

ORIGINAL ARTICLE

Retinal Nerve Fiber Layer Thickness Measured by Optical Coherence Tomography in Non-glaucomatous Taiwanese

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Background/Purpose: Assessment of the peripapillary retinal nerve fiber layer (RNFL) is essential for neuroretinal diseases, especially for early prediction of glaucomatous damage. The purpose of this study was to measure RNFL thickness by Stratus optical coherence tomography (OCT) in normal Taiwanese subjects according to age group.

Methods: The thickness of the RNFL around the disc of normal subjects was obtained, after pupil dilation, by means of 3.4 mm diameter fast mode circle OCT scan. Data for one randomly selected eye of each subject were used for statistical analysis. Differences in RNFL thickness were determined by ANOVA.

Results: A total of 162 participants were evaluated: 61 male and 101 female, with mean age of 41.3 ± 20 years (range, 6–74 years). Mean RNFL thickness was $108.7 \pm 9.4 \mu\text{m}$ (range, 85.5–133.7 μm). The RNFL was thickest in the inferior ($135.8 \pm 16.3 \mu\text{m}$) and superior ($133.9 \pm 18.0 \mu\text{m}$) quadrants, followed by the nasal ($82.6 \pm 16.0 \mu\text{m}$) and temporal ($82.4 \pm 17.8 \mu\text{m}$) quadrants ($F = 551.9$, $p < 0.001$).

Conclusion: RNFL thickness was determined by OCT for a normal Taiwanese population aged 6–74 years. The normative data from this study may offer valuable information to enable comparisons of ocular diseases involving RNFL across different populations. [*J Formos Med Assoc* 2008;107(8):627–634]

Key Words: nerve fibers, optical coherence tomography, visual fields

Assessment of the retinal nerve fiber layer (RNFL) is crucial for a variety of neuroretinal diseases, especially glaucoma. For decades, monochromatic fundus photography has been used to evaluate RNFL structural changes.¹ RNFL loss from mild slit defect to severe generalized atrophy usually precedes the occurrence of visual field (VF) defects.^{2,3} However, monochromatic fundus photography requires a clear medium and a dilated pupil to yield a good image. Moreover, interobserver conflicts often exist. Recently, noninvasive and non-contact devices such as scanning laser polarimetry (SLP),⁴ retinal thickness analyzer (RTA),⁵ and

optical coherence tomography (OCT)⁶ have emerged to allow computerized quantification of RNFL thickness. These advanced technologies are more objective and reproducible than observation with the naked eye. Among these techniques, OCT is capable of producing stratified, cross-sectional tomographic images of the retina based on the reflectivity of the different layers within it. Recent literature has suggested that OCT possesses adequate reproducibility and sensitivity to assess RNFL thickness.^{7,8} Therefore, OCT is a promising tool to detect early RNFL defects in glaucoma^{9–11} and other optic nerve diseases such as ischemic optic

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neuropathy, traumatic optic neuropathy,¹² Leber's hereditary optic neuropathy,¹³ and others.¹⁴⁻¹⁶

Stratus OCT is the most recent model of OCT (the third generation). It has established a normal range of RNFL thickness since version 4.0, by using an age-matched database of 328 normal subjects aged > 18 years. Although the normal thickness distributions of RNFL among different populations have been investigated worldwide,^{10,11,17,18} the normative values are not yet available for a Taiwanese population. We know that the differences in optic nerve head characteristics and central corneal thickness may probably account for the high risk of primary angle-open glaucoma in Blacks.^{19,20} However, for Chinese, susceptible to primary angle-closure glaucoma, the biometric predisposing factors are not well established.^{21,22} A fuller understanding of the structural associations that underlie the diseases may optimize detection and management.

Therefore, the aim of this study was to evaluate the peripapillary RNFL thickness in normal Taiwanese by means of OCT. The results of this study provide data for comparison of normal RNFL parameters with those of other races, and offer relevant information on the probability of abnormalities that affect the RNFL.

Methods

A group of normal subjects were enrolled in this study between September 2005 and August 2006. They were recruited from the community, staff and family members of patients. Thorough ophthalmologic examinations including history-taking, refraction, intraocular pressure (IOP) measurement by noncontact tonometer (XPERT PLUS Advanced Logic Tonometer; Reichert, Depew, NY, USA), slit lamp biomicroscopic evaluation, and dilated fundus check-up with 78D Volk lens were performed. Mydriasis was achieved with 1% tropicamide. To be eligible for the study, each subject had to meet the following criteria: no evidence of corneal or retinal diseases; no previous ocular laser treatment or surgery; normal optic disc and

RNFL appearance; no major neurologic and diabetic disorders; best corrected visual acuity (spherical equivalent between +4.0 and -6.0 D and cylinder correction < 3.0 D) better than 6/12; and IOP < 21 mmHg. If nuclear cataract was detected, a degree of opacity over grade 2 severity, according to the guidelines for cataracts of the Lens Opacities Classification System II (LOCS II), excluded participation in the study.²³

Third-generation OCT (version 4.0.2; Stratus, Carl Zeiss Meditec, Dublin, CA, USA) was used to measure RNFL thickness. This instrument submits an 820-nm near infrared illumination to create a high-resolution cross-sectional (~10 µm) image of the eye. It is therefore similar to ultrasound. Fast RNFL scan mode and internal fixation were chosen for this study. To obtain the average value, three circumferential peripapillary 3.4-mm RNFL thickness scans were acquired and compressed into one scan. The circular scans were aimed at the optic disc, which measured RNFL at 256 points in a linear fashion. Only scans with good quality (signal strength ≥ 7), as well as the presence of a centered, circular ring around the optic disc, were accepted. OCT examinations were undertaken for all participants after pupil dilatation. RNFL thickness was evaluated as a full-circle average, in various quadrants, and at 30° intervals (each hour of the clock). Quadrants were defined as follows: superior (46-135°), inferior (226-315°), temporal (316-345°), and nasal (136-225°). Other parameters measured included superior maximum (Smax, thickest point in the superior quadrant), inferior maximum (Imax, thickest point in the inferior quadrant), Imax/Smax (ratio between maximum inferior and maximum superior quadrant thickness), Smax/Imax (ratio between maximum superior and maximum inferior quadrant thickness), Smax/Tavg (ratio between maximum superior and average temporal quadrant thickness), Imax/Tavg (ratio between maximum inferior and average temporal quadrant thickness), Smax/Navg (ratio between maximum superior and average nasal quadrant thickness), and Max-Min (difference between thickest and thinnest points along the peripapillary circle).

To determine the intraclass correlation coefficient (ICC), which usually represents a parameter of reliability, a substudy was carried out. The criteria for subjects recruited in this substudy were the same as for the main study. However, these subjects underwent three measurements at the first visit, with brief breaks between each measurement. All the subjects were scanned an additional three times at the second visit within a 2-week period. A total of six measurements gave the RNFL measurements intersubject, intervisit and intravisit variances. The ICC equaled the intersubject components of variance divided by total variance. Two-way random ANOVA was carried out to calculate ICC values.

Data in this study were all presented as mean \pm standard deviation. The values for one eye from subjects were selected randomly for analysis. The principle of selection was as follows: each participant was numbered according to the order he/she presented to the outpatient clinic. Then, 200 numbers were chosen from a table of random numbers. The subject's number was matched to the number from the table. Data from the right eye were chosen with an odd number and those from the left eye with an even number. Statistical analyses were carried out using SPSS version 12.0 (SPSS Inc., Chicago, IL, USA). The Shapiro–Wilk test was used to measure RNFL thickness in each age group to establish whether these subjects were drawn from a normally distributed population. ANOVA was conducted to determine the significance of differences between RNFL thickness according to gender and age. A value of $p < 0.05$ was considered statistically significant.

Results

Table 1 shows the demographic characteristics and age distribution of subjects in this study. Of the 162 normal participants, two-thirds were female. Mean age was 41.3 ± 20 years (range, 6–74 years). The mean spherical equivalent was -0.5 ± 2.0 D (range, -6 to 2.75 D). IOP was < 21 mmHg in all subjects.

Table 1. Demographic characteristics of the study group*

Total number	162
Age (yr)	41.3 ± 20 (6–74)
< 18	28
18–30	29
31–40	14
41–50	25
51–60	37
61–70	22
> 70	7
Gender	
Male	61 (37.9)
Female	101 (62.1)
Spherical equivalent, D	-0.5 ± 2.0 (-6.0 to 2.75)
Intraocular pressure (mmHg)	14.4 ± 2.6 (9–20)

*Data presented as n or mean \pm standard deviation (range) or n (%).

Table 2 shows the various RNFL thickness parameters measured by OCT. The average RNFL thickness along the entire circumference was 108.7 ± 9.4 μ m. Regional differences in RNFL thickness were found (by one-way repeated measures ANOVA: $F = 551.9$, $p < 0.001$) with the mean RNFL thickness higher in the inferior (135.8 ± 16.3 μ m) and superior (133.9 ± 18.0 μ m) quadrants (with no statistical significance between these two quadrants), followed by nasal (82.6 ± 16.0 μ m) and temporal (82.4 ± 17.8 μ m) quadrants (with no statistical significance between these two quadrants). No statistical significance was found between the average RNFL thickness and gender ($t = 1.21$, $p = 0.23$). In terms of the ratios between different quadrants, both the I_{max}/S_{max} and S_{max}/I_{max} were ~ 1.0 , while S_{max}/T_{avg} , I_{max}/T_{avg} and I_{max}/N_{avg} were ~ 2.0 .

The subjects were divided into seven groups according to age. The average RNFL thickness and in the various four quadrants according to age group is shown in the Figure. The mean RNFL thickness increased from 107.3 ± 10.2 μ m in individuals aged < 18 years to 111.4 ± 8.9 μ m in individuals aged 18–30 years. It then slowly declined with age: 31–40 years, 111.1 ± 9.1 μ m; 41–50 years, 110.3 ± 10.2 μ m; 51–60 years, 107.9 ± 8.4 μ m;

Table 2. Retinal nerve fiber layer (RNFL) thickness measured by optical coherence tomography in a normal Taiwanese population

RNFL thickness parameters (μm)	Mean	SD	Max	Min
Average	108.7	9.4	133.7	85.5
Superior	133.9	18.0	187.0	82.0
Nasal	82.6	16.0	126.0	51.0
Inferior	135.8	16.3	177.0	98.0
Temporal	82.4	17.8	142.0	46.0
Imax/Smax	1.1	0.2	1.6	0.7
Smax/Imax	1.0	0.1	1.5	0.6
Smax/Tavg	2.0	0.4	3.1	1.1
Imax/Tavg	2.1	0.4	3.2	1.3
Smax/Navg	2.2	0.5	4.0	1.3
Max-Min	131.8	20.0	183.0	82.0
Smax	167.2	20.7	234.0	112.0
Imax	174.9	21.8	232.0	126.0
Clock hour				
1	133.5	26.5	202.0	66.0
2	98.8	20.4	161.0	53.0
3	66.1	14.4	110.0	37.0
4	84.0	21.6	199.0	49.0
5	133.3	31.0	219.0	68.0
6	142.7	26.2	220.0	87.0
7	132.0	28.9	218.0	68.0
8	84.4	21.0	147.0	44.0
9	66.4	14.3	116.0	41.0
10	95.5	23.8	170.0	49.0
11	135.6	26.4	222.0	77.0
12	132.5	25.7	204.0	62.0

Imax/Smax = ratio between maximum inferior and maximum superior quadrant thicknesses; Smax/Imax = ratio between maximum superior and maximum inferior quadrant thicknesses; Smax/Tavg = ratio between maximum superior and average temporal quadrant thicknesses; Imax/Tavg = ratio between maximum inferior and average temporal quadrant thicknesses; Smax/Navg = ratio between maximum superior and average nasal quadrant thicknesses; Max-Min = difference between thickest and thinnest points along the peripapillary circle; Smax = thickest point in the superior quadrant; Imax = thickest point in the inferior quadrant.

61–70 years, $106.1 \pm 9.6 \mu\text{m}$; and >70 years, $104.2 \pm 9.1 \mu\text{m}$. No statistical significance was found between average RNFL thickness in the various age groups ($F = 1.37$, $p = 0.23$). The p values from the Shapiro–Wilk test for the average and four quadrants RNFL thickness in various age groups were all non-significant, which indicated that the RNFL thickness in various age groups were drawn from normally distributed populations.

Seventeen subjects were enrolled in the ICC substudy. Based on six measurements of OCT-generated RNFL data, the predicted ICCs are shown in Table 3. If we measure RNFL thickness once in the future, the ICCs for the mean, superior, nasal, inferior and temporal quadrants will be 0.82, 0.53, 0.57, 0.73 and 0.67, respectively. The ICCs were higher with repeated measurements ($n = 2, 3$ and 6). Within each measurement,

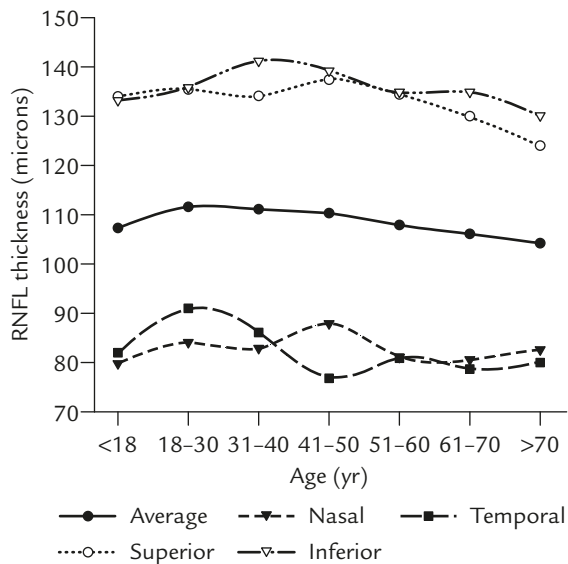


Figure. Average retinal nerve fiber layer (RNFL) thickness and RNFL thickness in the four regional quadrants, according to age group. RNFL thickness increased from the <18-year-old to the 18–30-year-old group. After that, RNFL thickness decreased with increasing age. * $p < 0.05$ when compared with 18–30-year-old group.

the ICCs were highest at average RNFL thickness, followed by inferior and temporal regions of RNFL. Measurements of superior quadrant RNFL had the lowest ICCs.

Discussion

The major purpose of this work was to assess the RNFL thickness in a normal Taiwanese population. The following principle findings emerged from this study. First, the RNFL thickness detected by Stratus OCT is reproducible and reliable. Second, age has no significant effect on RNFL thickness. Third, repeated OCT scans are required to increase accuracy when regional RNFL thickness is assessed. Last, the performance of OCT is good for subjects younger than 18 years old. Because it is difficult to perform visual field tests on children or adolescents, OCT measurements of RNFL thickness might aid in the diagnosis or long-term follow-up of these patients.

The new Stratus OCT affords better resolution by measuring 128 to 512 A-scans, with 1024 samples per scan, in comparison with previous OCT

Table 3. Intraclass correlation coefficient (ICC) for retinal nerve fiber layer (RNFL) thickness by average and quadrants

Number of measurements	ICC	95% CI
Average RNFL		
1	0.82	0.69–0.92
2	0.90	0.82–0.96
3	0.93	0.87–0.97
6	0.96	0.93–0.99
Superior RNFL		
1	0.53	0.33–0.75
2	0.69	0.50–0.86
3	0.77	0.60–0.90
6	0.87	0.75–0.95
Nasal RNFL		
1	0.57	0.37–0.78
2	0.73	0.54–0.87
3	0.80	0.64–0.91
6	0.89	0.78–0.95
Inferior RNFL		
1	0.73	0.56–0.87
2	0.84	0.72–0.93
3	0.89	0.79–0.95
6	0.94	0.88–0.98
Temporal RNFL		
1	0.67	0.48–0.83
2	0.80	0.65–0.91
3	0.86	0.74–0.94
6	0.92	0.85–0.97

CI = confidence interval.

1 and OCT 2000, which measures 100 A-scans, with 500 samples per scan.²⁴ The double-hump configuration of the peripapillary RNFL, because of the large number of nerve fibers converging on the vertical section of the optic nerve head, was found in all our participants. The RNFL thickness obtained by Stratus OCT has been reported to be underestimated within 20 μm when compared with OCT 2000.²⁴ Therefore, one should be cautious when interpreting RNFL values assessed by OCT, as they are liable to vary with different modalities and software versions. Table 4^{25–28} presents a comparison of RNFL thickness by using the current Stratus OCT version 4.0 from different studies. The average overall RNFL thicknesses at 360° around the optic disc ranges from 91 μm to

Table 4. Comparison of retinal nerve fiber layer (RNFL) thickness by recent Stratus optical coherence tomography in various studies

Study	N	Population	Software version	Age (yr)*	RNFL thickness (μm)				
					A	S	N	I	T
Hoh et al ²⁵	17	Black & White	4.0	53 ± 13 (27–72)	91	NP	NP	NP	NP
Fisher et al ²⁶	72	Caucasian (88%)	4.0	38 ± 10 (NP)	105	NP	NP	NP	NP
Ramakrishnan et al ²⁷	118	Indian	4.0.1	NP (21–74)	104	138	85	129	66
Sihota et al ²⁸	160	Indian	4	NP (20–70)	102	129	84	130	67
Peng & Lin (this study)	162	Taiwanese	4.0.2	41 ± 20 (6–74)	109	134	82	136	83

*Data presented as mean ± standard deviation (range). N = number of eyes; A = average; S = superior; N = nasal; I = inferior; T = temporal; NP = not published.

109 μm. These values are indeed lower than those with OCT 2000 (110–133 μm)^{17,18,29} and OCT 1 (153 μm).⁷ In a prior study by Chen et al,³⁰ which used Stratus OCT (version 2.0) to discriminate early glaucoma from normal eyes in Taiwan, the overall mean value of normal RNFL thickness was 100.9 ± 20.04 μm, and 120.8 ± 25.3 μm in the superior, 82.1 ± 23.0 μm in the temporal, 122.3 ± 31.3 μm in the inferior, and 77.0 ± 21.4 μm in the nasal quadrants. These values are thinner than our results. We suppose that the discrepancy in RNFL thickness between these two studies may have been caused by the different algorithms applied. The higher resolution or other unknown factors in new generations of software might have accounted for the distinction.³¹ However, to the best of our knowledge, there is no evidence-based data available that compares measurements according to the version of Stratus OCT used.

Age is recognized as a significant factor that affects RNFL thickness, with most previous publications demonstrating that RNFL decreases with age.^{18,32} A large study by Varma et al¹⁸ with 312 subjects showed a nearly 20-μm difference in RNFL thickness between participants aged 40–49 years and those aged >70 years. Another study by Hougaard et al³³ also showed a decrease of 2.6–2.9 μm in RNFL thickness with every 10-year increase in age. However, the opposite conclusion is reached when controlling for refraction, where age no longer has a significant effect on RNFL thickness.³¹ This observation was supported in a recent study by Ramakrishnan et al²⁷ and also by our findings. In addition, the association between

RNFL thickness and age in our study was not linear. The slope of the line was flat before age 50 and then dropped (Figure), which indicates that RNFL thinning is more significant in older people. In order to determine whether refractive status is a confounding factor, analysis of covariance was performed, in which age and refraction were covariates. Whether average or regional RNFL thickness was used for analysis, the *p* values did not reach a significant level (data not shown). Hence, the results of our study support the notion that age is not an important factor for RNFL thickness.

On the other hand, very few studies have investigated RNFL thickness in subjects < 18 years old. It is uncertain whether OCT examination can be carried out in young children. Based on our study and that of Salchow et al,³¹ the mean RNFL thickness is slightly thinner in children and adolescents (age < 18 years) than in adults. At the same time, the reproducibility and reliability of these two studies were adequate. Therefore, OCT is valuable for the diagnosis of glaucoma^{34,35} and other neuroretinal disorders in this special population.

ICC commonly represents an index of reliability: the higher the ratio, the better is the reliability. This means that the inter- and intravisit variances are small, and the major variance is intersubject. Often, an ICC ratio > 0.6 is considered to be acceptable.³⁶ According to our results (Table 3), the predictive ICCs after six measurements of RNFL thickness, on average and in four quadrants, were > 0.87, which demonstrates the excellent reproducibility of OCT. Thus, the measurement errors in our study are mostly derived

from intersubject variation. Blumenthal et al⁹ have also reported that the main source of variance in OCT results from differences between patients (79%). Other factors were visits (5%), operator (2%) and session (1%). If RNFL thickness was measured once, the ICCs for the superior and nasal quadrants were < 0.6. The superior quadrant appears to be the least reproducible of the four. This finding is compatible with the study conducted by Budenz et al,³⁷ in which variability was higher for quadrants and clock-hour sectors, with the nasal region being the least reliable. On the other hand, the reliability for measurements of average and inferior quadrant RNFL thickness is perfect even with one single examination. It also documents that OCT is a suitable instrument for discriminating inferior quadrant involvement in glaucoma examination. Because increasing the number of scans can improve the reproducibility of RNFL measurements, three repetitive measurements are recommended for assessing superior and nasal quadrants of RNFL, in an attempt to acquire more accurate data, as well as saving time.

In summary, the normal RNFL thickness for Taiwanese measured by Stratus OCT in the current study was largely consistent with studies in other countries. Regional differences occur in circumpapillary RNFL thickness. It is essential to develop normograms in each institute because a number of factors lead to variability in the results. Also, a normative dataset reference is valuable to identify any signs of presymptomatic stages of ocular diseases to allow early treatment.

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