

Comparative Analysis of Peripapillary Retinal Nerve Fiber Layer and Macular Parameters Using Spectral-Domain Optical Coherence Tomography in Primary Open-Angle Glaucoma and Normal Eyes: A Cross-Sectional Observational Study

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ABSTRACT

Purpose: The purpose of the study is to compare peripapillary retinal nerve fiber layer (RNFL) thickness and macular parameters measured by spectral-domain optical coherence tomography (SD-OCT) between normal subjects and patients with primary open-angle glaucoma (POAG) and to correlate RNFL thickness with age, spherical equivalent, central corneal thickness (CCT), disc area, and rim area. **Materials and Methods:** This is a cross-sectional study of 109 eyes (55 normal, 54 POAG). All subjects underwent perimetry, pachymetry, and Cirrus SD-OCT (fast RNFL and macular cube protocols). Groups were compared using the unpaired *t*-test/Mann-Whitney U test; correlations were assessed using Pearson's coefficient. **Results:** Mean RNFL thickness was lower in POAG than in normal eyes (68.13 ± 10.9 vs. 96.64 ± 8.3 μm ; $P < 0.001$), as were all four quadrants ($P < 0.001$ each). Average macular thickness, central macular thickness, and average macular volume were all significantly reduced in POAG ($P \leq 0.011$). RNFL thickness correlated negatively with age ($r = -0.86/-0.84$) and positively with spherical equivalent ($r = 0.76/0.74$) in both groups ($P < 0.001$), with no significant CCT correlation. **Conclusion:** SD-OCT-derived macular volume, macular thickness, and peripapillary RNFL thickness are all significantly reduced in POAG, and macular parameters track RNFL loss closely enough to serve as a useful adjunct to disc and RNFL assessment.

Keywords: Central corneal thickness, Cup-disc ratio, Indian population, Macular thickness, Macular volume, Optic nerve head, Primary open-angle glaucoma, Retinal nerve fiber layer, Rim area, Spectral-domain optical coherence tomography

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INTRODUCTION

Glaucoma is a chronic optic neuropathy characterized by progressive retinal ganglion cell loss and a corresponding reduction in retinal nerve fiber layer (RNFL) thickness, and remains one of the leading causes of irreversible blindness worldwide.^[1] Because visual field testing and clinical disc assessment only detect glaucomatous damage after a substantial proportion of ganglion cell axons have already been lost, considerable effort has gone into imaging techniques that can identify structural loss earlier and more objectively.

The ganglion cell layer and RNFL together account for roughly a third of retinal thickness at the macula, where ganglion cells are most densely packed – which is the rationale for using macular thickness and volume, in addition to peripapillary RNFL thickness, as structural markers of glaucomatous damage. Spectral-domain optical coherence tomography (SD-OCT) improved on time-domain systems by offering faster acquisition and higher axial resolution, translating into better reproducibility of both peripapillary RNFL and macular thickness measurements, which is particularly relevant for detecting early, pre-perimetric disease.

Reported normative RNFL values vary meaningfully across populations and OCT platforms, and Indian normative data in particular have not always tracked closely with Western cohorts – which limits how directly Western reference ranges can be applied to Indian patients. This study was designed to compare peripapillary RNFL thickness and macular parameters (central macular thickness, average macular thickness, and average macular volume) measured by SD-OCT between normal and primary open-angle glaucoma (POAG) eyes in an Indian population and

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to characterize how RNFL thickness relates to age, refractive error, corneal thickness, and optic disc morphology within this cohort.

Aims and Objectives

Research question

Are there significant differences in RNFL thickness, macular volume, central macular thickness, and average macular thickness between normal subjects and POAG patients as measured by SD-OCT?

Primary outcome

Comparative analysis of macular volume, macular thickness, and RNFL thickness using SD-OCT in POAG and normal subjects.

Secondary outcomes

Association of RNFL thickness with (i) spherical equivalent, (ii) central corneal thickness, (iii) age, (iv) disc area, and (v) rim area.

MATERIALS AND METHODS

Study Design and Setting

This was a cross-sectional observational study conducted in the General Outpatient and Glaucoma Departments of Aravind Eye Hospital and Post Graduate Institute of Ophthalmology, Tirunelveli, Tamil Nadu, India, over 25 months (November 2012–November 2014). The study protocol and informed consent were approved by the Institutional Review Board of the same institute, and the study adhered to the tenets of the Declaration of Helsinki.

Study Population

A total of 109 eyes of 109 adults meeting the eligibility criteria were recruited and divided into a normal group ($n = 55$) and a POAG group ($n = 54$). One eye per subject was selected using a computer-generated randomization table.

Inclusion Criteria

Normal

Intraocular pressure (IOP) <21 mmHg; open angles on gonioscopy; normal optic nerve head (ONH) on slit-lamp biomicroscopy with a +90D lens; normal visual fields on 24-2 achromatic automated perimetry.

POAG

IOP >21 mmHg on two occasions at least 24 h apart; open angles on gonioscopy; ONH changes consistent with glaucomatous atrophy; visual field loss on 24-2 perimetry corresponding to the disc changes on two consecutive visits, satisfying at least two of Anderson and Patella's criteria.

Exclusion Criteria

Spherical equivalent outside the range -4.00 D to $+3.50$ D; secondary open-angle glaucoma; angle-closure suspects or angle-closure glaucoma; unreliable visual fields; media opacity precluding good-quality OCT scans; retinal pathology; prior intraocular or refractive surgery; neurologic disease; congenital disc anomalies; or intolerance to topical anesthetics/mydriatics.

Examination Protocol

All subjects underwent a detailed history, Snellen visual acuity, refraction (converted to spherical equivalent), slit-lamp anterior segment examination, Goldmann applanation tonometry, dilated stereoscopic biomicroscopy of the ONH with a +90D

lens, Humphrey Field Analyzer (720i, 24-2 program) perimetry, ultrasound pachymetry (PacScan 300p, Sonomed), and SD-OCT.

SD-OCT imaging was performed with the Cirrus high-definition OCT system (Model 4000, software version 5.1.1.4; Carl Zeiss Meditec, Dublin, CA) after pharmacological pupil dilation. Peripapillary RNFL thickness was measured using the fast RNFL (3.4 mm circle, 256 A-scans) protocol, with internal fixation used for better reproducibility; RNFL thickness was recorded as quadrant averages, clock-hour averages, and 360° average. Macular scans used six 100-pixel radial scans centered on the fovea, generating a nine-sector ETDRS-style thickness map (1 mm central disc, 2.22 mm inner ring, 3.45 mm outer ring) from which central macular thickness, average macular thickness, and average macular volume were derived. Scans with an involuntary saccade within a 1.73 mm radius, or signal strength below 7, were discarded and repeated. All scans were acquired by a single experienced operator.

Sample size

Based on prior studies, a sample of 52 eyes per group was calculated to be sufficient to detect a clinically important between-group difference, assuming standard deviations (SDs) of 20 and 16 in the two groups, a mean difference of 10, 80% power, and a 5% significance level.

Statistical analysis

Continuous variables are reported as mean $\pm$ SD and categorical variables as frequency (%). The Chi-square or Fisher's exact test was used for categorical comparisons and the unpaired Student's *t*-test (or Mann-Whitney U-test where indicated) for continuous variables. Pearson's correlation coefficient was used to assess associations between RNFL thickness and age, spherical equivalent, CCT, disc area, and rim area. $P < 0.05$ was considered statistically significant. Analyses were performed separately within the normal and POAG groups using (Insert Statistical Software and Version, e.g., Statistical Package for the Social Sciences v.XX, IBM Corp.).

RESULTS

Of the 109 eyes analyzed, 55 were in the normal group and 54 in the POAG group. Gender distribution was comparable between groups [Table 1].

Age, spherical equivalent, and CCT were comparable between groups, as expected for an age- and refraction-matched design; IOP was, as expected, significantly higher in the POAG group [Table 1].

Table 1: Baseline demographic and clinical characteristics

Parameter	Normal ($n=55$)	Primary open-angle glaucoma ($n=54$)	P-value
Female, n (%)	28 (50.9)	30 (55.6)	—
Male, n (%)	27 (49.1)	24 (44.4)	—
Age (years)	57.18 ± 7.2	58.65 ± 6.0	0.251
Spherical equivalent (D)	0.30 ± 1.9	0.35 ± 1.8	0.894
Central corneal thickness (μm)	551.09 ± 43.5	562.69 ± 40.8	0.154
Intraocular pressure (mmHg)	14.31 ± 2.2	25.20 ± 4.2	<0.001

The normal group showed the classic “double-hump” pattern with the inferior and superior quadrants thickest; in the POAG group, the superior quadrant was relatively best preserved, but every quadrant was significantly thinner than in the normal group [Table 2].

Rim area was significantly smaller and cup volume and cup-disc ratios significantly larger in the POAG group; disc area was also modestly larger in POAG eyes [Table 3].

All three macular parameters were significantly reduced in the POAG group. Central macular thickness showed the smallest between-group difference of the three, consistent with the relative absence of ganglion cells at the fovea [Table 4].

Eyes with more advanced RNFL loss ($<53 \mu\text{m}$) had significantly lower macular volume and thickness than eyes with relatively preserved RNFL [Table 5].

RNFL thickness correlated negatively with age and positively with spherical equivalent (i.e., thinner RNFL with increasing myopia) in both groups [Table 6, Figures 1 and 2]. Correlations with

rim area and disc area were positive and statistically significant [Figures 3 and 4], whereas no significant correlation was found with CCT in either group [Figure 5].

DISCUSSION

The rationale for using macular parameters alongside peripapillary RNFL thickness in glaucoma rests on simple retinal anatomy: Ganglion cells and their axons make up roughly a third of retinal thickness at the macula, where ganglion cell density is highest, so meaningful ganglion cell loss should be detectable as macular thinning even though the outer retina – unaffected by glaucoma – makes up the bulk of the remaining thickness. Prior work evaluating RNFL, ONH, and macular thickness together for glaucoma detection supports this rationale,^[2] and later comparisons between time-domain and spectral-domain platforms showed that the latter had a clear reproducibility advantage, particularly for sectoral RNFL and macular measurements – the very measurements that matter most for catching early disease. Macular thickness measurements have

Table 2: Peripapillary RNFL thickness: Overall and by quadrant

Parameter	Normal	POAG	P-value
360° average RNFL (μm)	96.64 $\pm$ 8.3	68.13 $\pm$ 10.9	<0.001
Superior quadrant (μm)	115.80 $\pm$ 12.7	81.24 $\pm$ 18.1	<0.001
Inferior quadrant (μm)	123.56 $\pm$ 15.0	78.33 $\pm$ 20.3	<0.001
Temporal quadrant (μm)	68.25 $\pm$ 10.0	54.50 $\pm$ 12.7	<0.001
Nasal quadrant (μm)	78.65 $\pm$ 9.3	61.11 $\pm$ 11.0	<0.001

POAG: Primary open-angle glaucoma, RNFL: Retinal nerve fiber layer

Table 3: Optic nerve head parameters

Parameter	Normal	Primary open-angle glaucoma	P-value
Rim area (mm^2)	1.35 $\pm$ 0.2	0.74 $\pm$ 0.2	<0.001
Disc area (mm^2)	1.96 $\pm$ 0.4	2.06 $\pm$ 0.3	0.043
Cup volume (mm^3)	0.19 $\pm$ 0.1	0.76 $\pm$ 0.1	<0.001
Average cup-disc ratio	0.54 $\pm$ 0.1	0.76 $\pm$ 0.1	<0.001
Vertical cup-disc ratio	0.54 $\pm$ 0.1	0.76 $\pm$ 0.1	0.031

Table 4: Macular parameters

Parameter	Normal	Primary open-angle glaucoma	P-value
Central macular thickness (μm)	257.16 $\pm$ 16.6	247.94 $\pm$ 20.5	0.011
Average macular thickness (μm)	272.13 $\pm$ 13.2	255.28 $\pm$ 18.2	<0.001
Average macular volume (mm^3)	9.84 $\pm$ 0.3	9.10 $\pm$ 0.6	<0.001

Table 5: Macular parameters stratified by RNFL thickness within the POAG group

Parameter	RNFL $<53 \mu\text{m}$	RNFL $>53 \mu\text{m}$	P-value
Average macular volume (mm^3)	8.38 $\pm$ 0.4	9.53 $\pm$ 0.6	<0.001
Average macular thickness (μm)	232.6 $\pm$ 10.2	265.28 $\pm$ 16.8	<0.001

POAG: Primary open-angle glaucoma, RNFL: Retinal nerve fiber layer

Table 6: Correlation of RNFL thickness with clinical and structural parameters

Parameter	Normal (r, P-value)	POAG (r, P-value)
Age	-0.856, <0.001	-0.843, <0.001
Spherical equivalent	0.756, <0.001	0.739, <0.001
Central corneal thickness	0.229, 0.092	0.027, 0.846
Rim area	0.271, 0.045	0.355, 0.008
Disc area	0.377, 0.005	0.390, 0.004

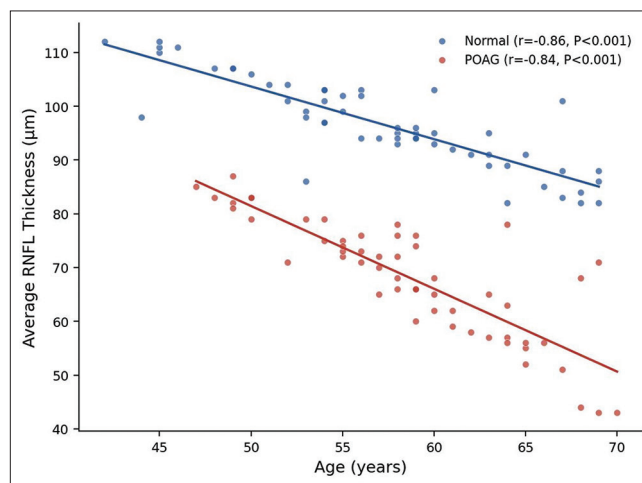


Figure 1: Correlation of age with average retinal nerve fiber layer thickness in normal and primary open-angle glaucoma groups

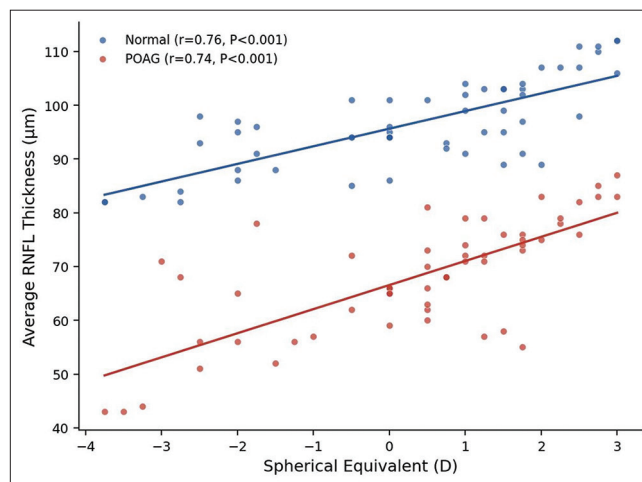


Figure 2: Correlation of spherical equivalent with average retinal nerve fiber layer thickness in normal and primary open-angle glaucoma groups

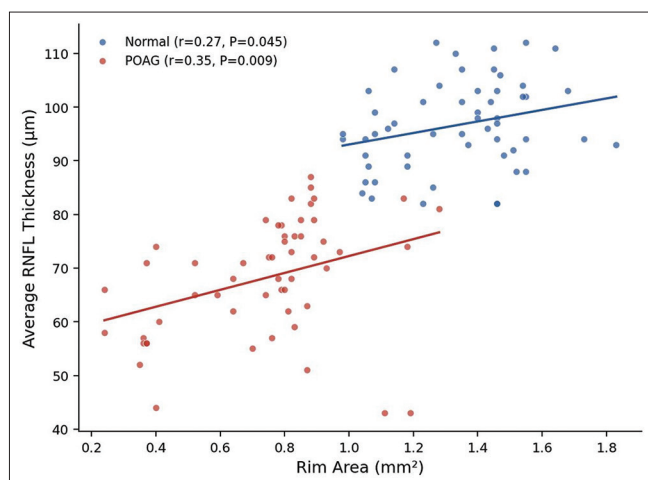


Figure 3: Correlation of rim area with average retinal nerve fiber layer thickness in normal and primary open-angle glaucoma groups

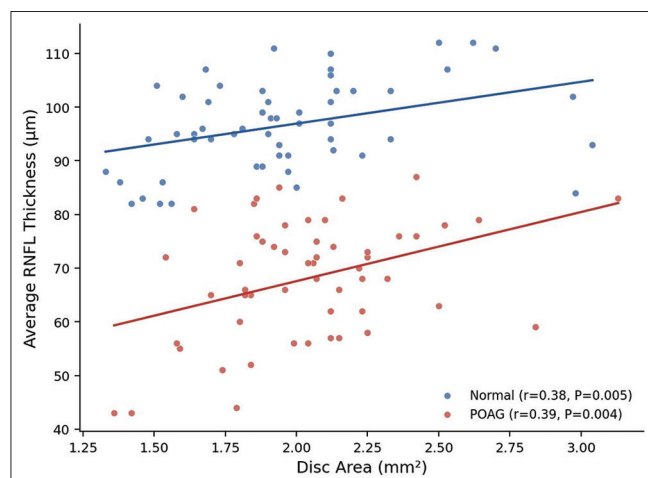


Figure 4: Correlation of disc area with average retinal nerve fiber layer thickness in normal and primary open-angle glaucoma groups

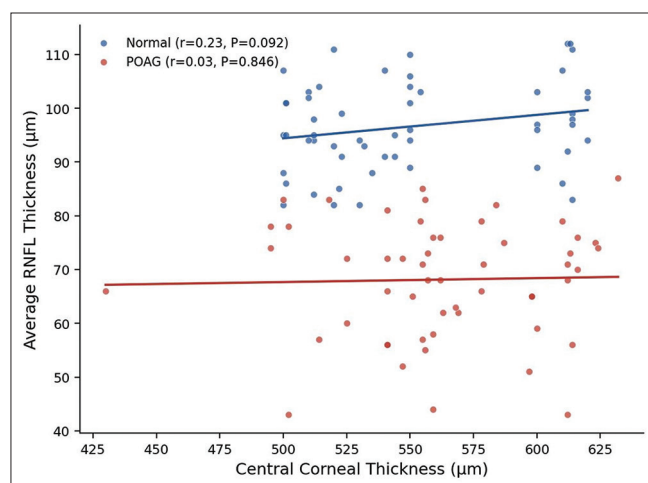


Figure 5: Correlation of central corneal thickness with average retinal nerve fiber layer thickness in normal and primary open-angle glaucoma groups

also been reported to perform comparably to peripapillary RNFL measurements for glaucoma detection in some series.^[3]

Subsequent studies using Fourier-domain OCT and ganglion cell complex (GCC) analysis reinforced this: GCC thickness tracked glaucoma progression and visual field change well, and combining GCC with standard RNFL parameters improved detection rates over RNFL alone in some reports. This study did not include GCC analysis, but the pattern in our data – clear, concordant reductions in RNFL thickness, macular thickness, and macular volume in POAG – is consistent with that broader literature: whichever of these parameters is available on a given OCT platform appears useful for following glaucoma status.

Our findings align closely with a 2018 comparison of ONH and macular parameters in POAG and ocular hypertension, in which average macular thickness was the macular parameter with the largest between-group separation – as it was in our cohort.^[4] That study also identified the neuroretinal rim as the single ONH parameter most strongly associated with functional loss, consistent with the positive rim area–RNFL correlation we found in both our groups and with earlier reports of a positive correlation between peripapillary RNFL thickness and rim area on SD-OCT.^[5] Our macular volume findings are also consistent with an earlier report that macular volume distinguishes normal from glaucomatous eyes^[6] and with an Indian series that similarly found total macular volume, and inner and outer macular thickness, significantly reduced in POAG relative to controls, with central macular thickness the least affected of the parameters – the same relative ordering we observed.^[7] That two independently conducted Indian cohorts, examined roughly a decade apart with different OCT platforms, arrive at the same ordering (macular volume and average thickness discriminating better than central thickness) strengthens the case that this is a real anatomical pattern rather than a platform-specific artifact.

The negative correlation between age and RNFL thickness in both groups mirrors the broader literature on age-related RNFL thinning, and the positive correlation with spherical equivalent – thinner RNFL with more myopic refraction – is consistent with reports that myopic eyes measure thinner on Cirrus SD-OCT independent of glaucoma status, an important confounder to account for when interpreting RNFL results in myopic populations.^[8] We found no significant correlation between CCT and RNFL thickness in either group; reports on this association have been mixed in the wider glaucoma literature, and our results are consistent with the studies reporting no significant relationship.

Normative RNFL values in this study's control group ($96.64 \pm 8.3 \mu\text{m}$, 360° average) were somewhat lower than those reported in some other Indian SD-OCT cohorts, such as one normative series (average RNFL $114.03 \pm 9.59 \mu\text{m}$ in 210 normal eyes)^[9] and an earlier Indian normative dataset.^[10] This spread across Indian studies itself illustrates why population- and even sub-population-specific normative databases matter, rather than eyes being read against a single "Indian" or "Asian" reference range; differences in OCT protocol (scan diameter, software version), operator technique, and regional/genetic variation in disc and RNFL anatomy likely all contribute, and are worth flagging explicitly as a limitation rather than an unexplained discrepancy.

How this Study Differs from Prior Work

Structural OCT comparisons between POAG and normal eyes are not new – they form a well-established sub-literature going back

to time-domain OCT, and several groups have published broadly similar cross-sectional designs.^[4,6,7,9] What this study adds, rather than a wholly novel hypothesis, is:

- A single Indian cohort in which peripapillary RNFL, all four RNFL quadrants, five ONH parameters, and three macular parameters (central thickness, average thickness, average volume) were all measured on the same Cirrus SD-OCT platform in the same subjects – several prior Indian papers report RNFL or macular parameters, but fewer report both panels together with the full ONH parameter set in one dataset.
- A direct stratification of macular volume and thickness by RNFL severity (<53 μm vs. > 53 μm) within the POAG group itself, which is a more clinically direct way of asking whether macular parameters track disease severity than a normal-versus-POAG comparison alone.
- Simultaneous correlation analysis of RNFL thickness against age, refractive error, CCT, disc area, and rim area within the same cohort, letting these potential confounders be weighed against each other rather than considered in isolation.
- A regional Indian normative comparison, since our control-group RNFL values differed from other published Indian cohorts – a finding worth reporting on its own, since it argues for caution in applying any single normative RNFL database across Indian sub-populations.
- Positioned honestly, this is a well-conducted confirmatory and regional-normative study rather than a paper reporting a new diagnostic parameter or a new mechanism – and it should be written up and submitted as such.

CONCLUSION

In this Indian cohort, SD-OCT-measured peripapillary RNFL thickness, macular thickness, and macular volume were all significantly reduced in POAG eyes compared with normal eyes, and macular volume tracked RNFL severity closely. RNFL thickness declined with age and with increasing myopia in both groups, and correlated positively with rim area and disc area, without a significant association with central corneal thickness. Macular parameters may serve as a useful adjunct to peripapillary RNFL assessment in glaucoma evaluation, particularly where disc assessment is limited.

STUDY SITE AND ETHICS APPROVAL

This study was conducted at Aravind Eye Hospital and Post Graduate Institute of Ophthalmology, Tirunelveli, Tamil Nadu, India, where the corresponding author was undergoing postgraduate/fellowship training at the time of data collection (November 2012 to November 2014). The study protocol and informed consent were approved by the Institutional Review Board of that institute,

and the study adhered to the tenets of the Declaration of Helsinki. Written informed consent was obtained from all participants.

AUTHORS' CONTRIBUTIONS

P.V. conceived and designed the study and drafted the manuscript. J.V. contributed to data collection and analysis, and critically reviewed the manuscript for important intellectual content. Both authors approved the final version and take responsibility for the integrity of the data and the accuracy of the analysis.

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