the reference plane in relation to the fixed anatomic landmarks—the cutoff ends of the retinal pigment epithelium (RPE) around the optic cup, and thus allows a consistent and reproducible estimation of cup-disc boundary and neuroretinal rim area. By default, the reference plane is located 150 μm above the level of RPE. However, this is only an arbitrary location, and no published investigation has been performed to assess the effect of shifting the position of the reference plane in the analysis of ONH parameters and at what level the reference plane should be positioned to yield the maximal performance in glaucoma detection or monitoring. The purpose of this study was to use the OCT to assess the impact of different reference planes on evaluation of glaucoma by varying the positions of the anterior offset from the RPE. We also compared the RNFL thickness and topographic ONH parameters in terms of their performances for detection of early...
glaucoma and their functional correlations with visual field mean sensitivities.

**MATERIALS AND METHODS**

**Subjects**

This study was a noninterventional, cross-sectional study. One eye was selected randomly from each of 41 normal subjects and from 50 with early glaucoma and 40 with advanced glaucoma, who met the inclusion criteria, as will be defined later. All recruited cases were examined in the department of Ophthalmology, Caritas Medical Centre, Hong Kong. Caritas Medical Centre is the ophthalmic referral center in the Kowloon West Cluster serving a population of ~1.2 million. The study was conducted in accordance with the ethical standards stated in the 1964 Declaration of Helsinki and approved by Hong Kong Hospital Authority Kowloon West Cluster Clinical Research Ethics Committee, with informed consent obtained.

The inclusion criteria were best corrected visual acuity not worse than 20/40, spherical refractive error within the range of −6.00 D to +3.00 D, and reliable Humphrey visual field test results (Carl Zeiss Meditec), defined as having fixation loss <20% and false-positive and false-negative errors <25%. Subjects were excluded if they had a history of any retinal disease, surgery or laser procedures, optic disc anomalies such as coloboma or optic disc drusen, or any kind of neurologic diseases that might cause visual field defects. Automated visual field tests were tested in all subjects (Humphrey Field Analyzer II, Carl Zeiss Meditec) using the central 30-2 threshold program. Normal subjects were individuals with no visual field defect, no structural optic disc abnormalities, and no history of intraocular pressure >21 mm Hg. Glaucoma patients were identified entirely based on the presence of visual field defects. A field defect was defined as having three or more significant (P < 0.05) non-edge-contiguous points with at least one at the P < 0.01 level on the same side of the horizontal meridian in the pattern deviation plot and classified according to normal limits in the glaucoma hemifield test. Any detected field defect was confirmed on at least one other attempt to have been considered abnormal. Subjects were then assigned to the early group if the severity of visual field defect, as reflected by the overall sensitivity index (MD), was at or better than −7.0 dB and to the advanced group if the MD was worse than −7.0 dB. Among all 70 subjects with glaucoma, 25 had low-pressure glaucoma, 10 had primary angle-closure glaucoma, 35 had primary open-angle glaucoma, and 2 had uveitic glaucoma.

**OCT Measurements**

The third-generation OCT (Stratus OCT or OCT 3; Carl Zeiss Meditec) was used in the study. The optical principles and applications of OCT have been described by Huang et al. ONH analysis was performed with the fast optic nerve scan protocol. Six radially linear scans centered over the ONH were analyzed cross-sectionally as shown in Figure 1. The algorithm detected and showed the location of the top and inner edges of RPE on each side of the optic disc where a line was joined and signified as the disc diameter (Fig. 1, green line). The reference plane (the cup offset) was then determined by tracing a line parallel to the disc diameter with an anterior offset of 95, 150, or 205 μm (Fig. 1, white line). The neuroretinal rim area in each cross-sectional scan was estimated by the area bounded using the reference plane as the posterior border and the lines extending perpendicularly from the ends of the disc diameter as the lateral boundaries (Fig. 1, red area). The nerve width at the disc on each side was a straight line from each disc reference point to the nearest point on the anterior surface (Fig. 1, yellow line). Data analyzed in each scan were then incorporated and formed the composite image measurements including integrated rim volume (calculated by integration of vertical cross-sectional rim area), integrated rim width (calculated by integration of average nerve width at the disc), disc area, cup area, rim area (disc area − cup area), cup-to-disc area ratio, cup-to-disc horizontal ratio, cup-to-disc vertical ratio, and cup volume. Retinal NFL thickness was measured by averaging the results of three sequential circular scans (512 scan points) with diameter 3.4 mm centered at the ONH. The NFL thickness was determined by the difference in distance between the vitreoretinal interface and a posterior boundary based on a predefined reflectivity signal level. A good-quality scan was one with a signal-to-noise ratio of >35, 100% accepted A-scan and good delineation of the anatomic boundaries. Subjects were not included in the study if the quality of the OCT image was suboptimal.
Statistical Analysis
Statistical analysis was performed on computer (SPSS ver. 11.0; SPSS, Chicago, IL). Differences in the retinal NFL thickness and ONH topographic parameters among the diagnostic groups were evaluated by one-way analysis of variance. Bonferroni correction was used for multiple comparisons. Area under the ROC curve (AUC) was used to assess the ability to differentiate suspected glaucomatous or glaucomatous eyes from normal eyes with each testing parameter. An AUC of 1.0 represents perfect discrimination, whereas an area of 0.5 represents chance discrimination. The method described by Hanley and McNeil was used to compare the AUCs. The relationship between measured parameters and visual field MD was studied with linear regression analysis. Correlation was expressed as the Pearson coefficient of correlation and the Cox and Snell coefficient of determination. The Hosmer-Lemeshow test was performed to evaluate the differences in correlations. In all statistical analyses, \( P < 0.05 \) was considered statistically significant.

RESULTS

Subject Characteristics
One eye was selected randomly from 41 normal subjects and 30 with early glaucoma and 40 with advanced glaucoma. All subjects were Hong Kong Chinese. No significant difference was found among the groups in age and refractive error (Table 1). The visual field MD of early glaucoma (\(-4.30 \pm 1.70 \text{ dB}, P < 0.001\)) and patients with advanced glaucoma (17.66 \( \pm 6.65 \text{ dB}, P < 0.001\)) were significantly different from the normal group.

Retinal NFL Thickness and ONH Topographic Measurements
RNFL thickness and ONH topographic measurements including integrated rim width, integrated rim volume, disc area, cup area, rim area, cup-to-disc area ratio, cup-to-disc horizontal and vertical ratio, and cup volume in the three diagnostic groups analyzed with different settings of the reference plane—namely, 150, 95, and 205 \( \mu \text{m} \) above the RPE—were analyzed and presented in Table 2 (normal group) and Table 3 (all diagnostic groups). Because RNFL thickness, integrated rim width, and disc area were calculated independent of the levels of the reference plane, their values remained constant. When the reference plane was shifted 55 \( \mu \text{m} \) above and below the default level (150 \( \mu \text{m} \) above the RPE), significant changes in the ONH topographic measurements were observed. For example, in integrated rim volume, there was a significant increase when the offset of the reference plane was lowered to 95 \( \mu \text{m} \) and a corresponding decrease when the offset was increased to 205 \( \mu \text{m} \) (Table 2). Irrespective of the level of the reference plane, all measured parameters in the early or advanced glaucoma groups showed statistically significant differences from normal subjects (Table 3). Analysis was also performed comparing normal individuals with patients with primary open-angle, primary angle-closure, or low-pressure glaucoma (Table 4). All parameters but disc area showed significant differences between normal and each type of glaucoma examined. Significant differences in disc area were found only when comparing patients with primary open-angle glaucoma (3.13 \( \pm 0.61 \text{ mm}^2 \)) and normal individuals (2.58 \( \pm 0.50 \text{ mm}^2 ; P < 0.001\)).

Diagnostic Sensitivity for Early Glaucomatous Damage
AUCs were used to compare the discriminating power of RNFL thickness and the ONH topographic parameters measured with different offsets of reference plane in detecting early glaucomatous damage (average visual field MD = \(-4.30 \text{ dB} \); Table 5). Integrated rim volume measured at the default reference plane showed the largest AUC (0.966). It was decreased significantly

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### Table 1. Demographic Characteristics of Study Subjects

<table>
<thead>
<tr>
<th></th>
<th>Normal (n)</th>
<th>Early Glaucoma (n)</th>
<th>Advanced Glaucoma (n)</th>
<th>( P^* )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subjects (n)</td>
<td>41</td>
<td>30</td>
<td>40</td>
<td></td>
</tr>
<tr>
<td>Age (y)</td>
<td>52.3 ± 13.1</td>
<td>59.1 ± 15.0</td>
<td>58.0 ± 14.3</td>
<td>0.096</td>
</tr>
<tr>
<td>Refraction (D)</td>
<td>-0.12 ± 2.17</td>
<td>0.09 ± 2.22</td>
<td>-0.37 ± 2.42</td>
<td>0.704</td>
</tr>
<tr>
<td>Visual field MD (dB)</td>
<td>-1.19 ± 1.17</td>
<td>-4.30 ± 1.70</td>
<td>-17.66 ± 6.56</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

\( \text{Age, refraction, and MD are expressed as the mean ± SD.} \)

\( ^* \text{Analysis of variance.} \)

\( \dagger \text{Analysis of variance with Bonferroni correction.} \)

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### Table 2. Comparisons of RNFL Thickness and Topographic ONH Parameters in Normal Subjects in Different Levels of Reference Plane

<table>
<thead>
<tr>
<th></th>
<th>Reference Plane Offset = 150 ( \mu \text{m} )</th>
<th>Reference Plane Offset = 95 ( \mu \text{m} )</th>
<th>Reference Plane Offset = 205 ( \mu \text{m} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>RNFL thickness (( \mu \text{m} ))</td>
<td>103.29 ± 9.52</td>
<td>103.29 ± 9.52</td>
<td>103.29 ± 9.52</td>
</tr>
<tr>
<td>Integrated rim width (( \text{mm}^2 ))</td>
<td>1.677 ± 0.178</td>
<td>0.460 ± 0.161 (( P &lt; 0.001 ))</td>
<td>0.186 ± 0.108 (( P &lt; 0.001 ))</td>
</tr>
<tr>
<td>Integrated rim volume (( \text{mm}^3 ))</td>
<td>0.309 ± 0.134</td>
<td>2.584 ± 0.503</td>
<td>2.584 ± 0.503</td>
</tr>
<tr>
<td>Disc area (( \text{mm}^2 ))</td>
<td>0.975 ± 0.464</td>
<td>0.751 ± 0.398 (( P = 0.108 ))</td>
<td>1.259 ± 0.550 (( P = 0.022 ))</td>
</tr>
<tr>
<td>Cup area (( \text{mm}^2 ))</td>
<td>1.612 ± 0.290</td>
<td>1.853 ± 0.367 (( P = 0.005 ))</td>
<td>1.328 ± 0.303 (( P &lt; 0.001 ))</td>
</tr>
<tr>
<td>Rim area (( \text{mm}^2 ))</td>
<td>0.362 ± 0.130</td>
<td>0.278 ± 0.116 (( P = 0.014 ))</td>
<td>0.471 ± 0.146 (( P = 0.001 ))</td>
</tr>
<tr>
<td>Cup-to-disc area ratio</td>
<td>0.638 ± 0.154</td>
<td>0.540 ± 0.148 (( P = 0.012 ))</td>
<td>0.741 ± 0.151 (( P = 0.008 ))</td>
</tr>
<tr>
<td>Cup-to-disc horizontal ratio</td>
<td>0.550 ± 0.112</td>
<td>0.494 ± 0.117 (( P = 0.084 ))</td>
<td>0.621 ± 0.112 (( P = 0.017 ))</td>
</tr>
<tr>
<td>Cup volume (( \text{mm}^3 ))</td>
<td>0.145 ± 0.106</td>
<td>0.111 ± 0.089 (( P = 0.477 ))</td>
<td>0.197 ± 0.126 (( P = 0.094 ))</td>
</tr>
</tbody>
</table>

Data are expressed as the mean ± SD. Probabilities were calculated with analysis of variance with Bonferroni correction compared with reference plane offset at 150 \( \mu \text{m} \).

\( ^* \text{Significant differences.} \)

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<table>
<thead>
<tr>
<th></th>
<th>Reference Plane Offset = 150 μm</th>
<th>Reference Plane Offset = 95 μm</th>
<th>Reference Plane Offset = 205 μm</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>RNFL thickness (μm)</strong></td>
<td>103.29 ± 9.52</td>
<td>75.90 ± 13.25</td>
<td>55.04 ± 14.60</td>
</tr>
<tr>
<td></td>
<td>(P &lt; 0.001)</td>
<td>(P &lt; 0.001)</td>
<td>(P &lt; 0.001)</td>
</tr>
<tr>
<td>**Integrated rim width</td>
<td>1.677 ± 0.178</td>
<td>1.277 ± 0.220</td>
<td>1.028 ± 0.230</td>
</tr>
<tr>
<td>(mm²)</td>
<td>(P &lt; 0.001)</td>
<td>(P &lt; 0.001)</td>
<td>(P &lt; 0.001)</td>
</tr>
<tr>
<td>**Integrated rim volume</td>
<td>0.390 ± 0.134</td>
<td>0.100 ± 0.069</td>
<td>0.047 ± 0.056</td>
</tr>
<tr>
<td>(mm³)</td>
<td>(P &lt; 0.001)</td>
<td>(P &lt; 0.001)</td>
<td>(P &lt; 0.001)</td>
</tr>
<tr>
<td>**Disc area (mm²)</td>
<td>2.584 ± 0.503</td>
<td>3.092 ± 0.505</td>
<td>3.007 ± 0.684</td>
</tr>
<tr>
<td></td>
<td>(P = 0.004)</td>
<td>(P = 0.001)</td>
<td>(P = 0.004)</td>
</tr>
<tr>
<td>**Cup area (mm²)</td>
<td>0.973 ± 0.464</td>
<td>2.136 ± 0.565</td>
<td>2.448 ± 0.755</td>
</tr>
<tr>
<td></td>
<td>(P &lt; 0.001)</td>
<td>(P &lt; 0.001)</td>
<td>(P &lt; 0.001)</td>
</tr>
<tr>
<td>**Rim area (mm²)</td>
<td>1.612 ± 0.290</td>
<td>0.965 ± 0.390</td>
<td>0.565 ± 0.285</td>
</tr>
<tr>
<td></td>
<td>(P &lt; 0.001)</td>
<td>(P &lt; 0.001)</td>
<td>(P &lt; 0.001)</td>
</tr>
<tr>
<td>**Cup-to-disc area ratio</td>
<td>0.362 ± 0.130</td>
<td>0.686 ± 0.128</td>
<td>0.805 ± 0.112</td>
</tr>
<tr>
<td></td>
<td>(P &lt; 0.001)</td>
<td>(P &lt; 0.001)</td>
<td>(P &lt; 0.001)</td>
</tr>
<tr>
<td><strong>Cup-to-disc horizontal ratio</strong></td>
<td>0.638 ± 0.154</td>
<td>0.856 ± 0.084</td>
<td>0.917 ± 0.060</td>
</tr>
<tr>
<td></td>
<td>(P &lt; 0.001)</td>
<td>(P &lt; 0.001)</td>
<td>(P &lt; 0.001)</td>
</tr>
<tr>
<td><strong>Cup-to-disc vertical ratio</strong></td>
<td>0.550 ± 0.112</td>
<td>0.794 ± 0.098</td>
<td>0.874 ± 0.078</td>
</tr>
<tr>
<td></td>
<td>(P &lt; 0.001)</td>
<td>(P &lt; 0.001)</td>
<td>(P &lt; 0.001)</td>
</tr>
<tr>
<td>**Cup volume (mm³)</td>
<td>0.145 ± 0.106</td>
<td>0.499 ± 0.235</td>
<td>0.584 ± 0.277</td>
</tr>
<tr>
<td></td>
<td>(P &lt; 0.001)</td>
<td>(P &lt; 0.001)</td>
<td>(P &lt; 0.001)</td>
</tr>
</tbody>
</table>

Data are expressed as the mean ± SD. Probabilities were calculated using analysis of variance with Bonferroni correction compared with normal group. All differences were statistically significant.
when the offset of the reference plane was lowered to 95 \( \mu \text{m} \) (0.966 vs. 0.941, \( P = 0.009 \); Fig. 2), whereas it was essentially unchanged when the offset was at the level of 205 \( \mu \text{m} \). No significant difference was observed in AUC comparing RNFL thickness and any of the ONH parameters except disc area.

**Functional Correlation with Severity of Visual Field Defect**

To assess the functional correlation between the measured parameters and the mean sensitivity of the visual field, coefficient of correlation, and coefficient of determination were calculated and are presented in Table 6. Whereas rim area measured with the default reference plane demonstrated the highest correlation with visual function (\( r = 0.742 \)) among all ONH-related measurements, it was evident that RNFL thickness achieved the strongest correlation among all the measured parameters (\( r = 0.793 \)). Apart from rim area (\( P = 0.08 \)), statistically significant differences were found in the coefficient of correlation comparing RNFL thickness and any of the ONH parameters (all with \( P < 0.05 \)). For rim area, a significant decrease in correlation coefficient was found when the reference plane offset was shifted to 95 \( \mu \text{m} \) (0.742 vs. 0.668, \( P = 0.001 \)) although the decrease was not significant when measured at 205 \( \mu \text{m} \) (0.742 vs. 0.720, \( P = 0.206 \)). Figure 3 presents scatterplots of rim area versus visual field MD in three different positions of reference plane. Collectively, among the three reference planes examined parameters measured at the reference plane located 150 \( \mu \text{m} \) above the level of RPE had the optimal functional correlation with visual function. Correlations between ONH topographic parameters and RNFL thickness were also evaluated, and integrated rim width was found to have the highest correlation with RNFL thickness (\( r = 0.895 \)).

**Table 4.** Comparisons of RNFL Thickness and Topographic ONH Parameters in Low-Pressure Glaucoma (LPG), Primary Angle-Closure Glaucoma (PACG), and Primary Open-Angle Glaucoma (POAG)

<table>
<thead>
<tr>
<th>Reference Plane Offset = 150 ( \mu \text{m} )</th>
<th>Normal ( (n = 41) )</th>
<th>LPG ( (n = 25) )</th>
<th>PACG ( (n = 10) )</th>
<th>POAG ( (n = 33) )</th>
<th>( P^* )</th>
</tr>
</thead>
<tbody>
<tr>
<td>RNFL thickness (( \mu \text{m} ))</td>
<td>103.29 ± 9.52</td>
<td>66.36 ± 18.80</td>
<td>64.85 ± 14.84</td>
<td>62.30 ± 17.42</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Integrated rim width (mm(^2))</td>
<td>1.677 ± 0.178</td>
<td>1.135 ± 0.262</td>
<td>1.179 ± 0.545</td>
<td>1.125 ± 0.282</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Integrated rim volume (mm(^3))</td>
<td>0.309 ± 0.134</td>
<td>0.078 ± 0.067</td>
<td>0.090 ± 0.108</td>
<td>0.060 ± 0.051</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Disc area (mm(^2))</td>
<td>2.584 ± 0.503</td>
<td>2.889 ± 0.589</td>
<td>3.092 ± 0.716</td>
<td>3.127 ± 0.607</td>
<td>=0.001</td>
</tr>
<tr>
<td>Cup area (mm(^2))</td>
<td>0.973 ± 0.464</td>
<td>2.125 ± 0.532</td>
<td>2.245 ± 0.975</td>
<td>2.249 ± 0.705</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Rim area (mm(^2))</td>
<td>1.612 ± 0.290</td>
<td>0.771 ± 0.568</td>
<td>0.847 ± 0.479</td>
<td>0.685 ± 0.384</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Cup-to-disc area ratio</td>
<td>0.362 ± 0.130</td>
<td>0.736 ± 0.117</td>
<td>0.707 ± 0.184</td>
<td>0.799 ± 0.127</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Cup-to-disc horizontal ratio</td>
<td>0.638 ± 0.154</td>
<td>0.885 ± 0.692</td>
<td>0.850 ± 0.130</td>
<td>0.906 ± 0.060</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Cup-to-disc vertical ratio</td>
<td>0.550 ± 0.112</td>
<td>0.825 ± 0.083</td>
<td>0.810 ± 0.128</td>
<td>0.857 ± 0.094</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Cup area (mm(^2))</td>
<td>0.145 ± 0.106</td>
<td>0.504 ± 0.231</td>
<td>0.551 ± 0.399</td>
<td>0.578 ± 0.244</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Visual field MD (dB)</td>
<td>−1.18 ± 1.17</td>
<td>−10.45 ± 7.53</td>
<td>−13.64 ± 10.97</td>
<td>−12.88 ± 8.29</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Data are expressed as the mean ± SD. Probabilities were calculated with analysis of variance with Bonferroni correction compared with the normal group. No significant difference was found in any of the ONH parameters and visual field MD among the different types of glaucoma.

\* Significant differences from normal.

**Table 5.** AUC of RNFL Thickness and Each of the Topographic ONH Parameters for Discriminating Early Glaucoma from Normal

<table>
<thead>
<tr>
<th>Reference Plane Offset = 150 ( \mu \text{m} )</th>
<th>Reference Plane Offset = 95 ( \mu \text{m} )</th>
<th>Reference Plane Offset = 205 ( \mu \text{m} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>RNFL thickness</td>
<td>0.957</td>
<td>0.957</td>
</tr>
<tr>
<td>Integrated rim width (mm(^2))</td>
<td>0.929</td>
<td>0.929</td>
</tr>
<tr>
<td>Integrated rim volume (mm(^3))</td>
<td>0.966</td>
<td>0.941</td>
</tr>
<tr>
<td>Disc area (mm(^2))</td>
<td>0.749</td>
<td>0.765</td>
</tr>
<tr>
<td>Cup area (mm(^2))</td>
<td>0.935</td>
<td>0.930</td>
</tr>
<tr>
<td>Rim area (mm(^2))</td>
<td>0.913</td>
<td>0.848</td>
</tr>
<tr>
<td>Cup-to-disc area ratio</td>
<td>0.960</td>
<td>0.943</td>
</tr>
<tr>
<td>Cup-to-disc horizontal ratio</td>
<td>0.921</td>
<td>0.925</td>
</tr>
<tr>
<td>Cup-to-disc vertical ratio</td>
<td>0.962</td>
<td>0.931</td>
</tr>
<tr>
<td>Cup volume (mm(^3))</td>
<td>0.921</td>
<td>0.907</td>
</tr>
</tbody>
</table>
However, a recent study by Medeiros et al.\cite{13} demonstrated the importance of rim shape and volume, and compared at three different reference planes located at 95, 150, and 205 μm above the RPE. This is in contrast to the HRT in which the reference plane is set below the variable reference plane along the z-axis defined and adopted by the HRT. In the linear discriminant analysis of Mikelberg et al.,\cite{12} the parameters for glaucoma detection. In Moorfield regression analysis, log neuroretinal rim area was found to have the highest sensitivity (84.3%) and specificity (96.3%) in separating patients with early glaucoma from normal subjects.\cite{11}

Accurate determination of the level of the reference plane is the most essential aspect of ONH analysis, because it has a direct impact on all the subsequent analyses of ONH parameters. In the Stratus OCT (Carl Zeiss Meditec), the default reference plane divides the rim–cup boundary at an arbitrary level, 150 μm above the RPE. To identify the optimal reference plane for evaluation of ONH parameters, analyses were performed and compared at three different reference planes located at 95, 150, and 205 μm above the RPE. Significant differences in ONH table 6. Comparison of Coefficients of Correlation and Determination between the Examined Parameters with Visual Field MD and RNFL thickness in Different Levels of Reference Plane

<table>
<thead>
<tr>
<th>Reference Plane Offset = 150 μm</th>
<th>Reference Plane Offset = 95 μm</th>
<th>Reference Plane Offset = 205 μm</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Visual Field MD</td>
<td>RNFL Thickness</td>
</tr>
<tr>
<td></td>
<td>( r/R^2 )</td>
<td>( r/R^2 )</td>
</tr>
<tr>
<td>RNFL thickness</td>
<td>0.793/0.629</td>
<td>—</td>
</tr>
<tr>
<td>Integrated rim width (mm²)</td>
<td>0.736/0.542</td>
<td>0.895/0.801</td>
</tr>
<tr>
<td>Integrated rim volume (mm³)</td>
<td>0.618/0.382</td>
<td>0.779/0.607</td>
</tr>
<tr>
<td>Disc area (mm²)</td>
<td>0.278/0.077</td>
<td>0.265/0.070</td>
</tr>
<tr>
<td>Cup area (mm²)</td>
<td>0.653/0.427</td>
<td>0.720/0.518</td>
</tr>
<tr>
<td>Rim area (mm²)</td>
<td>0.742/0.551</td>
<td>0.871/0.758</td>
</tr>
<tr>
<td>Cup-to-Disc area ratio</td>
<td>0.727/0.529</td>
<td>0.856/0.773</td>
</tr>
<tr>
<td>Cup-to-Disc horizontal ratio</td>
<td>0.609/0.370</td>
<td>0.737/0.547</td>
</tr>
<tr>
<td>Cup-to-Disc vertical ratio</td>
<td>0.698/0.487</td>
<td>0.830/0.688</td>
</tr>
<tr>
<td>Cup volume (mm³)</td>
<td>0.604/0.365</td>
<td>0.664/0.440</td>
</tr>
</tbody>
</table>

\( r \), coefficient of correlation; \( R^2 \), coefficient of determination.
measurements were observed when the reference plane offset was changed (Table 2). In this study, relatively high sensitivities and specificities for detection of early glaucoma were evident in most of the ONH parameters examined (Table 5). Integrated rim volume, cup-to-disc area ratio, and vertical cup-to-disc ratio analyzed with the default reference plane attained the largest AUCs (AUC = 0.966, 0.960, and 0.962, respectively) among all the ONH measurements. These findings were consistent with an earlier report by Schuman et al.\textsuperscript{17} Among five individual ONH parameters examined by OCT3 (disc area, cup-to-disc area ratio, cup area, cup volume, and rim volume), they found cup-to-disc area ratio and rim volume had the largest AUCs when comparing the glaucoma group with the normal and suspected glaucoma groups (AUCs = 0.79 and 0.77, respectively). In terms of correlation with visual function, integrated rim width, rim area, and cup-to-disc area ratio had the highest correlation (all with \( r > 0.700 \)). The correlations were weakened in rim area and cup-to-disc area ratio when the level of reference plane was changed. The coefficients of correlation remained the same for integrated rim width, as its calculation was independent of the levels of reference plane. Our results showed that the default reference plane had the optimal performance in terms of glaucoma detection and correlation with visual function.

RNFL thickness, in contrast to ONH parameters, is less controversial, and numerous reports have arrived at the consistent conclusion that it is a useful surrogate marker for assessment of structural damage in glaucoma.\textsuperscript{1–6} Depending on the selection of subjects and the severity of visual field defect at the time of inclusion, the diagnostic sensitivity of RNFL thickness could achieve a level as high as \( >90\% \) at a specificity of 90\%. Yet, it is still uncertain whether RNFL thickness or ONH measurements is a better clinical indicator for the assessment of glucomatous damage. In the present study, both RNFL thickness and integrated rim volume demonstrated comparable performance for detection of early glaucoma in each of the reference planes tested. Because all the optic nerve fibers finally converge toward the ONH, one would expect a corresponding change in neuroretinal rim area/volume when there is reduction in RNFL thickness. (This was reflected by the high correlation between RNFL thickness and the integrated rim width/volume/rim area as shown in Table 6.) This concurred with the findings that both RNFL thickness and most of the ONH measurements attained similar performance in diagnostic sensitivity for glaucoma. However, a stronger correlation with visual function was evident in RNFL thickness compared with all the ONH measurements (Table 6). Theoretically, RNFL is a more direct measure of ganglion cell function in contrast to neuroretinal rim volume, which comprises other supportive connective tissue structures and glial cells besides the RNFL. Moreover, the characteristic cupping of ONH in glaucoma involves not only loss of the axons but also change of supporting tissue integrity in the lamina cribrosa. Therefore, structural thinning of the RNFL is more closely related to the real-time functional loss of ganglion cells and then to the visual field defect. It is interesting to note that although integrated rim volume demonstrates the best performance in detection of early glaucoma, it is the rim area that shows the best correlation with visual function among all the ONH measurements. The observed difference may also be explained by the fact that whereas integrated rim volume is a three-dimensional measurement of the bulk of neuroretinal rim consisting of both nerve fiber bundles and their supporting tissues, rim area is a two-dimensional measurement, calculated by subtracting the cup area from the disc area and therefore reflecting more contribution from the nerve fiber bundles in proportion to supporting tissues. This is in agreement with the finding that rim area and integrated rim width had the best correlation with RNFL thickness compared with all ONH measurements. Collectively, although both RNFL and ONH measurements are useful for diagnosing glaucoma, RNFL thickness may be a better surrogate marker for monitoring progression of glucomatous damage compared with ONH measurements. A prospective study would be useful to address this question fully.

We did not find any difference in ONH parameters among primary open-angle, primary angle-closure, and normal-tension glaucoma, although significant differences were evident in all ONH measurements, except the optic disc area, between normal and the three glaucoma subgroups. Controversy still exists regarding the relationship between optic disc size and glaucoma. Although some studies have reported larger optic disc size in glaucoma when using the HRT or OCT,\textsuperscript{17,19} others did not find differences in optic disc size between normal subjects and those with glaucoma.\textsuperscript{11,19} In the Blue Mountains Eye Study with 3654 subjects examined, larger optic disc size was found in patients with glaucoma, and a significant difference in mean disc diameter was found only when comparing the normal and high-pressure glaucoma groups, but not the low-pressure group.\textsuperscript{20} In agreement with the Blue Mountains Eye Study, the Reykjavik Eye Study (1040 study subjects) also reported larger disc size in glaucoma patients.\textsuperscript{21} Using OCT in the present study, our results echoed the findings in these two epidemiology studies. Whether it has any role as an indicator of susceptibility for primary open-angle glaucoma has yet to be determined.
Study Limitations

In the present study, the AUCs observed in most of the parameters examined were relatively high. The AUC represents the probability that a random pair of normal and abnormal parameters will be correctly ranked as to their disease state. Therefore, the performance of the ROC curve of any particular testing parameter is dependent on the diagnostic criteria of the disease state. In terms of visual field changes, glaucoma can be classified as mild (MD better than −6 dB), moderate (MD between −6 and −12 dB), or severe (MD worse than −12 dB), according to the classification by Hodapp et al. A higher AUC is derived when one compares a normal group to a glaucoma group with more severe mean visual field defects. This difference was exemplified in a recent study by Reus and Lemij. Using confocal scanning laser polarimetry, they found the parameter nerve fiber indicator could achieve an AUC of 0.96 when comparing normal and mild glaucoma subjects (average visual field MD, −2.01 dB). The AUC further increased to 0.99 when they compared the normal group with the moderate glaucoma group (average visual field MD, −5.44 dB). With the confocal scanning laser ophthalmoscope, Ahn and Kee reported that with the parameter cup-to-disc ratio, 79.3% of subjects who had mild visual field loss (defined as MD better than −6 dB) were identified as having glaucoma. Whereas in the group of moderate visual field loss (defined as MD between −6 and −12 dB), the ratio of classifying them as glaucomatous reached 100%. In the present study, −7 dB was selected as the cut off to divide the glaucoma subjects into early and advanced groups. If the Hodapp et al grading scale were applied, the early glaucoma group would include individuals with mild to moderate glaucomatous visual field defect. We believe the high AUCs observed in most of the parameters examined can be explained by the relatively low visual field MD in our selected sample. Because the primary objective of the present study was to assess the impact of different reference planes on evaluation of glaucoma and to compare the diagnostic performance of RNFL thickness and topographic ONH parameters in the selected samples, emphases were on the comparison of the relative values of AUCs rather than their absolute figures. AUCs obtained in individual parameters should only be extrapolated to other population samples with caution.

Nevertheless, even without the aid of any imaging devices, clinical assessment of the ONH has been found to achieve high values of AUC. In the study by Greaney et al, they found an AUC of 0.93 for detection of glaucoma, using qualitative assessment of ONH stereophotographs (average visual field MD of the glaucoma group, −3.9 dB). Uchida et al also found an AUC of 0.93 for detecting subjects with glaucoma by using qualitative optic disc evaluation (average visual field MD of the glaucoma group, −4.8 dB). It is hoped that by quantifying the cup-to-disc measurements with imaging devices, the diagnostic performance for glaucoma detection can be enhanced.

Because the calculation of the ONH parameters was estimated based on six linear scans cutting cross-sectionally over the optic disc, disc areas lying between the scan lines were not subjected to the analysis. As a result, the sensitivity in detecting a highly localized optic disc abnormality may be reduced. Second, with the current version of the analysis software, it is not yet possible to get individual clock-hour ONH measurements. Future enhancement of the analysis protocol by increasing the number of linear scans and incorporation with sectorial measurements in the analysis software may increase the measurements’ accuracy and their diagnostic performance in glaucoma detection.

CONCLUSIONS

Our study has demonstrated that OCT analysis of the ONH can differentiate normal subjects from those with glaucoma. The level of reference plane has a significant impact on the values of the ONH measurements and should remain constant during serial analysis. The default reference plane at 150 μm above the RPE layer was found to be optimal in terms of diagnostic performance in glaucoma detection and correlation with visual function. Although both RNFL thickness and the ONH measurements were found to be useful diagnostic markers for glaucoma, RNFL thickness may be a superior indicator in reflecting the retinal ganglion cell function and thus in monitoring the disease progression.

References


