Relationship Between Age and the Thickness of the Retinal Nerve Fiber Layer in Normal Subjects

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Purpose: To determine if there are any age-dependent changes in the thickness of the retinal nerve fiber layer (RNFL) in the peripapillary area.

Methods: Sixty normal volunteers (31 men, 29 women) (120 eyes) whose ages ranged from 23–75 years (mean 48.4 years) participated in this study. The thickness of the RNFL was determined using a scanning laser polarimeter along the peripapillary area with a 1.75 disc diameter and along another ring 0.8 mm away from the disc margin.

Results: The thickness of the RNFL was not significantly correlated with age in either of the two ring areas. However, the RNFL thickness ratio of total/nasal area decreased significantly with increase in age in both rings. There was an increase in the difference of RNFL thickness between the right and left eyes of the same individual with aging, in both rings.

Conclusions: It was suggested that the RNFL thickness determined along both rings demonstrated almost identically the relationship between age and RNFL thickness in normal subjects. **Jpn J Ophthalmol 1999;43:180–185** © 1999 Japanese Ophthalmological Society

Key Words: Age, normal eyes, retinal nerve fiber layer, scanning laser polarimeter.

Introduction

The scanning laser polarimeter (Nerve Fiber Analyzer; NFA) now enables us to make objective and quantitative measurements of the thickness of the retinal nerve fiber layer (RNFL) in vivo.[1–13] Several investigators used the instrument and reported that measurements of the RNFL thickness were highly reproducible.[2,4–8,10] Measurements with this instrument approximately those obtained by histological examination,[2,4,14–16] and the peripapillary RNFL thickness in the superior and inferior quadrants has been shown to exceed that in the temporal and nasal quadrants. Also, RNFL thickness has been shown to decrease as the distance from the margin of the optic disc increases. Chi et al[4] reported that the RNFL thickness, as measured along the peripapillary ring with a 1.5 disc diameter (DD), significantly decreased with the increase in age. The RNFL thickness is measured with NFA along the peripapillary ring, which is concentric to the outer margin of the optic disc, at diameters of 1.5, 1.75, or 2.0 DD.[4,5] This means that the distance from the outer margin of the optic disc to the ring varies depending on the size of the optic disc being measured, because the size of the optic disc differs from person to person. It is still controversial whether the RNFL thickness should be measured along the ring that is more distal to the outer margin of the optic disc in patients with larger optic discs, or whether it should be measured along the ring at a set distance, regardless of the size of the optic disc. In the present study, we measured the RNFL thickness along two distinct rings, one with a diameter of 1.75 DD and the other one 0.8 mm away from the disc margin, and compared the data from both rings in a correlation between RNFL thickness and age.

Materials and Methods

A total of 120 normal eyes of 60 Japanese volunteers (31 men and 29 women) were studied. Subjects ranged in age from 23–75 years, mean ± standard

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deviation (SD) 48.4 ± 16.2 years. There were 10 subjects in each of six groups classified by age: 20–29, 30–39, 40–49, 50–59, 60–69, and 70–79 years. Informed consent was obtained from each subject. The subjects studied had no history of systemic diseases, such as diabetes mellitus or hypertension, and no significant ocular diseases found by routine ophthalmological examination. All subjects had a best corrected visual acuity above 20/25, normal intraocular pressure ≤21 mm Hg, and satisfactory results of visual field measurement (fixation loss <20% false-negative and false-positive <25%) performed on the central 24-2 or 30-2 program of the Humphrey visual field analyzer (Allergan-Humphrey, San Leandro, CA, USA). Results of the glaucoma hemifield test were within normal limits for each eye. *P* values for mean deviation (MD) and pattern SD were not <0.05. The average of the refractive error (spherical equivalent), intraocular pressure, MD, and corrected pattern SD in the subjects were −0.85 ± 1.58 (−4.75–2.25) diopters, 15.4 ± 2.4 (11–21) mm Hg, −0.07 ± 1.50 (−3.22–2.50) dB, and 1.01 ± 0.74 (0.00–3.48) dB, respectively.

The retinal nerve fiber layer thickness (RNFLT) was measured using a scanning laser polarimeter (Nerve Fiber Analyzer, version 2, 1.15 alpha; Laser Diagnostic Technologies, San Diego, CA, USA). The basic principles and technical characteristics have been described extensively.[1–5,11–13] In brief, measurement of RNFLT is based on the assumption that the RNFL possesses birefringent properties that change (retard) the state of polarization of an illuminating laser beam. This change can be measured by determining the phase shift between extraordinary and ordinary beams. Because the extent of retardation is linearly correlated with the RNFLT, the NFA can provide a measurement of the RNFLT. The NFA consists of a confocal scanning laser ophthalmoscope with a polarization modulator, a cornea polarization compensator, and a polarization detection unit. The light source consists of a near-infrared diode laser (wavelength, 780 nm) in which the state of polarization is modulated. Polarized light penetrates the birefringent RNFL and is partially reflected from the deeper layer of the retina. It is separated from the illuminating light beam by a nonpolarizing beam splitter. The state of polarization of the light is analyzed by the polarization detection unit; then the resulting electrical signal from the detector is digitized and stored in the memory of a personal computer for later analysis. During the actual measuring procedure, the subject was seated in front of the scanning head of the NFA, and instructed to focus his nonexamined eye on the fixation light. A laser beam was irradiated to the center of the pupil of the eye being measured. The measurement region was determined on the liquid crystal monitor, then the NFA was focused and adequate intensity of the laser beam determined. A total of 65,536 retinal locations (256 × 256 pixels) were tested, enabling us to create a retardation map in which the RNFLT was measured for each retinal location. The margin of the optic disc was approximated by an ellipse placed around the inner margin of the peripapillary scleral ring, then the RNFLT along the peripapillary ring, having the same diameter as the disc, was automatically measured by the computer. Variations resulting from use by different examiners may be decreased in the present NFA more than in the previous procedure because this Version 2 instrument possesses a dual detection system that automatically sets the intensity of the laser beam.

In the present study, all analyses were performed by the same operator. The pupils were not dilated. A 15° field size was used and the optic disc was centered in the middle of the image for all image acquisitions. All measurements were obtained from a mean of three images. The average SD of the RNFLT in retinal locations (pixels) in the mean image of the three images was within 8 μm in each eye. The RNFLT was measured within two distinct bands, both 10 pixels wide. One was located concentrically to the optic disc margin at 1.75 disc diameters (1.75 DD ring); the other one was also located concentrically to the optic disc margin with an average distance from the optic disc margin to the vertical pole (superior and inferior poles) and average distance from the optic disc margin to the horizontal pole (temporal and nasal poles) or 0.8 mm (0.8 mm ring). The average of the RNFLT was determined within the entire peripapillary circular band including its four 90° quadrants (superior, temporal, inferior, and nasal). To calculate absolute values in the vertical and horizontal diameters, errors in magnification were corrected using Bengtsson's correction with axial length.[17] If the distance from the optic disc margin is set too long, the ring may extend to the limit of the measuring monitor because of its 15° field size. Therefore, in the present study, we arbitrarily selected the distance of 0.8 mm. Axial length was measured by A-scan ultrasonography (Echo Scan US-1600; Nidek, Tokyo). The average of the axial length in all subjects was 23.91 ± 1.02 (21.54–27.17) mm.

For our first analysis, one eye of each of the 60 subjects was chosen at random. We studied the correlation between age and average RNFLT within the

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entire peripapillary circular band and its four 90° quadrants. According to a previous report by Tjon-Fo-Sang et al[7] that the RNFLT ratio based on RNFLT in the nasal quadrant, ie, superior/nasal RNFLT ratio or inferior/nasal RNFLT ratio, decreased with age in the normal subjects, we estimated the ratio of total RNFLT/nasal RNFLT (RNFLT ratio) and then studied the correlation between age and RNFLT ratio. Next, to investigate the difference between the eyes of one individual in the decrease of RNFLT with age, the correlation between age and the "difference of total RNFLT in both eyes/average total RNFLT in both eyes" (difference/average RNFLT) was investigated. The correlation between age and the "difference of RNFLT ratio in both eyes/average RNFLT ratio in both eyes" (difference/average RNFLT ratio) was also investigated.

Data are reported as mean ± SD. Linear regression analysis was used to calculate correlation coefficients. To compare the difference in the correlation coefficients, the comparison of two correlation coefficients were used. A level of $P < .05$ was considered statistically significant, and $P < .1$ was considered marginally significant.

Results

The average distance from the optic disc margin to the 1.75 DD ring, measured both in the vertical and horizontal directions, was 0.62 ± 0.08 mm in the 60 subjects. The average size of the 0.8 ring in all subjects was 1.98 ± 0.12 DD. The RNFLT over the entire 1.75 DD ring, and superior, temporal, inferior, and nasal quadrants was 62.5 ± 12.5 μm, 78.3 ± 17.8 μm, 39.9 ± 8.4 μm, 80.0 ± 16.4 μm, and 51.6 ± 12.7 μm, respectively. RNFLT over the entire 0.8 mm ring, and superior, temporal, inferior, and nasal quadrants was 46.7 ± 9.8 μm, 58.5 ± 13.9 μm, 29.8 ± 6.4 μm, 59.4 ± 12.4 μm, and 38.4 ± 9.8 μm, respectively.

There was no significant correlation between axial length and total RNFLT along either of the rings (1.75 DD ring: R = 0.138, P = .293; 0.8 mm ring: R = 0.162, P = .216). Also, there was no significant correlation between the refractive error (spherical equivalent) and total RNFLT along either ring (1.75 DD ring: R = −0.147, P = .262; 0.8 mm ring: R = −0.152, P = .246).

There was no significant correlation between age and total RNFLT along either ring (1.75 DD ring: R = −0.103, P = .434; 0.8 mm ring: R = −0.106, P = .420) (Figure 1). The correlation between age and RNFLT in each quadrant is shown in Table 1. There was no significant correlation between age and RNFLT in any quadrant in either ring.

The average RNFLT ratio along both the 1.75 DD and 0.8 mm rings was 1.23 ± 0.12. The RNFLT ratio along both rings decreased significantly with age (1.75 DD ring: R = −0.333, P = .009; 0.8 mm ring: R = − 0.370, P = .004) (Figure 2). There was no significant difference in either correlation coefficient (P = .818).

The mean of the difference in total RNFLT in both eyes of an individual was 8.0 ± 5.7 μm in the 1.75 DD ring and 6.5 ± 4.2 μm in the 0.8 mm ring. The mean of the "difference/average of RNFLT" was 0.14 ± 0.10 in the 1.75 DD ring and 0.15 ± 0.10 in the 0.8 mm ring. The "difference/average RNFLT," measured along both rings, tended to increase with

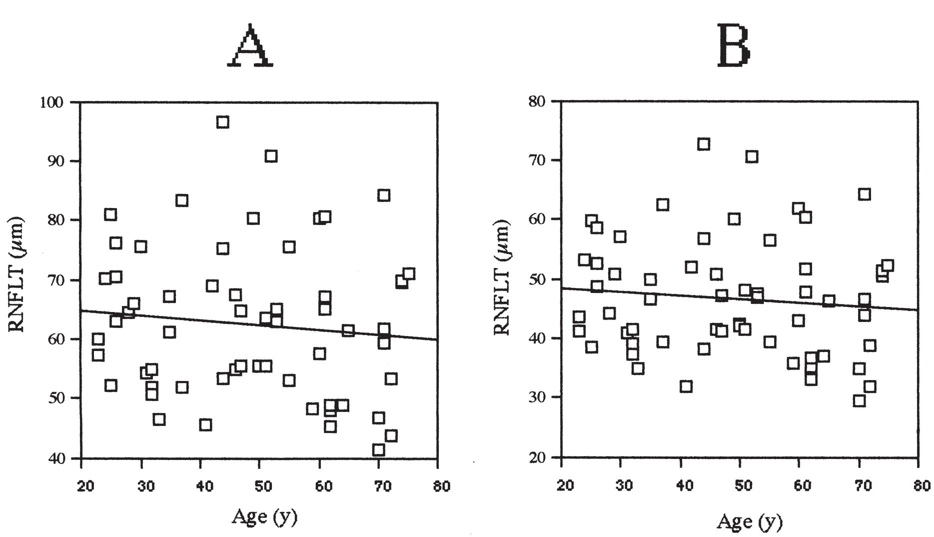

Figure 1. Correlation between age (X) and total retinal nerve fiber layer thickness (RNFLT) (Y) in 60 normal eyes. (**A**) 1.75 DD ring. R = −0.103, P = .434, Y = −0.079X + 66.383, n = 60. (**B**) 0.8 mm ring. R = −0.106, P = .420, Y = −0.064X + 49.770, n = 60.

Table 1. Correlation Between Age (X) and Retinal Nerve Fiber Layer Thickness in Superior, Temporal, Inferior, and Nasal Quadrants (Y)

Quadrant	1.75 DD ring (n = 60)	0.8 mm ring (n = 60)
Superior	R = −0.078 (*P* = .554)	R = −0.055 (*P* = .676)
	Y = −0.085X + 82.382	Y = −0.047X + 60.762
Temporal	R = −0.152 (*P* = .247)	R = −0.154 (*P* = .240)
	Y = −0.078X + 43.655	Y = −0.061X + 32.708
Inferior	R = −0.207 (*P* = .113)	R = −0.210 (*P* = .108)
	Y = −0.210X + 90.203	Y = −0.161X + 67.166
Nasal	R = 0.053 (*P* = .688)	R = 0.061 (*P* = .643)
	Y = 0.041X + 49.632	Y = 0.037X + 36.633

the increase in age (1.75 DD ring: R = 0.247, *P* = .057; 0.8 mm ring: R = 0.234, *P* = .072) (Figure 3). There was no significant difference in the correlation coefficients (*P* = .944).

The mean of the difference in RNFLT ratio in both eyes of an individual was 0.13 ± 0.09 in the 1.75 DD ring and 0.13 ± 0.10 in the 0.8 mm ring. The mean of the "difference/average RNFLT ratio" was 0.10 ± 0.07 in the 1.75 DD ring and 0.11 ± 0.08 in the 0.8 mm ring. There was no significant correlation between age and "difference/average RNFLT ratio," measured along either ring (1.75 DD ring: R = 0.135, *P* = .304; 0.8 mm ring: R = 0.143, *P* = .276) (Figure 4).

Discussion

In the present study, we obtained results indicating that RNFLT in the superior and inferior quadrants was greater than in the temporal and nasal quadrants when measured along both the 1.75 DD and 0.8 mm rings, which corresponded to previous reports.[2,4,10,14,15] We found no significant correlations between the axial length and total RNFLT, nor between refractive error and total RNFLT in the present study, which also corresponded to the results obtained by Chi et al.[4]

It has been reported from histological studies that the annual loss of optic nerve fiber is 4000–6723 axons,[18–22] with clinical studies using NFA, corroborating evidence that the RNFLT decreases with age.[2,4,7] However, Repka and Quigley[23] reported that there was considerable interindividual variability in optic nerve fiber counts even within the same generation, with no significant correlation between age and optic nerve fiber count in histological investigation. In the present study, no significant correlation was found between age and RNFLT measured along either the 1.75 DD or 0.8 mm ring. However, Chi et al[4] indicated that RNFLT, measured along the 1.5 DD ring using NFA, decreased significantly with age in 75 normal eyes. Although the reason for this difference between their results[4] and ours is not completely clear, the effect of aging may not have shown up in the present study because of our smaller sample size. Or possibly, the effect of aging may not have shown up because of the wide variation in RNFLT among individuals in the present study; several investigators have reported wide variation among normal individuals measured by scanning laser polarimetry.[2,4,6,7,10] The optical axonal counts of human fetuses revealed a peak of 3,700,000 axons in a fetus of 16- to 17-week gestation, after which the number stabilized at 1,100,000 axons at 29 weeks of gestation.[24] Variation

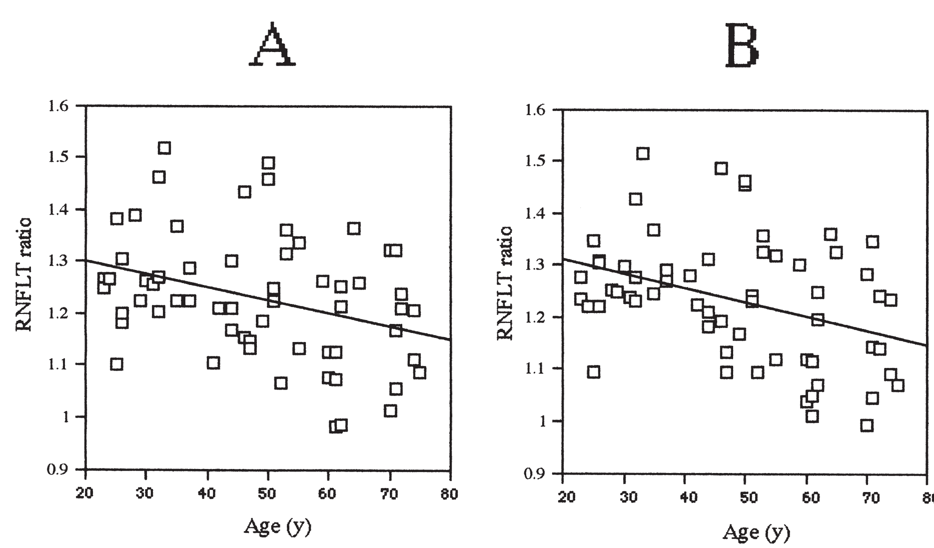

Figure 2. Correlation between age (X) and RNFLT ratio (Y) in 60 normal eyes. (**A**) 1.75 DD ring. R = − 0.333, *P* = .009, y = −0.002X + 1.350, n = 60. (**B**) 0.8 mm ring. R = −0.370, *P* = .004, Y = −0.003X + 1.365, n = 60.

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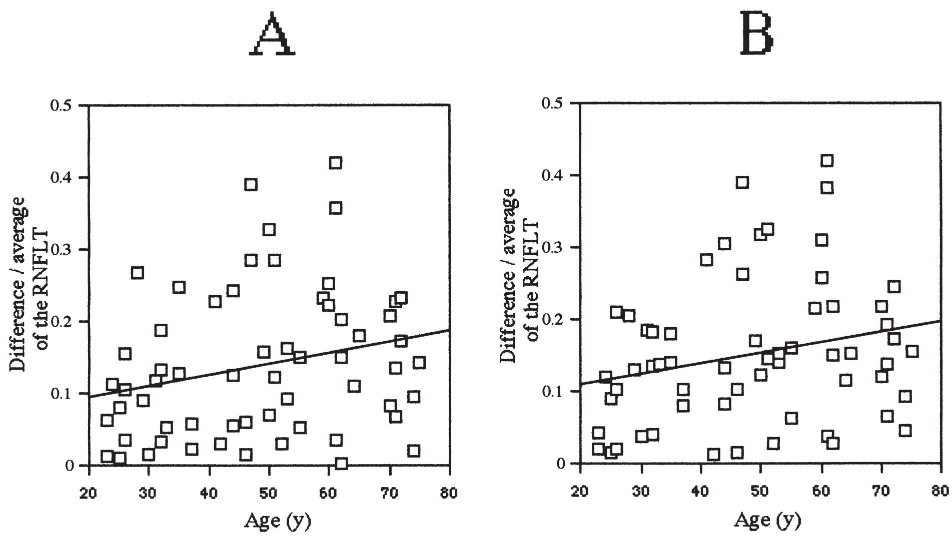

Figure 3. Correlation between age (X) and "difference/average RNFLT" (Y) in 60 normal eyes. (**A**) 1.75 DD ring. R = 0.247, *P* = .057, Y = 0.002X + 0.063, n = 60. (**B**) 0.8 mm ring. R = 0.234, *P* = .072, Y = 0.001X + 0.080, n = 60.

in RNFLT may be due to variables in the prenatal regression of retinal ganglion cells of an individual.[7]

In the present study, we estimated the RNFLT ratio based on the thickness in the nasal quadrant. We then investigated the correlation between age and the RNFLT ratio, with reference to the previous report of Tjon-Fo-Sang et al[7] that the ratio based on the thickness in the nasal quadrant, such as "superior RNFLT/nasal RNFLT" or "inferior RNFLT/nasal RNFLT," significantly decreased with age. It was revealed that the RNFLT ratio, measured along both the 1.75 DD and 0.8 mm rings, significantly decreased with age. We speculated that the significant decrease in RNFLT with age appeared because the interindividual variability was corrected to some degree by estimating the ratio of total to nasal RNFLT.

We found no significant decrease in nasal RNFLT with increase in age in the present study, but Chi et al[4] reported that RNFLT in the inferior and nasal quadrants decreased significantly with age. Therefore, further investigation is necessary to determine whether the ratio based on nasal RNFLT can be used for correcting the wide variation among normal individuals. Because there was no significant difference in the correlation coefficients between age and the RNFLT ratio measured along either the 1.75 DD or 0.8 mm ring, it was suggested that the effect of aging on the RNFLT ratio was demonstrated identically in both rings.

The difference in the RNFLT between the eyes of an individual has been described previously. Schuman et al[25] reported that the correlation coefficients

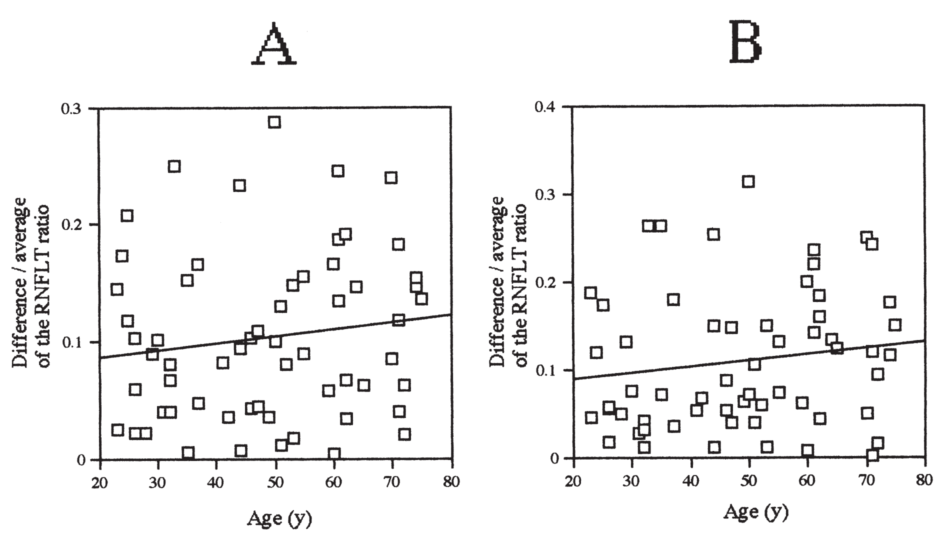

Figure 4. Correlation between age (X) and "difference/average RNFLT ratio" (Y) in 60 normal eyes. (**A**) 1.75 DD ring. R = 0.135, *P* = .304, Y = 0.001X + 0.075, n = 60. (**B**) 0.8 mm ring. R = 0.143, *P* = .276, Y = 0.001X + 0.076, n = 60.

between the RNFLT in the right and left eyes in normal individuals were 0.59 (P = .003) in the superior quadrant, 0.31 (P = .15) in the temporal quadrant, 0.52 (P = .01) in the inferior quadrant, and 0.05 (P = .80) in the nasal quadrant. Tjon-Fo-Sang et al[7] reported that the correlation coefficient between the ratio of "superior RNFLT/nasal RNFLT" of the right and left eyes was 0.40, and the correlation coefficient between the ratio of "inferior RNFLT/nasal RNFLT" was 0.45, which they speculated might be due to variables in the prenatal regression of retinal ganglion cells. In the present study we found that "difference/average RNFLT," as measured along both 1.75 DD and 0.8 mm rings, tended to increase with age. Although these results may indicate that the decrease in RNFLT related to aging is not necessarily identical in each eye of an individual, we need to investigate further the difference between eyes, because we did not find a significant correlation between age and the "difference/average RNFLT ratio."

In summary, using scanning laser polarimetry, we demonstrated clinically that, although there is considerable variation among normal individuals in RNFLT, the RNFLT ratio does indeed decrease with age. Further, it was shown that the thickness measured along both 1.75 DD and 0.8 mm rings demonstrated an almost identical relationship between age and RNFLT in normal subjects.

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