

Title: Difference in retinal nerve fiber layer thickness between normal and glaucomatous eyes

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Abstract

Background: Screening of the retinal nerve fiber layer (RNFL) is valuable in the early stages of glaucoma, because RNFL changes may precede functional loss.

Aim to study: The purpose of this study was to assess the RNFL thickness in normal and glaucomatous eyes. Difference in the RNFL thickness was also determined between the groups.

Methods: Sixty healthy subjects and 30 subjects with glaucoma participated in this cross-sectional study. Healthy volunteers free of glaucoma were divided into 2 groups, younger (age \leq 35 years) and older (age $>$ 35 years) participants. All subjects underwent complete ophthalmic evaluation, including visual acuity, refraction, slit-lamp, intraocular pressure (IOP) measurement, and RNFL thickness measurement by Optical Coherence Tomography (OCT). Unpaired t-test was performed to compare RNFL thickness between the eyes of younger and older participants. One way analysis (ANOVA) test was employed to compare RNFL thickness among the 3 groups (normal young, normal old & glaucomatous eyes).

Results: Mean values of RNFL thickness in the younger, older and glaucoma populations were $109.3 \pm 15 \mu\text{m}$ (95% CI 103.7-114.06 μm), $106.8 \pm 14 \mu\text{m}$ (95% CI 101.49-112.02) and $92.8 \pm 29 \mu\text{m}$ (95% CI 82.11-103.47), respectively. There was a significant difference among the 3 groups ($p=0.0045$). The RNFL was thinner in older participants than younger participants ($p=0.0067$).

Conclusions: Screening of the RNFL is valuable in the early stages of glaucoma.

Key Words: Glaucoma, Retinal nerve fiber layer thickness.

Introduction

In glaucomatous eyes, major structural changes take place before visual functional loss is detected. The situation may nowadays be reversed, with functional loss detected before structural loss can be observed (Schacknow PN , 2010). Progressive optic neuropathy presents with loss of retinal ganglion cells, resulting in visual field abnormalities. Loss of vision results if it is not detected and managed in the early stages. It has been demonstrated that structural damage to the optic nerve head and retinal nerve fiber layer (RNFL) can occur well before there is any detectable functional visual loss. It is for this reason that objective and quantitative assessment of the optic nerve is of great importance in terms of early diagnosis and monitoring of glaucoma progression. Methods of measuring RNFL thickness are under active development to serve these purposes (Javier et al. 2009).

Screening of the RNFL is valuable in the early stages of glaucoma, because RNFL changes may precede visible atrophy of the optic disc and/or functional loss (Akiyasu K, 2006).

However, a clinically detectable change in the optic cup likely represents loss of thousands of axons. Furthermore, testing of visual function, at least with conventional techniques, is a relatively insensitive measure of glaucoma damage, because studies have shown that 25% to 35% of retinal ganglion cells may be lost before an abnormality appears on standard achromatic perimetry (Leung CK et al. 2004).

Although visual field testing has a high specificity for detection of glaucoma, the loss of optic nerve fibers usually precedes the development of reproducible glaucomatous visual field defects. Therefore, detection of early glaucoma using ocular imaging technology has gained much clinical interest (Karen G et al. 2007).

Methods

This was a prospective, cross-sectional study carried out according to the Helsinki Declaration. Informed consent was obtained from each participant, after a verbal description of the procedures involved in the study, possible side effects and the rights of the subjects during and after the study were explained. This study included normal volunteers who were staff and patients with glaucoma at the Ophthalmology & Optometry Clinic, King Fahad Medical City Hospital, Riyadh, Saudi Arabia.

All subjects underwent complete ophthalmic evaluation, including refraction, slit-lamp biomicroscopy, intraocular pressure (IOP) measurement, gonioscopy, dilated optic disc fundus evaluation (direct & indirect ophthalmoscope) and RNFL thickness measurement.

Participants

Inclusion criteria for participants with glaucoma were: > 35 years of age for any type of glaucoma, best visual acuity $\geq 20/40$, refractive error $< \pm 5.00$ D sphere and < -3.00 D cylinder, any detectable visual field defect appearing with automated perimeter, IOP ≥ 22 mm Hg in either eye, or the IOP controlled with medication into the normal range, or the patient has been diagnosed with normal tension glaucoma.

Inclusion criteria for normal subjects were: younger ≤ 35 years, older < 35 years, match controlled to the glaucoma group with IOP < 22 mm Hg, best visual acuity $\geq 20/40$, refractive error $\leq \pm 3.00$ D sphere & < -2.00 D cylinder. Negative history for diabetic retinopathy, macular edema, retinal disease or optic nerve abnormality in either eye were required. Patients with contraindications to dilation or intolerance to topical anesthetics or mydriatics were excluded (Budenz DL et al. 2007 & Budenz DL, 2008)

Our study included a total of 90 subjects with both eyes intact (90 right eyes, 90 left eyes). They were categorized equally into 3 groups (healthy younger, healthy older & participants with glaucoma). Among the thirty patients, 2 patients had had lasik surgery, 4 had pseudophakic glaucoma (intraocular lens) and 3 patients had cataract formation starting.

The procedure was done for all the subject included in this study. It was begun by auto refraction for both eyes by taking three readings; the average was calculated to determine the best visual acuity. After that, slit lamp biomicroscopy was done to examine the entire anterior segment.

Anesthetic eye drops were instilled in both eyes (0.4% Oxybuprocaine Hydrochloride). After 15 seconds, the Portable Tono-Pen (Medtronic/Solan, Jacksonville, Fla, USA) was applied and three readings taken at the same time. The Goldman applanation tonometer (manufactured by Zeiss) was started using 2% fluorescein sodium dye; an average of three readings were taken at the same time of the day to avoid diurnal fluctuation of IOP. All measurements were taken in the morning from 9:00 am to 12:00 pm. To avoid bias in the IOP measurement, we randomly selected which eye to measure first. Patients who had his/her file number ending with an odd number started with his/her right eye first, while those who had a file number ending with an even number had the left eye measured first.

We performed the Gonioscopy by Volk 4 Mirror Gonio Lens. The cornea was anesthetized before we applied the IOP measurements. After that the special lens was inserted, and viewed.

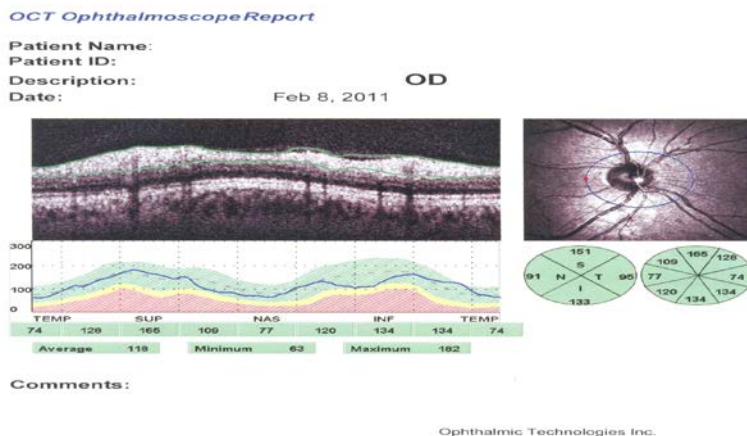
The ocular fundus was examined by direct ophthalmoscope after dilating the pupil by applying 3 drops of mydriatic agent (topical instillation of 2.5% phenylephrine and 1.0% tropicamide) and waiting for 30 minutes to let the pupil dilate.

Finally, Speed Domain-OCT (OTI Spectral OCT/SLO, Ophthalmic Technologies Inc) was obtained after dilation of the pupil by mydriatic agent as described above. During the scanning mode, the Spectral OCT/SLO produced and displayed the cross-sectional OCT image and confocal fundus image through the same optics at the same time. These images were displayed simultaneously on the computer screen (Figure1).

The RNFL thickness was measured with RNFL scanning mode. All the subjects from the three groups were asked to look at the internal fixation target, and the circular scan with a diameter of 3.4 mm was centered around the optic disc while the location was observed on the scanning laser ophthalmoscope image to ensure proper positioning of the scan in relation to the optic nerve head. The system captured three sequential OCT images.

The RNFL analysis used an automated OCT software algorithm to identify anterior and posterior margins of RNFL and average the measurements around the circular scan to obtain the average RNFL thickness globally for four quadrants (superior, nasal, inferior, and temporal). To be acceptable for inclusion, scans must have been properly centered on the optic disc as shown in Figure 1.

Figure 1: RNFL Analysis for Normal



Data Analysis

The Graphpad Instat software (version 3.05) was used to perform all analysis and determine the distribution of numerical data. Unpaired *t* test was performed to compare RNFL thickness between normal eyes of younger and older subjects.

We compared the characteristics displayed by the OCT printout in each group, including the overall average thickness of the RNFL and the thickness of each of the 4 sectors (Inferior, Superior, Nasal and Temporal). One way analysis (ANOVA) test was employed to compare RNFL thickness among the 3 groups (normal young, normal old & glaucoma).

Independent samples *t*-test was used to compare the differences in the RNFL thickness between male and female participants and between the RNFL thickness of the right and left eyes.

Pearson correlation analysis was performed with mean RNFL thickness as the dependent variable and age as independent variable. In all tests a *p* value less than 0.05 was considered statistically significant.

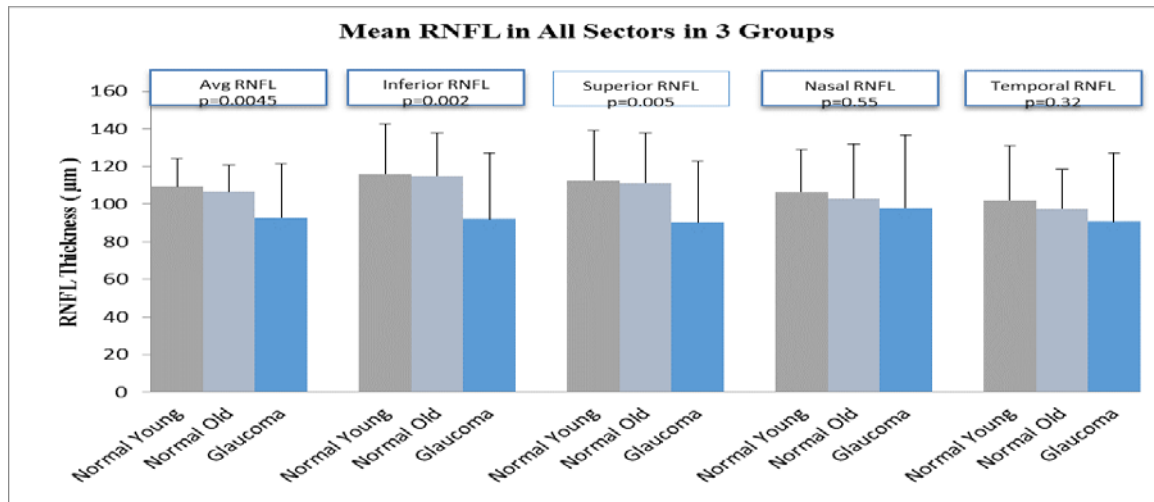
Results

Table 1: Demographic data of all subjects

	Normal		Glaucoma
	Younger	Older	
Subjects, N	30	30	30
Age (years) mean \pm SD	27.4 \pm 3.9	47.9 \pm 8.9	55 \pm 9
Spherical equivalent (Diopter) mean \pm SD	-0.78 \pm 0.97	0.021 \pm 1.35	-0.079 \pm 1.74
Mean IOP (mm Hg) by Goldmann Applanation tonometry mean \pm SD	12.91 \pm 1.54	13.02 \pm 1.69	16.28 \pm 3.93
Mean IOP (mm Hg) by Portable Tono-Pen mean \pm SD	17.24 \pm 1.03	17.63 \pm 1.33	18.48 \pm 3.93
C/D ratio, (subjectively determined it by Direct Ophthalmoscope) mean \pm SD	0.28 \pm 0.07	0.28 \pm 0.08	0.55 \pm 0.16

Demographic data of all participants are presented in Table 1. There was a significant difference in average RNFL among these three groups according ($p=0.0045$) as shown in Figure 2 except for the nasal & temporal sectors.

Figure 2: Mean RNFL Thickness (μm) in All Sectors for All Three Groups.



No significant difference in mean RNFL thickness with age was found (Pearson correlation coefficient $r = -0.089$, $p = 0.494$).

RNFL thickness & gender

We found no significant difference between the RNFL thickness of male and female participants in the three groups. In the normal young group, normal old group and glaucoma group, the p values were $p=0.79$, $p=0.83$ & $p=0.65$, respectively.

RNFL Thickness between the right and left eyes

We found no significant difference in the thickness of RNFL between the right and left eyes in all thirty subjects among normal young, normal old & glaucoma groups. The p values were, $p=0.61$, $p=0.15$ and $p=0.09$, respectively.

Discussion

To our knowledge, this was the first study that compared the RNFL thickness in normal young, normal old & glaucoma populations by Spectral-Domain-OCT (OTI Spectral Optical coherence tomography OCT versus Scanning Laser Ophthalmoscopy SLO).

Because there have been no longitudinal studies of RNFL thickness measured with the OCT in normal individuals, we made the assumption that a cross-sectional analysis was a good surrogate for change over time in an individual. Cross-sectional studies of RNFL thickness using Spectral-Domain-OCT have found a decrease in RNFL thickness with age (Bendschneider D et al 2010 & Budenz DL 2008). The study demonstrates that the mean RNFL is thinner in normal old than in normal young eyes. This was the same as was found in studies by Budenz DL et al (2007), Bendschneider D et al (2010) and Toshiyuki et al (2007) which reported OCT measures of the RNFL as thinner in older persons, with a decline of approximately 2 μm per decade.

We found that the RNFL thickness decreased significantly with increasing age when we compared RNFL thickness between normal young & normal old ($p=0.0067$), and ($p=0.0045$) with glaucomatous eye. This was not similar to Bendschneider D et al (2010), those found a significant decrease in RNFL thickness with age. (Pearson correlation coefficient $r = -0.214$, $p = 0.005$), Due to small sample size in these 2 groups.

The human RNFL loses approximately 5000 axons per year from birth to death, approximately 2500 per year before age 50 and 7500 per year after 50.3 years. It was not surprising, then, that RNFL thickness decreases with age. In our study we found that RNFL was thickest in the inferior quadrant followed by superior, nasal and temporal quadrants, respectively, in both normal young and normal old. This called ISNT rule. (Thicker in Inferior, then, Superior,

Nasal& Temporal). Between the groups, RNFL thickness in all 4 sectors differed at the $p<0.05$ level, which corresponded to a double hump pattern of RNFL thickness. This was the same finding in the two studies of Tarannum et al (2010) and Bendschneider D et al (2010). The temporal quadrant RNFL thickness was thinner than the nasal quadrant ($p<0.05$) except in the glaucoma group, where the nasal sector was the thickest.

We found that the mean peripapillary RNFL thickness and superior and inferior sector thickness were significantly ($p< 0.05$) thinner in glaucomatous eyes than in normal eyes. This also was similar to what Tarannum et al (2010) reported except for the nasal and temporal sectors ($p> 0.05$ for both sectors). This also was the same as reported by Anton et al (2007) where the mean RNFL thickness and superior and inferior RNFL thickness were significantly thinner in glaucomatous eyes than in eyes with ocular hypertension or normal eyes.

Several studies using OCT have identified the average and inferior average RNFL thickness as the best discriminators between normal and glaucomatous eyes. Tarannum et al (2010) and Toshiyuki et al (2007) found inferior quadrant RNFL thickness to be the best parameter to discriminate between normal and glaucomatous eyes. The finding that initial RNFL thinning occurs at the inferior quadrant in normal eyes was of interest because visual field defects associated with glaucoma usually occur initially in the superior visual field corresponding to a defect in the inferior pole of the optic disc. In agreement with Daniel et al (2010), Budenz DL et al (2007) &Toshiyuki O et al. (2007), eye type (right\left) and gender do not need to be considered when determining what constitutes normal RNFL measurements.

Conclusion

We found that the RNFL decreased with increasing age, and there were no significant differences in RNFL thickness between male and female participants or between the right and left eyes.

Conflict of interest: None declared

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