

Retinal Nerve Fiber Layer Thickness Analysis using Spectral Domain OCT in Glaucomatous Eyes in Central India

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Abstract: Aim: To study the retinal nerve fiber layer thickness(RNFL) using spectral domain optical coherence tomography (SD-OCT) in normal and glaucomatous eyes. Design: cross sectional study. Methods: 50 eyes from 50 normal controls, 44 eyes of 26 ocular hypertension (OHT) and 46 eyes of 27 patients with primary open angle glaucoma (POAG) were included. Age between 40 to 80 years was selected with BCVA 6/12 or more with normal Visual Field. We studied average RNFL thickness, RNFL in each four quadrant, frequency distribution of BCVA and IOP with RNFL defect. Results: Average RNFL was 121.66 ± 12.16 , 106.89 ± 6.91 and 90.41 ± 11.14 μm in normal, OHT and POAG eyes respectively. Quadrant wise RNFL thickness in normal controls was greatest in inferior quadrant(141.96 ± 14.59), and gradually lesser in superior (138.04 ± 13.06), nasal (104.60 ± 12.00) and temporal quadrants (100.92 ± 11.34) μm . In OHT patients, RNFL were greatest in superior (125.89 ± 7.13) and thinned in the inferior (119.16 ± 8.87), nasal(92.98 ± 7.09) and temporal quadrants(88.98 ± 7.03). Similarly in POAG group, RNFL was greatest in the superior quadrant(119.09 ± 11.20 μm) and followed by inferior(107.22 ± 12.74 μm), then nasal(73 ± 14.07 μm) and then temporal quadrant(61.98 ± 11.20 μm). 75% RNFL defect was seen in inferior quadrant in OHT group and (19.23% and 32.31%) defect was in inferior quadrant and temporal quadrant in POAG group. The mean (SD) IOP was 13.88 (1.98), 28.59 (4.11) and 29.91(6.10) in group I, group II and group III respectively. Conclusion: SD-OCT has the ability to detect early glaucomatous change in the form of thinning of RNFL especially in inferior and temporal quadrants even in patients with BCVA of 6/6 and when the IOP was not very high.

Keywords: Quadrant wise Retinal Nerve Fiber layer Thickness, Glaucoma, Optical Coherence Tomography.

1. Introduction

Glaucoma is one of the major causes of blindness worldwide [1]. The mean prevalence for combined open angle glaucoma and angle closure glaucoma worldwide in 2020 will be 2.86%. [2] By 2020, India will become second overall in number with glaucoma, surpassing Europe. [2]

Glaucoma causes loss of retinal ganglion cells resulting in characteristic of the retinal nerve fiber layer (RNFL) loss, optic nerve cupping and visual field defects. These structural changes precede VF defects as measured by standard automated perimetry. The peripapillary Retinal Nerve Fiber Layer (RNFL) thickness evaluation is a useful method to detect the early structural damage of glaucoma. Optical Coherence Tomography (OCT) provides an objective and quantitative measurement of RNFL thickness by measuring echo time delay and intensity of backscattered light from different retinal layers using a low coherence interferometry. [3]

The OCT was first reported by Huang et al. in 1991 [4] and since then, this device has been evolving rapidly. The most recent technology, spectral domain or Fourier domain OCT uses a spectrometer as a detector of OCT signal [5]. Spectral domain OCT (SD-OCT) has benefits over the time domain OCT (TD-OCT) such as higher axial resolution (3 to 6 μm), up to 200 times faster scanning speed and better reproducibility [6]. The higher resolution can provide image subtle abnormalities or progression currently not visible with TD-OCT and would potentially allow improved segmentation and greater accuracy in measurements of retinal layers. [3] In this study, we used the

spectral domain OCT (SD-OCT) which is the most advanced OCT method available commercially for clinical use.

The present study was undertaken to evaluate the RNFL thickness measured by spectral domain optical coherence tomography (SD-OCT) in normal eyes and in glaucomatous eyes.

2. Material and Methods

This study was a cross sectional, non-interventional observation study conducted over a period of 24 months in a tertiary eye care hospital. All patients attending the ophthalmology outpatient department (OPD) of the tertiary eye care hospital who met the inclusion criteria were counseled regarding the disease and the study and those willingly consenting to participate in the study were selected. Informed and written consent was obtained from all patients with consent form approved by the Institutional ethical committee.

A total of 113 subjects were consecutively recruited for the study. Ten subjects were excluded because of the poor signal strength in the eye. Thus, a total of 140 eyes of 103 subjects were included in the analysis and were classified into three groups.

All patients included in our study were between 40 and 80 years old and were not under any treatment. If both eyes from the same patient were eligible for the study, both eyes were included in the analysis.

Group I consisted of 50 eyes of 50 normal controls.
Group II consisted of 44 eyes of 26 patients of ocular hypertension (OHT).
Group III consisted of 46 eyes of 27 patients with primary open angle glaucoma (POAG).

Inclusion criteria: Patients attending OPD, diagnosed as glaucoma suspects, controls were selected from healthy volunteers, age between 40 and 80 years along with written informed consent, Visual acuity 6/12 or better with spherical refraction up to 5 dioptres and cylinder refraction up to 3.0 D.

Exclusion criteria: Diagnosed case of Primary and secondary glaucoma, associated anterior and posterior segment pathology, Best corrected visual acuity (BCVA) worse than 6/12, inability to view the optic nerve head due to media opacity, SD-OCT scans with poor signal strength.

All subjects underwent a thorough ophthalmic examination including visual acuity, refraction, slit-lamp examination, fundus examination by direct and indirect ophthalmoscopy, Intraocular pressure (IOP) measurement by Applanation tonometry, Gonioscopy, stereoscopic photographs of the optic disc, and visual field testing prior to OCT image acquisition.

Visual Field Diagnosis Criteria

Classification as a glaucomatous or healthy subject was based on visual field results, without structural information. Humphrey 24-2 Swedish Interactive Thresholding Algorithm visual field testing was used. The diagnosis was glaucoma if visual field mean deviation or pattern standard deviation was below 5% cutoffs, or glaucoma hemifield test was outside normal limits reproducibly in at least 2 reliable visual field tests. Visual fields were considered reliable if fixation losses, false negatives, and false positives were less than 30%, and if defects were present in a consistent location between tests. Subjects were declared healthy if visual field mean deviation, pattern standard deviation, and glaucoma hemifield test were all within normal limits for at least 2 reliable visual field tests. If glaucoma hemifield test was borderline, mean and pattern standard deviation were <10% but > 5%, or defects were inconsistent between visual fields, the subject was categorized as "glaucoma suspect" and was removed from the data set.

All included subjects were scanned with the spectral domain OCT by a single operator. It was excluded that an image with a minimum signal strength 6/10 and below. One of the 3 scans, obtained the same day, with maximum signal strength was included. For this study, we analyzed the global average RNFL thickness, average RNFL thickness in the superior, inferior, nasal and temporal quadrants.

Sample size in our study was calculated from OpenEpi version 3.03(2014) software by Sample Size for Comparing Two Means with 95 % confidence interval and 90% power. The minimum sample size required was $n=37$ in each group^[7]. However, with this sample size the percentile could easily have been influenced by outlier, so the minimum sample size was increased.^[8]

The data were imported into OpenEpi version 3.03(2014) software; all analyses were performed with this software. Demographic and ocular characteristics of the study group were summarized with means and standard deviations (SDs) for interval level variables and with percentages for categorical variables. Normal ranges for differences were established as within 95% confidence interval; the Gaussian approximations of the means ± 1.96 SDs were also calculated. A χ^2 test was used for the analysis of categorical variables. Comparison with two groups was done by t test for continuous variable and chi-square test for categorical variable. Comparison between three groups was done by ANOVA test. Z-test was applied for properties within the group comparison. A p value of less than 0.05 was considered statistically significant.

3. Observations and Results

A total of 113 subjects were selected for the study. Ten subjects were excluded because of the poor signal strength in the eye. Thus, 103 subjects qualified for the study.

All patients included in our study were between 40 and 80 years old. The mean (SD) age was 55.46 (8.73), 57.86 (8.23), 58.17 (8.49) years in group I, group II and group III respectively. Of these 103 patients, 52 were males while 51 were females. In all groups males were slightly more in number than the females except in group III where females were more than males, though the difference was not statistically significant ($p>0.05$).

Of these 140 eyes evaluated, 69 were right eyes (OD) while remaining 71 were left eyes (OS). There was no statistical difference in eye chosen for examination in the three groups ($p>0.05$).

All normal controls had best corrected visual acuity (BCVA) of 6/6. Amongst ocular hypertensive 72.73 % eyes had a BCVA of 6/6 while remaining 27.27% had a BCVA of 6/9. Amongst glaucomatous eyes, majority had a BCVA of 6/6 (50%) followed by 6/9 and 6/12. There was statistically significant difference in the BCVA of all three groups. ($p<0.05$)

The mean (SD) IOP was 13.88 (1.98), 28.59 (4.11), and 29.91(6.10) in group I, group II and group III respectively. A mean vertical C: D ratio was 0.28, 0.30 and 0.69 in normal eyes, ocular hypertensive eyes and POAG eyes respectively. There was no statistically significant difference in the C:D between normal or ocular hypertensive eyes. ($p=0.107$)

The global average RNFL thickness, average RNFL thickness in four quadrants measured by OCT were compared in all groups. Table 1 summarized RNFL thickness values in all parameters measured by OCT.

Analysis of RNFL thickness in the four quadrants in the POAG, ocular hypertensive, and normal eyes separately revealed a characteristic "double-hump pattern". Figure 1 showed that in POAG as well as in ocular hypertension, the double hump pattern of RNFL distribution was maintained however it was significantly depressed as compared to normal.

In our study, we have determined the frequency of subjects in each group having RNFL defect in the average RNFL and quadrant-wise RNFL measurement – which was taken as the RNFL value (μm) less than the minimum RNFL value in normal subjects.(table 2)

Hence, we consider patients eyes to have a RNFL defect if they have RNFL values: $<112.44 \mu\text{m}$, $<113.37 \mu\text{m}$, $< 81.08 \mu\text{m}$, $< 78.69 \mu\text{m}$, $<97.83 \mu\text{m}$ for Superior, Inferior, Nasal, Temporal Quadrant and Average (360°) RNFL thickness respectively.

Table 3 summarized frequency distribution of RNFL defect in ocular hypertensive and POAG eyes. Amongst ocular hypertensive eyes, of all RNFL defects detected, maximum defect was seen in the inferior quadrant. Patients of glaucomatous eyes (POAG) show RNFL defects in all quadrants, more so in temporal and inferior quadrant than in the nasal and superior quadrant.

In ocular hypertensive eyes, of all RNFL defects detected in inferior quadrant, 50% of eyes showed RNFL values less than the minimum RNFL value in normal subjects with the best corrected visual acuity (BCVA) of 6/6. Amongst POAG eyes with a BCVA of 6/6, RNFL defect was more in temporal and inferior quadrants than nasal and superior quadrants.

In ocular hypertensive eyes, majority of RNFL defect seen in inferior quadrant had an IOP in the range of 26 to 30 mm Hg. In POAG eyes; majority had an IOP in the range of 26-30 mm Hg with RNFL defect more in temporal and inferior quadrants than nasal and superior quadrants.

4. Discussion

As there is considerable evidence that RNFL loss precedes visual field loss and optic nerve head defects in patients with glaucoma^[9] it is of interest to quantify this loss in patients with suspected glaucoma prior to the development of visual field or optic neuropathy. The ability to quantify changes in RNFL thickness early in the course of glaucoma is one of the features that make OCT appealing as a diagnostic instrument for glaucoma.^[8]

The RNFL thickness was greatest in the Inferior quadrant and gradually lesser in the superior, nasal and temporal quadrants on normal subjects. Thus the RNFL measurements followed the “ISNT” rule. These results were in agreement with the OCT studies by Bowd et al (2000)^[10], kanamori et al (2003)^[11] and the histologic study done by Varma and associates.^[12]

The RNFL profile demonstrated “double-hump” pattern with RNFL thickness peaks in the superior and inferior quadrants and troughs in the temporal and nasal quadrants. [Figure 1]

Ramakrishnan et al (2006)^[13], in their study on normal subjects, found the RNFL thickness for superior, inferior, nasal and temporal quadrants to be 138.2 ± 21.74 (95% CI: 134.3-142.1), 129.1 ± 25.67 (95% CI: 124.5-133.7), 85.71 ± 21 (95% CI: 81.9-89.5), and 66.38 ± 17.37 (95% CI: 63.3-69.5) μm , respectively. Sony et al (2004)^[14] found superior

quadrant had an average thickness of $131.09 \pm 14.31 \mu$ (range 85-171 μ), inferior quadrant $132.34 \pm 14.70 \mu$ (range 90-180 μ), temporal quadrant $67.10 \pm 12.77 \mu$ (range 35-145 μ) and nasal quadrant $85.93 \pm 17.85 \mu$ (range 44-150 μ). Both studies suggest that the “ISNT rule” applied to Sony et al but not to the Ramakrishnan et al group of Indian population.

We noticed a significant variation in the average RNFL thickness measured in normal controls in our study as compared to the other studies. The comparison and summary of previous reports of RNFL thickness in normal individuals is provided in table 4. This difference can be owing to different populations studied, difference in sample size, and changing OCT machine parameters.

Both in ocular hypertensive and POAG group, the RNFL thickness was greatest in the superior quadrant and thinned in the inferior, nasal and temporal quadrant. Bowd et al (2000)^[10] and Kanamori et al (2003)^[11] found similar results in their study except that the RNFL thickness in temporal quadrant was more than that in nasal.

Quigley et al^[18] showed preexisting RNFL defects in 57% of OHT eyes that converted from normal to defective visual fields. Pre-existing RNFL defects also were present in 35% of non-converted eyes. Moreover, the risk of conversion to defective visual fields in OHT eyes increased with increases in RNFL damage.^[19]

Gazzard et al (2002)^[20] found that in subjects with glaucomatous group (POAG), the superior hemi field was more severely affected than the inferior. This suggests that inferior RNFL will be damaged more in POAG. This correlates with our study. Optic nerve defects associated with glaucoma often occur initially at the inferior pole.^[21] Finally, visual field defects associated with glaucoma usually occur initially in the superior visual field (corresponding to inferior pole defects).^[22]

Any diagnostic imaging technology in glaucoma has to be capable of differentiating normal eyes from those with early to moderate glaucoma. Our study showed that in all study groups in all four quadrants, the RNFL thickness was more in normal > ocular hypertensive eyes > glaucomatous eyes.

In OHT group, of all RNFL defects detected, 75% defect was seen in inferior quadrant. There was statistically significant thinning in RNFL thickness in superior, inferior, nasal, temporal and average (360%) RNFL in ocular hypertensive eyes than in normal eyes. ($p < 0.05$)

Subbiah et al (2007)^[7] found thinning of RNFL in nasal ($p < 0.001$), temporal ($p < 0.001$), inferior ($p < 0.001$), superior ($p = 0.02$), and average RNFL ($p = 0.008$) in ocular hypertensive group when compared to normal.

Table 3 shows that, retinal nerve fiber layer showed thinning in all four quadrants. However, majority of the defect was seen in inferior and temporal quadrants and average RNFL thickness.

Subbiah et al (2007)^[7] in their study found that the mean thickness of the RNFL in glaucomatous (POAG) patients

(52.96 ± 31.10) and in ocular hypertensive patients (82.87 ± 17.27) was significantly less than in normal subjects (94.26 ± 12.36). They found that in glaucomatous eyes, the RNFL thickness in the inferior quadrant was $64.41 \pm 43.68 \mu\text{m}$ and that superior quadrant was $73.45 \pm 39.57 \mu\text{m}$, which was significantly thinner than in normal subjects, wherein RNFL thickness in the inferior quadrant was $120.15 \pm 14.32 \mu\text{m}$ ($p < 0.001$) and in superior quadrant was $116.19 \pm 19.97 \mu\text{m}$ ($p < 0.001$).

Hoh et al (2000)^[23] reported that the mean RNFL thickness measured with OCT was significantly less in glaucomatous eyes ($56.9 \pm 21.5 \mu\text{m}$) than in ocular hypertensive ($83.70 \pm 16.57 \mu\text{m}$) and normal ($90.86 \pm 14.17 \mu\text{m}$) eyes; although RNFL thickness tended to be greater in normal than in ocular hypertensive eyes, this difference was not statistically significant.

Amongst ocular hypertensive eyes with a BCVA of 6/6, there was RNFL defect in the inferior quadrant. Thus, this suggests that inferior quadrant RNFL is most susceptible to glaucomatous damage in ocular hypertension.

Guedes et al (2003)^[17] reported that the inferior RNFL was the only parameter in which a statistically significant difference was observed between normal subjects and glaucoma suspect groups.

Subbiah et al (2007)^[7] found that in the group clinically assessed as ocular hypertensive, RNFL thickness in the inferior quadrant was $107.87 \pm 25.79 \mu\text{m}$ and in the superior quadrant was $106.25 \pm 33.54 \mu\text{m}$, which was significantly thinner ($p < 0.001$) than the value observed in normal controls, suggesting an early glaucomatous damage to the inferior RNFL. Thus it can be inferred that the inferior were the first to be damaged by glaucoma.

Taliantzis et al (2009)^[24] showed that in cases of ocular hypertension and pre-perimetric glaucoma, the correlation of average RNFL thickness with the global indices of the VF is not very strong. The calculated correlation is stronger in eyes with pre-perimetric glaucoma than in eyes with ocular hypertension. Also that when the structural alternation is detected with OCT in patients with suspected glaucoma, the functional sensitivity seems also to be decreased, although it still ranged within the normal limits of the white perimetry. Thus, stressing on the importance of pre-perimetric diagnosis of glaucoma.

Amongst POAG eyes with a BCVA of 6/6, RNFL defect was more in temporal and inferior quadrants than nasal and superior quadrants.

Jones et al (1993)^[25] showed that the infero-temporal segment of the neuroretinal rim (NRR) is typically most susceptible to early damage in primary open angle glaucoma (POAG). This corresponds to the finding in our study.

Among ocular hypertensive eyes, majority of RNFL defect seen in inferior quadrant had an IOP in the range of 26 to 30 mm Hg. In POAG eyes, majority had an IOP in the range of 26-30 mm Hg with RNFL defect more in temporal and inferior quadrants than nasal and superior quadrants.

This shows that in spite of having a relatively less IOP of 26-30 mm Hg RNFL defects are seen, especially inferior and temporal quadrant. Current theories postulate a mixed mechanism of optic nerve damage in POAG, with elements of pressure-sensitive and pressure-independent damage responsible for the characteristic patterns of glaucomatous optic neuropathy.^[26]

5. Conclusion

SD-OCT detects RNFL thinning prior to the development of visual field or optic neuropathy especially in the inferior and temporal quadrant. Segmental RNFL thickness, particularly in the inferior quadrant seems to be a more reliable index for the early diagnosis of glaucoma. In early glaucoma, RNFL defects can be detected even in patients with a BCVA of 6/6 and even when IOP is not very high. Thus, SD-OCT has the ability to detect early glaucomatous change in ocular hypertensive and glaucomatous eyes in the form of thinning of RNFL.

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Table 1: The mean and standard deviation values of RNFL thicknesses in four quadrants and average thickness measured by SD-OCT in control, OHT and POAG groups.

OCT parameters	Control group	OHT group	POAG group
Average	121.66 ± 12.16 µm	106.89 ± 6.91 µm	90.41 ± 11.14 µm
Quadrants			
Superior	138.04 ± 13.06 µm	125.89 ± 7.13 µm	119.09 ± 11.20 µm
Temporal	100.92 ± 11.34 µm	88.98 ± 7.03 µm	61.98 ± 11.20 µm
Inferior	141.96 ± 14.59 µm	119.16 ± 8.87 µm	107.22 ± 12.74 µm
Nasal	104.60 ± 12.00 µm	92.98 ± 7.09 µm	73 ± 14.07 µm

OCT: optical coherence tomography, OHT: ocular hypertension, POAG: primary open angle glaucoma

Table 2: Range of RNFL thickness (µm) in normal controls (95% confidence interval)

Quadrant	Superior	Inferior	Nasal	Temporal	Average
Range					
Maximum	163.67	170.55	128.11	123.14	145.49
minimum	112.44	113.37	81.08	78.69	97.83

Table 3: Frequency distribution of RNFL defect in Group II and Group III

Rnfl Defect	Group II	Group III
Superior (<112.44 µm)	0	11
Inferior (<113.37 µm)	12	25
Nasal (<81.08 µm)	0	21
Temporal (<78.69 µm)	2	42
Average (<97.83 µm)	2	31

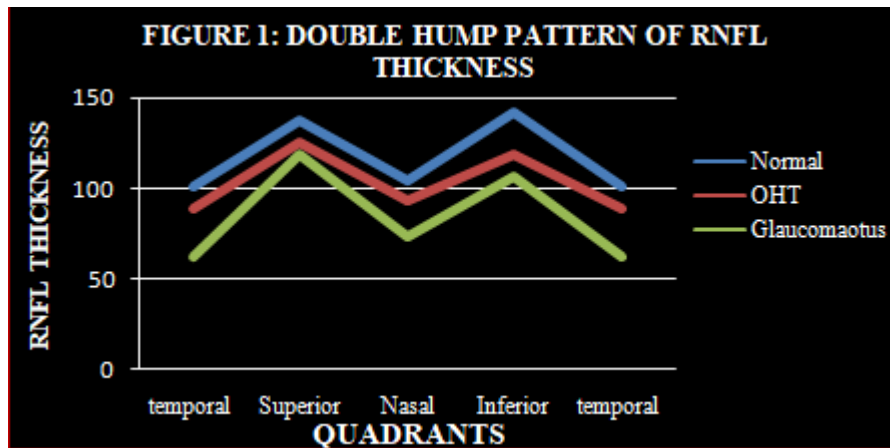


Table 4: Comparison of mean RNFL (μm) in normal subjects in various studies

STUDY (Year)	OCT machine	Average RNFL thickness (mean \pm SD) in μm	No. of patients	Significance
Mitselberger et al (1999) ^[15]	Not mentioned	90.86 \pm 14.77	17	lower
Bowd et al (2000) ^[10]	OCT 2000	85.8	30	lower
Soliman et al (2002) ^[16]	OCT 2000	127.11 \pm 25	25	higher
Guedes et al (2003) ^[17]	OCT 1	114.8 \pm 13.1	18	lower
Kanamori et al (2003) ^[11]	OCT 2000	120 \pm 12.9	160	lower
Sony et al (2004) ^[14]	OCT 3000	104.27 \pm 8.51	146	lower
Ramakrishnan et al(2006) ^[13]	OCT 3000	104.87 \pm 38.81	118	lower
Subbiah et al(2007) ^[7]	OCT 3000	94.26 \pm 12.36	30	lower
Our study	SD-OCT	121.66 \pm 12.16	50	-