Original Article

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Retinal Nerve Fibre Layer and Ganglion Cell Complex Measurement and their Correlation with Visual Field Changes among Glaucoma Patients

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ABSTRACT

Introduction: Glaucoma is an optic neuropathy that causes loss of Retinal Ganglion Cell (RGC) and changes in the visual field. The loss in RGC is determined by Optical Coherence Tomography (OCT), and visual field defects are evaluated with perimetry. A study of RGC loss and visual field defects together might help to detect glaucoma earlier.

Aim: To determine the relationship between Retinal Nerve Fibre Layer (RNFL) and Ganglion Cell Complex (GCC) thickness measured with OCT, and visual field sensitivity evaluated with Standard Automated Perimetry (SAP) in glaucoma patients of varying severity.

Materials and Methods: This was a cross-sectional study, conducted in a tertiary care hospital, Ajmer, Rajasthan from October 2018 to January 2020. Sixty glaucoma patients were recruited for ophthalmic evaluation. The two test SAP and SD-OCT were performed on all patients. Patients were categorised into three groups i.e., early, moderate and severe, based on severity. The RNFL, Mean Deviation (MD) and Pattern Standard Deviation (PSD) were compared among these three groups. One way Analysis of Variance (ANOVA) was used to compare the mean values of RNFL, GCC, MD and PSD across the three glaucoma groups. Post hoc analysis was done by Tukey's range test. Correlation was assessed using Spearman's correlation coefficient.

Results: The mean (SD) age of participants were 54.43 (10.07) years. Total 15% of patients belonged to early stage glaucoma, 40% to moderate and 45% to severe glaucoma. The difference in mean RNFL (both superior and inferior) was found significant among the three groups. In bivariate analysis, MD was found significantly correlated with superior and inferior RNFL (r=0.70, p-value <0.01 and r=0.47, p-value <0.01, respectively). The MD was not found to correlate with superior and inferior GCC whereas, PSD was found weakly correlated with GCC superior and inferior (r=-0.26, p-value=0.04 and r=-0.27, p-value=0.03, respectively), though there was inverse correlation with both GCC superior and GCC inferior. PSD was not found to correlate with superior and inferior RNFL.

Conclusion: Superior and inferior RFNL were associated with MD, an indicator of the severity of glaucoma. The RNFL thickness was the best indicator to differentiate the severity of glaucoma. Further exploration is needed to develop specific indications of RNFL and GCC measurement in the management of glaucoma.

Keywords: Optical coherence tomography, Optic neuropathy, Perimetry, Retinal ganglion cell

INTRODUCTION

Glaucoma is an optic neuropathy that causes loss of RGC and changes in visual field [1]. It is one of the leading causes of blindness [2]. Diagnosis of glaucoma is based on the classical triad of elevated Intraocular Pressure (IOP), glaucomatous optic atrophy or cupping, and visual field loss [3]. However, the progression of glaucoma is monitored by visual field defect evaluation [1,4]. SAP is the gold standard for visual field testing [5]. However, functional defects are revealed after significant structural damage, and compliance to SAP depends on patient attention levels [1,4,6].

Evaluation of structure and function together might help to detect glaucoma earlier. RNFL imaging and GCC imaging from OCT has been found useful to detect and monitor glaucomatous changes in various studies [1,4-12]. Their relation with perimetry parameters has been studied over the last few years [1,4-12]. Localised or diffused thinning of RNFL represents the loss of RGCs of varying degrees. It is determined by OCT, which distinguishes normal eyes from glaucomatous eyes. Macular Ganglion Cell Complex (GCC) is also known to be affected in glaucoma. Glaucoma targets the cells in the area of their highest concentration i.e., GCC. The relevance of GCC thickness to diagnosing glaucoma was studied by many researchers [4,6,8,9,12].

In glaucoma, structural changes precede loss of visual function. Therefore, it is important to study the structural and functional changes together to detect glaucoma at its various stages. There are limited studies available from India which has studied the structural and functional changes together to detect glaucoma. So, the present study aimed to determine the relationship between visual field sensitivity evaluated with SAP, RNFL and GCC thickness measured with OCT in glaucoma patients of varying severity.

MATERIALS AND METHODS

This was a cross-sectional study, conducted in the Department of Ophthalmology in JLN Medical College in central Rajasthan from October 2018 to January 2020 with the purposive sampling technique. Total sixty glaucoma patients were recruited for the study. The study was approved by the Institutional Review Board of JLN Medical College, Ajmer, Rajasthan. (No. 979/2019). The study followed all the tenets of the Declaration of Helsinki. Written informed consent was obtained from each participant before data collection.

Inclusion criteria: Patients more than 40 years, Best Corrected Visual Acuity (BCVA) atleast finger count from six meters, glaucomatous optic neuropathy, good corneal clarity, no significant cataract, and pupil diameter greater than 3 mm were included in the study.

Exclusion criteria: Subjects with evidence of any anterior segment pathology, any lens pathology, congenital glaucoma, history of any other known systemic or ocular disease, and patients with unreliable

visual fields defined as false negative >33%, false positive >33%, and fixation errors >20% were excluded from the study.

Each participant underwent ophthalmic evaluation, visual acuity testing, IOP, slit lamp biomicroscopy, ophthalmoscopy, visual field testing and OCT.

Study Procedure

Distant and near visual acuity was assessed by Snellen's Chart [13]. Refraction was done with an auto refractometer. The IOP was taken with goldmann applanation tonometer and values were presented in mmHg. Anterior segment was evaluated with slit lamp bio microscopy. Angle was assessed by gonioscopy. Fundus was evaluated with direct and indirect ophthalmoscope.

Visual field was examined with automated perimetry using Swedish Interactive Thresholding Algorithm (SITA) Standard 30-2 perimetry. The criteria to define glaucomatous defect were, a threshold sensitivity loss of two or more contiguous test locations on the pattern deviation plot with p<0.01, three or more such contiguous test locations with p<0.05 with atleast one of the points depressed to p<0.01, or a 10 dB difference across the nasal horizontal midline at two or more test locations [14]. Humphrey global parameters, MD and PSD were used for comparison and correlation [15].

Optic disc changes were evaluated with spectral domain OCT. RNFL thickness was assessed by Optic Nerve Head (ONH) mode with a 3.45 mm diameter around the optic disc.

The GCC was assessed with a focus of 1 mm temporal to the fovea and a square grid (7×7 mm) on the central macula. Superior and inferior GCC thicknesses were calculated. The pupil size of each subject was assessed under ambient conditions before the scan. Those with a diameter of less than 4 mm were dilated with 0.5% tropicamide.

Patients were categorised into three groups i.e., early, moderate and severe, based on severity according to Hodapp, Parrish and Anderson classification [16].

STATISTICAL ANALYSIS

Statistical analysis was done using Statistical Package for Social Sciences (SPSS) version 20.0. Categorical variables such as gender and type of glaucoma were presented as proportion, and continuous variables such as age, RNFL/GCC thickness, MD, PSD were presented as mean (SD). Bivariate correlation between RNFL/GCC thickness and visual field indices (MD and PSD) were assessed with Spearman's correlation coefficient. The mean values of RNFL, GCC, MD and PSD were compared using One way ANOVA. Post hoc analysis was done by Tukey's range test. Receiver Operating Characteristic (ROC) curves were used to assess the accuracy of RNFL and GCC thickness to diagnose glaucoma. The p<0.05 was considered statistically significant for all statistical analyses.

RESULTS

A total of 67 subjects were recruited for the study, out of which seven were excluded due to poor OCT imaging. Sixty patients were included to determine the relationship between RNFL/GCC thickness and visual field sensitivities.

The mean (SD) age of participants were 54.43 (10.07) years. Out of sixty participants, 40 were male and 20 were female. The participants had 25%, 48.33% and 26.67% open, narrow and close angle glaucoma, respectively. The mean (SD) IOP in the study sample was 23.12 (4.01) mmHg. The mean (SD) RNFL thickness for superior and inferior region were 81.35 (26.97) um and 78.15 (29.6) um, respectively. Average superior and inferior GCC thickness were found 80.27 (30.98) um and 77.07 (32.03) um, respectively. The mean (SD) MD and PSD were found to be -14.23 (7.73) dB and 7.77 (2.77) dB, respectively [Table/Fig-1].

Characteristic	Parameters	Value
Age (Years), Mean (SD)		54.43 (10.07)
Gender n (%)	Male	40 (66.67)
	Female	20 (33.33)
Glaucoma n (%)	Open angle	15 (25.0)
	Narrow angle	29 (48.33)
	Close angle	16 (26.67)
IOP (mmHg), Mean (SD)		23.12 (4.01)
RFNL (um), Mean (SD)	Superior	81.35 (26.97)
	Inferior	78.15 (29.6)
GCC (um), Mean (SD)	Superior	80.27 (30.98)
	Inferior	77.07 (32.03)
MD (dB), Mean (SD)		-14.23 (7.73)
PSD (dB), Mean (SD)		7.77 (2.77)
CPSD (dB), Mean (SD)		7.06 (2.77)

[Table/Fig-1]: Characteristics of participants (n=60).
IOP: Intraocular pressure; RNFL: Retinal nerve fibre layer; GCC: Ganglion cell complex; MD: Mean deviation; PSD: Pattern standard deviation; CPSD: Corrected PSD

About 9 (15%) of patients belonged to early stage glaucoma, 24 (40%) to moderate and 27 (45%) to severe glaucoma. The difference in mean RNFL (both superior and inferior) was found significant among the three groups. On post hoc analysis, a significant difference in RNFL superior was found between early and severe, and between moderate and severe. Mean of RNFL inferior was found significantly different between moderate and severe glaucoma in post hoc analysis. Mean of MD and PSD were also found significantly different among the three groups. In the post hoc analysis, this difference was observed across the groups [Table/Fig-2,3].

Glaucoma	Early (n=9, 15%)	Moderate (n=24, 40%)	Severe (n=27, 45%)	p-value
IOP (mmHg)	22.16 (3.83)	22.74 (2.81)	23.77 (4.89)	0.492
RNFL superior (um)	108.67 (9.18)	90.25 (24.59)	64.33 (21.41)	<0.01*
RNFL inferior (um)	86.33 (23.16)	87.17 (31.67)	67.41 (26.96)	0.037*
GCC superior (um)	80.0 (12.34)	77.79 (24.65)	82.56 (39.79)	0.864
GCC inferior (um)	78.44 (16.23)	73.54 (24.91)	79.74 (40.97)	0.786
CCT (um)	516.56 (20.24)	511.67 (48.10)	492.26 (34.47)	0.128
MD (dB)	-5.51 (0.42)	-9.15 (1.76)	-21.66 (5.04)	<0.01*
PSD (dB)	5.44 (1.93)	6.73 (2.54)	9.46 (2.16)	<0.01*

[Table/Fig-2]: Comparison of mean (SD) values of OCT and visual field parameters according to severity of glaucoma.
*Statistically significant, One way ANOVA test was applied

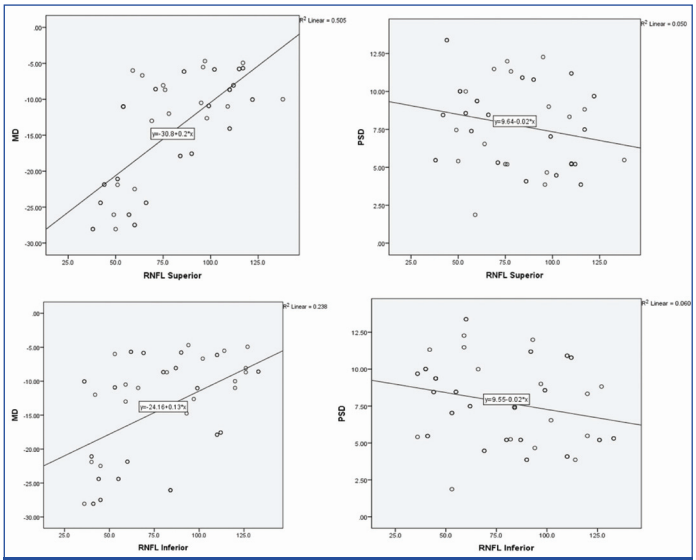
Dependent variable	A	B	Mean difference (A-B)	Std. Error	p-value
RNFL superior	Early	Moderate	18.42	8.42	0.082
	Early	Severe	44.33	8.29	<0.01*
	Moderate	Severe	25.92	6.04	<0.01*
RNFL inferior	Early	Moderate	-0.83	11.13	0.997
	Early	Severe	18.92	10.96	0.204
	Moderate	Severe	19.75	7.99	0.043*
MD	Early	Moderate	3.61	1.40	0.033*
	Early	Severe	16.12	1.38	<0.01*
	Moderate	Severe	12.51	1.01	<0.01*
PSD	Early	Moderate	-1.29	0.89	0.325
	Early	Severe	-4.02	0.88	<0.01*
	Moderate	Severe	-2.72	0.64	<0.01*

[Table/Fig-3]: Post hoc test following one way ANOVA to assess mean difference among the groups.
*Statistically significant, Tukey's post hoc test was applied

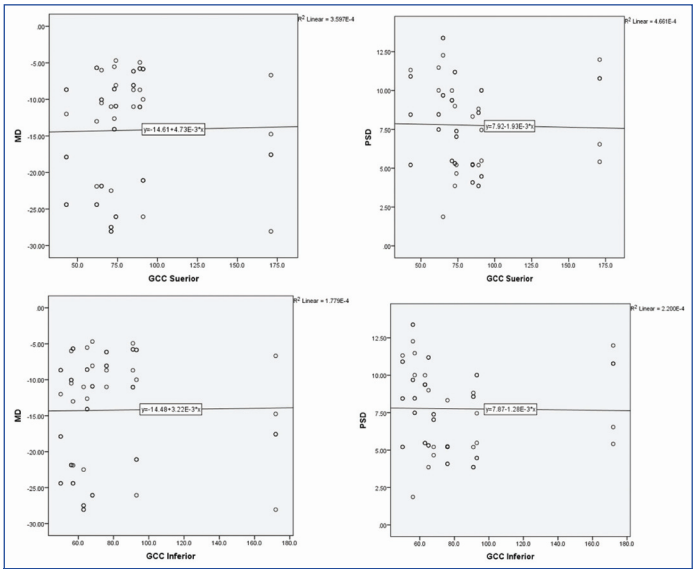
In bivariate analysis, MD was found significantly correlated with superior and inferior RNFL (r=0.70, p-value <0.01 and r=0.47, p-value <0.01 respectively). The MD was not found correlated with superior and inferior GCC. The PSD was found weakly correlated with GCC superior and inferior (r=-0.26, p-value=0.04 and r=-0.27, p-value=0.03, respectively). Since there was a negative correlation, it is inferred that as the glaucoma advances GCC decreases and PSD increases. The PSD was not found correlated with superior and inferior RNFL [Table/Fig-4-6].

Parameters	MD		PSD	
	r	p-value	r	p-value
RFNL superior	0.70	<0.01*	-0.23	0.073
RFNL inferior	0.47	<0.01*	-0.22	0.058
GCC superior	0.19	0.156	-0.26	0.045*
GCC inferior	0.18	0.169	-0.27	0.036*

[Table/Fig-4]: Bivariate correlation of OCT parameters with visual field parameters (n=60).
r-Spearman's correlation coefficient, *statistically significant



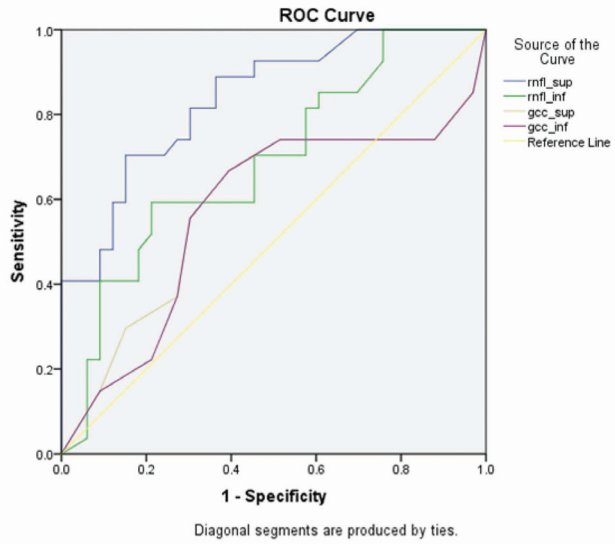
[Table/Fig-5]: Scatter plot showing bivariate correlation of RNFL thickness with MD and PSD.



[Table/Fig-6]: Scatter plot showing bivariate correlation of GCC with MD and PSD.

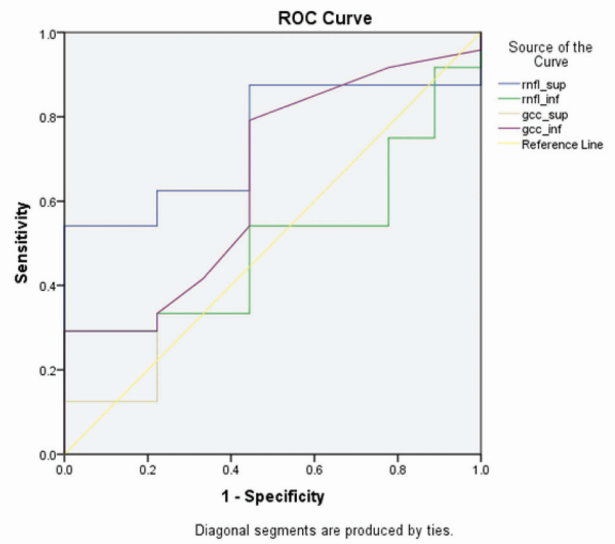
To assess a differentiation based on RNFL and GCC, ROC curves were generated with various sensitivities at fixed specificity. Results are shown in [Table/Fig-7,8]. The Area Under the Receiver Operating Characteristic Curve (AUROC) were found 0.839 and 0.687 for RNFL superior and inferior respectively to differentiate severe glaucoma from other types of glaucoma. It was observed 0.745 and 0.505 for RNFL superior and inferior respectively to differentiate moderate glaucoma from early glaucoma. The AUROC of GCC superior and inferior were found 0.585 and 0.572 respectively to differentiate severe glaucoma, and 0.616 and 0.653, respectively to differentiate moderate glaucoma from early glaucoma [Table/Fig-7,8].

Area under the curve	
Variable	Area
RNFL superior	0.839
RNFL inferior	0.687
GCC superior	0.585
GCC inferior	0.572



[Table/Fig-7]: Receiver operating characteristics (ROC) curve to differentiate severe glaucoma from early and moderate glaucoma.

Area under the curve	
Variable	Area
RNFL superior	0.745
RNFL inferior	0.505
GCC superior	0.616
GCC inferior	0.653



[Table/Fig-8]: Receiver operating characteristics (ROC) curve to differentiate moderate glaucoma from early glaucoma.

DISCUSSION

Detection of glaucomatous changes is usually done by observing structures (RNFL and GCC) affected by glaucoma and by assessing visual function using perimetry. It is crucial to understand the link between structural and functional glaucoma changes. In this study, authors observed structure (RNFL and GCC) and visual function (MD

and PSD) of 60 glaucoma patients. The SAP points were converted to a linear scale before calculating the correlation, as RNFL and GCC values were also collected in linear scale (in microns).

It was found that the thickness of the RNFL and the thickness of the GCC had different structure function relationship with visual field sensitivity and varied diagnostic values to identify glaucoma severity in the current study. The RNFL and GCC thickness, on the other hand, had the same structure function relationship with VF sensitivity and diagnostic values for glaucoma detection, according to Kim NR et al., [4]. In the present study, RNFL (superior and inferior) thickness was found significantly different in three glaucoma groups. Similar findings were reported by Kim NR et al., [4]. The GCC thickness was not found significantly different in three glaucoma groups. It was not consistent with the study done by Kim NR et al., in which GCC was found better predictor to differentiate the severity of glaucoma [4]. The present study was in agreement with other studies, in which RNFL was found as a better predictor than GCC [10-12].

When compared to GCC thickness, RNFL thickness was found to be a superior diagnostic sign for glaucoma, and the AUROC difference was similarly in line. We found distinct AUROC outcomes for glaucoma distinguishing between RNFL and GCC thickness in the current study. The diagnostic value of RNFL and GCC measures has been evaluated in a few studies, and the results have demonstrated that GCC diagnosis is comparable to RNFL diagnosis [8,9].

So, more research on the relevance of mean GCC thickness in the diagnosis of early glaucoma is needed. The RNFL thickness also showed a relationship with severity of disease, whereas GCC thickness did not show any relationship. Other studies had shown role of both RNFL and GCC in the severity of the disease [1,4,6,10]. This could be due to the fact that only about half of the RGC are found in the macula, whereas virtually all of the RGC are examined in a peripapillary OCT RNFL scan [4]. Because glaucoma is a diffuse illness, the ability to assess damage throughout the eye may provide RNFL assessment an edge with GCC thickness evaluation in identifying glaucoma. Moreover, RGCs measurement at the periphery revealed greater absolute thickness changes than at the macula [4]. Another major advantage of RNFL over macular GCC thickness assessment is that it is not affected by non glaucomatous macular pathology. Diabetes and macular degeneration, for example, have a direct effect on macular thickness and may restrict or accentuate the findings seen with glaucoma. Such major issues were not observed in the RNFL evaluation [4,8].

Though, the glaucomatous changes often occur initially in the inferior pole and that change associated with glaucoma usually manifests first in the superior visual field, corresponding to abnormalities in the inferior pole [1,7,8]. The present study showed that the superior RNFL was the most strongly associated parameter with glaucoma status because it had mostly moderate and severe glaucoma patients.

The thickness of the RNFL as evaluated by OCT was shown to be correlated with visual field parameters like MD in the present study. The RNFL loss can occur without any change in the visual field in early stage glaucoma and is better diagnosed by OCT whereas, in advanced stages, visual field variations may be used to detect the progression of glaucomatous damage since the variation in RNFL thickness is too tiny to detect and the changes in the visual field is larger and easier to notice [1]. The PSD is also thought to be a more accurate indicator of localised changes in the visual field, and it has been shown to duplicate a good correlation with glaucoma in thinner portions of the papillary rim [1,12,17].

Though, in current study it was found that RNFL thickness had a higher sensitivity than GCC thickness in differentiating glaucoma severity. But, macular GCC thickness also has a significant role in the detection of glaucoma [1,6,8,12]. New algorithms for the specific indication of RNFL and GCC measure, in combination or alone, should be developed. The relevance and benefits of RNFL and GCC thickness assessment in glaucoma is relatively a novel approach and should be further amplified for the evaluation and management of the disease.

Limitation(s)

The study had few limitations, including small sample size. The longitudinal link between structural and functional factors could not be determined in this cross-sectional study. Although the linear correlation was found, the spatial distribution of structural characteristics for the changes in functional loss was not investigated. The present study excluded controls and the full spectrum of glaucomatous damage including suspected glaucoma. The precision of the RNFL and GCC thickness was not assessed, which could alter diagnostic abilities depending on glaucoma severity. The role of RNFL and GCC in glaucoma management should be further explored and specific indications, in combination or alone should be developed.

CONCLUSION(S)

The optic disc findings on SD-OCT were correlated with visual field parameters in glaucoma patients. Superior and inferior RFNL was strongly correlated with MD. The RNFL thickness can differentiate the severity of glaucoma in early glaucoma patients. These parameters should be used together to improve the understanding of glaucomatous changes.

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