Influence of Correction of Ocular Magnification on Spectral-Domain OCT Retinal Nerve Fiber Layer Measurement Variability and Performance

Sara Nowroozizadeh,[1] Nila Cirineo,[1] Navid Amini,[1] Shane Knipping,[1] Ted Chang,[2] Tom Chou,[3] Joseph Caprioli,[1] and Kouros Nouri-Mahdavi[1]

[1]Glaucoma Division, Stein Eye Institute, David Geffen School of Medicine, University of California, Los Angeles, California, United States
[2]Department of Computer Sciences, University of California San Diego, San Diego, California, United States
[3]Department of Biomathematics, David Geffen School of Medicine, University of California, Los Angeles, California, United States

Correspondence: Kouros Nouri-Mahdavi, 100 Stein Plaza, Los Angeles, CA 90095, USA; nouri-mahdavi@jsei.ucla.edu.

Submitted: January 2, 2014
Accepted: April 21, 2014

Citation: Nowroozizadeh S, Cirineo N, Amini N, et al. Influence of correction of ocular magnification on spectral-domain OCT retinal nerve fiber layer measurement variability and performance. *Invest Ophthalmol Vis Sci*. 2014;55:3439–3446. DOI:10.1167/iovs.14-13880

Purpose. To analyze the influence of ocular magnification on the peripapillary retinal nerve fiber layer (RNFL) thickness measurement and its performance as acquired with spectral-domain optical coherence tomography (SD-OCT).

Methods. Spectral domain OCT measurements from 108 normal eyes (59 subjects) and 72 glaucoma eyes (58 patients) were exported and custom software was used to correct RNFL measurements for ocular magnification. Retinal nerve fiber layer prediction limits in normal subjects, structure-function relationships, and RNFL performance for detection of glaucoma were compared before and after correction for ocular magnification (Bennett's formula). Association of disc area with cross-sectional RNFL area was explored.

Results. The median (interquartile range, [IQR]) visual field mean deviation and scaling factor were 0 (−0.85 to 0.73) dB and 0.96 (0.93–1.00) in normal eyes and −4.0 (−6.0 to −2.2) dB and 0.99 (0.95–1.03) in the glaucoma group ($P < 0.001$ and $P = 0.003$, respectively; average correction 3%). Correction for ocular magnification caused a reversal of the negative relationship between the cross-sectional RNFL area and axial length (slope = −0.022 mm^2/mm, $P = 0.015$ vs. = 0.22 mm^2/mm, $P = 0.007$). However, such correction did not change RNFL prediction limits (except in superior and nasal quadrants), improve global or regional structure-function relationships, or enhance the ability of RNFL measurements to discriminate glaucoma from normal eyes ($P > 0.05$). The cross-sectional RNFL area was not correlated with optic disc area ($P = 0.325$).

Conclusions. Correction of RNFL measurements for ocular magnification did not improve prediction limits in normal subjects or enhance the performance of SD-OCT in this group of eyes in which the axial length did not deviate significantly from average values. The cross-sectional area of the RNFL was not related to the optic disc area.

Keywords: ocular magnification, spectral-domain OCT, glaucoma imaging

Retinal nerve fiber layer (RNFL) thickness analysis with optical coherence tomography (OCT) is widely used to assess structural damage in glaucoma patients. Schuman et al.[1] showed that measurement of RNFL thickness along a fixed circle diameter of 3.4 mm was optimal and offered the best reproducibility; however, the use of the same circle size for all eyes without taking into account factors affecting the magnification of the eye, such as the axial length or refractive error, could lead to increased limits of variability of RNFL measurements. Optical coherence tomographs have been set to measure RNFL thickness at a fixed angular distance (approximately 12°) centered on the optic disc. However, the magnification of the ocular optical system is known to impact the actual location of the measurement circle on the peripapillary retina.[2] A longer eye will result in a larger measurement circle diameter, thereby measuring the RNFL at a farther distance from the optic disc center. The reverse would apply to smaller eyes. Kang and colleagues[3] demonstrated that the ocular magnification significantly affected the average RNFL thickness measurements. However, correction for magnification was done by extrapolating RNFL measurements rather than with direct measurements potentially available on some spectral domain OCTs (SD-OCT).

In this study, we used a new approach for correcting for the effect of ocular magnification, making optimal use of the extensive data gathered by SD-OCT machines. The purpose of this study is to determine whether correcting for ocular magnification improves 95% prediction limits for RNFL thickness measurement among normal subjects, strengthens structural-functional relationships, or enhances detection of glaucoma. Our study group consisted of normal subjects and glaucoma patients enrolled at a tertiary referral center in which the ocular axial length did not deviate significantly from the average axial length in the general population as assumed by the SD-OCT device. We also explored the relationship between the optic disc size (i.e., Bruch's membrane opening area

corrected for ocular magnification) and cross-sectional RNFL area.

Methods

Subjects of this cross-sectional study were prospectively enrolled as part of the UCLA OCT Imaging Study. Details of the study have been published elsewhere.[4] The study was reviewed and approved by the Institutional Review Board at the University of California Los Angeles (UCLA) and was performed in adherence to the tenets of the Declaration of Helsinki. Patients with a clinical diagnosis of open-angle glaucoma made by an attending ophthalmologist and who met the following criteria were prospectively enrolled in the study: age $\geq$ 30 years, open angles, best corrected visual acuity $\geq$ 20/80, visual field mean deviation ≥ -15 dB, refractive error $\leq$ 8.0 diopters (D) and astigmatism $\leq$ 3 D. Eyes with evidence of retinal or neurological diseases or prior glaucoma surgery were excluded. All patients had at least one prior visual field test before being enrolled in the study. Normal subjects were recruited by advertising at UCLA's campus, placing fliers in the clinics, and soliciting spouses and friends of patients seen at Jule Stein Eye Institute's Glaucoma Clinic. The enrolled normal subjects were required to have open angles, corrected visual acuity of 20/25 or better, normal eye exam including normal visual fields, and no definitive evidence of optic nerve glaucomatous damage (see below).

All subjects underwent a thorough eye exam on the day of imaging that included visual acuity, automated refraction, IOP measurement, gonioscopy, slit lamp exam, dilated fundus exam, and standard achromatic perimetry (SAP) or short-wavelength automated perimetry (SWAP). Axial length and K readings were measured with an optical biometer (IOLMaster; Carl Zeiss-Meditec, Dublin CA, USA). Central corneal thickness (CCT) measurements were measured with a pachymeter (DGH 55 Pachmate; DGH Technology, Inc., Exton, PA, USA). Stereoscopic disc photographs and optic disc cube 200 $\times$ 200 SD-OCT imaging (Cirrus HD-OCT; Carl Zeiss-Meditec) were carried out after pupillary dilation. All the images were reviewed afterward by one of the investigators and images with signal strength $<$ 7, obvious motion or blinking artifact, or incorrect segmentation were excluded. The 200 $\times$ 200 disc cube provides the segmented RNFL thickness for a square area of A-scan measurements centered on the optic nerve head (ONH), which measures 6 $\times$ 6 mm in an emmetropic eye (2 mm in depth). The area within the ONH is not segmented. A graph of RNFL thickness measurements (Temporal–Superior–Nasal–Inferior-Temporal [TSNIT] graph) is ordinarily provided along a standard measurement circle, 3.46 mm in diameter, which is placed and centered on the ONH centroid automatically by the machine. The angular size of the measurement circle is approximately 12.5° in an emmetropic eye, in which every mm corresponds to 3.5 degrees on the fundus. The SD-OCT measurements are not corrected for the magnification of the ocular optical system and this correction must be performed post hoc.

The Cirrus HD-OCT defines the ONH border as the inner edge of the Bruch's membrane. Recent research supports use of the Bruch's membrane opening (BMO) as the most consistent landmark for defining the disc margin (or more precisely, the anterior opening of the neural canal) in humans.[5,6] En face SD-OCT images (BMP format), where the measurement circle is marked by the device as a purple circle, were exported to a personal computer. The corresponding 200 $\times$ 200 grid of RNFL measurements for each eye (as segmented by Cirrus HD-OCT; Carl Zeiss Meditec) and TSNIT curve values were also exported as spreadsheet files (Excel; Microsoft Corp., Redmond, WA, USA). The RNFL data grid was then superimposed on the en face image and RNFL thickness values were retrieved after adjusting the diameter of the measurement circle for ocular magnification with Bennett's formula.[7,8] Bennett's formula is one of the most accurate and practical formulas available for this purpose. It relies on the axial length to correct for ocular magnification. The ocular magnification is approximated based on the estimated location of the second principal point and its usual spatial relationship to the nodal point. The relationship between a measured SD-OCT image (the measurement circle in this case) and its actual size can be expressed as $t = p \times q \times s$, where t is the actual size of an object on the retina, s is the SD-OCT measurement, p is the magnification factor related to the OCT's camera, and q is the magnification factor related to the eye. Given the default axial length (AL = 24.46 mm) and refraction (zero D) for a magnification of 1 with the SD-OCT system (i.e., $t = s$), p can be calculated as $1/[0.01306 \times (24.46 - 1.82)] = 3.382$. The q factor based on axial length would be $0.01306 \times (\text{AL} - 1.82)$ according to Bennett's formula where 1.82 is a constant representing the distance between corneal apex of a three-surface schematic eye to its second principal plane. The product $p \times q$ represents the scaling factor. The corrected diameter of the measurement circle was calculated by adjusting it by the scaling factor, (i.e., the diameter of the measurement circle was *reduced* by the same percentage in eyes with a scaling factor $>$ 1 and *increased* in eyes with a scaling factor $<$ 1). The RNFL thickness values were retrieved along the newly defined corrected measurement circle (one measurement per degree or 360 for the entire measurement circle) with custom software. In case there were less than 360 measurements available on the measurement circle, the value from the closest neighboring point (interpolating in a linear fashion) was used to avoid duplicate measurements.

We estimated the cross-sectional RNFL area by calculating the average RNFL thickness along the TSNIT curve and multiplying it by the circumference of the corrected measurement circle. To validate our approach, we measured the RNFL cross-sectional area for the corrected TSNIT curve derived from our method and compared the results with those measured from the TSNIT curve derived from the SD-OCT machine (exported as a spreadsheet file [Microsoft Corp.]). All left eye data were converted to right eye format.

Statistical Methods

Bivariate scatter plots were used to explore the relationships of variables of interest such as RNFL thickness versus axial length, spherical equivalent, or disc area. Continuous variables were compared with unpaired t-test or nonparametric equivalent tests depending on the distribution of individual variables. Categorical variables were compared with the χ^2 test. For comparing changes in 95% prediction limits for RNFL thickness in normal eyes, the absolute differences from the mean in the paired groups of data (i.e., RNFL thickness uncorrected measurements and measurements corrected for ocular magnification) were compared with Wilcoxon signed-rank test. Spearman correlations were used to explore structure-function relationships between average RNFL thickness versus visual field mean deviation (MD) as well as the RNFL thickness in the superior and inferior hemiretinas versus the mean deviation for corresponding hemifields. The areas under receiver operating characteristic curves (ROC) were used to compare the performance of corrected RNFL thickness measurements to those of uncorrected measurements for discriminating glaucoma from normal eyes. Correlation between the two eyes of the same subjects, where applicable, was taken into account with appropriate statistical tests. For analyses where only one eye of

Investigative Ophthalmology & Visual Science

Table 1. Demographic and Clinical Characteristics of Glaucoma Patients and Normal Control Subjects Enrolled in the Study

Demographic Data	Total	Normal	Glaucoma	*P* Value
Number of eyes (patients)	180 (117)	108 (59)	72 (58)	
Age (mean ± SD), y	61.5 (±8.6)	59.0 (±9.0)	64.1 (±7.4)	0.001*
Sex (female/male)	73/44	39/20	34/24	0.404†
Ethnicity				
White	79 (67.5%)	41 (69.5%)	38 (65.5%)	0.981‡
African-American	13 (11.1%)	6 (10.2%)	7 (12.1%)	
Hispanic	12 (10.3%)	6 (10.2%)	6 (10.4%)	
Asian	12 (10.3%)	6 (10.2%)	6 (10.4%)	
Indian	1 (0.9%)	0 (0%)	1 (1.7%)	
Visual acuity (median and IQR), logMAR	0 (0–0.1)	0 (0–0.1)	0.05 (0–0.1)	0.002§
Spherical equivalent (median and IQR), D	0 (−2–1.1)	0 (−1.4–1.1)	−0.13 (−3.07–1.07)	0.232§
Axial length (median and IQR), mm	23.9 (23.2–24.7)	23.6 (22.9–24.3)	24.1 (23.4–25.2)	0.004§
Central corneal thickness, (mean ± SD), μm	555.8 (±38.7)	560.3 (±38.4)	549.0 (±38.4)	0.056*
Keratometry reading (mean ± SD), D	44.0 (±1.5)	44.1 (±1.4)	43.8 (±1.6)	0.238*
Mean deviation (median and IQR), dB	−0.9 (−3.3–0.2)	0 (−0.9–0.7)	−4.00 (−6.0 to −2.2)	<0.001§
Pattern SD (median and IQR), dB	1.9 (1.6–4.3)	1.6 (1.4–1.9)	5.5 (3.0–7.9)	<0.001§
Intraocular pressure on the examination day (median and IQR), mm Hg	14.0 (12–16)	15.0 (13–16)	13.3 (11–15)	0.001§
Scaling factor (median and IQR)	0.97 (0.94–1.01)	0.96 (0.93–1.00)	0.99 (0.95–1.03)	0.003§

* 2-sample *t* test.
† χ^2 test.
‡ Fisher's exact test.
§ Wilcoxon rank-sum test.

each subject was included, we chose the right eye of patients who had both eyes eligible.

Results

One hundred and eighty phakic eyes of 117 subjects (72 eyes of 58 glaucoma patients and 108 eyes of 59 normal subjects) were included in the current study. Table 1 shows the demographic and clinical characteristics of the enrolled subjects. The glaucoma patients were somewhat older than normal subjects (average age 64.1 ± 7.4 vs. 59.0 ± 9.0 years, respectively; $P = 0.001$) and had longer axial length (median and interquartile range [IQR]: 24.1 [23.4–25.2] vs. 23.6 [22.9–24.3] mm, respectively; $P = 0.004$) whereas the distribution of refractive error was similar between the two groups (median [IQR]: –0.13 [–3.07 to 1.07] vs. 0 [–1.4 to 1.1] D, respectively). Since the scaling factor derived from Bennett's formula is directly related to the axial length, the scaling factors were also significantly different. The median (IQR) scaling factor for normal and glaucoma patients was 0.96 (0.93–1.00) and 0.99 (0.95–1.03), respectively ($P = 0.003$). Figure 1 shows the frequency distribution of the scaling factors for normal and glaucoma groups.

A comparison of the machine-exported RNFL cross-sectional area and uncorrected RNFL cross-sectional area calculated with our method showed excellent agreement between the two ($r = 0.999$; $P < 0.001$), confirming the validity of our approach (Fig. 2). The scatter plots in Figure 3 illustrate the relationship between the RNFL cross-sectional area and axial length before (Fig. 3A) and after (Fig. 3B) correction for ocular magnification in normal subjects taking into account the effect of age. A significant negative association was found between exported RNFL cross-sectional area and axial length with the area decreasing with longer axial lengths (slope = –0.022 mm^2/mm, $P = 0.015$). After correction for ocular magnification, the relationship between the RNFL cross-sectional area and the axial length became positive (slope = 0.22 mm^2/mm, $P = 0.007$).

We then calculated the corrected disc area as measured by the HD-OCT device (Carl Zeiss Meditec) with Bennett's formula as previously reported.[9] The corrected disc area was not correlated with the cross-sectional RNFL area in normal subjects before or after correction for ocular magnification ($\beta = 0.04$ mm^2/mm^2, $P = 0.917$ before correction for ocular magnification and $\beta = 0.43$ mm^2/mm^2, $P = 0.325$ after correcting for ocular magnification; Fig. 4). The magnitude of the change in RNFL cross-sectional area as a function of scaling factor is demonstrated in Figure 5; the change in the area was linearly related to the scaling factor and was slightly larger in normal subjects compared with glaucoma subjects. Similar results were found when average RNFL thickness values were used instead of RNFL cross-sectional area (data not shown).

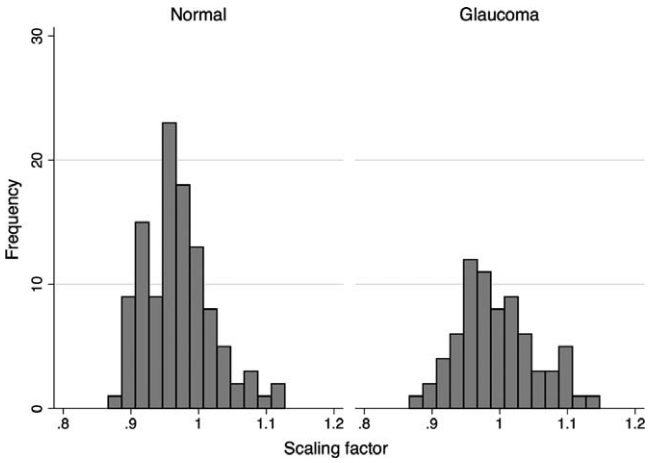

Figure 1. Frequency distribution of the scaling factors used to correct for the ocular magnification and adjust the size of the measurement circle as calculated with Bennett's formula in the normal and glaucoma groups.

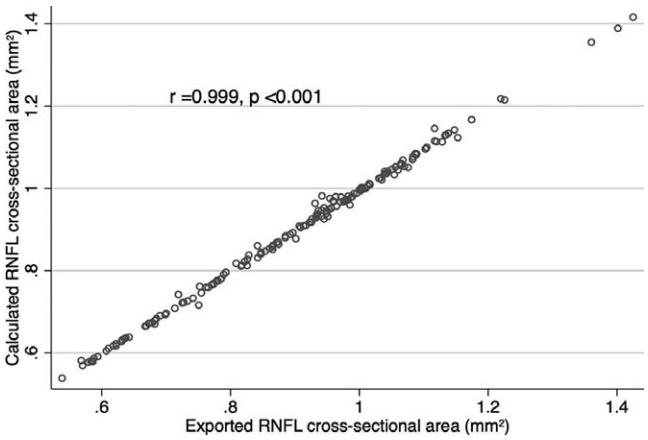

FIGURE 2. Scatter plot demonstrating the correlation between uncorrected calculated cross-sectional retinal nerve fiber layer area versus exported cross-sectional retinal nerve fiber layer area (both uncorrected for ocular magnification). There was excellent correlation between the two measurements ($r = 0.999$, $P < 0.001$), which validates our technique.

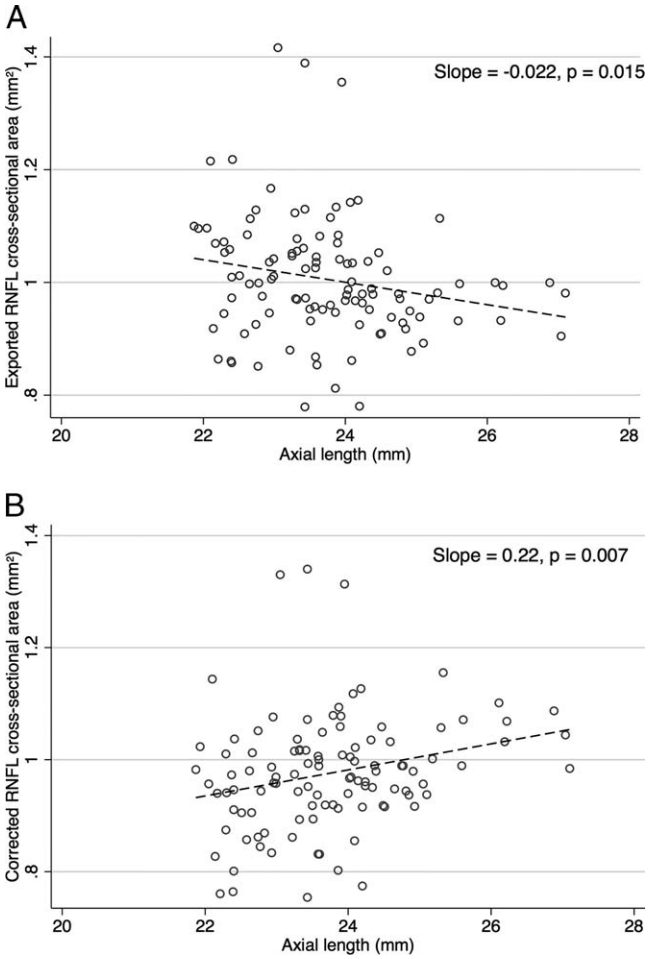

FIGURE 3. The relationship between the retinal nerve fiber layer (RNFL) cross-sectional area and axial length in normal subjects. (**A**) Bivariate scatter plot showing a negative association between the exported cross-sectional RNFL area and axial length in normal subjects (slope = –0.022 mm²/mm, $P = 0.015$). (**B**) After correction for ocular magnification with Bennett's formula taking into account the effect of age, the relationship between the corrected cross-sectional RNFL area and the axial length reverses (slope = 0.22 mm²/mm, $P = 0.007$).

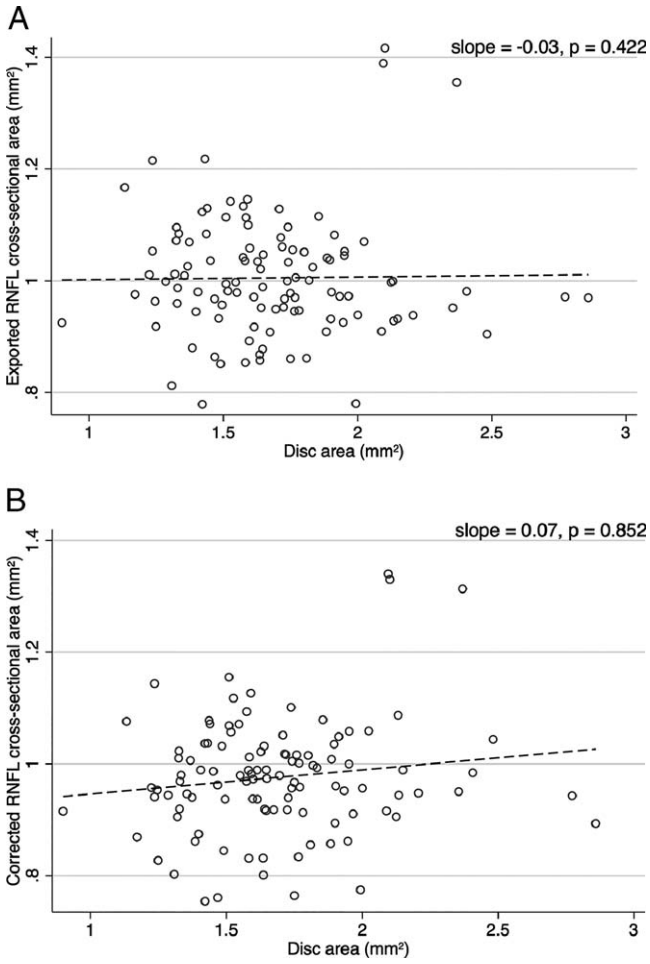

FIGURE 4. There was no association between the disc area as determined by the HD-OCT device (Carl Zeiss Meditec; defined as Bruch's membrane area) and cross-sectional RNFL area before (**A**) or after (**B**) correction for ocular magnification with Bennett's formula. The disc areas in both plots were corrected for ocular magnification.

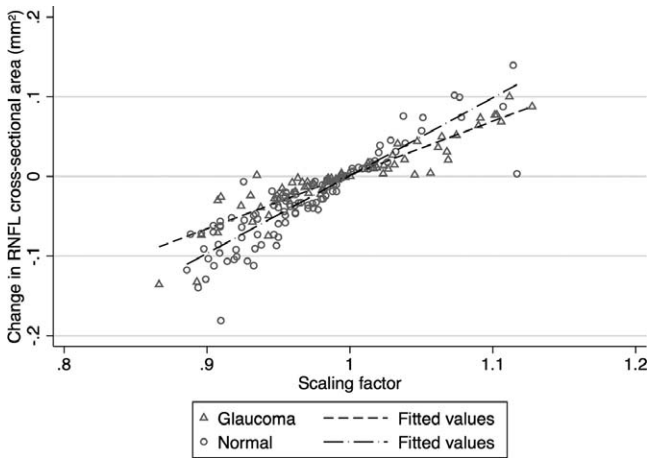

FIGURE 5. The bivariate scatter plot demonstrated the change in retinal nerve fiber cross-sectional area (i.e., corrected minus uncorrected retinal nerve fiber cross-sectional area) as a function of the scaling factor used to correct for ocular magnification. The scaling factors were calculated according to Bennett's formula.

Investigative Ophthalmology & Visual Science

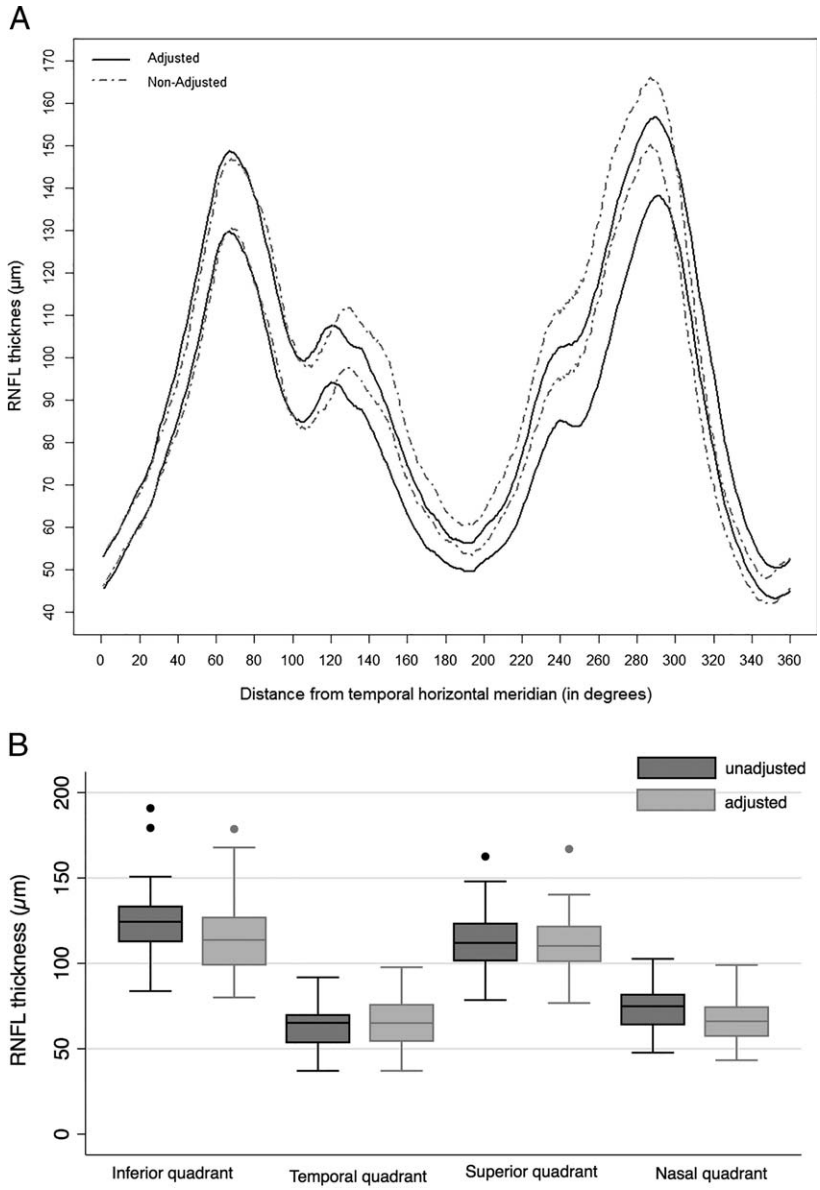

Figure 6. Changes in retinal nerve fiber layer (RNFL) variability in normal subjects after correction for ocular magnification. (**A**) Comparison of 95% prediction limits between uncorrected cross-sectional RNFL area and those corrected for ocular magnification in normal subjects (59 eyes of 59 subjects). (**B**) The *box* and *whisker* plots show the median, interquartile range, and 95% prediction limits for RNFL thickness measurements in four quadrants before and after correction for ocular magnification in one eye of normal subjects (59 eyes).

Changes in RNFL Prediction Limits and its Performance After Correction for Ocular Magnification

The 95% prediction limits for distribution of RNFL thickness measurements for 59 eyes of 59 normal subjects are demonstrated in Figure 6A before and after correction for ocular magnification. The prediction limits were qualitatively similar before and after correction for the ocular magnification. Figure 6B depicts the median, IQR, and 95% prediction limits of RNFL thickness measurements in four quadrants before and after correction for ocular magnification in the normal group. The *P* value for the change in prediction limits was significant for the superior and nasal quadrants ($P < 0.002$ and 0.032, respectively, with corrected measurements having narrower 95% prediction limits) whereas the change in limits of prediction was not significant or of borderline significance for the inferior and temporal quadrants ($P = 0.147$ and 0.566, respectively) or average RNFL ($P = 0.049$).

Global structure-function relationships were explored by correlating the visual field mean deviation with the average RNFL thickness ($\rho = 0.647$ before correction for magnification, and = 0.641 after correction; $P = 0.245$ for the difference). Regional structure-function relationships were investigated by correlating the superior and inferior hemifield MD with the average RNFL thickness in the corresponding hemiretinas (superior RNFL thickness versus inferior MD: $\rho = 0.516$ before correction for magnification and = 0.512 after correction, $P = 0.965$; inferior RNFL versus superior MD: $\rho = 0.554$ vs. = 0.526; $P = 0.04$). The hemifield MDs were calculated after converting the dB values to 1/Lambert values, averaging them, and reconverting to dB values (Fig. 7).

Finally, we compared the performance of the RNFL thickness measurements for detection of glaucoma before

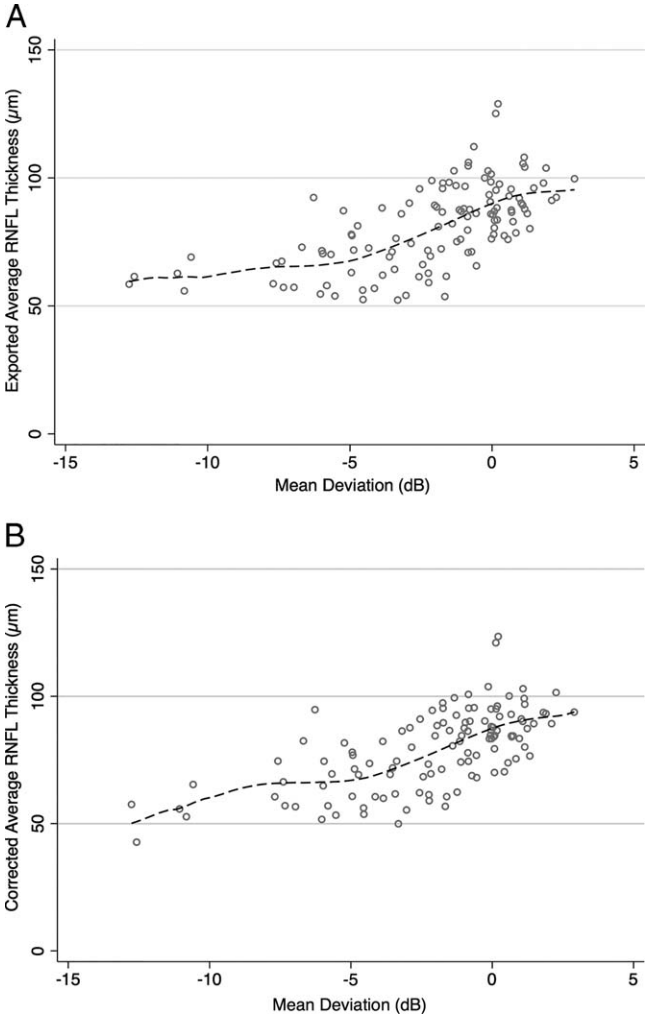

Figure 7. Scatter plots demonstrate the correlation between the visual field mean deviation and average RNFL thickness before (**A**) and after correction for ocular magnification with Bennett's formula (**B**). The fitted curves represent the best polynomial fit.

and after correction for ocular magnification. The areas under the receiver operating characteristics (ROC) curves (AUC) were calculated for the average RNFL thickness and RNFL thickness at all clock hour sectors and quadrants on the measurement circle before and after correction for ocular magnification (Table 2). Overall, there was a trend for uncorrected RNFL thickness measures to perform better than measurements corrected for ocular magnification. Figure 8 demonstrates the ROC curves for detection of glaucoma for the regional thickness measure with the highest AUC (RNFL thickness at 7 o'clock) with uncorrected data and with data corrected for ocular magnification ($P = 0.674$). The corresponding sensitivities at 95% specificities were 74.1% and 79.6% for the 7 o'clock sector.

Discussion

Retinal nerve fiber layer thickness measurement with SD-OCTs has become an important tool for assessment of glaucomatous structural damage. Traditionally, a measurement circle 12° in diameter has been used to measure circumpapillary RNFL. However, the real size of this circle on the retinal surface depends on the ocular magnification, which is a function of the

Table 2. Area Under ROC Curves (AUCs) for Detection of Glaucoma With Global and Regional RNFL Outcome Measures Before and After Correction for Ocular Magnification With Bennettã s Formula

Outcome Measure	AUCs Before Correction	AUCs After Correction
Clock hour 1	0.82 (0.76–0.88)	0.82 (0.75–0.87)
Clock hour 2	0.75 (0.69–0.82)	0.70 (0.63–0.77)
Clock hour 3	0.58 (0.51–0.66)	0.51 (0.44–0.59)
Clock hour 4	0.67 (0.59–0.74)	0.61 (0.54–0.68)
Clock hour 5	0.85 (0.78–0.89)	0.72 (0.65–0.79)
Clock hour 6	0.92 (0.87–0.96)	0.85 (0.79–0.90)
Clock hour 7	0.96 (0.92–0.98)	0.96 (0.92–0.98)
Clock hour 8	0.70 (0.62–0.76)	0.69 (0.61–0.75)
Clock hour 9	0.59 (0.51–0.66)	0.51 (0.44–0.59)
Clock hour 10	0.77 (0.70–0.83)	0.72 (0.65–0.78)
Clock hour 11	0.87 (0.81–0.92)	0.84 (0.78–0.89)
Clock hour 12	0.82 (0.76–0.88)	0.82 (0.76–0.88)
Inferior quadrant	0.97 (0.93–0.99)	0.94 (0.90–0.97)
Nasal quadrant	0.71 (0.64–0.78)	0.65 (0.57–0.71)
Superior quadrant	0.91 (0.86–0.95)	0.91 (0.86–0.95)
Temporal quadrant	0.72 (0.65–0.79)	0.67 (0.59–0.74)
Average RNFL	0.95 (0.91–0.98)	0.93 (0.89–0.97)

The numbers in parentheses represent 95% confidence intervals.

distance between the second principal plane of the eye and the retina.[7] Axial length has been commonly used as a proxy for the latter. Bennett's formula subtracts a constant (1.82) from the axial length to estimate the distance from the second principal plane of the eye to the fovea. It should be noted that the results are still an approximation of the magnification of the ocular optical system. Another caveat is that the axial length may not exactly represent the distance from the secondary plane of the eye to the peripapillary retina especially in highly myopic eyes. This fact could have led to a partial overcompensation of the magnification correction and have been a reason for the reversal of the relationship between the RNFL cross-sectional area and axial length. The actual size of the measurement circle is larger in eyes with larger axial length and smaller in eyes with a shorter axial length. We hypothesized that correcting the location of the measurement circle with the axial length could decrease 95% prediction limits for RNFL measurements in normal subjects, enhance

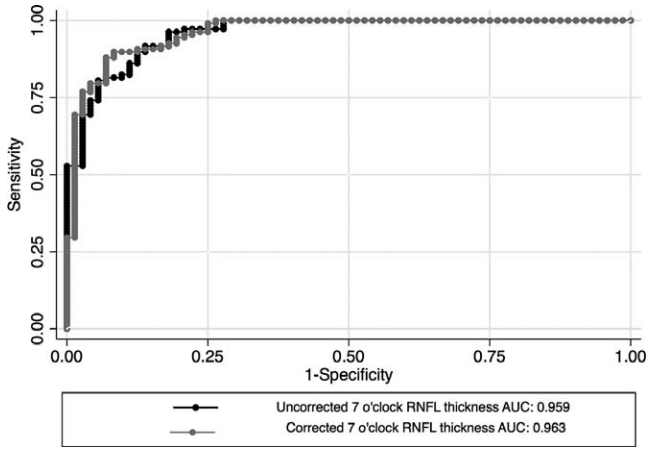

Figure 8. Receiver operating characteristic curves comparing the performance of the retinal nerve fiber layer thickness measurements at 7 o'clock (the sector with the highest area under the ROC curve in this study) for detection of glaucoma before and after correction for ocular magnification ($P = 0.674$).

structure-function relationships in glaucoma and normal subjects, and improve the ability of the SD-OCT RNFL measurements to detect glaucoma.[2,3,10,11] We found that normalizing the location of the measurement circle (i.e., measuring RNFL thickness exactly on a 3.46 mm-diameter circle on the retina for all eyes) did not improve most of the outcome measures of interest. Our results showed that neither the strength of structure-function relationships nor the discriminatory ability of SD-OCT for detection of glaucoma was significantly improved. However, we observed a small improvement in 95% prediction limits for the average RNFL and the RNFL thickness in the superior and nasal quadrants.

All current SD-OCT machines are able to acquire a cube of measurements around the optic disc at varying levels of resolution in a matter of seconds. The HD-OCT device (Carl Zeiss Meditec) used in this study scans the peripapillary area with a resolution of 200 × 200 axial scans over an area measuring 6 × 6 mm in an emmetropic eye (considered an eye with an axial length of 24.46 mm). The peripapillary RNFL is then segmented with a custom algorithm. Such data can be exported and the RNFL thickness map rebuilt with imaging software. We used customized imaging software to recalculate the RNFL thickness on a measurement circle normalized to a diameter of 3.46 mm. To test the accuracy of our technique, we compared our calculated RNFL cross sectional area with the those derived from TSNIT curves exported from the HD-OCT device (Carl Zeiss Meditec) and found that the two were very highly correlated ($r = 0.999$; $P < 0.001$); therefore, validating our measurements as being as accurate as those automatically provided by the machine.

Most prior studies of the effects of ocular magnification on RNFL measurements focused on correcting the RNFL thickness for axial length without adjusting for the location of such measurements.[2,11] A study by Kang et al.[3] used extrapolations of RNFL thickness measurements to recalculate the RNFL thickness in a large group of younger, mostly male Korean subjects. They assumed that the RNFL cross-sectional area would be constant within the vicinity of the optic disc and therefore used this value to calculate the adjusted RNFL thickness values. The authors reported that the thinning of the RNFL as a function of longer axial length and increasing myopia likely resulted from the larger size of the measurement circle around the ONH since it disappeared after adjusting for ocular magnification with Bennett's formula. However, as the investigators discussed, the two issues with such an approach are the assumption of a constant RNFL cross-sectional area and radial course of the RNFL. Our findings actually argue against the former assumption (Fig. 5). We found that the cross-sectional RNFL area actually decreased with increasing distance from the disc. However, it is not clear how much of this change is due to nonneural components such as blood vessels and how much constitutes real attrition of the axonal content.[10] Our novel approach addresses both of the above issues by measuring the RNFL thickness on a measurement circle of exactly the same diameter (3.4 mm) on the retina across all the study eyes. Prior studies had also suggested, mostly indirectly, that the apparent thinning of the RNFL with longer axial length was an artifact of ocular magnification since the correlation of RNFL thickness with axial length or myopia decreased or disappeared after correction for such confounding factors.[10–14] Patel and colleagues[10] reported similar findings in monkeys when they adjusted for axial length and used elliptical scan patterns placed approximately 500 μm from the disc margin. We believe that our findings prove that thinner RNFL measurements in myopic eyes are a result of the larger size of the measurement circle on the retina.

It is reassuring that despite applying the best practical correction for ocular magnification, most of the explored outcome measures remained unchanged in this group of patients in whom axial length measurements did not deviate substantially from the average assumed in one SD-OCT device. That means that RNFL measurements as provided by SD-OCT machines are appropriate for clinical decision making in eyes that do not significantly deviate from the average eye. However, the influence of other factors such as the fovea-disc axis angle, the location and course of main retinal blood vessels, and proper centering of the RNFL measurement circle still need to be explored.[15–17] In an earlier study, the distribution of peripapillary RNFL thickness was investigated by Gabriele et al.[18] who found significant variability as a function of the distance of the peak measurements from the edge of the ONH. The choice of a measurement circle with a diameter of 3.4 mm was corroborated by the investigators as they found that measurements performed at that distance from the disc center demonstrated the lowest limits of variability among study eyes. Our study confirms the 3.4 mm diameter measurement circle to be an appropriate choice although some authors have suggested using a fixed distance from the clinical disc margin for RNFL measurements.[19]

We found that the changes in 95% prediction limits (i.e., the scatter among normal subjects) of the global and regional RNFL measurements were mostly negligible except in the superior and nasal quadrants and possibly for average RNFL thickness where correction for ocular magnification led to decreased intersubject variability although statistical significance for the nasal quadrant and average RNFL was not as large as in the superior quadrant (Fig. 6). We speculate that this indicates that other sources of RNFL variability, such as the fovea-disc axis angle, location of blood vessels, etc., are likely more important sources of variability than ocular magnification. Furthermore, we found that not only the structure-function relationships and the ability for discriminating glaucoma from normal subjects did not improve after correction of ocular magnification, but that both of the above measures actually demonstrated mild worsening after such correction (Table 2). One explanation could be the magnitude of the size of correction in our sample size. The scaling factors estimated in our study were overall small (median: 3% in the entire study sample). The magnification correction exceeded 10% in only 8% of normal subjects and 11% of glaucoma patients. By including more myopic patients, our study might have shown a more prominent effect; however, generalizability to average glaucoma patients would have been more limited. One other possibility for lack of a significant change in the RNFL performance is that the focusing mechanism of the SD-OCT machine could to some extent correct for the refractive error of individual eyes and hence partially adjust for the effect of ocular magnification.[20]

We did not find a statistically significant correlation between the disc area and RNFL cross-sectional area, taking into account the age and axial length despite correction of both for ocular magnification. Savini et al.[21] found a positive relationship between increasing ONH size and the RNFL thickness with Stratus OCT. The findings were confirmed by Budenz et al.[2] when they used the same device in a large number of normal subjects. On the other hand, in another study, Savini and colleagues[19] found that the increase in RNFL thickness with increasing disc size with Stratus OCT was an artifact of changing distance to the disc margin (i.e., larger discs were associated with thicker RNFL thickness measurements because the measurement was actually performed closer to the disc margin). When RNFL measurements were carried out at a fixed distance from the optic nerve head, this correlation disappeared. More recently, Huang and colleagues[14] reported that the disc size, as defined with scanning laser ophthalmoscopy, was not related to average RNFL thickness measured with SD-OCT adjusted for ocular magnifi-

cation. After reintroducing the magnification error, a weak positive association could be detected. One confounding factor with regard to detection of any association between the disc size and the RNFL complement of individual eyes could be that, in most prior studies, the RNFL thickness rather than the total cross-sectional RNFL area was used. Retinal nerve fiber layer thickness measurements depend on the size of the measurement circle. However, we found that even the cross-sectional RNFL area demonstrated a sizable change after correction of the location of the measurement circle in our study. The current thinking with regard to definition of disc area has evolved as a result of recent findings of mismatches between the BMO and the clinical disc margin.[6] We speculate that another reason for the discrepancies reported in the literature with regard to the association between the disc size and RNFL thickness is probably the inconsistent definition of the disc area in previous studies. However, this discrepancy has also been observed among histological studies.[22–25]

In summary, we used the large array of data of the SD-OCT to correct RNFL thickness measurements for the effect of ocular magnification. Our findings suggest that, within the range of commonly observed variations in axial length in patients at a tertiary glaucoma clinic, correction for ocular magnification does not significantly improve performance of the RNFL thickness measurements derived from SD-OCT and hence, such corrections are not necessary in such eyes.

Acknowledgments

Previously presented as a poster at the annual meeting of the American Glaucoma Society, March 2013.

Supported by an Early Career Clinician-Scientist Award from the American Glaucoma Society (KN-M).

Disclosure: **S. Nowroozizadeh**, None; **N. Cirineo**, None; **N. Amini**, None; **S. Knipping**, None; **T. Chang**, None; **T. Chou**, None; **J. Caprioli**, Allergan (C), Heidelberg Engineering (R); **K. Nouri-Mahdavi**, Allergan (C), Heidelberg Engineering (R)

References

1. Schuman JS, Pedut-Kloizman T, Hertzmark E, et al. Reproducibility of nerve fiber layer thickness measurements using optical coherence tomography. *Ophthalmology*. 1996;103:1889–1898.
2. Budenz D, Anderson DR, Varma R, et al. Determinants of normal retinal nerve fiber layer thickness measured by Stratus OCT. *Ophthalmology*. 2007;114:1046–1052.
3. Kang SH, Hong SW, Im SK, Lee SH, Ahn MD. Effect of myopia on the thickness of the retinal nerve fiber layer measured by Cirrus HD optical coherence tomography. *Invest Ophthalmol Vis Sci*. 2010;51:4075–4083.
4. Nouri-Mahdavi K, Nowroozizadeh S, Nassiri N, et al. Macular ganglion cell/inner plexiform layer measurements by spectral domain optical coherence tomography for detection of early glaucoma and comparison to retinal nerve fiber layer measurements. *Am J Ophthalmol*. 2013;156:1297–1307.
5. Chauhan BC, Burgoyne CF. From clinical examination of the optic disc to clinical assessment of the optic nerve head: a paradigm change. *Am J Ophthalmol*. 2013;156:218–227.
6. Reis AS, Sharpe GP, Yang H, Nicolela MT, Burgoyne CF, Chauhan BC. Optic disc margin anatomy in patients with glaucoma and normal controls with spectral domain optical coherence tomography. *Ophthalmology*. 2012;119:738–747.
7. Bennett AG, Rudnicka AR, Edgar DF. Improvements on Littmann's method of determining the size of retinal features by fundus photography. *Graefes Arch Clin Exp Ophthalmol*. 1994;232:361–367.
8. Garway-Heath DF, Rudnicka AR, Lowe T, Foster PJ, Fitzke FW, Hitchings RA. Measurement of optic disc size: equivalence of methods to correct for ocular magnification. *Br J Ophthalmol*. 1998;82:643–649.
9. Moghimi S, Hosseini H, Riddle J, et al. Measurement of optic disc size and rim area with spectral-domain OCT and scanning laser ophthalmoscopy. *Invest Ophthalmol Vis Sci*. 2012;53:4519–4530.
10. Patel NB, Garcia B, Harwerth RS. Influence of anterior segment power on the scan path and RNFL thickness using SD-OCT. *Invest Ophthalmol Vis Sci*. 2012;53:5788–5798.
11. Savini G, Barboni P, Parisi V, Carbonelli M. The influence of axial length on retinal nerve fibre layer thickness and optic-disc size measurements by spectral-domain OCT. *Br J Ophthalmology*. 2012;96:57–61.
12. Bayraktar S, Bayraktar Z, Yilmaz OF. Influence of scan radius correction for ocular magnification and relationship between scan radius with retinal nerve fiber layer thickness measured by optical coherence tomography. *J Glaucoma*. 2001;10:63–169.
13. Kim MJ, Lee EJ, Kim TW. Peripapillary retinal nerve fibre layer thickness profile in subjects with myopia measured using the Stratus optical coherence tomography. *Br J Ophthalmol*. 2010;94:115–120.
14. Huang D, Chopra V, Lu AT, et al. Does optic nerve head size variation affect circumpapillary retinal nerve fiber layer thickness measurement by optical coherence tomography? *Invest Ophthalmol Vis Sci*. 2012;53:4990–4997.
15. Chung JK, Yoo YC. Correct calculation circle location of optical coherence tomography in measuring retinal nerve fiber layer thickness in eyes with myopic tilted discs. *Invest Ophthalmol Vis Sci*. 2011;52:7894–7900.
16. Hood DC, Raza AS, de Moraes CG, Liebmann JM, Ritch R. Glaucomatous damage of the macula. *Prog Retin Eye Res*. 2013;32:1–21.
17. Yamashita T, Asaoka R, Tanaka M, et al. Relationship between position of peak retinal nerve fiber layer thickness and retinal arteries on sectoral retinal nerve fiber layer thickness. *Invest Ophthalmol Vis Sci*. 2013;54:5481–5488.
18. Gabriele ML, Ishikawa H, Wollstein G, et al. Peripapillary nerve fiber layer thickness profile determined with high speed, ultrahigh resolution optical coherence tomography high-density scanning. *Invest Ophthalmol Vis Sci*. 2007;48:3154–3160.
19. Savini G, Barboni P, Carbonelli M, Zanini M. The effect of scan diameter on retinal nerve fiber layer thickness measurement using stratus optic coherence tomography. *Arch Ophthalmol*. 2007;125:901–905.
20. Sanchez-Cano A, Baraibar B, Pablo LE, Honrubia FM. Magnification characteristics of the optical coherence tomograph STRATUS OCT 3000. *Ophthalmic Physiol Opt*. 2008;28:21–28.
21. Savini G, Zanini M, Carelli V, Sadun AA, Ross-Cisneros FN, Barboni P. Correlation between retinal nerve fibre layer thickness and optic nerve head size: an optical coherence tomography study. *Br J Ophthalmol*. 2005;89:489–492.
22. Johnson BM, Miao M. Age-related decline of human optic nerve axon populations. *Age*. 1987;10:5–9.
23. Jonas JB, Muller-Bergh JA, Schlotzer-Schrehardt UM, Naumann GO. Histomorphometry of the human optic nerve. *Invest Ophthalmol Vis Sci*. 1990;31:736–744.
24. Quigley HA, Coleman AL, Dorman-Pease ME. Larger optic nerve heads have more nerve fibers in normal monkey eyes. *Arch Ophthalmol*. 1991;109:1441–1443.
25. Varma R, Skaf M, Barron E. Retinal nerve fiber layer thickness in normal human eyes. *Ophthalmology*. 1996;103:2114–2119.