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Study of the Effects of Aging, Refraction and Intraocular Pressure Levels on Retinal Nerve Fiber Layer Thickness of Normal Healthy Eyes

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1. Introduction

Glaucomatous optic neuropathy (GON), clinically characterized by progressive visual field defects that correspond with glaucomatous optic disc changes, is known as one of the major causes of irreversible blindness worldwide (Quigley, 1999). Thus far, there is no known cure for GON. Thus, early diagnosis and treatment to slow down the disease progression is considered of most importance. To diagnose earlier stages of GON, a sensitive and reliable methodology to detect the specific glaucomatous optic disc changes required. To this point, retinal nerve fiber layer (RNFL) thickness measurements by optical coherent tomography (OCT) has been widely utilized (Bowd et al., 2000; Katai et al., 2003). However, the optic disc appearance is quite variable within even healthy populations. In fact, recent studies have revealed that its incidence exclusively varies with races, ages and others, and therefore, these factors should be taken into account to perform better analyses through OCT (Katai et al., 2006). The purpose of the present study is to investigate the effects of aging, refraction, and intraocular pressure levels on RNFL thickness. To do so, the eyes of healthy Japanese individuals were evaluated and compared with those of Europeans.

1.1 Subjects and methods

1.1.1 Subjects

246 eyes of 126 healthy Japanese subjects (53 men, 73 women) over 18 years of age were recruited from 2002 to 2009 at Sapporo Medical University Hospital. The present study protocol was approved by the Ethics Committee of the Sapporo Medical University School of Medicine and conducted in accordance with the Declaration of Helsinki. After an explanation of the study's purpose and its protocol were provided, written informed consent was obtained from all participants before inclusion. To ensure that they had no systemic and ocular diseases including ocular surface diseases, uveitis, and retinal vascular diseases other than, early cataract, all subjects were asked to provide their past medical histories. Next, their eyes were examined by a slit-lamp biomicroscope, and they also underwent a fundus examination. The mean age, spherical equivalent (SE), and intraocular pressure (IOP) of the subjects were 45.5 ± 16.5 years (22 – 87 years), +2.2 ± 3.1 D (-12.8 - +2.9 D), and 14.2 ± 2.9 mmHg (7.0 - 20.0 mmHg), respectively. The best corrected visual acuity

ranged between 16/20 to 30/20. IOP of the subjects was measured by non-contact tonometer or Goldmann applanation tonometry.

When examining the effects of aging on the RNLF thickness, 152 eyes of 80 healthy subjects were further divided in-to 6 groups: 20 to 29 years (20 eyes), 30 to 39 years (35), 40 to 49 years (25), 50 to 59 years (29), 60 to 69 years (18), and 70 to 79 years (25).

For evaluation of the effects of myopia, subjects were initially divided into two groups according to their SE values: a myopic eyes group (SE ≤-3.0 D) and a non-myopic eyes group (-3.0 D < SE) (Mitchell et al., 1999). Myopic eyes consisted of 88 eyes of 47 subjects (mean age 37.1 ± 10.3 years (22 – 63 years)) and non-myopic eyes comprised 158 eyes of- 83 subjects (mean age 50.1 ± 17.6 years (22 - 87 years)). To study the effects of refractive errors on RNFL thickness, 47 myopic eyes and 83 non-myopic eyes from subjects over 40 years old were selected and included in the analysis. No significant difference was found in average age distribution and IOP levels between the two groups.

To investigate the ocular hypertension on RNFL thickness, other subjects with ocular hypertensive eyes with more than 21 mmHg IOP were also recruited at the Sapporo Medical University Hospital. Before inclusion in the present study, in addition to ocular examinations described above, a visual field test by a Humphrey Field Analyzer (HFA: HFA 30-2 SITA fast; Humphrey-Zeiss, San Leandro, CA) was performed to make sure that the subjects' eyes had no glaucomatous visual field changes (The Japan Glaucoma Society [JGS], 2006), thereafter (JGS, 2006). 45 ocular hypertensive eyes of 25 subjects (7 men, 18 women, mean age 59.9 ± 12.1 (36 - 83 years), mean SE -1.2 ± 2.5 D (-6.8 - +4.1 D), mean IOP 23.5 ± 2.0 mmHg (21.0 - 30.0 mmHg)) were included in the current study. As a control, 147 eyes of 75 healthy subjects (30 men, 45 women, mean age 55.9 ± 13.2 years (38 - 87 years), mean SE -1.6 ± 3.2 D (-11.6 - +3.0 D), mean IOP 14.1 ± 2.7 mmHg (7.0 - 20.0 mmHg)) were randomly selected and employed among the healthy eyes described above.

1.1.2 OCT (Optical Coherence Tomography) measurement

To measure RNFL thickness, OCT3 (Optical Coherence Tomography 3000; Zeiss-Humphrey, Dublin, CA) was used for. This contained an interferometer that resolved retinal structures by measuring the echo delay time of light that was reflected and backscattered from different microstructural features in the retina. The OCT3 projected a broad bandwidth near-infrared light beam (820 nm) onto the retina from a super luminescent diode. The light reflected from the retina consisted of multiple echoes and the distance traveled by various echoes was determined by varying the distance to the reference mirror. This produced a range of time delays of the reference light for comparison.

The OCT3 software package included 18 scan acquisition protocols and 19 analysis protocols. The fast RNFL thickness scan mode was used, and this protocol acquired three 3.4 mm diameter circle scans in 1.92 seconds of scanning. The RNFL thickness average analysis protocol calculated peripapillary RNFL thickness as the distance between vitreoretinal interface and the anterior surface of the retinal pigment epithelium/choriocapillaris region. These values were then averaged to yield 12 clock-hour thickness, four quadrant thickness, and a global average RNFL thickness measurement (360° measure) (Figure 1).

These values were then compared against a normative database of age-matched controls to derive percentile values. Statistical determinations of confidence bands at 1 %, 5 %, 95 %, and 99 % were then calculated. The OD and OS graphics displayed red, yellow, green, and white colored bands, corresponding to the Normal Distribution Percentiles as estimated by the RNFL Normative Database. The subject ethnicity of this normative database is comprised of 95 % whites, blacks, and Hispanic, classified as European, with the remaining 5 % classified as other races. Asians comprise only 3 % (Patella, 2003) (Figure 2).

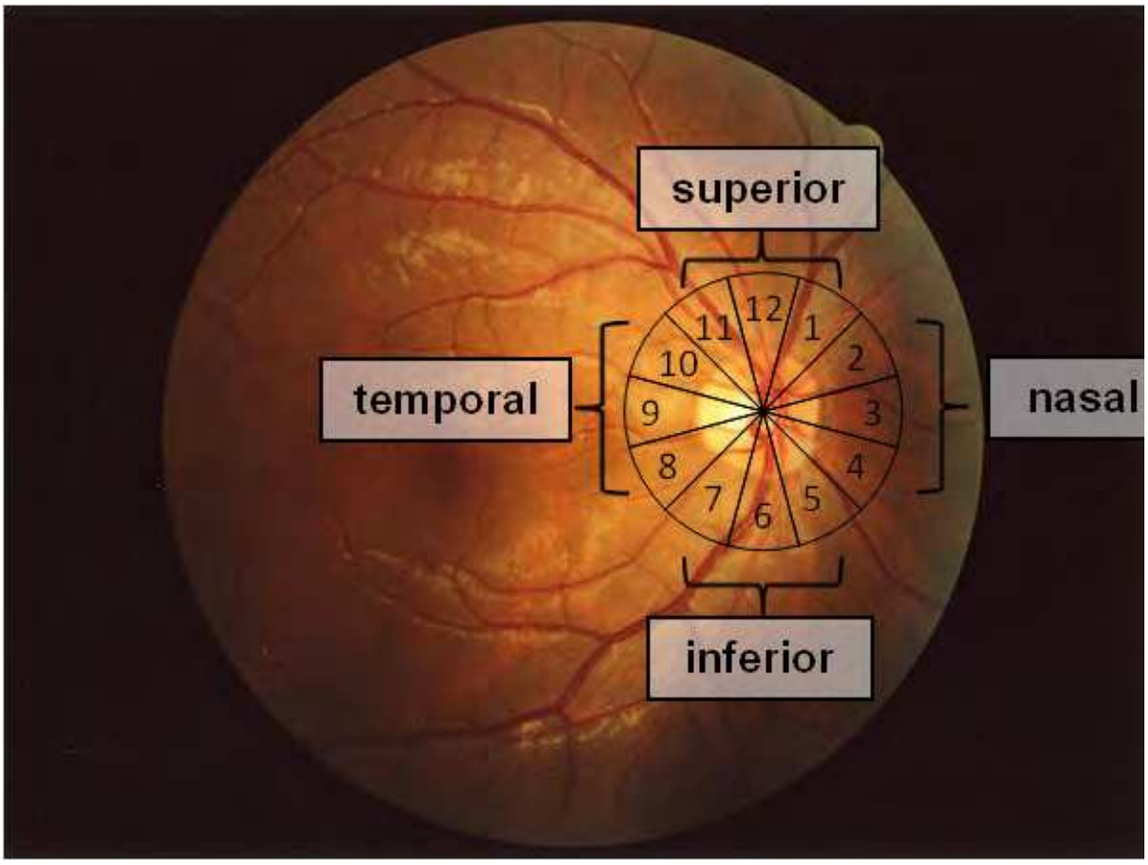


Fig. 1. Twelve 30-degree clock-hour sector of peripapillary RNFL (Right eye). Using OCT3, average RNFL thickness and each of the twelve 30-degree clock-hour sector averages were measured. Superior quadrant: 11, 12, 1 o'clock, Temporal quadrant: 8, 9, 10 o'clock, Inferior quadrant: 5, 6, 7 o'clock, Nasal quadrant: 2, 3, 4 o'clock.

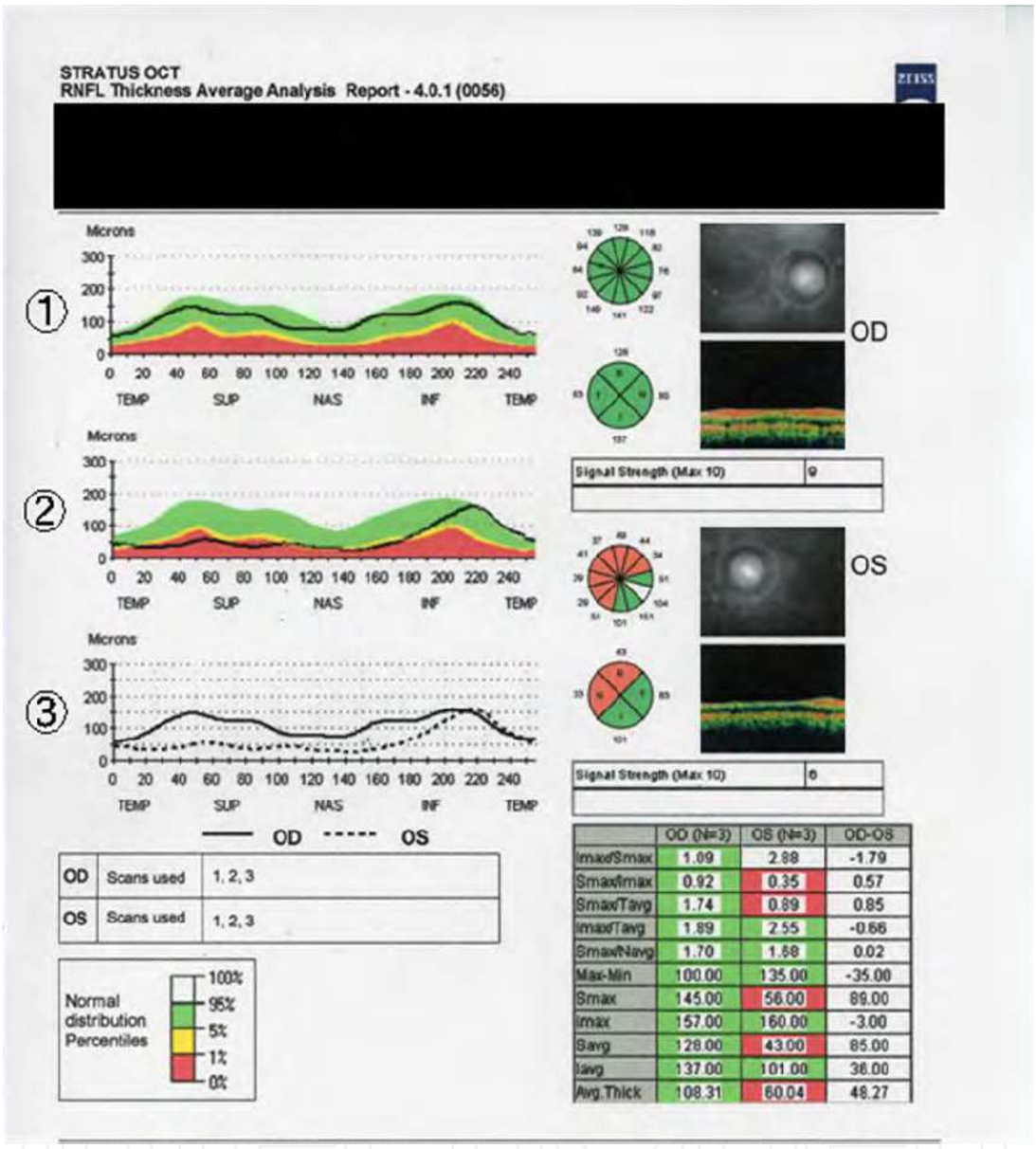


Fig. 2. Representative a sample OCT3 display data. RNFL was measured and analyzed using normative database of OCT3. (1) Right eye, (2) Left eye, (3) Comparison of both eyes (a solid line represented right eye, a dotted line represented left eye). X axis of these graphs indicated superior-temporal segment, superior segment, inferior segment, and inferior-temporal segment from the left. Y axis of these graphs indicated RNFL thickness (micrometer). Green, yellow and red colors indicated normal distribution percentiles of 5-95%, 1-5%, and 0-1%, respectively using normative database installed.

1.1.3 Statistical analysis

Statistical comparisons were performed using Mann-Whitney's U test. Mann-Whitney's U test was also known as Wilcoxon Rank sum test, and basically a non-parametric version of t

test. Multiple comparisons between groups were conducted using analysis of variance (ANOVA). ANOVA was a method to compare multiple linear models, a very common way to use an ANOVA test was to test the difference in means among more than two groups. So, the intuition was a t test which accommodated more than two groups to compare. Where any significant change was identified by ANOVA, post hoc analysis using Tukey, Scheffe, and Bonferroni multiple comparisons was utilized to confirm any significant differences among the groups (Field, 2005). $P<0.05$ was considered statistically significant.

1.2 Mean RNFL thickness in normal Japanese eyes

The 3rd generation of OCT3 installed a normative database in which Caucasians and Hispanics composed of 95 % of the samples, with the remaining 5 % comprised of other races. In our previous preliminarily studies, we evaluated RNFL thickness of patients with glaucoma using OCT3 and found that that of some of the patients were within the normal distribution percentiles of 5-95 %. We therefore suggested that the normal distribution percentiles of 5-95 % of RNFL thickness of normal Japanese population may differ from the normative database installed with the OCT3.

In the current study, we measured the optic nerve head appearance by OCT3 of 246 eyes (158 non-myopic and 88 myopic eyes) from 126 normal healthy Japanese volunteers whose ages ranged from 22 to 87 years. As shown in Figure 3 and Table 1, mean global RNFL thickness was 106.3 ± 12.0 μm and thus mean RNFL thickness in normal Japanese eyes was thicker than that in European eyes. Furthermore, 12 clock-hour thickness showed double apical shapes which were thicker within upper and lower segments and thinner within nasal and temporal segments.

	Clock-hour	Total n=246	Non-myopic n=158	Myopic n=88	OH n=45
superior	11 o'clock	140.0 ± 24.7	137.9 ± 24.1	143.8 ± 25.4	128.3 ± 25.7
	12 o'clock	128.3 ± 25.1	131.7 ± 25.3	122.0 ± 23.7	119.5 ± 32.2
	1 o'clock	109.1 ± 27.2	113.7 ± 27.8	100.9 ± 24.4	107.6 ± 31.5
nasal	2 o'clock	84.2 ± 26.3	88.4 ± 26.9	76.6 ± 23.7	80.0 ± 22.0
	3 o'clock	68.9 ± 16.1	71.9 ± 16.8	63.4 ± 13.3	60.1 ± 12.8
	4 o'clock	84.2 ± 23.1	90.4 ± 23.9	73.1 ± 16.7	69.6 ± 17.0
inferior	5 o'clock	117.3 ± 26.8	125.0 ± 25.2	103.5 ± 24.1	93.9 ± 19.6
	6 o'clock	142.4 ± 27.1	146.8 ± 25.8	134.5 ± 27.7	126.7 ± 27.0
	7 o'clock	131.8 ± 39.1	124.4 ± 40.4	145.0 ± 33.1	135.3 ± 23.5
temporal	8 o'clock	82.1 ± 25.5	74.6 ± 19.9	95.4 ± 28.8	80.5 ± 22.3
	9 o'clock	76.2 ± 20.3	73.0 ± 19.4	82.0 ± 20.5	61.8 ± 15.6
	10 o'clock	110.8 ± 28.4	107.9 ± 28.0	116.1 ± 28.4	92.2 ± 25.8

OH: Ocular hypertensive eyes

Table 1. RNFL thickness (mean ± SD μm) of normal eyes and ocular hypertensive eyes.

This observation suggested that some of the glaucoma patients could be excluded from the glaucoma screening of Japanese people by the OCT3 and that a revised normative database composed of Japanese for more precise glaucoma screening (Katai et al., 2006).

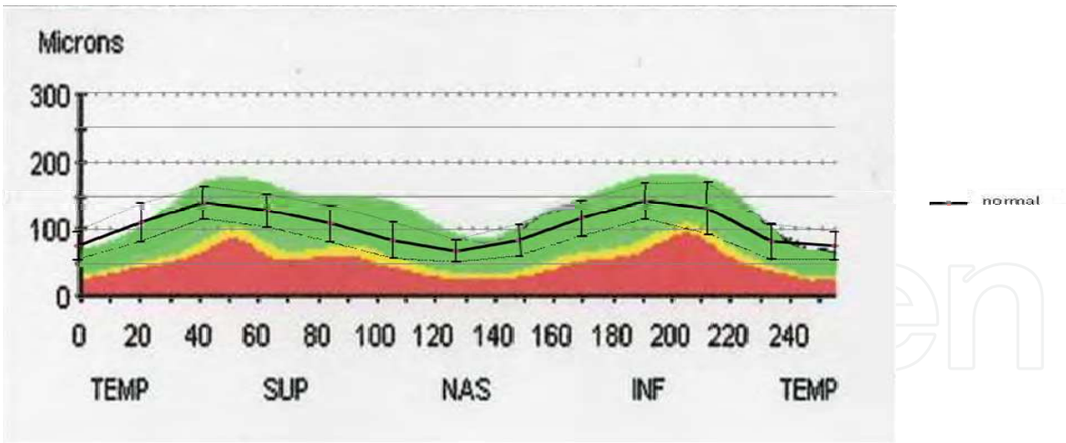


Fig. 3. Normal Japanese mean RNFL thickness and the RNFL normative database of OCT3. In most of the segments, (mean – SD) RNFL thickness of normal Japanese eyes was thicker than the RNFL normative database of OCT3, especially in temporal and nasal quadrants.

1.3 Effect of Aging on the mean RNFL thickness in normal non-myopic Japanese eyes

In the previous pilot studies, through an analysis of age-related change in the 4 quadrants of RNFL thickness, we observed a significant decrease in RNFL thickness within the temporal region in healthy Japanese eyes (Katai et al., 2004a). Therefore, it is of great interest to pursue a more detailed analysis of the effects of aging by 12 clock-hour thickness of RNFL.

To investigate the effects of aging on RNFL thickness in normal non-myopic Japanese eyes, its global and 12 clock-hour thickness was compared among the subjects with different ages, ranging from 40 years through 79 years. As shown in Figure 4-7, there was significant decrease in superior (11, 12 and 1 o'clock) and inferior-temporal (6, 7, 8 o'clock) quadrants with the advancement of age (ANOVA, $P < 0.05$).

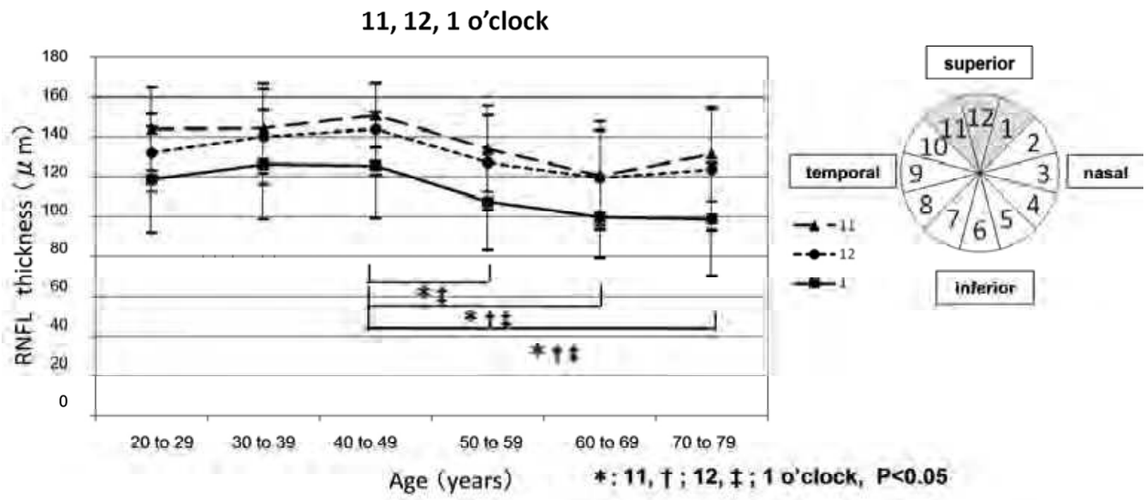


Fig. 4. RNFL thickness in the superior (11, 12, 1 o'clock) of normal non-myopic eyes stratified by age category.

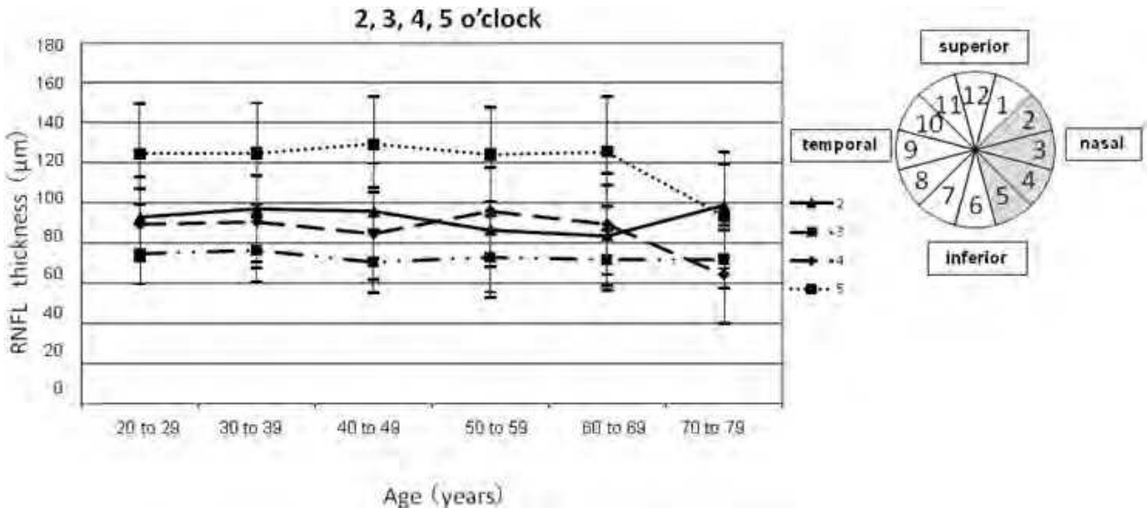


Fig. 5. RNFL thickness in the nasal (2, 3, 4, 5 o'clock) of normal non-myopic eyes stratified by age category.

In terms of effects of aging on the RNFL thickness, we found significant decreases within the superior (11, 12, 1 o'clock) (Figure 4) and inferior-temporal segments (6, 7, 8 o'clock) (Figure 6), which are known to be the most susceptible to glaucomatous optic nerve damage, in older subjects (JGS, 2006). In contrast, RNFL thickness in the other segments, nasal (2, 3, 4, 5 o'clock) (Figure 5), and temporal (9, 10 o'clock) (Figure 7) were not affected by aging.

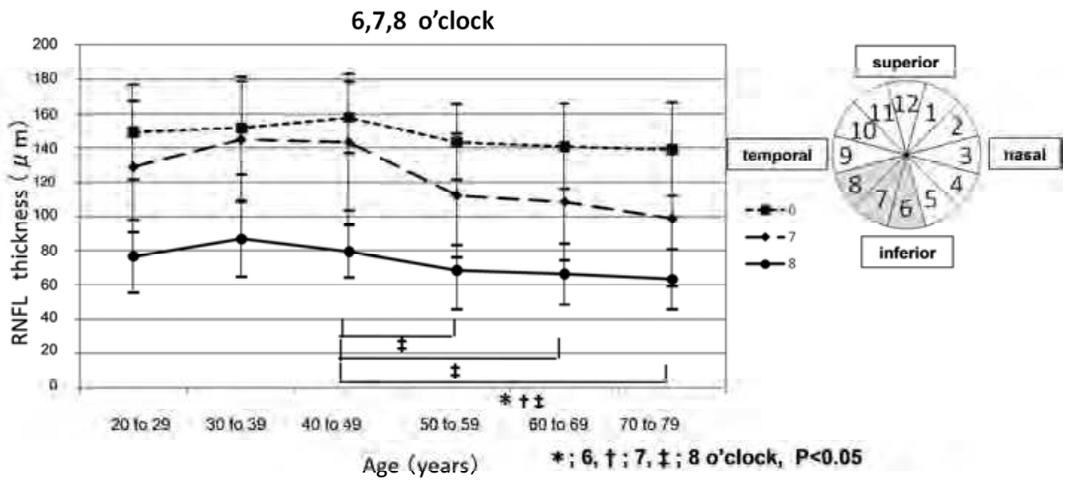


Fig. 6. RNFL thickness in the inferior-temporal (6, 7, 8 o'clock) of normal non-myopic eyes stratified by age category.

As mentioned in several previous glaucoma surveys, such as the Tajimi study (Table 2), the prevalence of glaucoma increases as people age (Suzuki et al.,2008). Thus, our present observation indicates that an age dependent natural decrease in RNFL thickness of normal Japanese may correlate with the age dependent increase in the prevalence of glaucoma.

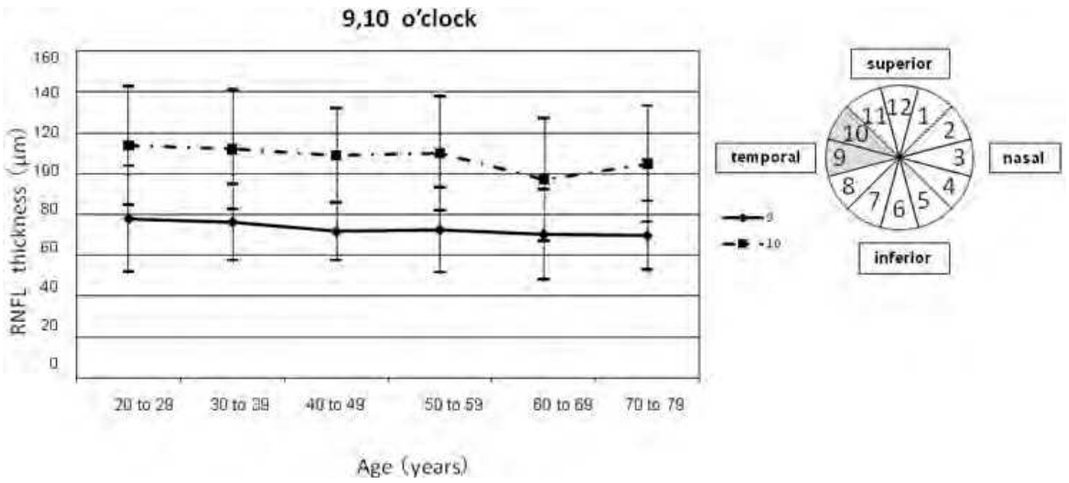


Fig. 7. RNFL thickness in the temporal (9, 10 o'clock) of normal non-myopic eyes stratified by age category.

Study	race	Glaucoma prevalence (%)	NTG prevalence (%)	Glaucoma prevalence of elderly more than 70 years (%)
Tajimi study[1)]	Japanese	5.0	3.6	10.8
Zulus[2)]	Black people	4.5	1.54	11.9
Ponza[3)]	Italian	3.8	—	—
Andhra Pradesh[4)5)]	Indian	2.8	0.7	9.5
Northern Mongolia[6)]	Mongolian	2.2	0.3	—
Proyecto VER[7)]	Latin American	2.1	1.58	—
Melbourne[8)]	Australian	2.0	—	—
Wroclaw[9)]	Caucasian	1.6	—	—
Northwest Alaska[10)]	Alaskan	0.65	—	11.7

NTG: Normal tension glaucoma —: not described

Table 2. Prevalence of glaucoma 1) Suzuki et al., 2008, 2) Rotchford & Johnson, 2002, 3) Cedrone et al., 1997, 4) Dandona et al., 2000a, 5) Dandona et al., 2000b, 6) Foster et al., 1996, 7) Quigley et al., 2001, 8) Wensor et al., 1998, 9) Nizankowska & Kaczmarek, 2005, 10) Arkell et al., 1987.

1.4 Effect of myopia on the mean RNFL thickness in normal Japanese eyes

Myopia has been reported as being a risk factor for glaucoma, and myopic fundus changes may complicate glaucomatous optic disk changes (Mitchell et al., 1999). Thus, this factor should be taken into account when evaluating RNFL changes of glaucoma in myopic eyes. Nevertheless, the normal population database for RFNL measurements, which was developed by the manufacturer and packaged within OCT3 software, did not include individuals with moderate or high degrees of myopia. Therefore, in the present study to investigate the effects of myopia on RNFL thickness in normal Japanese eyes, its global and 12 clock-hour thickness was compared in myopic and non-myopic eyes. Mean global mean RNFL thickness of non-myopic eyes (111.6 ± 10.1 μm) was significantly greater than that of myopic eyes (104.9 ± 10.6 μm) ($P < 0.01$). In the 2 clock-hour thickness analysis, mean RNLF thickness of the segment from 12 o'clock through 6 o'clock of myopic eyes was significantly greater than that in non-myopic eyes, and conversely that of the other segment from 7 o'clock through 11 o'clock was smaller in myopic eyes than in non-myopic eyes ($P < 0.01$) (Figure 8). Both average RNFL thickness and RNFL thickness of nasal half-quadrants in myopic eyes were thinner than those in non-myopic eyes ($P < 0.01$). Conversely, but with the exception of the 7, 10 and 11 o'clock positions, RNFL thickness of temporal half-quadrants in whole segments of myopic eyes was thicker than that in non-myopic eyes ($P < 0.01$).

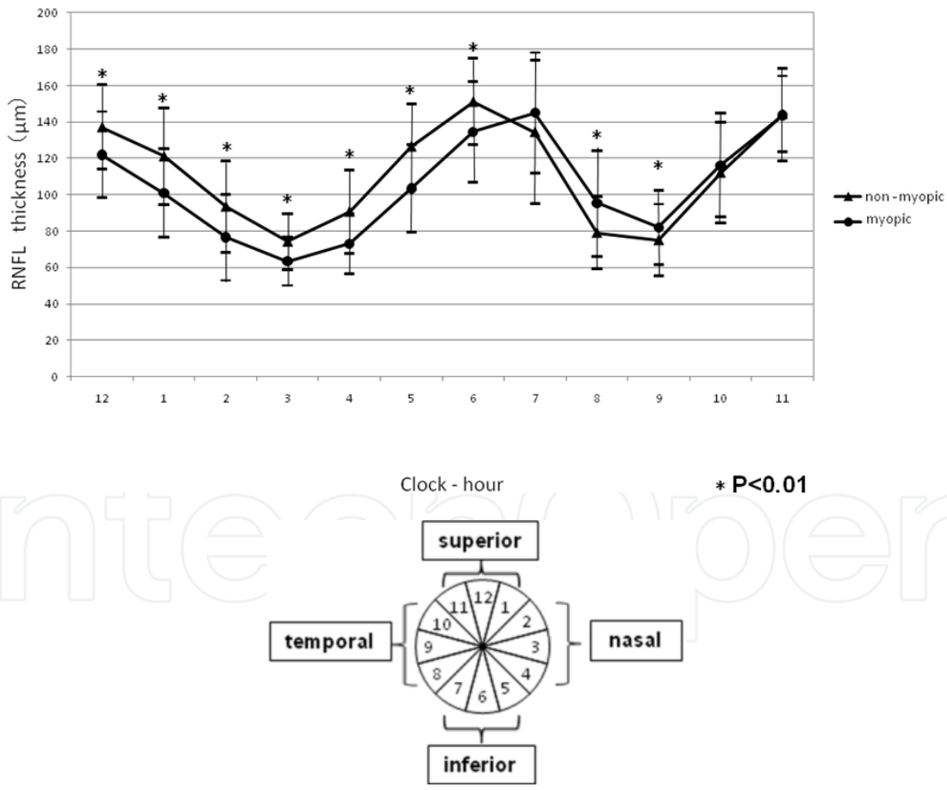


Fig. 8. RNFL thickness of myopic and non-myopic eyes. Mean RNLF thickness of the segment from 12 o'clock through 6 o'clock of myopic eyes was significantly greater than that in non-myopic eyes, and conversely that of the other segment from 7 o'clock through 11 o'clock was smaller in myopic eyes than in non-myopic eyes (Mann-Whitney's U test, $P < 0.01$).

Several previous studies have suggested that no significant correlation is associated between myopia and RNFL thickness (Bowd et al., 2002). However, these studies may have been limited by the poorer resolution of earlier generation OCT and confocal laser devices which had lower sensitivity. Using 3rd generation OCT3, it has been reported that RNFL thickness increased with increasing hyperopia (1.7 microns per diopter) in children (91 % Hispanic, mean age 10 years, range 4-17) (Salchow et al., 2006). Alternatively, significant decrease in RNFL thickness with increasing axial length in myopia has been reported. However, its decreasing ratios varied (2-7 microns per mm) in reports; as such, its changes of RNFL thickness may depend exclusively on different races and age distributions (Hoh et al., 2006).

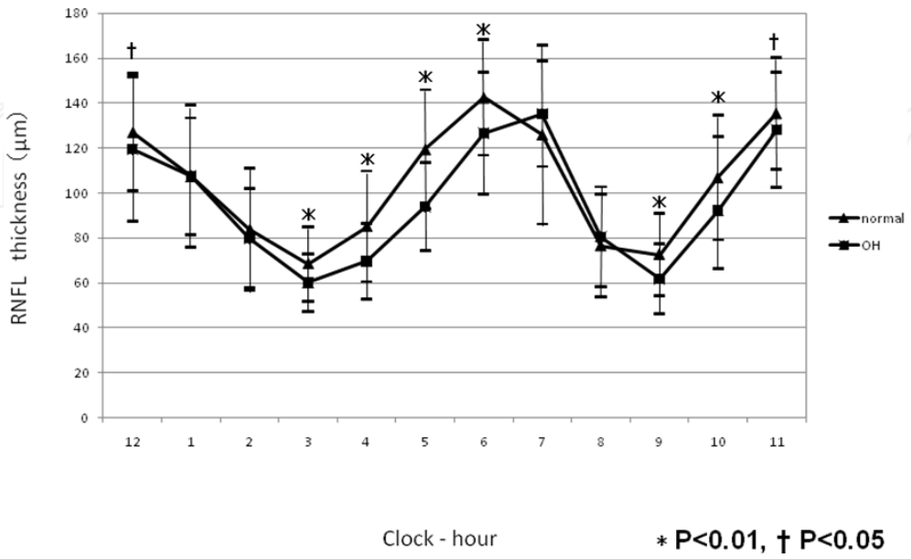
We demonstrated that RNFL thickness in myopic eyes appeared thicker in the temporal segments and thinner in the nasal segments in comparison to that of non-myopic eyes. It is generally known that tilted disc is more prevalent in myopic eyes, and thus this distortion of the optic nerve fibers at the myopic disc may cause a mechanical stress toward the optic nerve fibers, eventually leading to the glaucomatous optic neuropathy (Nakazawa et al., 2008). Therefore, our present data may support this mechanical theory in myopic eyes.

1.5 Comparison of mean RNFL thickness between ocular hypertensive and normal eyes in Japanese subjects

Bowd et al. has reported that global RNFL thickness of ocular hypertensive eyes is significantly thinner than it is in normal healthy eyes, especially within its inferior and nasal segments (Bowd et al., 2000). Similarly, Kamal et al. has reported significant thinning of the superior and inferior optic disc rims of ocular hypertensive eyes (Kamal et al., 2000).

To explore the effects of elevated IOPs on RNFL thickness, mean RNFL thickness was compared between ocular hypertensive and normal eyes in Japanese subjects. Global mean RNFL thickness of ocular hypertensive eyes (96.3 ± 14.1 μm) was significantly smaller than that of normal eyes (104.3 ± 12.0 μm) ($P < 0.01$), and average RNFL thickness, inferior-nasal segments, and superior-temporal segments were significantly thinner in ocular hypertensive eyes than in normal eyes ($P < 0.05$) (Figure 9).

RNFL thickness is well known to be different within the part of the ONH in a normal disc's so called "ISN'T rule". Specifically, the inferior is the thickest, followed by the superior, nasal and temporal. Since the RNFL is thickest in the inferior and superior portions of the optic nerve head, the relative blood perfusion within these portions is lower among the optic nerve head, and thus, these portions are known to be the most susceptible to glaucomatous optic nerve damage (Harris et al., 2003). It has been shown that the upper and lower visual field defects are more commonly involved in open angle glaucoma (Katai et al., 2004b). If blood perfusion is the lowest in these portions of optic nerve head as has been suggested, elevated intraocular pressure may suppress ocular blood circulation, thereby causing glaucomatous changes within the inferior and superior portions of the optic nerve head.



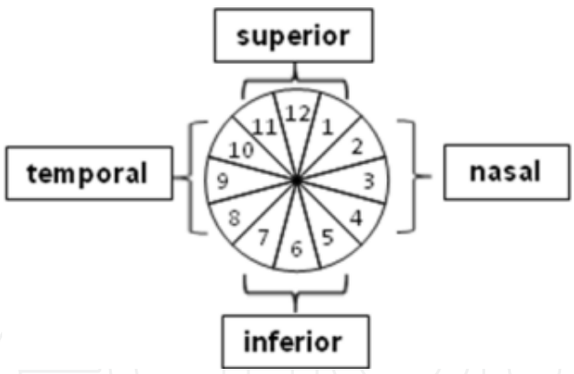


Fig. 9. RNFL thickness of normal eyes and ocular hypertensive eyes. Mean RNFL thickness, inferior-nasal segments, and superior-temporal segments were significantly thinner in ocular hypertensive eyes than in normal eyes (Mann-Whitney's U test, $P < 0.05$)

2. Conclusions

The present study demonstrates that RNFL thickness of normal Japanese eye is thicker than that of normal European eyes, and is significantly affected by aging, refraction and IOP levels. As a result, specific control data of normal Japanese eyes should be utilized in order to detect glaucoma in its early stage.

3. Acknowledgement

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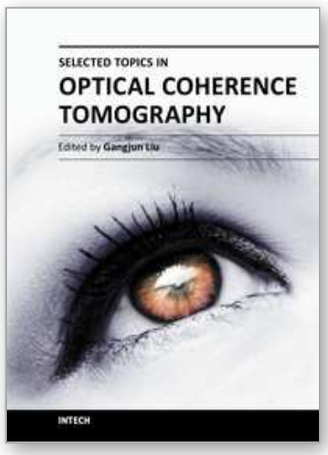
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