·Clinical Research·

Analysis of peripapilary retinal nerve fiber layer thickness of healthy Chinese from northwestern Shanghai using Cirrus HD−OCT

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Abstract

·**AIM:** To investigate peripapillary retinal nerve fiber layer (RNFL) thickness of healthy Chinese individuals from northwestern Shanghai using Cirrus HD−OCT (Carl Zeiss Meditec, Inc. Dublin, CA, USA).

·**METHODS:** The peripapillary RNFL thickness of 720 eyes from 360 healthy Chinese participants were measured using the Optic Disc Cube 200×200 protocol. Each eye was scanned 3 times. Global and each quadrant's RNFL thickness around the optic nerve were compared between genders, and interocular differences were analyzed. The correlation between global RNFL thickness and age were also assessed in this study.

·**RESULTS:** The mean global, superior, nasal, inferior and temporal RNFL thickness of all the eyes were 96.04 ± 7.40 μm, 118.36 ± 13.52 μm, 67.63 ± 8.60 μm, 125.17 ± 13.48 μm, 72.49 ± 10.70 μm, respectively. When analyzing between genders, the mean nasal RNFL thickness of male and female were 68.29 ± 8.44 μm and 66.97 ± 8.70 μm, with statistically significant difference ($P = 0.038$), while the data of global, superior, inferior and temporal quadrant showed no significant difference (all $P > 0.05$). When analyzing interocular differences, the mean RNFL thickness of all the right eyes and all the left eyes were 116.46 ± 13.17 μm and 120.27 ± 13.61 μm in superior quadrant ($P < 0.001$); 68.74 ± 8.80 μm and 66.52 ± 8.25 μm in nasal quadrant ($P < 0.001$); 73.16 ± 10.95 and 71.83 ± 10.41 in temporal quadrant ($P < 0.001$), all having statistically significant differences. There were no statistically significant interocular differences of global and inferior RNFL thickness (both $P > 0.05$). There was a significantly negative correlation ($r = -0.618$, $P < 0.001$) between the mean global RNFL thickness and the age.

·**CONCLUSION:** In healthy Chinese from northwestern Shanghai, there were no significant differences detected interocular difference and between genders in the mean global RNFL thickness. Nevertheless, significant difference existed in the nasal quadrant between genders, and interocular differences existed in the superior, nasal and temporal quadrants. The RNFL thickness appeared to gradually decrease with age.

·**KEYWORDS:** retinal nerve fiber layer; optical coherence tomography; Chinese

INTRODUCTION

Retinal nerve fiber layer (RNFL) thickness is a vital ingredient for diagnosing optic neuropathy diseases, such as glaucoma, diabetic neuropathy, ischemic or traumatic optic neuropathy [1-3]. Since Huang *et al*[4] introduced optical coherence tomography (OCT), a high resolution, noncontact, noninvasive, quantitative biological tissue imaging technology, to scan retinal structure *in vivo* in 1991, it has developed rapidly in clinical ophthalmology over the past twenty years. Since the change of the RNFL thickness may indicate the progress of damage or healing, measuring RNFL thickness has been one of the important applications of OCT technology. As the latest fourth generation, spectral domain OCT (SD-OCT) obtains light echo signals transformed from time-domain into frequency-domain through the Fourier commutation, increases the scanning speed and axial resolution to 60 times and 5 times of the third generation (time domain OCT, TD-OCT). Generating clearer images more than ever, SD-OCT has become an unparalleled guide for objective and accurate diagnosis and follow-up in clinical ophthalmology.

Throughout the world, millions of patients are suffering diverse optic neuropathy diseases like glaucoma. Data showed that glaucoma has become a serious blinding disease according to its second highest morbidity in 2010, and nearly half (about 47%) of the patients will be Asian in 2020 [5].

Nevertheless, there are no standard values of RNFL thickness of healthy Chinese in the database of SD-OCT, which challenge the diagnosis of retinal disease in China. Due to the giant population of Chinese patients, it becomes an urgent affair to establish the standard database of healthy Chinese RNFL thickness. In this study, Cirrus HD-OCT (Carl Zeiss Meditec, Inc. Dublin, CA, USA) were used to measure the RNFL thickness of healthy Chinese individuals from northwestern Shanghai. Difference between genders, interocular difference and variation of RNFL thickness along with age were also analyzed, which expected to provide the standard database for recognizing the RNFL thickness changes when diagnosing and treating optic neuropathy diseases in China.

SUBJECTS AND METHODS

Subjects Totally 720 eyes of 360 outpatients from northwestern Shanghai (197 females and 163 males) between 22 and 80 years old (average 51.6 ± 17.0) were randomly enrolled in this study. The participants consisted of the volunteers who visited our clinic for ophthalmic examination and patients with minor refractive disorders from March 2010 to June 2012. All study procedure followed the tenets of the Declaration of Helsinki and the study protocol had been approved by the Institutional Review Board of Tongji Hospital Affiliated to Tongji University. Written informed consents were obtained from all the participants. All the participants got routine ophthalmic examination. Inclusion criteria were the following [6]: best corrected visual acuity above 20/25, refractive error between -3.0 and +3.0 diopters of sphere, binocular anisometropia less than +1.5 diopters of sphere, transparent ocular media (allowing lens density increase when the age above 50 years old), intraocular pressure <21 mm Hg measured by non-contact tonometer, binocular orthophoria and normal foveal fixation; these participants did not have optic nerve diseases and ocular funds, had not undergone intraocular surgery, and had no systemic diseases with possible ocular involvement, such as diabetes mellitus. All the participants were subdivided into 6 groups by age: group A (>20 to ≤30 years old), group B (>30 to ≤40 years old), group C (>40 to ≤50 years old), group D (>50 to ≤60 years old), group E (>60 to ≤70 years old) and group F (>70 to ≤80 years old).

Methods All the scans were completed by the same experienced ophthalmic technician using Cirrus HD-OCT (software version 3.0.0.64). Before each scan, 0.5% tropicamide eyedrop was used to every participant to make sure binocular mydriasis (pupil dilated to 6 mm at least). Every participant was seated with the mandible fixed on the jaw-frame on the Cirrus HD-OCT. The technician adjusted the jaw-frame to correct eye position and to compensate for the refractive error. Images were obtained with internal fixation. The Optic Disk Cube 200×200 axial protocol was

Table 1 Peripapillary retinal nerve fiber layer thickness　(n=720)

Quadrant	Minimum (μm)	Maximum (μm)	Mean±SD (μm)
Global	74	116	96.04±7.40
Superior	77	159	118.36±13.52
Nasal	50	103	67.63±8.60
Inferior	84	164	125.17±13.48
Temporal	43	112	72.49±10.70

SD: Standard deviation.

used for scanning, with the light source of 840 nm wavelength, axial resolution of 5 μm, lateral resolution of 2 μm, and scanning depth of 2 mm and scan speed of 27 000 A-scans/s respectively. With the existing program, the measurement area was automatically identified by placing a diameter of 3.46 mm red calculation circle evenly around the center of the optic disc. After high-quality image was obtained, the RNFL thickness was calculated automatically using the existing algorithms. The optic disc was divided into four quadrants including superior, inferior, nasal and temporal by two vertical lines through the center of optic disc with a 45° angle between the line and horizontal line. On the analysis page, the RNFL thickness (global and four quadrants'), signal strength scaled from 0 (worst) to 10 (best), and binocular symmetr (%) were displayed. To make sure the accuracy of the results, only the signal strength higher than 6 and the binocular symmetry more than 85% were accepted in our study. Each patient was scanned 3 times. The global, superior, nasal, inferior and temporal RNFL thickness of each eye was respectively recorded in our database. The mean RNFL thickness of different genders and eyes in global and each quadrant were respectively calculated and analyzed. The correlation between the global mean RNFL thickness and age were analyzed as well.

Statistical Analysis All the data ran the normal distribution and homogeneity of variance tests before analysis and they were expressed as mean ±standard deviations (SD) in this article. SPSS 17.0 (SPSS Inc., Chicago, IL, USA) was used for statistical analysis. Interocular comparison of global and each quadrant's RNFL thickness were analyzed using the paired samples t-test, while comparison between genders were analyzed using the independent samples t-test. The correlation between RNFL thickness and age were analyzed by correlation analysis. $P<0.05$ was considered statistically significant.

RESULTS

Of 720 eyes, the mean global, superior, nasal, inferior and temporal RNFL thickness were 96.04 ± 7.40 μm, 118.36 ± 13.52 μm, 67.63 ± 8.60 μm, 125.17 ± 13.48 μm and 72.49 ± 10.70 μm, respectively. The RNFL thickness decreased in the order of inferior, superior, temporal and nasal in healthy eyes (Table 1).

The mean global RNFL thickness of 163 males was 96.07±

Table 2 Global and quadrant's RNFL thickness (µm) of male, female and both eyes

RNFL thickness (µm)	Gender		[1]P	Eye		[2]P
	M (n=163)	F (n=197)		Right (n=360)	Left (n=360)	
Global	96.07±7.11	96.00±7.68	0.900	96.18±7.72	95.89±7.06	0.229
Superior	119.02±13.16	117.71±13.85	0.191	116.46±13.17	120.27±13.61	<0.001
Nasal	68.29±8.44	66.97±8.70	0.038	68.74±8.80	66.52±8.25	<0.001
Inferior	124.54±13.62	125.80±13.32	0.213	125.43±13.49	124.92±13.48	0.352
Temporal	72.31±10.04	72.67±11.34	0.654	73.16±10.95	71.83±10.41	<0.001

RNFL: Retinal nerve fiber layer; [1]Independent samples t test; [2]Paired samples t test.

Table 3 Mean retinal nerve fiber layer thickness in six age-groups

Groups	n	Average RNFL thickness (µm)	Subset			
			1	2	3	4
A	101	102.12±4.82	102.12			
B	122	99.24±5.49		99.24		
C	135	99.67±5.11		99.67		
D	129	94.43±5.77			94.43	
E	118	93.23±5.67			93.23	
F	115	87.82±5.69				87.82
P	-	-	1.000	0.537	0.088	1.000

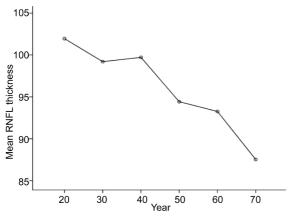Subset: From student Newan Keuls test (SNK-test) in SPSS 17.0.

Figure 1 Correlation between retinal nerve fiber layer thickness and age.

7.11 µm, and of 197 females was 96.00±7.68 µm, with no statistically significant difference between genders (P >0.05). There were no significant differences in superior, inferior and temporal RNFL thickness between males and females (all P >0.05), while the nasal RNFL thickness was detected with significant differences between genders (P =0.038). The mean global RNFL thickness of all the right eyes was 96.18± 7.72 µm, and of all the left eyes was 95.89±7.06 µm, with no statistically significant interocular difference (P >0.05). However, in the analysis of each quadrant, significant interocular differences were detected in the superior, nasal and temporal (P <0.05) except the inferior quadrant (P >0.05). The RNFL thickness of right eyes was thinner than the left eyes in superior quadrant, while it was thicker than the left in nasal, inferior and temporal quadrant (Table 2).

The mean global RNFL thickness of different age-groups showed that the maximum was the mean RNFL thickness of the > 20 to ≤30y group, and the minimum was the > 70 to ≤80y group. Although the results of SNK-test indicated no significant difference between Group B vs C (P =0.537) and Group D vs E (P =0.088) (Table 3), the RNFL thickness appeared to have a declined trend as age increasing (Figure 1), with a significant negative correlation between RNFL thickness and age (r =-0.618, P <0.001).

DISCUSSION

Compared with other ophthalmic facilities, OCT is a noninvasive technology that can provide objective, real time, cross-sectional measurements of various layer of retina, including RNFL. All the three previous generations of OCT were TD-OCT, which dynamic observation, image resolution and clarity were limited as consequence of the scan speed limitation. Application of SD-OCT was like a new milestone thanks to its high resolution that distinguishes the retinal structure clearly; its high speed that avoids artifacts caused by eyeball movement; and its three-dimensional technology that allows to generate plenty informations in defined area. All the advantages above have made the in $vivo$ detection of retinal neuropathy closer to the level of histopathological examination, which will definitely enhance the re-understanding and re-assessment of diverse optic neuropathy diseases.

Since there is close relationship between RNFL thickness and visual acuity, accurate measurement of the peripapillary RNFL thickness has a great significance on the diagnosis of glaucoma and other optic nerve diseases. Thus, the diagnosis based on the RNFL thickness is effected by the value of the healthy populations. In recent years, most of the reports about the RNFL thickness in healthy populations used former generations [7-9]. Previous studies also showed that the race seemed to be an influence factor of the RNFL thickness[10,11]. In this study, we randomly selected 360 healthy Chinese from different age groups (>20 to ≤80 years old) in northwestern Shanghai, hoping to provide a normal reference value of the RNFL thickness in healthy Chinese population. It will definitely assist the initial diagnosis and long term follow-up clinic of optic nerve diseases.

The mean global RNFL thickness of the healthy northwestern Shanghai individuals using Cirrus HD-OCT was 96.04±7.40 µm, which was different from other races. It

was 97.3 ±8.80 μm in Koreans, and 95.21 ±12.45 μm in Italians[12,13]. The results from different areas in America were 98.68±10.89 μm and 92.0±10.80 μm, also with difference[10,14]. Additionally, there were obvious differences between the results using different machines. Huang *et al*[15] reported the mean global RNFL thickness of Chinese was 110.74±9.43 μm using TD-OCT　(Stratus OCT), while it was 106.75±6.64 μm using Topcon 3D-OCT. The mean RNFL of Taiwanese reported by Hsu and Tsai[7] was 107.4±17.8 μm using Stratus OCT. All the different results indicated that the normal reference value varied in races, different inspection facilities, and even in different areas of the same country.

The RNFL consist of the axons of retinal ganglion cells from different sectors spread towards the optic disc. Since more cells walking towards the superior and inferior optic disc, the peripapillary RNFL are thicker in the inferior and superior quadrants, which were also proved by the studies of ours and others'[8]. The result from Ozden showed a little difference that the nasal RNFL was thicker than temporal[16,17]. Except for nasal quadrant in gender, there were no statistically significant differences in other three quadrants and global in our result. Both Mansoori *et al*[18] and Alasil *et al*[19] reported that there were no significant gender-related differences in RNFL thickness in various countries.

There was no significant interocular difference in global RNFL thickness in our study, which was different from Park *et al*'s[20] study. In his study, the average RNFL of right eyes was significantly thicker than the left eyes. And what needs to be emphasized in our study was the significant interocular difference in the superior, nasal and temporal quadrant only except the inferior quadrant. They were highly consistent with the results of Mwanza *et al*'s study[21] except the marginally interocular difference in average thickness was also detected. Due to that, he speculated that an interocular difference of mean RNFL thickness exceeding 9 μm might be considered statistically significant asymmetry or a symbol of early glaucomatous damage. Although his study contained people of white, black, Hispanic, Asian and others, the speculation still need large sample to prove.

Repka and Quigley[22] found no statistically significant difference in the number of nerve fiber between young and old individuals. On the contrary, our result of a negative correlation between the RNFL thickness and age indicated the global RNFL got thinner with age increasing, which was in agreement with Balazsi *et al*[23] and Johnson *et al*[24]. The probable reason could be a wide range　(from 500 to 7000 axons) of axonal loss per year[25]. It seems that the age-related degeneration of peripapillary RNFL thickness should be considered in diagnosis of optic neuropathy diseases, not only in Chinese, but also in other races. In addition, we found no statistically significant difference between age group B　(>30 to ≤40y)　*vs* C　(>40 to ≤50y), and D　(>50 to ≤60y)　*vs* E

(>60 to ≤70y). It still needs larger scale sample study to certificate whether these results were the characteristic of Chinese population.

Some studies showed that pupil size, scanning speed, and experience of the operators would affect the repeatability of measurement[15,26-30]. In our consideration, the pupil was dilated before measuring RNFL thickness was mean to increase image quality and binocular symmetry. It would not affect the accuracy of results from the studies of Zafar *et al*[26] and Polito *et al*[27], that no significant difference were found between the results in healthy subjects measured before and after mydriasis. We could also believe the repeatability of the measuring values using Cirrus HD-OCT from a big scale study that measurement variation was greatly reduced due to high scanning speed[15]. The scanning time was 1/100s, which greatly reduces the variation due to epiphora. All of the measurements in this study were completed by the same operator to avoid the impacts from different operators. Nonetheless, not like Stratus OCT which is hard to manually control the scan ring locating in the optic disc center, the system of Cirrus HD-OCT locate the scan ring automatically, also to avoid the impact from the operator. With all above, the results of our study measured by Cirrus HD-OCT were reliable and repeatable.

Although larger scale sample study needs to be carried out in China, the results of this preliminary study still showed crucial guidance about characteristics of the RNFL thickness among the healthy Chinese population, since there is no Chinese Data from the database in Cirrus-OCT. We truly hope this study to be a good start of the future large scale sample researches on RNFL and optic neuropathy diseases in Chinese population.

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