Correlation Between Retinal Nerve Fiber Layer Thickness and Visual Field Sensitivity: Diffuse Atrophy Imaging Study

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BACKGROUND AND OBJECTIVE: To identify the correlation between retinal nerve fiber layer (RNFL) thickness and visual field sensitivity in healthy eyes with preperimetric and perimetric glaucoma and to estimate the functional RNFL loss in eyes with preperimetric glaucoma.

PATIENTS AND METHODS: One hundred and two eyes with glaucoma and diffuse RNFL atrophy and 102 healthy eyes were enrolled. The correlation between optical coherence tomography (OCT)-measured RNFL thickness of the superior (clock-hour segments 10, 11, 12, 1, 2, and 3) and inferior (clock-hour segments 5, 6, 7, and 8) area and the average total deviations of the inferior and superior hemifields in standard automated perimetry (SAP) were evaluated using the simple linear model, respectively. The OCT-measured RNFL thickness was assumed to comprise functional and residual thicknesses; the residual thickness was calculated from the simple linear model and the eyes with severe diffuse RNFL atrophy. Functional RNFL thickness was compared between groups.

RESULTS: Twenty-seven eyes had preperimetric and 75 eyes had perimetric glaucoma. The coefficient of determination ($R^2$) of the simple linear model was 0.71 to 0.77 for the correlation between RNFL thickness and total deviation of SAP. The estimated residual thickness was 50.4 to 56.5 µm. On comparison with normal eyes, eyes with preperimetric glaucoma were estimated to have 37% to 41% functional RNFL loss.

CONCLUSION: The correlation between RNFL thickness and SAP sensitivity was well explained by the simple linear model. Approximately 40% loss of the functional RNFL was found in preperimetric glaucoma.

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INTRODUCTION

Glaucoma is characterized by progressive loss of retinal ganglion cells (RGCs) and the retinal nerve fiber layer (RNFL), with corresponding visual field defects. In early glaucoma, visual field defects may not be present in standard automated perimetry (SAP), although structural damage, such as a glaucomatous optic disc change or RNFL defect, is evident. Conversely, function loss can also occur despite statistically normal structure.

An experimental study with monkey eyes showed that visual sensitivity losses in SAP were not correlated with RGC losses until a substantial number of neurons had been lost. In addition, normal SAP results have been reported despite a substantial loss of RGCs in human eyes with glaucoma. Thus, visual field defects observed on SAP might be associated with a significant loss of RGCs and RNFL.

To date, only a few histologic studies on human eyes have investigated the extent of structural change detectable by SAP. These showed that 20% to 50% of RGC loss could be associated with visual field defects in SAP. However, these studies were performed using a limited number of human autopsy eyes and did not evaluate RNFL loss. In clinical practice, loss of RGCs cannot be measured directly, but several imaging techniques are currently available for detecting and quantifying RNFL damage. Using these devices, it is of interest to investigate the RNFL thickness loss, which can be associated with the change from a preperimetric to a perimetric glaucomatous stage.

In this study, we investigated the correlation between RNFL thickness and visual field sensitivity in healthy eyes with preperimetric and perimetric glaucoma and estimated the RNFL loss in eyes with preperimetric glaucoma. For this purpose, we assumed that RNFL thickness was composed of functional and residual thicknesses. Residual thickness is known to be attributable to glial cells and blood vessels, and does not change with decrease in functional thickness. Then, we compared the functional RNFL thickness measured by optical coherence tomography (OCT) between normal eyes and eyes with preperimetric glaucoma and diffuse RNFL atrophy.

PATIENTS AND METHODS

This investigation was part of the Diffuse Atrophy Imaging Study, which enrolled patients who had glaucoma with diffuse RNFL atrophy and healthy individuals at the Glaucoma Clinic of Seoul National University Boramae Hospital. The details of the study design have been published elsewhere. The study was approved by the Institutional Review Board of the Seoul National University Boramae Hospital, and it followed the tenets of the Declaration of Helsinki. In cases in which both eyes of a subject were eligible for the study, only 1 eye was randomly chosen for inclusion.

Study Participants

Inclusion Criteria for the Diffuse Atrophy Imaging Study. All participants underwent a complete ophthalmologic examination, including visual acuity determination, manifest refraction, intraocular pressure (IOP) measurements by Goldmann applanation tonometry, slit-lamp examination, gonioscopy, dilated fundus examination, color disc and red-free RNFL photography (TRC-50IX; Topcon, Tokyo, Japan), Swedish interactive thresholding algorithm (SITA) 30-2 perimetry (Humphrey Field Analyzer II; Carl Zeiss Meditec, Inc., Dublin, CA), and OCT (Sta-tus OCT; Carl Zeiss Meditec, Inc.). All examinations were conducted within a period of 3 months. All participants had a best-corrected visual acuity of 20/40 or better, a spherical equivalent refractive error within ± 5.00 diopters, astigmatism within ± 3.00 diopters, an open anterior chamber angle, good quality of color disc and red-free RNFL photographs, and reliable visual field tests.

Exclusion Criteria for the Diffuse Atrophy Imaging Study. Participants were excluded if they had a history of intraocular surgery other than uncomplicated cataract surgery. We also excluded all participants with nonglaucomatous secondary causes of elevated IOP (e.g., uveitis and trauma), other intraocular eye diseases, other diseases affecting the visual field (e.g., diabetic retinopathy, retinal vein occlusion, ischemic optic neuropathy, and demyelinating diseases), or diseases that may affect the peripapillary area from where OCT measurements are obtained (e.g., large peripapillary atrophy, chorioretinal coloboma, and peripapillary staphyloma). Any subject with poor photographic quality and unacceptable quality of OCT images was excluded.

Study Participants and Control Subjects. Eyes with diffuse RNFL atrophy were defined as those hav-
ing a generalized loss of RNFL visibility of the upper or lower retina without localized wedge-shaped RNFL defects regardless of their width. All eyes with diffuse RNFL atrophy were required to have a glaucomatous optic disc. Glaucomatous disc appearance was defined as either cup/disc asymmetry between fellow eyes of greater than 0.2, rim thinning, notching, or excavation.

Eyes with diffuse RNFL atrophy were divided into two groups: eyes with diffuse RNFL atrophy, accompanied by normal SAP results (preperimetric group) and eyes with diffuse RNFL atrophy, accompanied by glaucomatous visual field defect in the corresponding hemifield location (perimetric group).

Healthy control eyes had an IOP of 21 mm Hg or less with no history of increased IOP, absence of glaucomatous disc appearance, no visible RNFL defect according to red-free RNFL photography, and a normal visual field by SAP. Normal control subjects who were age-matched with participants with diffuse RNFL atrophy were selected for analysis.

Diagnostic Tests

**Red-Free RNFL Photography and Grading Methods for Diffuse RNFL Atrophy.** Red-free RNFL photographs were acquired using a digital fundus camera after maximum pupil dilation. Fifty-degree views of the fundus, which were carefully focused on the retina using the built-in split-line focusing device, were obtained and reviewed on an LCD monitor. RNFL photographs were evaluated using a semiquantitative method described by Quigley et al. Diffuse atrophy was divided into mild (D1), moderate (D2), and severe (D3) grades according to the brightness, texture, and covering of blood vessels by nerve fibers in superior and inferior arcuate bundles. A score of D0 indicated healthy nerve fibers, whereas a score of D3 indicated severe RNFL damage, with clearly visible blood vessels and no visible RNFL fibers. In this study, each photograph was assessed by two observers in a random order and masked fashion without knowledge of the clinical information. The details of the grading system have been published elsewhere.

**OCT.** Stratus OCT was performed using a previously described technique. Participants were examined using the peripapillary fast RNFL scan of Stratus OCT, analyzed with software version 4.0. Only good and well-focused images with signal strengths of 6 or greater and the presence of a centered circular ring around the optic disc were included in the analyses. During a single scan, the RNFL thickness was determined three times at 256 points around a circle with a set diameter (3.4 mm). RNFL thicknesses were measured in 12 clock-hours. All data were aligned with respect to the orientation of the right eye, so that clock-hour 3 and clock-hour 9 of a peripapillary scan represented the nasal and temporal side, respectively. Visual field maps reflecting superior or inferior RNFL thicknesses measured by OCT were constructed (Figure 1) according to the methods of Ferreras et al. and Lee et al. Superior RNFL thickness was defined as the average data from clock-hours 10, 11, 12, 1, 2, and 3 and inferior RNFL thickness as the average of clock-hours 5, 6, 7, and 8.

**Visual Field Testing.** Visual field analysis was performed using the SITA standard of the Humphrey Field Analyzer II 750 using the central full-threshold program 30-2. Glaucomatous visual field loss was defined as the consistent presence of a cluster of three or more non-edge points on the pattern deviation plot with a probability of occurring in fewer than 5% of the normal population ($P < .05$), with one of these points having the probability of occurring in less than 1% of the normal population ($P < .01$), a pattern standard deviation with a $P$ value of less than .05, or a glaucoma hemifield test result outside the normal limits. Visual field defects had to be repeatable on at least two consecutive tests at two separate visits. Visual fields were

![Figure 1. Structure-function map between (A) superior retinal nerve fiber layer (RNFL) and inferior RNFL areas and (B) corresponding retinal sensitivity of the Humphrey Field Analyzer II with 24-2 program (Carl Zeiss Meditec, Inc., Dublin, CA) for a right eye. The superior RNFL (clock-hour segments 10, 11, 12, 1, 2, and 3) and inferior RNFL (clock-hour segments 5, 6, 7, and 8) correspond to the inferior and superior hemifields in the total deviation map, respectively. Mean retinal sensitivity (MS) was the average of 26 points from each hemifield in the total deviation map. Corresponding sectors are grayscaled.](image-url)
evaluated for reliability and were excluded if (1) either the false-positive or false-negative rate was 33% or greater or (2) fixation loss was 20% or greater. Superior retinal sensitivity was defined as the average of 26 data points from the superior hemifield of the total deviation map, excluding the blind spot, and inferior retinal sensitivity was the average of 26 data points from the inferior hemifield of the total deviation map, excluding the blind spot (Figure 1). To obtain the average retinal sensitivity, the decibel (dB) scale of each point on the total deviation map was first converted to a linear scale by using the following formula: \( \frac{1}{\text{Lambert}} = 10^{0.1 \times \text{dB}} \). Further, the values from test points in the superior or inferior sectors were averaged. The averaged value was again converted to the dB scale for data analysis.4

Data Analysis

One-way analysis of variance or the chi-square test was used to compare demographic features among healthy control eyes, eyes with preperimetric glaucoma, and eyes with perimetric glaucoma.

The correlation between visual field sensitivity and RNFL thickness was plotted according to superior and inferior RNFL areas. The structure-function correlation was investigated using locally weighted scatterplot smoothing (LOWESS) curves and the simple linear model. LOWESS describes the relationship graphically, without the need for a specific function. The simple linear model proposed by Hood and Kardon4 assumes that OCT-measured RNFL thickness (R) has two components, functional (s) and residual (b) thickness, and R = s + b. The loss in functional thickness is linearly related to the loss in retinal sensitivity on a linear scale. Therefore, the equation can be represented as R = s₀ × 10^{0.1 \times D} + b for D of 0 or less and R = s₀ + b for D of 0 or greater, where D is the loss of visual sensitivity on the dB scale on the total deviation map. The variables of the simple linear model were estimated using linear regression for the superior RNFL sector and the inferior RNFL sector. The coefficient of determination (R²) was also analyzed.

Residual thickness was obtained from the average RNFL thickness of the D3 subgroup and from the simple linear model. By subtracting the residual thickness from the OCT-measured RNFL thickness, we calculated the loss of functional thickness in glaucomatous eyes compared to the normal eyes. All analyses were performed using SPSS for Windows version 15.0 (SPSS, Inc., Chicago, IL) and SAS 9.2 (SAS Institute, Cary, NC). A P value of less than .05 was accepted as statistically significant.

RESULTS

Study Participants

We analyzed 102 eyes of 102 patients with diffuse RNFL atrophy and 102 healthy eyes of 102 age-matched participants who fulfilled the inclusion and exclusion criteria. Details of the qualifying process have been published elsewhere.11 Initially, 431 eyes of 431 participants (247 patients with glaucoma and 184 healthy control subjects) were enrolled. Of these 431 eyes, 29 (6.7%) with unacceptable Stratus OCT scans were excluded from further analysis, leaving a sample of 402 eyes of 402 participants (231 patients with glaucoma and 171 healthy control subjects). Of the 231 eyes of 231 patients with glaucoma, 129 eyes had localized RNFL defects (n = 99) or provided ambiguous information (n = 30) and were excluded from further analysis. From the eyes of control subjects, 102 eyes of 102 participants who were age-matched with the participants who had diffuse RNFL atrophy were selected. In the diffuse atrophy group, 27 eyes were included in the preperimetric group and 75 eyes were included in the perimetric group (Table 1). Mean age, gender distribution, refraction, and initial IOP were similar between the three groups. In the preperimetric group, 18 eyes had a superior RNFL defect, 7 eyes had an inferior RNFL defect, and 2 eyes had both a superior and an inferior RNFL defect.

Correlation Between RNFL Thickness and Visual Field Sensitivity by the Simple Linear Model

The simple linear model and LOWESS fit to the superior and inferior RNFL areas in the current data (Figure 2). Using linear regression analysis, s₀ was estimated to be 37.3 ± 3.9 (95% confidence interval [CI], 29.6 to 45.0; P < .01) for the superior RNFL sector and 51.3 ± 4.2 (95% CI, 42.9 to 59.7; P < .01) for the inferior RNFL sector. Residual thickness (b) was estimated to be 56.5 ± 1.8 (95% CI, 52.8 to 60.1; P < .01) for the superior RNFL sector and 54.4 ± 1.6 (95% CI, 51.3 to 57.6; P < .01) for the inferior RNFL sector. The R² of the simple linear model was 0.71 and 0.77 for the superior and inferior RNFL sectors, respectively.
Residual RNFL Thickness by RNFL Photograph Grading

The agreement of RNFL photographic grading has been evaluated in our previous study. The kappa value was 0.760 to 0.777 with substantial agreement. The specificity of the RNFL photograph grading process was 94.1%. Seventeen eyes had severe RNFL atrophy (grade D3) in the superior RNFL area, and 24 eyes exhibited grade D3 in the inferior RNFL area. The mean corresponding retinal sensitivity in the total deviation plot was -24.8 and -26.0 dB for the superior D3 and inferior D3 groups, respectively. The average thickness in grade D3 was 50.4 ± 5.8 μm for the superior RNFL area (clock-hour segments 10, 11, 12, 1, 2 and 3) and 51.0 ± 4.7 μm for the inferior RNFL area (clock-hour segments 5, 6, 7 and 8). These values were also regarded as the residual thicknesses for each area.

**Estimation of Functional RNFL Thickness Loss in Preperimetric and Perimetric Glaucoma**

Tables 2 and 3 show that, on average, 37% to 42% functional RNFL loss was present in the preperimetric group. In the perimetric group, 78% to 89% functional RNFL loss was found.
DISCUSSION

By evaluating a large number of normal and glaucomatous eyes, our study showed that substantial loss of functional RNFL thickness was present in eyes with glaucoma that had normal SAP findings. To our knowledge, this is the first study to estimate the functional RNFL loss in preperimetric glaucoma. This result implies that approximately 40% or more loss of functional RNFL thickness is associated with the clinical detection of visual field defects in SAP.

In this study, we first plotted RNFL thickness against the corresponding average total deviation of SAP. Several previous studies have used a similar method and found a linear or non-linear correlation in the plot. However, no study showed the relative position of eyes with preperimetric and perimetric glaucoma in the plot. In this study, we showed the conversion of preperimetric to perimetric glaucoma in the plot. In addition, previous researchers did not assume that there was a minimum value beyond which the OCT-measured RNFL thickness could not be reduced. In this study, the curve was exponential and converged to an asymptotic value of RNFL thickness. Therefore, as Hood and Kardon and Hood et al. proposed, it was reasonable to assume that the OCT-measured RNFL thickness was composed of two components: the thickness due to the RGC axons (functional RNFL) and everything else (residual RNFL). The residual RNFL is composed of glial cells and blood vessels, which are

TABLE 2
Estimation of Functional RNFL Thickness Loss in the Superior RNFL Area (Clock-Hour Segments 10, 11, 12, 1, 2 and 3) by the SLM and RNFL Photograph Grading

<table>
<thead>
<tr>
<th>Variable</th>
<th>Normal Group</th>
<th>Preperimetric Group</th>
<th>Perimetric Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average RNFL thickness (µm)</td>
<td>106.8 ± 11.4</td>
<td>86.1 ± 8.1</td>
<td>62.2 ± 13.6</td>
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<tr>
<td>Average functional RNFL thickness by SLM (µm)</td>
<td>50.3</td>
<td>29.6</td>
<td>5.7</td>
</tr>
<tr>
<td>Average functional RNFL thickness by RNFL grading (µm)</td>
<td>56.4</td>
<td>35.7</td>
<td>11.8</td>
</tr>
<tr>
<td>% reduction compared to normal by SLM</td>
<td>–</td>
<td>41.2%</td>
<td>88.7%</td>
</tr>
<tr>
<td>% reduction compared to normal by RNFL grading</td>
<td>–</td>
<td>36.8%</td>
<td>79.1%</td>
</tr>
</tbody>
</table>

RNFL = retinal nerve fiber layer; SLM = simple linear model.

aFunctional RNFL thickness was obtained by subtracting the residual RNFL thickness (56.5 ± 1.8 µm) of the simple linear model from the average RNFL thickness.

bFunctional RNFL thickness was obtained by subtracting the residual RNFL thickness (50.4 ± 5.8 µm) of the RNFL grading from the average RNFL thickness.

TABLE 3
Estimation of Functional RNFL Thickness Loss in the Inferior RNFL Area (Clock-Hour Segments 5, 6, 7 and 8) by the SLM and RNFL Photograph Grading

<table>
<thead>
<tr>
<th>Variable</th>
<th>Normal Group</th>
<th>Preperimetric Group</th>
<th>Perimetric Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average RNFL thickness (µm)</td>
<td>124.0 ± 14.2</td>
<td>94.9 ± 5.7</td>
<td>66.9 ± 17.3</td>
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<tr>
<td>Average functional RNFL thickness by SLM (µm)</td>
<td>69.6</td>
<td>40.5</td>
<td>12.5 µm</td>
</tr>
<tr>
<td>Average functional RNFL thickness by RNFL grading (µm)</td>
<td>56.4</td>
<td>35.7</td>
<td>11.8</td>
</tr>
<tr>
<td>% reduction compared to normal by SLM</td>
<td>–</td>
<td>41.8%</td>
<td>82.0%</td>
</tr>
<tr>
<td>% reduction compared to normal by RNFL grading</td>
<td>–</td>
<td>39.8%</td>
<td>78.2%</td>
</tr>
</tbody>
</table>

RNFL = retinal nerve fiber layer; SLM = simple linear model.

aFunctional RNFL thickness was obtained by subtracting the residual RNFL thickness (56.5 ± 1.8 µm) of the simple linear model from the average RNFL thickness.

bFunctional RNFL thickness was obtained by subtracting the residual RNFL thickness (50.4 ± 5.8 µm) of the RNFL grading from the average RNFL thickness.
invariably included in the segmentation algorithm of OCT. The distribution of glial cells has been shown to remain unchanged in cases of long-standing ocular hypertension. In addition, blood vessel shrinkage has been shown to be relatively small, approximately 15% of the diameter, in progressed glaucoma. Therefore, we assumed that the residual RNFL thickness does not change with glaucoma progression, as proposed by Hood and Kardon and Hood et al.

We evaluated the structure-function correlation by using the simple linear model and LOWESS. The predicted simple linear model fit the data well ($R^2 = 0.71$ to 0.77). However, approximately 30% of normal eyes were above the 95-percentile prediction interval of the model. A further study involving a large number of participants is required to elucidate this finding. The LOWESS curve obtained in this study was similar to the curve predicted by the simple linear model.

In this study, residual thickness was estimated using the RNFL photograph grading and the simple linear model. Because no RNFL fibers were detected in grade D3 RNFL photographs, the RNFL thickness of the grade D3 group was regarded as the residual RNFL thickness. The severely depressed retinal sensitivity in the grade D3 group (average = -25 to -26 dB) supported the observation that a functional RNFL was rarely present in these cases. These values were also similar to the residual thicknesses, which were estimated from the simple linear model.

Kerrigan-Baumrind et al. compared the number of RGCs of 17 post-mortem human eyes with glaucoma to that of normal eyes and found that at least 25% to 35% of RGC loss was associated with a statistical abnormality in SAP. Harwerth et al. compared RGC counts to SAP sensitivity in monkey eyes with experimental glaucoma. They found that visual sensitivity losses were not correlated with RGC losses until 50% of RGCs were lost. Thus, the authors concluded that there was a 50% RGC reserve for visual sensitivity. Our results show that approximately 40% functional RNFL loss was present in preperimetric glaucoma. Although previous studies counted RGC numbers, the data from our study, which measured the loss of RNFL thickness, are in accordance with previous studies. However, our results do not suggest that 40% loss of functional RNFL is the absolute cut-off value for differentiating between eyes with perimetric and preperimetric glaucoma. Because intersubject variability in RNFL thickness is large, this cut-off value might vary with each eye. Hence, further studies are warranted.

Our results do not support that structural tests always detect glaucomatous damage earlier than functional tests. As Hood and Kardon proposed, the detection of glaucomatous damage was found to depend on the sensitivity and confidence interval of the tests. Because the range of normal RNFL thicknesses is wide, eyes with a greatly decreased RNFL thickness may nevertheless be classified as normal if RNFL thickness does not reach the fifth percentile value of a normative database. Therefore, functional loss can also occur despite the RNFL having a statistically normal structure. However, if structural change is evaluated using a sensitive qualitative test, fewer will show functional loss and normal structure. In this study, we used qualitative methods (optic disc and RNFL photography), which are more sensitive than statistical analysis for detecting structural change to diagnose glaucoma.

This study has several limitations. We did not include the participants with normal RNFL and visual field defects. Therefore, although the eyes were evaluated by sensitive qualitative methods (optic disc and RNFL photography) in this study, we cannot exclude the possibility of selection bias and overestimation of the change in RNFL thickness in eyes with preperimetric glaucoma. In addition, although we included a large number of normal eyes and eyes with glaucoma, the number of eyes with preperimetric glaucoma was relatively small, especially for the inferior RNFL area. Further studies with a large number of patients with preperimetric glaucoma are warranted. We also used conventional criteria to categorize the visual field results. Depending on the classification criteria, the RNFL loss of eyes with preperimetric glaucoma may change. Because we used sensitive criteria to identify perimetric glaucoma (visual fields that fulfilled any of three criteria were defined as abnormal), our results may represent the minimal value for the RNFL loss of eyes with preperimetric glaucoma. The current study is also limited by use of time-domain OCT. Further study with spectral-domain OCT should be performed to confirm our results.

We are the first to show that patients with glaucoma who have normal SAP already exhibited substantial RNFL loss compared to normal participants. These findings may provide an approximation for the detection threshold of RNFL loss in SAP.
REFERENCES


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