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Original Article

Ophthalmology Section

Retinal Nerve Fibre Layer Thickness Analysis using Spectral-domain Optical Coherence Tomography in Normal Eyes, Ocular Hypertension, Glaucoma Suspects, and Early Primary Open-Angle Glaucoma: A Cross-sectional Comparative Study

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ABSTRACT

Introduction: Structural alterations of the Retinal Nerve Fibre Layer (RNFL) are among the earliest detectable manifestations of glaucomatous optic neuropathy and may precede measurable visual field defects. Spectral Domain Optical Coherence Tomography (SD-OCT) enables objective quantification of these changes and facilitates early detection of glaucoma. This study compared RNFL thickness among normal eyes, ocular hypertensives, glaucoma suspects, and eyes with early Primary Open-Angle Glaucoma (POAG).

Aim: To compare retinal nerve fibre layer thickness using spectral-domain optical coherence tomography among normal eyes, ocular hypertensive eyes, glaucoma suspects, and early primary open-angle glaucoma eyes, and to evaluate its association with intraocular pressure.

Materials and Methods: This hospital-based cross-sectional study was conducted at the Outpatient Department (OPD) Ophthalmology of a Tertiary Care Hospital in Tamil Nadu, India, from August 2024 to October 2025. This study included 120 consecutively recruited participants categorised into normal eyes (n=30), ocular hypertension (n=30), glaucoma suspects (n=30), and early POAG eyes (n=30). All participants underwent a comprehensive ophthalmic evaluation, including intraocular pressure measurement by Goldmann applanation tonometry, gonioscopy, Humphrey visual field analysis, optic nerve head assessment, and spectral-domain optical coherence tomography-based RNFL analysis. Average and quadrant-wise RNFL thicknesses were compared among the groups using One-way Analysis of Variance (ANOVA). Receiver Operating Characteristic (ROC) analysis was used to evaluate the diagnostic performance of OCT-derived RNFL parameters.

Results: Average RNFL thickness showed a significant progressive decline from normal eyes (102.9±5.6 µm) to ocular hypertensives (95.8±5.9 µm), glaucoma suspects (82.0±6.2 µm), and early POAG eyes (72.1±6.1 µm) ($p<0.01$). Quadrant-wise analysis showed more pronounced RNFL reduction in the inferior and superior regions than in the nasal and temporal quadrants. OCT demonstrated high diagnostic accuracy, with area under the curve values of 0.96 for average RNFL thickness and 0.97 for inferior quadrant RNFL thickness.

Conclusion: Spectral-domain OCT-derived RNFL thickness may serve as a useful structural biomarker for early glaucomatous damage and may aid in risk stratification and early glaucoma detection.

Keywords: Glaucomatous optic neuropathy, Intraocular pressure, Optic disc, Perimetry, Retinal ganglion cells

INTRODUCTION

Glaucoma is one of the leading causes of irreversible blindness worldwide and remains a major contributor to visual disability, particularly in developing countries where a substantial proportion of affected individuals remain undiagnosed. POAG, the most common subtype, is characterised by progressive optic neuropathy associated with retinal ganglion cell degeneration and corresponding visual field deterioration. Because glaucomatous damage is irreversible, identifying structural alterations before advanced functional loss is essential to preventing permanent visual impairment [1-3].

Structural injury in glaucoma primarily involves degeneration of retinal ganglion cell axons within the RNFL. Previous studies have demonstrated that RNFL thinning may precede detectable visual field abnormalities by several years, with nearly 25–40% of retinal ganglion cells lost before reproducible perimetric defects become evident [4,5]. Consequently, objective structural biomarkers capable of identifying pre-perimetric damage have become increasingly important in glaucoma evaluation.

Optical Coherence Tomography (OCT) is a valuable non invasive imaging modality for quantitative assessment of RNFL thickness and optic nerve head architecture. Spectral Domain OCT (SD-OCT), owing to its high axial resolution and reproducibility, permits detection of subtle structural alterations that may not be clinically appreciable [6,7]. Several studies have reported that thinning of the superior and inferior RNFL quadrants demonstrates a strong association with early glaucomatous optic neuropathy and may differentiate healthy eyes from glaucomatous eyes with high diagnostic accuracy [8-10].

Ocular hypertensive individuals and glaucoma suspects represent clinically important high-risk groups because they may subsequently develop glaucomatous optic neuropathy despite the absence of definitive visual field abnormalities at presentation. Identification of early structural compromise in such patients may facilitate closer surveillance and timely intervention. OCT-derived RNFL measurements and diagnostic thresholds, however, may vary according to ethnicity, disease severity, and imaging protocols [11-13]. Although previous

Indian studies have evaluated OCT parameters in glaucomatous eyes, most have primarily focused on isolated comparisons between normal and primary open-angle glaucoma groups or evaluated limited structural parameters. Comparative analysis simultaneously assessing normal eyes, ocular hypertensives, glaucoma suspects, and early primary open-angle glaucoma within a uniform clinical and imaging framework remains limited in the Indian population [14-16]. Recent Indian studies [14-16] have primarily evaluated isolated OCT parameters or comparisons between healthy and glaucomatous eyes, while comprehensive evaluation across the glaucoma spectrum remains limited, highlighting the need for further population-specific evidence. Since RNFL thickness profiles and diagnostic cut-offs may demonstrate ethnic and population-specific variability, region-specific data may improve risk stratification and interpretation of OCT findings in routine ophthalmic practice.

Previous studies have also demonstrated significant correlations between progressive RNFL loss and worsening visual field indices, supporting the role of OCT in glaucoma detection and monitoring [17-20]. Furthermore, Receiver Operating Characteristic (ROC) analysis of RNFL parameters has demonstrated promising diagnostic utility in distinguishing glaucomatous from non glaucomatous eyes [21-23].

The present study was therefore undertaken to compare OCT-derived RNFL thickness among normal eyes, ocular hypertensive eyes, glaucoma suspects, and early POAG eyes in an Indian tertiary-care setting and to evaluate its association with intraocular pressure.

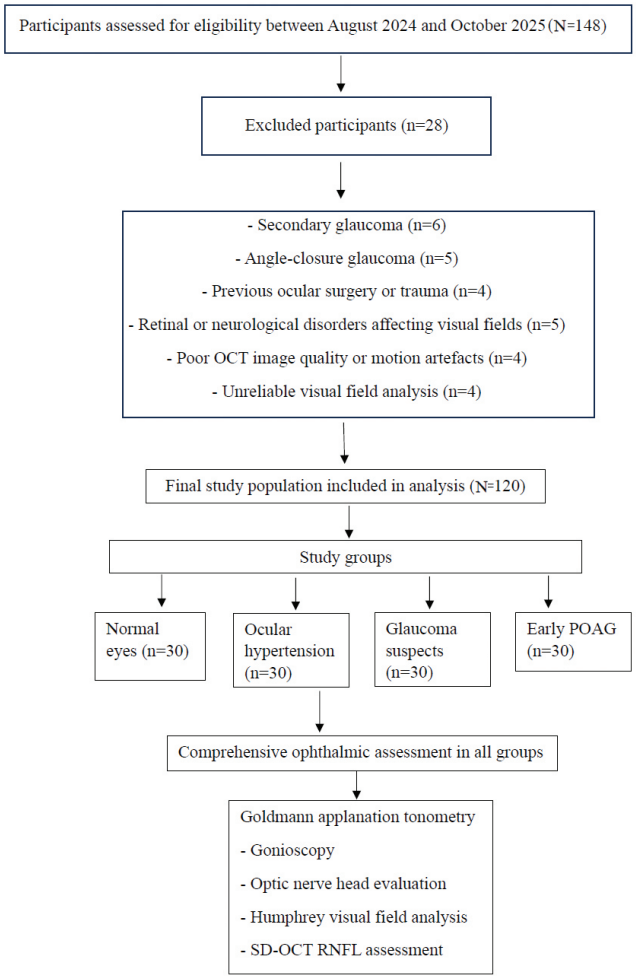
MATERIALS AND METHODS

This hospital-based cross-sectional study was conducted in the OPD of Ophthalmology at a Tertiary Care Hospital SRM Medical College Hospital and Research Centre, SRMIST, Chennai, Tamil Nadu, India, from August 2024 to October 2025. The study protocol was approved by the SRM Institutional Ethics Committee, SRM Medical College Hospital and Research Centre, Tamil Nadu (Ethics Clearance No: SRMIEC-ST0724-1560; approved on 30.07.2024). The study adhered to the tenets of the Declaration of Helsinki [24]. Written informed consent was obtained from all participants after explaining the nature and purpose of the study in their native language prior to enrollment.

Sample size calculation: The sample size was calculated based on the expected difference in mean retinal nerve fibre layer thickness between normal and glaucomatous eyes reported by Godar ST, Kaini KR [25]. They reported a mean RNFL thickness of 94.73±6.76 µm in normal eyes and 88.73±9.59 µm in glaucoma-suspect eyes. For the present sample-size calculation, a mean difference of 6.00 µm and a common standard deviation of 6.74 µm, giving a standardised mean difference of 0.89. For a four-group comparison, with an alpha level of 5% ($Z\alpha$=1.96) and 80% power ($Z1-\beta$=0.84), the minimum required sample size was 29 participants per group. This was rounded to 30 participants per group, yielding a final sample size of 120 participants.

A total of 120 participants aged 21–65 years were included and categorised into four groups comprising normal eyes (n=30), ocular hypertension (n=30), glaucoma suspects (n=30), and early primary open-angle glaucoma (POAG) (n=30). Eligible participants attending the Ophthalmology OPD during the study period were enrolled using a consecutive sampling method based on predefined eligibility criteria. Only one eye per participant was included in the final analysis. In participants in whom both eyes fulfilled the eligibility criteria, the eye corresponding to the predefined diagnostic category was selected for analysis to avoid inter-eye correlation and maintain statistical independence [Table/Fig-1].

Inclusion criteria: Participants with clear ocular media and open anterior chamber angles on gonioscopy were included. Normal eyes demonstrated normal IOP, healthy optic discs, normal RNFL, and absence of glaucomatous visual field defects. Ocular hypertensive eyes had an IOP greater than 21 mmHg without structural or functional glaucomatous damage [16]. Glaucoma suspects demonstrated suspicious optic disc or retinal nerve fibre layer changes, including



[Table/Fig-1]: Study flow diagram.

a vertical cup-to-disc ratio ≥0.6, asymmetry of the cup-to-disc ratio >0.2 between eyes, focal neuro-retinal rim thinning, or violation of the ISNT rule, without definitive, reproducible glaucomatous visual field defects. Early POAG eyes demonstrated glaucomatous optic neuropathy with corresponding reproducible visual field defects on Humphrey 24-2 automated perimetry, open anterior chamber angles on gonioscopy, and mean deviation better than –6 dB according to the Hodapp–Parrish–Anderson classification [26].

Exclusion criteria: Patients with secondary glaucoma, angle-closure glaucoma, advanced glaucoma, previous ocular surgery or trauma, retinal pathology, non glaucomatous optic neuropathy, significant media opacity that affected image quality, neurological disorders that affected visual fields, or unreliable visual field tests were excluded from the study.

Study Procedure

All participants underwent a detailed ophthalmic examination, including best-corrected visual acuity assessment, slit-lamp biomicroscopy, Goldmann applanation tonometry, gonioscopy, pachymetry, and dilated fundus examination. Central corneal thickness and anterior chamber depth were documented using standard methods.

Optic nerve head evaluation included assessment of cup-to-disc ratio, neuro-retinal rim characteristics, rim area, and cup volume. Visual field assessment was performed using the Humphrey Field Analyser 24-2 automated perimetry, and mean deviation values and glaucomatous field defects were recorded.

Optical coherence tomography assessment: The retinal nerve fibre layer thickness was measured using spectral-domain optical coherence tomography APPA OCT-2015 system (Appasamy Associates, Chennai, India) [6,7]. RNFL assessment was performed using a standard circular scan protocol centred on the optic disc. Average RNFL thickness and quadrant-wise RNFL thicknesses involving the

superior, inferior, nasal, and temporal quadrants were recorded. Scans demonstrating segmentation errors, motion artefacts, or poor signal strength (<7) were excluded from analysis, and repeat scans were obtained whenever necessary to ensure adequate image quality.

Outcome measures: The primary outcome was the comparison of average and quadrant-wise retinal nerve fibre layer thickness among the four study groups. The secondary outcome was the diagnostic performance of OCT-derived retinal nerve fibre layer parameters for identifying early primary open-angle glaucoma.

STATISTICAL ANALYSIS

Data were analysed using Statistical Package for the Social Sciences (SPSS) software version 29.0. Continuous variables were expressed as mean±standard deviation, whereas categorical variables were presented as frequency and percentage. Normality of continuous variables was assessed using the Shapiro–Wilk test, and the data were considered approximately normally distributed. One-way Analysis of Variance (ANOVA) was used to compare continuous variables between groups, while categorical variables were analysed using the chi-square test. ROC curve analysis was used to determine the diagnostic performance of OCT-derived parameters. A p-value <0.05 was considered statistically significant.

RESULTS

A total of 120 participants were included in the analysis, comprising normal eyes (n=30), ocular hypertension (n=30), glaucoma suspects (n=30), and early primary open-angle glaucoma (POAG) eyes (n=30). Baseline demographic and clinical characteristics are summarised and average and quadrantwise RNFL data plus optic nerve head parameters are shown in [Table/Fig-2]. No significant difference in age or gender distribution was observed between the study groups.

Variables	Normal eyes (n=30)	Ocular hypertension (n=30)	Glaucoma Suspects (n=30)	Early POAG (n=30)	p-value
Age (in years), (Mean±SD)	43.9±13.6	41.7±12	41.6±11.6	43.4±13.2	0.85
Male n (%)	12 (40.0)	11 (36.7)	11 (36.7)	13 (43.3)	0.94
Central corneal thickness (µm), (Mean±SD)	533.6±10.8	533.3±11.3	529.1±10.5	531.6±12.3	0.40
IOP (mmHg), (Mean±SD)	16.3±2.7	24.3±2.3	22.2±4.6	27.1±3.9	0.01

[Table/Fig-2]: Baseline characteristics of study participants.
Footnote: RNFL: Retinal nerve fibre layer; POAG: Primary open-angle glaucoma; SD: Standard deviation; IOP: Intraocular pressure

IOP was lowest in normal eyes and highest in early POAG, though the ocular-hypertension group showed a numerically higher mean IOP than the glaucoma-suspect group. Central corneal thickness did not differ significantly between the study groups.

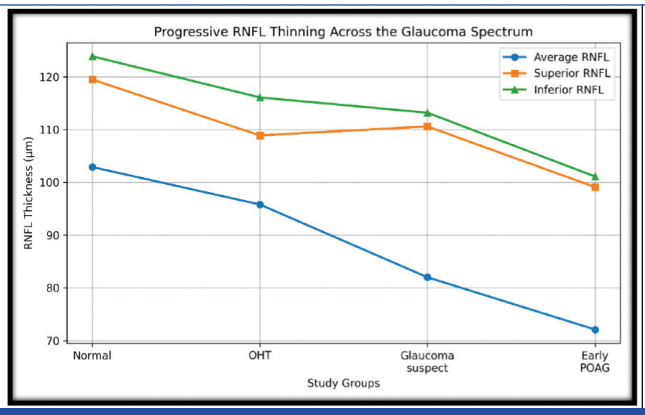
RNFL thickness demonstrated significant structural deterioration across the glaucoma spectrum [Table/Fig-3]. Average RNFL thickness progressively declined from normal eyes to ocular hypertensive eyes, glaucoma suspects, and early POAG eyes (p<0.001). RNFL thinning was observed across all quadrants. The superior and inferior quadrants demonstrated the greatest reduction in RNFL thickness across the study groups, while nasal and temporal quadrant changes were comparatively less pronounced [Table/Fig-4].

Optic nerve head evaluation revealed corresponding progressive structural alterations, including an increasing cup-to-disc ratio and cup volume, with a reduction in neuro-retinal rim area from normal eyes to early POAG eyes.

Receiver operating characteristic analysis comparing eyes with early primary open-angle glaucoma (n=30) with normal eyes (n=30)

Parameters	Normal eyes (n=30)	Ocular hypertension (n=30)	Glaucoma suspects (n=30)	Early POAG (n=30)	p-value
Average RNFL Thickness (µm)	102.9±5.6	95.8±5.9	82.0±6.2	72.1±6.1	<0.001
Superior RNFL Thickness (µm)	119.5±6.5	108.9±5.8	110.6±5.8	99.1±5.9	<0.001
Inferior RNFL thickness (µM)	123.9±5.2	116.1±5.3	113.2±6.7	101.1±5.9	<0.001
Nasal RNFL thickness (µm)	75.2±5.1	67.5±5.3	69.2±4.6	59.9± 6.5	<0.001
Temporal RNFL thickness (µm)	69.6±5.0	61.7±4.5	62.0±4.2	55.3±3.9	<0.001
Cup-to-disc ratio	0.45±0.24	0.61±0.06	0.74±0.05	0.72±0.05	<0.001
Rim area (mm^2)	1.49±0.06	1.23±0.09	1.20±0.07	0.99±0.13	<0.001
Cup volume (mm^3)	0.19±0.02	0.33± 0.05	0.32±0.06	0.51±0.06	<0.001

[Table/Fig-3]: Comparison of RNFL and optic nerve head parameters among study groups.
RNFL: Retinal nerve fibre layer; OCT: Optical coherence tomography



[Table/Fig-4]: Comparison of average and quadrant-wise RNFL thickness among normal eyes, ocular hypertensive eyes, glaucoma suspects, and early POAG eyes.

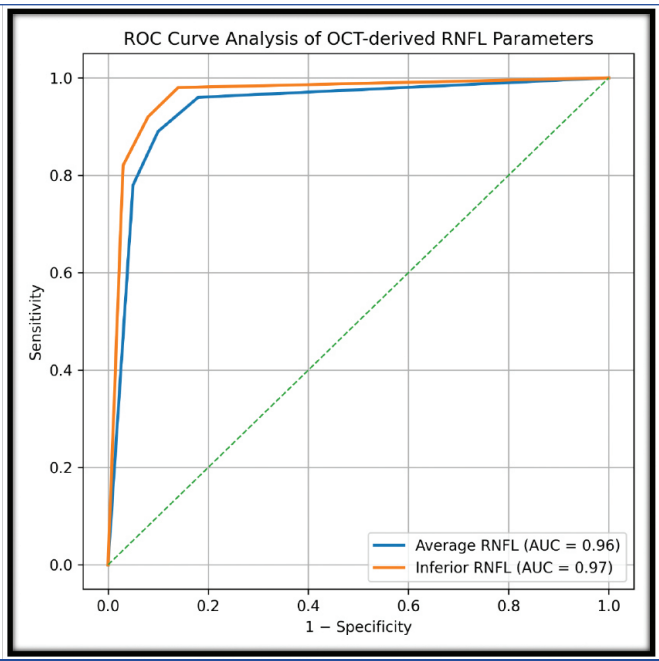
demonstrated excellent diagnostic performance of OCT-derived retinal nerve fibre layer parameters [Table/Fig-5]. Inferior quadrant retinal nerve fibre layer thickness showed the highest diagnostic accuracy (AUC=0.97), followed by average retinal nerve fibre layer thickness (AUC=0.96) [Table/Fig-6].

OCT parameters	AUC (95% CI)	Sensitivity (%)	Specificity (%)	Youden Index	Optimal cut-off for early POAG (µm)
Average RNFL thickness	0.96 (0.92-0.99)	96.7	90.0	0.867	<80
Superior RNFL thickness	0.93 (0.88-0.97)	66.7	97.8	0.645	<102
Inferior RNFL thickness	0.97 (0.95-1.00)	76.7	96.7	0.734	≤105
Nasal RNFL thickness	0.87 (0.80-0.93)	70.0	82.2	0.522	<65
Temporal RNFL thickness	0.90 (0.85-0.96)	70.0	90.0	0.600	<58

[Table/Fig-5]: Receiver Operating Characteristic (ROC) Analysis of OCT-derived retinal nerve fibre layer parameters for differentiating early primary open-angle glaucoma eyes from normal eyes.
The optimal cut-off represents the RNFL thickness threshold providing the maximum Youden Index for discrimination between the two groups. AUC: Area under the curve; CI: Confidence interval; RNFL: Retinal nerve fibre layer

DISCUSSION

The present study demonstrated a consistent reduction in RNFL thickness across the spectrum of early glaucomatous disease, with progressive structural deterioration observed from normal eyes to ocular hypertensive eyes, glaucoma suspects, and early POAG. The findings highlight the utility of Spectral-domain OCT (SD-OCT) in identifying early structural damage even before advanced functional visual field impairment becomes clinically evident.



[Table/Fig-6]: Receiver operating characteristic (ROC) curves of OCT-derived retinal nerve fibre layer parameters for differentiating early primary open-angle glaucoma from normal eyes.

One important observation in the present study was measurable RNFL thinning among glaucoma suspects despite relatively preserved visual field parameters. This finding reinforces the concept that structural neuronal injury precedes detectable functional loss in glaucoma. Histological studies have previously demonstrated that a substantial proportion of retinal ganglion cells may be lost before abnormalities become apparent on standard automated perimetry [4,5]. The present findings further support the growing clinical reliance on OCT-derived structural biomarkers for identifying pre-perimetric glaucomatous damage. Similar observations have been reported by Medeiros FA et al., and Leung CK et al., who demonstrated superior diagnostic sensitivity of RNFL analysis in early glaucoma compared with isolated visual field assessment [27,28].

Among the quadrant-wise measurements, the superior and inferior RNFL regions showed the largest overall reduction in thickness when normal eyes were compared with early POAG eyes. These findings suggest that structural damage may be particularly prominent within these regions during the early stages of glaucomatous optic neuropathy. An earlier study by Nakatani Y et al., and Mwanza JC et al., similarly identified thinning in the inferior and superior quadrants as among the early detectable OCT abnormalities in glaucomatous optic neuropathy [29,30]. The reproducibility of OCT-derived RNFL measurements has also been demonstrated in a previous study, supporting the reliability of these structural measurements for assessing RNFL changes [30]. However, these previous studies did not perform a specific head-to-head comparison of the diagnostic sensitivity of individual OCT parameters. Therefore, the present findings provide a comparative assessment of the diagnostic performance of the evaluated OCT-derived parameters within the current study population. The preferential involvement of the superior and inferior quadrants may provide clinically useful information for identifying structural changes in early glaucomatous optic neuropathy

Another clinically relevant observation was the intermediate structural profile demonstrated by ocular hypertensive eyes and glaucoma suspects. Although these groups did not exhibit the magnitude of RNFL loss observed in established glaucoma, measurable differences in RNFL thickness compared with healthy eyes were evident. As shown in [Table/Fig-2], however, the pattern was not uniformly progressive across all RNFL parameters. The average and inferior RNFL measurements showed a stepwise reduction from normal eyes through ocular hypertension, glaucoma suspects, and early POAG, whereas the superior, nasal, and temporal quadrants showed some variation between the ocular-hypertension and glaucoma-suspect groups. In particular, the glaucoma-suspect group showed numerically higher mean values than the ocular-hypertension group for the superior, nasal, and temporal quadrants. This non monotonic pattern may reflect biological variability and overlap between these clinically defined groups and should therefore not be interpreted as evidence of a uniform sequence of structural loss across all RNFL regions. The ocular hypertension treatment study previously established that not all ocular hypertensive individuals progress to glaucoma; however, the presence of structural alterations in the optic nerve substantially increases the risk of future conversion [31]. The present findings suggest that OCT may assist in characterising structural differences and potentially in risk stratification within these clinically ambiguous groups, although longitudinal follow-up would be required to determine whether the observed structural differences predict subsequent glaucomatous conversion. ROC analysis in the present study demonstrated excellent diagnostic performance of OCT-derived retinal nerve fibre layer parameters for differentiating early primary open-angle glaucoma from normal eyes. Inferior quadrant RNFL thickness demonstrated the highest diagnostic accuracy, followed by average RNFL thickness, indicating that structural alterations in these regions may serve as reliable biomarkers for early glaucomatous damage. Similar observations have been reported in recent studies, which demonstrated that retinal nerve fibre layer and macular ganglion cell complex measurements obtained using spectral-domain optical coherence tomography provide high diagnostic accuracy for early glaucoma detection and complement conventional clinical examination. Furthermore, recent advances in OCT imaging have improved the ability to detect subtle structural changes before clinically significant functional loss becomes evident, thereby facilitating earlier diagnosis, risk stratification, and longitudinal monitoring of glaucoma progression [32-36]. The findings of the present study further support the incorporation of SD-OCT into routine glaucoma evaluation, particularly for individuals at high risk of disease progression, including ocular hypertensive patients and glaucoma suspects.

An additional strength of the present study lies in the simultaneous evaluation of normal eyes, ocular hypertensives, glaucoma suspects, and early POAG eyes within a uniform analytical framework. Many previous studies have focused primarily on clinically manifest glaucoma, whereas the present analysis provides a broader understanding of structural alterations across varying stages of glaucoma risk and early POAG. Furthermore, the study contributes population-specific OCT data from an Indian tertiary-care setting, which may have practical relevance because RNFL thickness profiles can vary across ethnic populations and imaging databases. Overall, the findings of the present study support the role of OCT-derived RNFL analysis as a sensitive structural marker for early glaucomatous damage. Early recognition of quadrant-specific RNFL thinning, particularly within the superior and inferior regions, may facilitate earlier diagnosis, closer surveillance, and more timely therapeutic intervention in eyes at risk of glaucomatous progression.

Limitation(s)

The present study has certain limitations. First, its cross-sectional design precluded longitudinal assessment of disease progression and conversion from ocular hypertension or glaucoma-suspect status to primary open-angle glaucoma. Second, the study was conducted at a single tertiary-care centre with a relatively modest sample size, which may limit the generalisability of the findings. Third, OCT measurements and clinical assessments were predominantly performed by a single observer, and interobserver reproducibility was not formally evaluated. Finally, ganglion cell complex analysis and longitudinal progression mapping were not assessed. Future multicentric prospective studies with larger sample sizes and serial OCT assessments are warranted to validate these findings.

CONCLUSION(S)

Spectral-domain optical coherence tomography appears to be an effective adjunctive tool for detecting early structural glaucomatous damage. Progressive retinal nerve fibre layer thinning was observed across ocular hypertensive eyes, glaucoma suspects, and early primary open-angle glaucoma, with predominant involvement of the superior and inferior quadrants. OCT-derived RNFL assessment demonstrated strong diagnostic performance and may aid in early detection, risk stratification, and monitoring of glaucoma progression.

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