

## Original Research Article

# Comparative study of retinal nerve fibre layer thickness in normal and glaucomatous human eyes as measured by optical coherence tomography

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**Received:** 08 February 2020

**Revised:** 18 February 2020

**Accepted:** 29 February 2020

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### ABSTRACT

**Background:** Glaucoma is a disease primarily associated with damage to the Retinal Ganglion Cell (RGC) bodies and axons, which causes characteristic patterns of Visual Field (VF) and changes in the appearance of the Optic Nerve Head (ONH). This Study Compares Nerve Fibre layer thickness in normal and Glaucomatous Human Eye.

**Methods:** It is a case control observational study carried out in 100 patients in a tertiary eye care hospital in the department of ophthalmology in the period from 1<sup>st</sup> January 2019 to 30<sup>th</sup> June 2019.

**Results:** The diagnostic ability for distinguishing between normal and glaucoma patients were same when authors consider average RNFL thickness and average GCC thickness. Diagnostic accuracy increases when authors consider both RNFL and GCC thickness. There was a significant difference in both RNFL and GCC thickness between normal and glaucoma patient ( $p < 0.001$ ). The mean deviation shows a significant correlation with all the parameters in eyes with glaucoma ( $< 0.001$ ).

**Conclusions:** RNFL thickness as measured by OCT showed statistically significant correlation with glaucoma. RNFL seems to have higher sensitivity and specificity for the detection of early glaucoma. Diagnostic accuracy increases when we include other parameters like RNFL and visual fields.

**Keywords:** Ganglion cells, Nerve fiber layer, Optical coherence tomography, Optic nerve head, Primary open angle glaucoma

### INTRODUCTION

Glaucoma is a disease primarily associated with damage to the Retinal Ganglion Cell (RGC) bodies and axons, which causes characteristic patterns of Visual Field (VF) defects and changes in the appearance of the Optic Nerve Head (ONH). The loss of RGCs, accompanied by thinning of the ONH neuro-retinal rim and the RGC-related layers of the retina, is associated with a loss of sensitivity in perimetric tests of the VF. Structural and functional tests are thus both indispensable tools in

assessing the extent of glaucomatous damage, especially in its early stage, and the progression of damage over time.<sup>1</sup> Asians will have 47% of disease worldwide by 2020.<sup>2</sup> Glaucoma accounts for 12% of blindness and 11.4% of low vision in India.<sup>3</sup> In fact, a number of studies have used Optical Coherence Tomography (OCT) to show that there are significant, strong correlations between Standard Automated Perimetry (SAP) determined VF sensitivity and OCT-determined Circumpapillary Retinal Nerve Fiber Layer (cpRNFL) thickness, macular Ganglion Cell Complex (GCC)

thickness, the combined thickness of the macular RNFL (mRNFL), Ganglion Cell Layer (GCL) and Inner Plexiform Layer (IPL).

The diagnosis of glaucoma in clinical practice largely depends on identification of characteristic structural changes to the optic nerve head, which are often accompanied by functional deficits on visual field testing.<sup>1,2</sup> Advances in imaging technologies, especially the development of Optical Coherence Tomography (OCT), provide a means for the objective evaluation of structural changes to the optic nerve head and retina in glaucoma, and offer the potential for improved detection of disease. It has been shown that circumpapillary Retinal Nerve Fiber Layer (RNFL) measurements from OCT have good ability to differentiate glaucomatous and healthy eyes.<sup>4-7</sup>

## METHODS

This study was a case control observational study conducted in a tertiary teaching hospital from January 2019 to June 2019. Institutional ethical clearance was taken, and participants were given detailed information and consent was taken. In this study all the patient coming to Eye OPD at Rohilkhand Medical College and Hospital with age more than 45 years, having the refractive error between -2 to +2 diopter, Media Opacity not more than nuclear cataract 3 cortical 3 as per locs 3 classification. and visual field defects suggestive of glaucoma were taken into study. All the other forms of Glaucoma, Refractive error beyond +2/-2 diopter, history of Diabetes Mellitus/Hypertension and all those patients who were not willing to participate in study were excluded from the study.

All the cases were divided into 2 groups - sample size was calculated assuming 95% confidence interval, margin of error 5 micrometer and SD of 21 using sample size formula. Group I consisted of 50 newly diagnosed POAG patients aged between 40 years to 70 years applying inclusion and exclusion criteria. Group 2 consisted of 50 non glaucoma subjects coming for routine ophthalmological checkup and were age and gender matched with the cases.

## Procedure

A detailed ocular examination was undertaken employing refraction and corneal curvature radius measurement with an automatic refractometer, best corrected visual acuity measurement, axial length measurement with the IOL Master (Carl Zeiss Meditec, Dublin, CA, USA), slit-lamp examination, Intraocular Pressure (IOP) measurement with a Goldman applanation tonometer (Haag-Streit, Koeniz, Switzerland), dilated funduscopy, and Visual field testing with the Humphrey Field Analyzer (HFA) 24-2 Swedish Interactive Threshold Algorithm Standard (SITA-S) program (Carl Zeiss Meditec).

## Statistical analysis

The SPSS 22.0 programme was used to analyze the result. To compare the measured parameters value between 2 groups and Analysis of Variance (ANOVA) was used. The p value <0.05 was considered significant. To look for RNFL thickness and macular thickness measurement difference between the 2 groups one-way ANOVA was applied. Method of regression analysis was utilized to establish the relationship between mean RNFL. To assess the correlation between continuous variables Pearson's correlation coefficients were used. ROC curves were used to differentiate glaucomatous eyes from healthy eyes using SD-OCT parameters (RNFL).

## RESULTS

In this study 50 patients were included as cases in group I and 50 subjects were included as controls group II (total of 200 eyes: 100 normal eyes and 100 POAG patients' eyes). Mean age in group I was 62.07±1.95 years and 57.98±2.14 years in Group II. In group I, 30 (60.00%) were males and 20 (40.00%) were females and in group II, 33 (66%) were males and 17 (34%) were females. No statistically significant difference was found in age distribution, sex, pachymetry distribution between the groups.

Defects in the retinal NFL may be an early sign of glaucoma, preceding optic disc and visual field changes. The NFL is composed primarily of RGC axons, neuroglia and astrocytes. Raw data obtained by OCT were analyzed using standard processing algorithms that measured total retinal and NFL thickness. In addition, the algorithms corrected for various artifacts, including eye movement, blood vessel shadows, blinks and media opacities. The location of the vitreoretinal interface and the retinal pigment epithelium defined the inner and outer boundaries respectively, of the neurosensory retina. These two boundaries were associated with the sharpest edges in each OCT A-scan because of high contrast in optical reflectivity between the relatively nonreflective vitreous and the reflective neurosensory retina and between the minimally reflective photoreceptor outer segments and the highly reflective retinal pigment epithelium/choriocapillaries.

RNFL thickness was significantly thinner in glaucomatous groups as compared to the control subjects. RNFL thickness showed significant difference with the Mean Deviation (MD) of the visual field in glaucoma subjects (p=0.01) (Table 1).

ROC curves were drawn for RNFL parameters as shown in (Figure 1). The area under the ROC (AROC) for average RNFL was 0.963. With a cut off value of 80.5 microns there was 93% sensitivity and 89% specificity as shown in table 2.

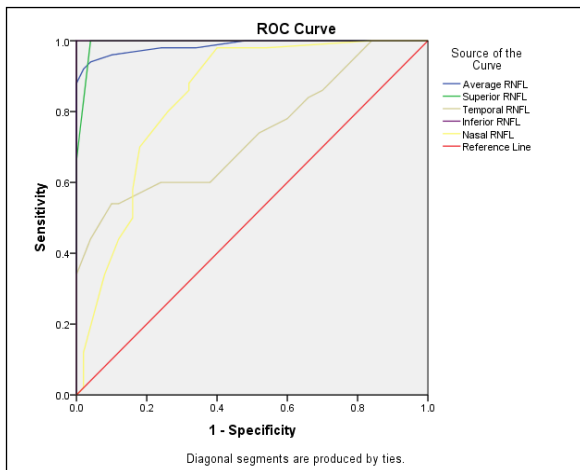
Overall GCC thickness analysis also showed significant difference in two groups where GCC overall thickness

was significantly thinner in glaucomatous groups as compared to the control subjects. GCC overall thickness showed significant difference with the MD of the visual field in glaucoma subject (p=0.01). GCC overall thickness decreased with increasing severity of Visual Fields (HAP classification). Average GCC values were higher in normal than POAG 83.22±5.52 u and 65.62±7.65 u respectively.

**Table 1: Average RNFL in cases and control.**

Variables		Mean	Std. deviation	p-value
Average RNFL	Case	66.02	±9.682	<0.001*
	Control	92.48	±8.286	
Superior RNFL	Case	76.38	±14.446	<0.001*
	Control	111.96	±11.047	
Temporal RNFL	Case	49.04	±10.445	<0.001*
	Control	60.36	±13.326	
Inferior RNFL	Case	55.24	±12.434	<0.001*
	Control	116.04	±10.431	
Nasal RNFL	Case	56.94	±12.940	<0.001*
	Control	73.04	±8.154	

\*p<0.05 consider statistically significant. Independent t-test was appropriate



**Figure 1: ROC curve for RNFL.**

**Table 2: Sensitivity and specificity of RNFL using one-way ANOVA analysis.**

Test variables	Area under ROC	Cut off value	Sensitivity	Specificity
Average RNFL	0.963	80.5	93	89
Superior RNFL	0.943	88.5	90	82
Inferior RNFL	0.931	90.5	89	81

In summary, studying the RNFL topology with the specific-domain OCT RNFL, thickness maps offer a

useful alternative to red free RNFL photography to detect RNFL defects and determine the pattern of RNFL progression with the added advantage of providing objective and quantitative analysis of change.

**DISCUSSION**

Glaucoma causes irreversible damage to structural and functional anatomy of patient’s eyes. By the time it is diagnosed, 40% of nerve tissues has already been damaged. Till now visual field remains the gold standard for diagnosing POAG. OCT is a newer diagnostic tool to see the status of various layer of the retina. OCT provides an objective as well as numeric measurement of RNFL thickness by measuring echo time delay by backscattering of light from different layer of the retinal layers.<sup>8</sup> The instrument is fast, less time consuming and patient friendly.<sup>9</sup> Hong S et al, has shown that cirrus SD-OCT able to analyse the peripapillary RNFL thickness was constantly reproducible with negligible variation in measurements in healthy eyes.<sup>10</sup> It is a known fact that RGCs are selectively lost in early glaucoma but how much GCC loss already occurred to record visual field defect is still in research. Studies in animal models have shown that GCC cell loss agrees with visual field defects.<sup>11</sup> Studies have also shown reduced central macular thickness in early glaucoma but has limited role. In recent years, imaging of macular ganglionic cell with high definition images showed a promising result as approximately 50% of RGCs are located 4-5 mm from the center of the fovea.<sup>12</sup> At 750-1100 um from the foveal centre, the ganglionic cell density is maximum. The cell density thickness may be 4-6 cell bodies.<sup>13</sup> For its thick areas, these are the sites of more initial damage to GCC. Present study pointed out, both the RNFL as well as GCC showed the maximum AROC among all of the parameters. Previous studies showed the RNFL has the highest AROC both with Frequency- domain OCT and time-domain OCT though most of the RNFL thickness parameters obtained from these two devices are quite different.<sup>14,15</sup> The superior and inferior sectors gather most of the RNFL and have the priority of glaucomatous damage and in early stage glaucoma, RNFL damage can occur either in superior and inferior region (or both) in a focal and diffuse way. Correspondingly, superior and inferior GCC have more priority of glaucomatous damage and in early glaucoma stage. Budenz DL et al, showed that if authors take ROC curve area of 0.793 for average RNFL analysis, authors can differentiate early glaucoma from normal eyes.<sup>16</sup>

In another study by Bowd C et al, showed AROC of 0.93 for inferior RNFL has best predictor value for differentiation for early glaucoma.<sup>17</sup> This study has shown that average RNFL thickness followed by superior and inferior RNFL thickness has the best ability discriminate between early onset glaucoma and healthy eyes with AROC of 0.963, 0.943 and 0.931, respectively. Sihota R et al, has also done the study in Indian eyes on the role of OCT to detect average difference in RNFL

thickness from healthy eyes and glaucomatous eyes.<sup>18</sup> Authors also use RNFL parameters to differentiate between different stages of glaucoma diseases. Study concluded that inferior quadrants and average RNFL parameters are among the most efficient parameters for making such a differentiation.

In the present study authors can found that RNFL thickness and GCC thickness had almost similar diagnostic values for glaucoma detection. The GCC parameters (average, superior and inferior thickness) readily identified glaucoma patients. Inferior GCC thickness appeared to be a better predictor of early glaucoma than superior and average GCC thickness.

This study shows that the RNFL and GCC thickness are lower in POAG than in normal eyes. The average RNFL thickness is a measurement of the global thickness of the RNFL and, therefore it is presumed important in the differentiation of glaucoma from healthy eyes. GCC thickness, as measured by OCT had higher sensitivity and specificity of early glaucoma.

Values of inferior GCC seems to be a better identifying tool for early glaucoma compared with RNFL thickness as GCC is a direct measure of RGC integrity. In glaucoma, RGC loss is earlier than axonal loss and therefore, macular GCC analysis may prove to be an earlier predictor than RNFL parameters. Secondly as in macular OCT scan, GCC is done within 6 mm x 6mm square area centered on macula and early glaucomatous changes occur in paracentral region (10o-20o of central field), this can be easily detected by GCC scan. Macular GCC and RNFL analysis may yield fallacious results in high myopes from the study. However, observational study by Lee JW et al, has shown that macular thickness is independent of the axial length and refractive status of the non-glaucomatous healthy children in china.<sup>19</sup>

Limitations of this study was small sample size with lack of a normal database of Indian eyes for measuring GCC and RNFL thickness. Large population-based studies are required to establish the GCC parameters in glaucomatous patients.

## CONCLUSION

SD-OCT was able to show significant differences of thickness between glaucoma and normal subjects. At present there is no consensus that which is the best structural parameter for early detection of glaucoma. However, no doubt RNFL measurements is of value in diagnosis of early glaucoma patients.

## ACKNOWLEDGEMENTS

Authors sincerely thanks statistician Mr. Mahendra Kumar for his help and support in making tables and data analysis.

*Funding: No funding sources*

*Conflict of interest: None declared*

*Ethical approval: The study was approved by the Institutional Ethics Committee*

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**Cite this article as:** Jain R, Choudhary N. Comparative study of retinal nerve fibre layer thickness in normal and glaucomatous human eyes as measured by Optical coherence tomography. *Int J Res Med Sci* 2020;8:1375-9.