Original Article

Structural evaluation of preperimetric and perimetric glaucoma

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Purpose: To evaluate diagnostic ability of macular ganglion cell layer–inner plexiform layer (GCL-IPL) for detection of preperimetric glaucoma (PPG) and perimetric glaucoma and comparison with peripapillary RNFL. **Methods:** Three hundred and thirty seven eyes of 190 patients were enrolled (127 normals, 70 PPG, 140 perimetric glaucoma). Each patient underwent detailed ocular evaluation, standard automated perimetry, and spectral domain optical coherence tomography. Diagnostic abilities of GCL-IPL and RNFL parameters were determined. Data were compared using one-way analysis of variance, Pearson's Chi-square test, and area under the curve (AUC). **Results:** After adjusting for age, gender, and signal strength, all GCL-IPL and RNFL parameters except mean thickness and disc area differed significantly. Among GCL-IPL thicknesses, inferotemporal had the highest AUC (0.865) for classifying perimetric glaucoma from normals, inferior (0.746) for PPG from normals, and inferotemporal (0.750) for perimetric glaucoma from PPG. When using RNFL, inferior thickness had the highest AUC (0.922) in discriminating POAG from normal, while the same parameter had lower AUC (0.813) in discriminating PPG from normal. The average thickness had maximum AUC (0.775) for discriminating POAG from PPG. For discriminating perimetric glaucoma and normals, inferotemporal GCL-IPL had the highest strength (sensitivity 81.43% and specificity 77.96%), slightly lower than inferior RNFL thickness (sensitivity 87.85% and specificity 84.26%). The same parameters were sensitive in discriminating perimetric glaucoma from PPG (87.14% and 92.85%, respectively). However, their specificities were poor (56.43% both). **Conclusion:** RNFL had better diagnostic ability, when compared with GCL-IPL for detecting PPG and perimetric glaucoma. However, difference was small and may not be clinically relevant.

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Glaucoma is one of the leading causes of irreversible blindness in the world. It is characterized by retinal ganglion cell (RGC) loss leading to classic optic nerve head (ONH) damage and distinctive retinal nerve fiber layer (RNFL) defects.[1] It is known that structural changes precede clinically detectable visual field (VF) defects and it is important to identify the structural changes associated with RGC loss as early as possible.[2-4]

With the advent of spectral domain optical coherence tomography (SD-OCT), structural evaluation can be performed before functional loss. SD-OCT makes the imaging of the inner retinal layers more comprehensive. Cirrus OCT (software version 6.0; Carl Zeiss Meditec, Dublin, CA, USA) has introduced an inbuilt ganglion cell analysis (GCA) algorithm that allows successful and reproducible segmentation of the inner macular layers [i.e., a combination of the ganglion cell layer and the inner plexiform layer (GCL-IPL)].[5,6]

As >50% of all RGCs are concentrated and multilayered in the macular area, macular thickness parameters such as total macular thickness, macular inner retinal layer thickness, and macular ganglion cell complex (GCC) thickness can be used as complementary methods.[5,6] Recent studies have garnered interest in the GCL-IPL and have postulated its early loss as an early sign of glaucomatous damage.[7] Studies with GCA have reported the diagnostic abilities of GCL-IPL parameters to be similar to RNFL parameters in diagnosing glaucoma. Studies have also reported good correlation between the GCL-IPL parameters and the retinal sensitivities in glaucoma.[5,6,8]

There are a handful of studies reported in Indian eyes.[7,9] This study was planned to determine the diagnostic ability of GCL-IPL parameters in preperimetric glaucoma (PPG) and perimetric and compare them with RNFL parameter in Indians.

Methods

This was a hospital-based, retrospective, cross-sectional study performed at a tertiary care center from Indian cohort and included patients who presented between December 2015 and May 2018. The study was approved by the Hospital Ethics Committee and adhered to the Declaration of Helsinki and

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Tokyo. All the patients underwent a medical history review, detailed ocular examination including visual acuity, cycloplegic refraction, slit-lamp examination, indirect ophthalmoscopy, intraocular pressure (IOP) with Goldmann's applanation tonometer, 4 mirror indentation gonioscopy with 4 mirror Sussman's gonioscope, and ONH evaluation with slit-lamp biomicroscopy using 78D noncontact lens. VFs were mapped using Humphrey Visual Field Analyzer (Carl Zeiss Meditec) with the 24-2 Swedish Interactive Threshold Algorithm standard program, and SD-OCT examinations were performed with Cirrus SD-OCT (Carl Zeiss Meditec).

The inclusion criteria were as follows: age more than 18 years, best-corrected visual acuity of Snellens >6/12 (logMAR < 0.3), refractive error (under cycloplegia) between −6 dioptre sphere (DS) myopia, +4 DS hyperopia and ± 3 DS of astigmatism, normal and quiet anterior chamber on slit-lamp examination, open anterior chamber angles on indentation gonioscopy with normal structures, quiet uncomplicated pseudophakic eyes, and standard automated perimetry (SAP) test with reliable indices. VF results were considered reliable if fixation losses were <15%, a false-positive <15%, and false-negative <15%. SD-OCT with signal strength ≥5 and absence of artifacts in the examination circle were included. Images with a signal strength <4, eye movements, blinking artifacts, and segmentation failure were excluded from the study.

Excluded were the patients with media opacity, history of trauma, history of any intraocular surgery including complicated pseudophakia, retinal pathology affecting macula, previous laser therapy, neurologic disease that could affect the VF, and moderate or severe glaucomatous damage.

Glaucoma was defined according to Anderson and Patella's criteria, which included glaucoma hemi-field test outside normal limits; a pattern standard deviation probability of <5%; or a cluster of three or more adjacent nonedge points in typical glaucomatous locations that did not cross the horizontal meridian, all of which were depressed on the pattern deviation plot at *P* <5%, and 1 of which was depressed at *P* <1%, on at least two consecutive examinations [Fig. 1].

PPG was defined as eyes with normal VF results and one or more localized RNFL defects (on red-free fundus photographs) that were associated with a glaucomatous disc appearance (e.g., notching or thinning of neuroretinal rim) and IOP more than 21 mmHg [Fig. 2].

Normals were eyes with no history of ocular disease, an IOP of ≤21 mmHg, a normal optic disc, SAP within normal limits, and normal OCT.

OCT procedure

OCT image acquisition was carried out after pupillary dilation by a single operator. Images with signal strength <5, lost data on the peripapillary ring, motion artifact, or incorrect segmentation were excluded. The Optic Disc Cube 200 × 200

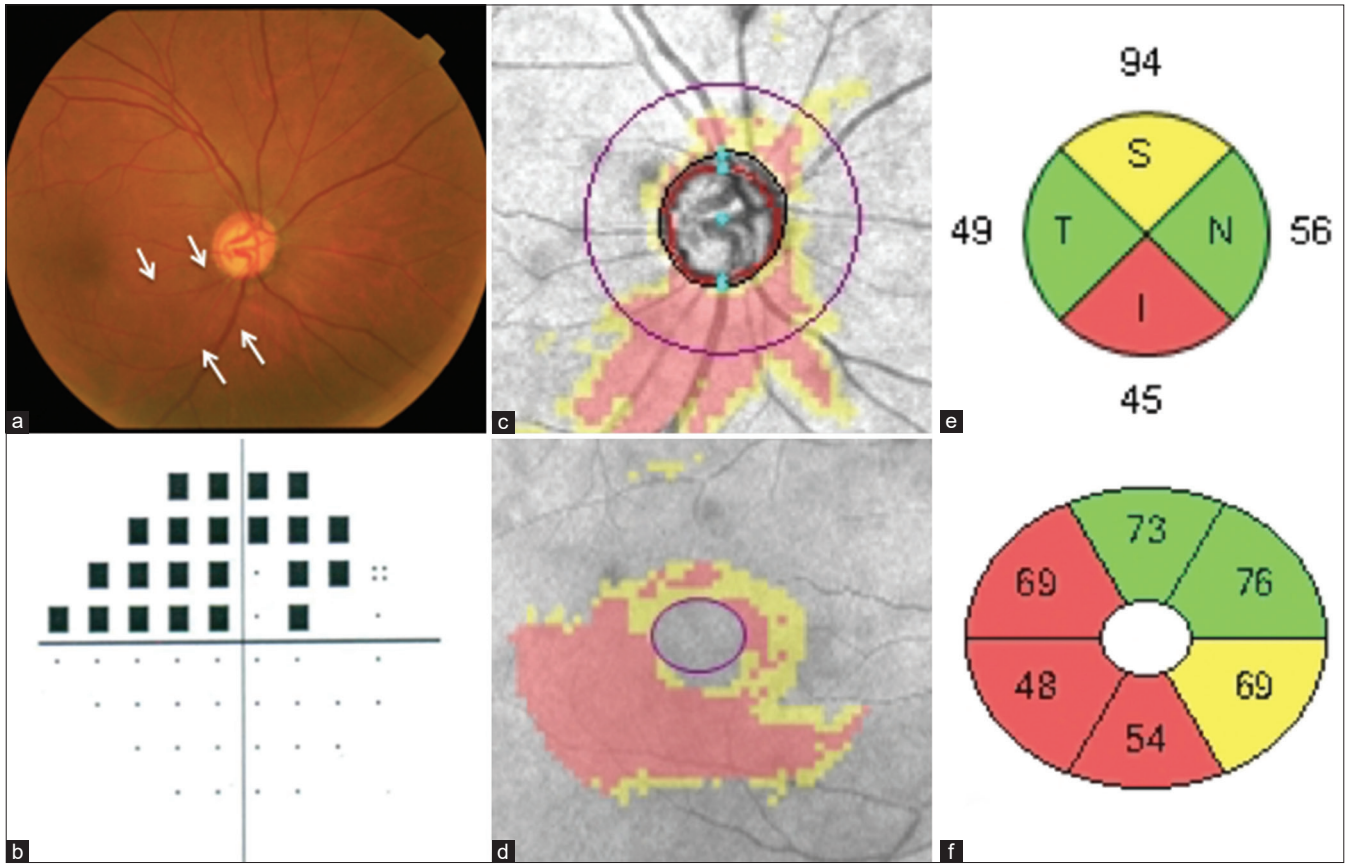

Figure 1: A 78-year-old male in the perimetric glaucoma group. (a) Fundus photography showing diffuse RNFL defect. (b) Superior visual field on automated perimetry. (c, d) Peripapillary RNFL and GC-IPL deviation maps showing thinning in superior and inferior region. (e, f) Defects on RNFL quadrant and GC-IPL sector analysis

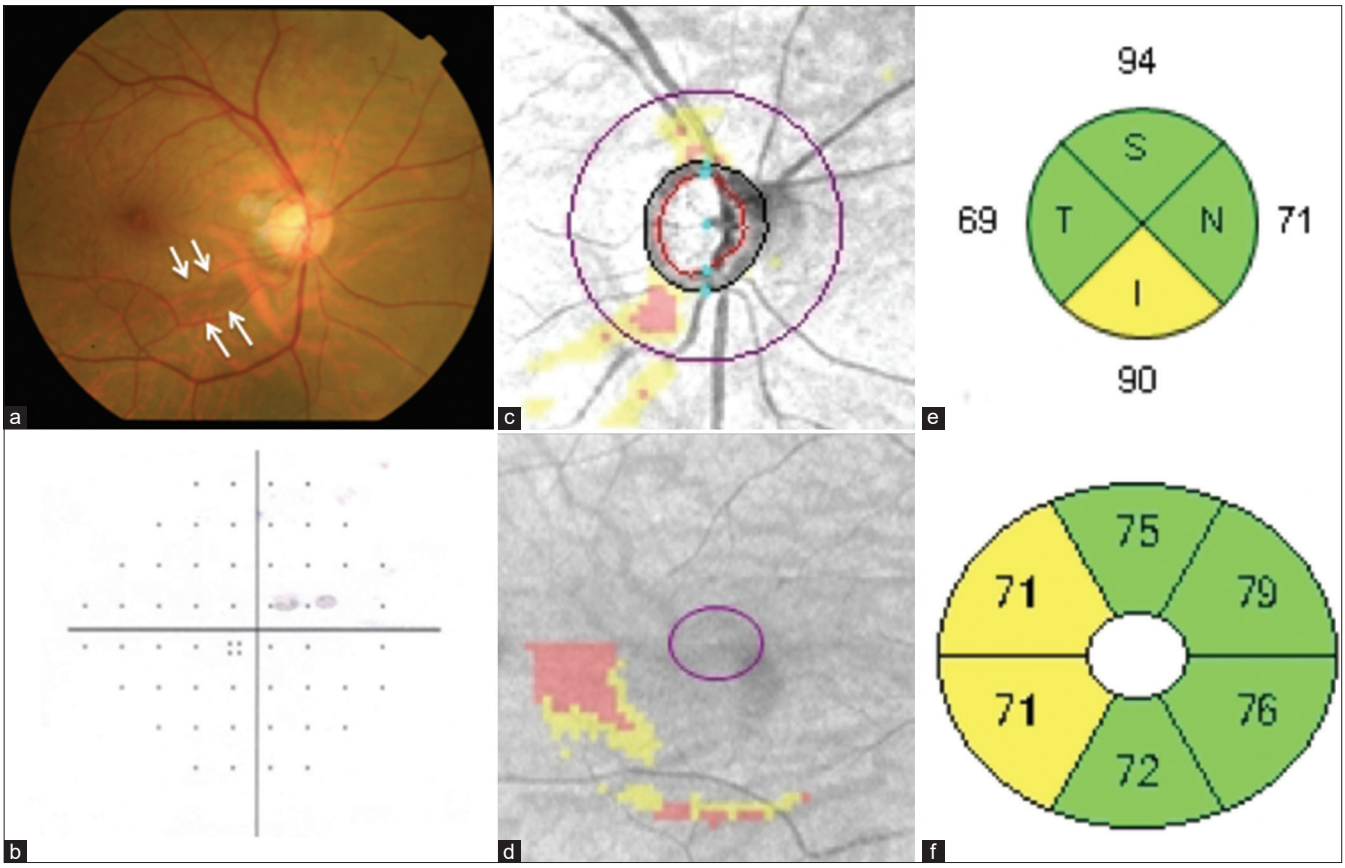

Figure 2: A 45-year-old male in the preperimetric glaucoma group. (a) Fundus photography showing localized RNFL defect. (b) Normal visual field on automated perimetry. (c, d) Peripapillary RNFL and GC-IPL deviation maps showing thinning in inferior region. (e, f) Defects on RNFL quadrant and GC-IPL sector analysis

consisted of 40,000 axial scans (in a 6 × 6 × 2 mm cube) centered on the optic disc. The average RNFL thickness and RNFL thickness in quadrants on a measurement circle 3.46 mm in diameter were calculated, and their deviation from a normative database was provided in a color-coded scheme. RNFL pseudocolor thickness maps and deviation maps for the 6 × 6 mm area were also provided. The ONH and RNFL parameters identified were average RNFL thickness, rim area, disc area, average C/D ratio, vertical C/D ratio, cup volume, and superior, inferior, temporal, and nasal RNFL quadrant thicknesses.

The GCL-IPL analysis available on the Cirrus software version 6.0 (or higher) measured the combined thickness of RNFL, GCL, and IPL in a 4.8 × 4.0 mm oval with a longer horizontal axis. It provided measurements in six wedge-shaped sectors after excluding the central foveolar region (1 mm in diameter) along with a pseudocolor scheme for the GCL-IPL thickness. A deviation map also flagged abnormally thin areas within the oval area as yellow ($P < 5\%$) or red ($P < 1\%$) superpixels. The parameters found were average GCL-IPL, minimum GCL-IPL and sector measurements (superonasal, superior, superotemporal, inferonasal, inferior, and inferotemporal).

Statistical analysis

Data were compared using one-way analysis of variance, Pearson's chi-square test, and area under the curve (AUC).

Results

The demographics and structural parameters of paramacular and peripapillary areas were obtained for patients diagnosed with perimetric glaucoma and PPG as well as the normal individuals. The mean age of patients in normal group was 54.25 ± 9.36 years, while in perimetric group was 61.22 ± 9.69 years and PPG group was 56.85 ± 10.52 years.

Both age and signal strength differed significantly across the groups and analysis of covariance was used to adjust the measurements of each parameter in each group [Tables 1 and 2]. All structural parameters showed significant difference of means across the groups, except mean thickness on GCL-IPL and temporal quadrant thickness on RNFL; with P <0.0001.

The ability of GCL-IPL and RNFL parameters in classifying three groups was determined based on area under the ROC curve [Table 3]. The AUCs and sensitivities at fixed specificities of the RNFL and GCL-IPL parameters to differentiate perimetric and PPG from control eyes are shown in Table 2.

Among GCL-IPL parameters, inferotemporal sector thickness had maximum AUC of 0.865 in classifying perimetric and normal groups and was followed by average inferior thickness (AUC 0.857), inferior sector thickness (AUC 0.835), and average GCL-IPL thickness (AUC 0.83). For discriminating PPG and normal groups, the average inferior thickness had maximum AUC of 0.746,

Table 1: Descriptive statistics for demographic parameters and signal strength of GCL-IPL and RNFL scans in three patient groups

Parameters	Diagnosis			Statistic	*P*
	Normal (*n*=127)	POAG (*n*=140)	PPG (*n*=70)		
Age in years (M±SD)	54.25±9.36	61.22±9.69	56.85±10.52	17.33	**<0.0001***
Gender [no. (%)]					
Male	63 (49.61)	85 (60.71)	33 (47.14)	4.84	**0.0891**†
Female	64 (50.39)	55 (39.29)	37 (52.86)		
GCL-IPL					
S/S (M±SD)	6.02±0.53^{a}	5.83±0.56^{bc}	6.00±0.74^{abc}	3.34	**0.0196***
RNFL					
S/S (M±SD)	5.87±0.39^{a}	5.57±0.7^{c}	5.77±0.68^{abc}	9.12	**<0.0001***

GCL-IPL=ganglion cell layer-inner plexiform layer; RNFL=retinal nerve fiber layer; PPG=preperimetric glaucoma; SD=standard deviation; S/S=signal strength, **P*-values obtained using one-way analysis of variance; †*P*-value obtained using Pearson's Chi-square test; POAG=Primary open angle glaucoma; Similar superscripts corresponding to means indicate statistical insignificance; Bold: Highlighted are the AUCs which are maximum in the category and the comparison, which are relevant to the study and discussion

Table 2: Descriptive statistics for structural features of GCL-IPL and RNFL in three patient groups after adjusting with age and signal strength

Parameters	Diagnosis			*F*	*P**
	Normal (*n*=127)	POAG (*n*=140)	PPG (*n*=70)		
GCL-IPL					
MT (M±SD)	254.12±11.42	255.77±13.51	258.28±12.00	2.48	0.0849
SN (M±SD)	81.45±8.46^{a}	71.82±14.33^{b}	72.15±14.38^{b}	27.22	**<0.0001**
S (M±SD)	78.87±8.74^{a}	69.49±14.6^{b}	71.92±12.26^{b}	23.36	**<0.0001**
Superior temporal (M±SD)	76.38±8.37^{a}	65.62±12.93^{b}	71.85±11.71^{c}	32.32	**<0.0001**
IN (M±SD)	79.12±9.52^{a}	66.63±14.23^{b}	72.72±12.00^{c}	37.76	**<0.0001**
I (M±SD)	76.73±10.5^{a}	62.62±13.38^{b}	68.94±13.17^{c}	45.27	**<0.0001**
IT (M±SD)	77.61±9.09^{a}	61.61±13.22^{b}	71.52±10.86^{c}	68.05	**<0.0001**
Avg GCL-IPL (M±SD)	78.39±8.21^{a}	66.31±12.72^{b}	71.48±11.30^{c}	43.88	**<0.0001**
Min GCL-IPL (M±SD)	72.64±11.42^{a}	56.41±15.32^{b}	65.29±15.71^{c}	49.23	**<0.0001**
Average GCL-IPL (M±SD)	0.29±0.05^{a}	0.22±0.06^{b}	0.25±0.06^{c}	47.02	**<0.0001**
Average S (M±SD)	0.31±0.03^{a}	0.27±0.05^{b}	0.28±0.05^{b}	32.66	**<0.0001**
Average I (M±SD)	0.31±0.04^{a}	0.25±0.05^{b}	0.28±0.04^{c}	62.51	**<0.0001**
Average S vs. I (M±SD)	1.02±0.09^{a}	1.10±0.15^{b}	1.02±0.11^{a}	14.31	**<0.0001**
RNFL					
Avg RNFL thickness (M±SD)	87.31±7.83^{a}	68.52±11.54^{b}	78.78±7.43^{c}	141.82	**<0.0001**
S (M±SD)	109.94±12.77^{a}	85.61±19.43^{b}	98.22±14.42^{c}	83.77	**<0.0001**
N (M±SD)	68.43±9.57^{a}	57.83±8.68^{b}	62.52±7.51^{c}	51.77	**<0.0001**
I (M±SD)	112.90±13.16^{a}	78.77±20.54^{b}	96.15±16.84^{c}	141.83	**<0.0001**
S/I (M±SD)	0.97±0.10^{a}	1.13±0.27^{b}	1.14±1.05^{ab}	3.59	**0.0287**
T (M±SD)	58.01±8.57^{a}	51.74±10.96^{b}	56.36±8.79^{ab}	5.49	**0.0045**

GCL-IPL=ganglion cell layer-inner plexiform layer; RNFL=retinal nerve fiber layer; PPG=preperimetric glaucoma; SD=standard deviation; MT=mean thickness; SN=superior nasal; S=superior; I=inferior; IT=inferior temporal; IN=inferior nasal; N=nasal; S/I=superior by inferior; T=temporal, Means obtained after adjusting age and signal strength; **P*-values obtained using one-way analysis of variance; Similar superscripts corresponding to means indicate statistical insignificance; Bold: Highlighted are the AUCs which are maximum in the category and the comparison, which are relevant to the study and discussion

followed by superonasal sector thickness (AUC 0.741), inferotemporal sector thickness (AUC 0.714), and inferior sector thickness (AUC 0.714). Inferotemporal sector thickness also provided best classification of perimetric glaucoma and PPG groups with maximum AUC of 0.750. The remaining parameters resulted in AUC near or less than 0.7.

While analyzing RNFL parameters, inferior quadrant thickness and average RNFL thickness had the best discriminating ability; AUC of 0.922 and 0.923, respectively, when comparing perimetric glaucoma to normals. Inferior quadrant thickness also had maximum AUC of 0.813 when classifying PPG from normals. The average RNFL thickness (AUC 0.775) and inferior quadrant thickness (AUC 0.758) had maximum strength in classifying perimetric from PPG group; the remaining parameters resulted in AUCs near or less than 0.7.

Thus, inferotemporal sector thickness and inferior quadrant thickness, which were the most discrimintive GCL-IPL and RNFL parameters, respectively, were considered for obtaining the cut-offs for three paired comparisons of groups. Sensitivity at 95% specificity was obtained for each cut-off [Fig. 3 and Table 4]. For diagnosing perimetric glaucoma, the cut-off for inferotemporal sector thickness was 73.39 µm, which had maximum sensitivity (81.43) and specificity (77.96%);

Table 3: AUC for GCL-IPL and RNFL parameters

Parameters	POAG/normal	PPG/normal	POAG/PPG
GCL-IPL			
MT	0.569 (0.500, 0.638)	0.614 (0.533, 0.695)	0.527 (0.447, 0.607)
SN	0.757 (0.699, 0.814)	0.741 (0.667, 0.816)	0.527 (0.445, 0.609)
S	0.735 (0.675, 0.796)	0.701 (0.623, 0.779)	0.559 (0.479, 0.639)
Superior temporal	0.787 (0.732, 0.841)	0.647 (0.565, 0.728)	0.667 (0.590, 0.743)
IN	0.798 (0.745, 0.851)	0.706 (0.630, 0.782)	0.640 (0.563, 0.717)
I	0.835 (0.786, 0.883)	0.714 (0.643, 0.786)	0.679 (0.602, 0.757)
IT	**0.865 (0.820, 0.910)**	**0.714 (0.642, 0.787)**	**0.750 (0.681, 0.819)**
Avg GCL-IPL	0.830 (0.781, 0.879)	0.736 (0.665, 0.807)	0.651 (0.574, 0.729)
Min GCL-IPL	0.842 (0.794, 0.890)	0.670 (0.593, 0.747)	0.711 (0.632, 0.789)
Average S	0.773 (0.717, 0.829)	0.736 (0.662, 0.810)	0.557 (0.477, 0.638)
Average I	0.857 (0.812, 0.902)	**0.746 (0.677, 0.815)**	0.688 (0.613, 0.763)
Average S vs. I	0.665 (0.598, 0.731)	0.517 (0.427, 0.607)	0.646 (0.570, 0.721)
RNFL			
Avg RNFL thickness	**0.923 (0.892, 0.953)**	0.798 (0.734, 0.862)	**0.775 (0.714, 0.837)**
S	0.867 (0.826, 0.909)	0.750 (0.677, 0.823)	0.701 (0.632, 0.771)
N	0.792 (0.738, 0.845)	0.690 (0.615, 0.765)	0.658 (0.583, 0.733)
I	**0.922 (0.890, 0.954)**	**0.813 (0.750, 0.876)**	**0.758 (0.693, 0.824)**
S/I	0.638 (0.572, 0.705)	0.576 (0.489, 0.662)	0.575 (0.497, 0.653)
T	0.686 (0.623, 0.749)	0.559 (0.474, 0.643)	0.634 (0.558, 0.710)

AU=area under the curve; GCL-IPL=ganglion cell layer-inner plexiform layer; RNFL=retinal nerve fiber layer; PPG=preperimetric glaucoma; MT=mean thickness; SN=superior nasal; S=superior; I=inferior; IT=inferior temporal; IN=inferior nasal; N=nasal; S/I=superior by inferior; T=temporal, AUC with 95% confidence interval in different subgroups; Bold: Highlighted are the AUCs which are maximum in the category and the comparison, which are relevant to the study and discussion

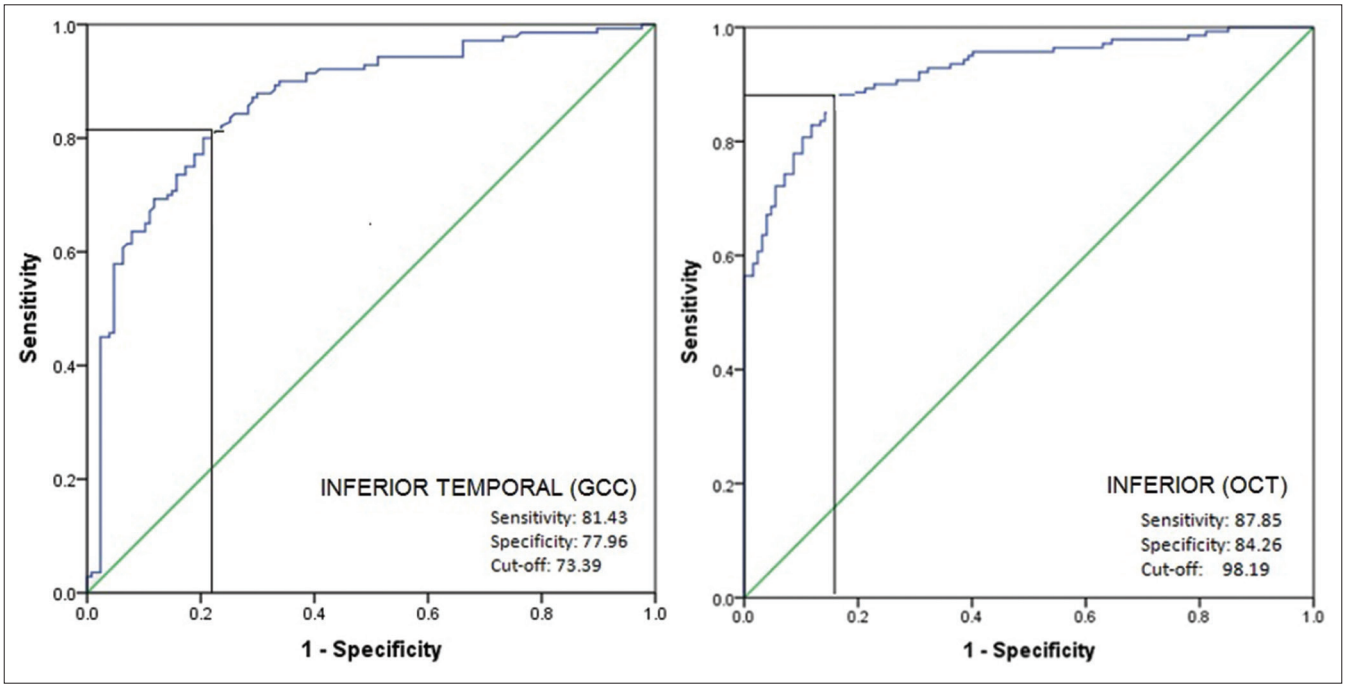

Figure 3: Left: ROC plot for inferior temporal GCC parameter for the comparison of POAG vs. normal. Right: ROC plot for inferior OCT parameter for the comparison of POAG vs. normal

Table 4: Sensitivity and specificity with IT and I of GCL-IPL and RNFL

Group	Cut-off (μm)	Sensitivity (95% CI)	Specificity (95%CI)
GCL-IPL - inferotemporal sector thickness			
POAG/normal	**73.39**	**81.43 (73.98, 87.50)**	**77.96 (69.74, 84.82)**
PPG/normal	75.98	65.71 (53.40, 76.65)	69.30 (60.41, 77.17)
POAG/PPG	64.46	87.14 (76.99, 93.95)	56.43 (47.80, 64.78)
RNFL - inferior quadrant thickness			
POAG/normal	**98.19**	**87.85 (81.27, 92.76)**	**84.26 (76.37, 90.11)**
PPG/normal	106.2	85.71 (75.29, 92.93)	67.72 (58.85, 75.74)
POAG/PPG	77.15	92.85 (84.11, 97.64)	56.43 (47.80, 64.78)

IT=inferior temporal; I=inferior; GCL-IPL=ganglion cell layer-inner plexiform layer; RNFL=retinal nerve fiber layer; CI=confidence interval; PPG=preperimetric glaucoma; Bold: Highlighted are the AUCs which are maximum in the category and the comparison, which are relevant to the study and discussion

however, the inferior RNFL quadrant thickness was 98.19 μm with higher sensitivity of 87.85% and specificity of 84.26%. For diagnosing PPG, the inferotemporal sector thickness cut-off was 75.98 μm with poor sensitivity and specificity. And the inferior quadrant thickness had a cut-off of 106.2 μm with fair sensitivity and poor specificity.

Discussion

This study confirmed that the diagnostic ability of peripapillary RNFL is better than macular GCL-IPL parameters in patients with both PPG and perimetric glaucoma.

In our study, we evaluated the diagnostic ability of GCL-IPL and RNFL parameters in identifying PPG and perimetric group from normal eyes. We found that age and signal strength varied significantly among the three groups and had to be adjusted before analysis. The cause of the latter could not be explained.

Our findings are similar to previous studies reported in literature in that all the AUCs of the RNFL parameters were better than GCL-IPL parameters in both the PPG and perimetric group.[7] It is known that the macula has the maximum density of RGCs; nevertheless, only 50% of the total retinal RGCs reside at the macula.[7] The GCL-IPL samples only that confined area, and any RGC damage outside the scan area is not identified. The RNFL scan includes axons of the RGCs at the entire peripapillary region, and hence has a better diagnostic power. The other reason, as cited by Begum *et al*., states that gold standard definition of glaucoma in all similar studies evaluating the diagnostic ability of macular parameters is based on the optic nerve and RNFL changes evaluated by the experts and not on macular changes.[7] This is because the glaucomatous changes at macula, unlike the changes at ONH and RNFL, are not detectable clinically. Such a bias is known to inflate the diagnostic abilities of ONH and RNFL parameters.[10]

Recently, Michelessi *et al*. studied the Spectralis SD-OCT and found that the minimum rim width and peripapillary RNFL analysis performed statistically and clinically better than macular analysis to discriminate early glaucoma from healthy eyes in an Italian cohort.[11]

When we compared the sensitivities at 95% specificity among the groups, the inferior quadrant thickness on RNFL performed better than the inferotemporal sector thickness on GCL-IPL. However, in contrast, Na *et al*. reported a significant difference in the macular GCL-IPL thickness between healthy control eyes and eyes with PPG, which suggested that macular GCL-IPL thickness might serve as an early indicator of glaucomatous structural damage.[12] There are studies that have found the inferior, inferotemporal, and minimum GCL-IPL sectors to be the ones showing thinning and, therefore, better AUCs and sensitivities to diagnose perimetric and PPG.[5,13-15] However, a majority of these studies were carried out in Korean eyes. It is known that the prevalence of normal tension glaucoma with parafoveal lesions is higher, and hence GCL-IPL analysis gave better AUCs. Our study results vary because of racial differences.

Hwang *et al*. found that all the GCL-IPL parameters showed good glaucoma diagnostic ability in their study. AUCs of average, superior, and inferior GCL-IPL thickness increased as the severity of glaucoma increased. Barua *et al*. found that GCC and RNFL parameters showed equal predictive capability in perimetric versus normal group. In early stage, inferior GCC was the best parameter.[16]

We have several limitations to our study. We included early, moderate, and advanced glaucoma in our perimetric group, which may have lead to potential skewing of data. However, on further analysis beyond the scope of this study, it was found that few eyes had advanced damage and majority had early to moderate glaucoma. We included only eyes with IOP more than 21 mmHg, and hence our results may not be applicable to normal tension glaucoma eyes. We excluded eyes with age-related macular degeneration; however, as glaucoma is generally seen in geriatric eyes, our results may not be applicable to them as well. We excluded patients with high ametropia. To represent the general population, further studies, including a wide range of refractive errors, are necessary.

As stated by Begum *et al*., PPG in our study was diagnosed based on a single evaluation of the ONH. There is a possibility of a few optic discs diagnosed as PPG actually being normal physiological variants.[7] Medeiros *et al*., therefore, have recommended longitudinal evaluation of optic discs, especially for progressive loss in rim area, for detecting change and definitively diagnosing PPG.[17]

Conclusion

In conclusion, the diagnostic ability of GCL-IPL parameters was less than RNFL when classifying perimetric and PPG in Indian cohort.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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