Original Article

Comparison of diagnostic ability of standard automated perimetry, short wavelength automated perimetry, retinal nerve fiber layer thickness analysis and ganglion cell layer thickness analysis in early detection of glaucoma

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Purpose: The aim of this study was to compare the diagnostic ability of macular ganglion cell layer (GCL) analysis using spectral domain optical coherence tomography against retinal nerve fiber layer analysis (RNFL), short-wavelength automated perimetry (SWAP), and standard automated perimetry (SAP) in early detection of glaucoma. **Methods:** Participants fulfilling the inclusion criteria were consecutively enrolled from the glaucoma clinic of tertiary care eye hospital in Western India from November 2015 to October 2016. The subjects underwent a detailed evaluation by trained glaucoma specialists. On suspicion of glaucoma, the patients underwent SAP, SWAP, and SD-OCT for GCL and RNFL analysis. **Results:** There were 91 patients in total of which experts classified 54 eyes into GON and 37 eyes into nonglaucomatous group. Sensitivity of SAP (42.59%) was significantly lower ($P < 0.05$) than that of average GCL thickness (79.63%) and average RNFL thickness (72.22%). Specificity and positive LR of SWAP (97.3% and 19.19, respectively) and SAP (94.6% and 7.88, respectively) were greater than those of GCL (81.08% and 4.21) and RNFL (67.57% and 2.23) parameters. Negative LR of average GCL thickness (0.25) was superior to that of average RNFL thickness (0.411), SWAP (0.495), and SAP (0.607). **Conclusion:** Macular GCL parameters perform better than RNFL parameters in patients with early glaucomatous damage. There is superior ability of SWAP over SAP in detecting glaucomatous changes in glaucoma suspect group. GCL thickness analysis has higher sensitivity and negative likelihood ratio, whereas SWAP had higher specificity and positive likelihood ratio. Thus, combining both tests can lead to better diagnostic ability for early glaucomatous damage.

Key words: Diagnostic ability, ganglion cell layer, glaucoma, retinal nerve fiber layer, short-wavelength automated perimetry, spectral-domain optical coherence tomography, standard automated perimetry

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Glaucoma is a group of disorders characterized by optic neuropathy with loss of retinal ganglion cells.[1] This results in characteristic changes to the optic nerve and macular ganglion cell layer (GCL) which leads to corresponding visual field (VF) defects on standard automated perimetry (SAP).[2] Glaucoma leads to usually irreversible and progressive vision loss. As such early diagnosis and treatment form an important tool in maintaining visual function and preventing vision loss.[3]

However, diagnosis of early glaucoma presents a dilemma for the clinicians and can be difficult. As many as 35%–50% of retinal ganglion cells can be lost before a visual field defect is detected.[4] As prior to VF defects appearing on SAP, structural damage might be detectable, several technologies aiming at objectively and quantitatively measuring the retina have been used to attempt to improve diagnostic accuracy and reproducibility.[1] However, despite the availability of tests that quantitatively measure structure and function relevant to glaucoma, there is currently no ''gold standard'' for diagnosis.[5]

In this study, we compared the diagnostic ability of SAP, short-wavelength automated perimetry (SWAP), and SD-OCT parameters viz. retinal nerve fiber layer thickness (RNFL) and GCL thickness. Sensitivity to blue light (mediated by blue cone photoreceptors) is adversely affected relatively early in glaucoma which is utilized by SWAP.[6] RNFL loss precedes VF loss and optic nerve head defects in patients with glaucoma. The GCL is thickest in the perimacular region and there is thinning of GCL in this region in glaucomatous eyes. Glaucoma shows structural anomalies in the GCA sector, deviation and thickness maps.[7,8]

Although several studies have been reported evaluating the diagnostic accuracy of imaging devices in glaucoma, the design of most studies has not replicated the situation in which these tests are used in clinical practice. In fact, a clinician is most interested in the ability of a test to provide additional information that can be helpful in a patient who presents suspicious findings for the disease, such as apparently large cup or neuroretinal rim thinning.[9] Thus the identification of the

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best early predictor of glaucoma can lead to early management and preservation of vision of the patient.

Methods

This was an observational, cross-sectional study of subjects referred to a tertiary eye care facility by general Ophthalmologists for a glaucoma evaluation. Informed consent was obtained from all subjects and the Ethics Committee of the hospital approved all methodology. All methods adhered to the tenets of the Declaration of Helsinki for research involving human subjects. Inclusion criteria were age 18 years and older, best corrected visual acuity of 20/40 or better, and refractive error within ±5D sphere and ±3D cylinder. Exclusion criteria were presence of any media opacities that prevented good-quality SDOCT imaging and any retinal/macular disease other than glaucoma. All participants underwent a comprehensive ocular examination which included a detailed medical history, BCVA, slit-lamp biomicroscopy, Goldmann applanation tonometry, gonioscopy, dilated fundus examination, SAP, and SDOCT imaging with Cirrus SD-OCT (Carl Zeiss Meditech, Dublin, CA). SAP and SWAP were performed using a Humphrey Field analyzer, model 750 (Zeiss Humphrey Systems, Dublin, California), with the Swedish interactive threshold algorithm standard Centre 24-2 test. All VFs were graded by a single expert masked to the optic disc classification, SDOCT findings, and the other eye status. VFs were classified as "glaucomatous" if the pattern SD had a *P* value of <5% and the glaucoma hemifield test result was outside normal limits. VFs were classified as "normal" otherwise. The expert also noted the VF classification as "repeatable" if the VF classification was similar for the 2 most recent VFs of an eye. SDOCT examination was performed with the Cirrus SD-OCT. The RNFL parameters generated by the software and analyzed in this study were the superior and inferior hemisphere averages and the overall RNFL average. The parameters generated by the GCL analysis are the average, superior, and inferior GCL thickness. A few of the Cirrus SD-OCT parameters are compared with the internal normative database within the software and one of the 3 diagnostic categorizations is provided. "Outside normal result" categorization indicates that the value is <99% confidence interval (CI) of the healthy, age-matched population. "Borderline" result indicates that the value is between the 95% and 99% CI, and a "within normal limits" indicates that the value is within the 95% CI. For the purpose of the current study, we clubbed the "borderline" result with the "outside normal limits" result to create a dichotomous classification. Only well-centered images with a signal strength index of ≥6/10 were used for analysis.

Optic disc evaluation was performed independently by two glaucoma experts (with at least 5-year experience of working as glaucoma specialists), who were masked to the clinical examination results of the subjects and also the SAP, SWAP, SDOCT, and other eye examination results. They classified the optic discs into glaucomatous optic neuropathy (GON) and nonglaucomatous groups based on the presence or absence of characteristic glaucomatous optic disc changes (focal or diffuse neuroretinal rim thinning, localized notching, or nerve fiber layer defects). Discrepancies between the 2 experts were resolved by consensus. Eyes, where a classification to either glaucoma or nonglaucoma group was not possible by either of the experts, were labelled as "suspects" and excluded from the analysis. Thus a total of 91 eyes were analysed of which 54 eyes were labelled as Glaucoma and 37 eyes were labelled as nonglaucomatous. Expert opinion was considered as the gold standard for diagnosis of glaucoma which has been used as the reference standard in various other studies too.[10] Sample size was determined to be 54 as calculated based on the following formula:

Sample size = 4 x prevalence X (100-prevalence)/error^2

Where prevalence = 3.51[11] and experimental error is taken to be 5.

Statistical analysis

Data were analyzed by using SPSS version 21. Mean and standard deviation were reported for normally distributed data. For continuous data, parametric tests (independent *t*-test) were applied and for categorical data, Chi-square test was applied. Sensitivity, Specificity, Likelihood ratios (LRs) were the outcome measures reported for the different tests with expert opinion as the gold standard. Sensitivity and Specificity was compared between the test using McNemar's test and LR was compared using LR regression methodology. A value of $P < 0.05$ was considered significant.

Descriptive statistics included mean and SD for normally distributed variables and median and interquartile range for nonnormally distributed variables. Sensitivities, specificities, and LRs were reported for the diagnostic classification of SAP (normal or glaucomatous) and SDOCT (outside normal limits or within normal limits) parameters to differentiate GON eyes from nonglaucomatous eyes. Sensitivity is the ability of the diagnostic test to pick up all those with the disease, whereas specificity is the ability of the diagnostic test to pick up all those without the disease. LR is the probability of a given test result in those with disease divided by the probability of the same test result in those without the disease. The LR for a given test result indicates how much that result will raise or lower the probability of disease.[10] A LR of 1 or close to 1 would mean that the test provides no additional information about the post-test probability of the disease. LRs >10 or <0.1 would be associated with large effects on post-test probability, LRs from 5 to 10 or from 0.1 to 0.2 would be associated with moderate effects, and LRs from 2 to 5 or from 0.2 to 0.5 would be associated with small effects.

Results

Of the 131 consecutive eyes undergoing glaucoma evaluation at our center between November 2015 and October 2016, 110 eyes had undergone 2 or more reliable VFs. Of these, three eyes in which the VF classification of the most recent VFs was inconsistent and seven eyes with signal strength index values on SDOCT of <6/10 were excluded. Nine eyes, where a classification to either GON or nonglaucomatous group was not possible by both the experts (labelled as suspects), were excluded from analysis, leaving 91 eyes of 51 subjects for the current analysis. Glaucoma experts, evaluating the optic disc photographs of these eyes in a masked manner classified 37 eyes as nonglaucomatous and 54 eyes as having GON. Age of the subjects was comparable between the two groups. VF and SDOCT parameters were significantly different in the nonglaucomatous compared with the GON group.

The age group of patients in present study varied from 20 to 79 years The mean age of the glaucoma patients and nonglaucomatous patients were 49.185 ± 15.16 years and 35.892 ± 13.13 years, respectively, with the range of glaucoma patients being 20–79 years and in nonglaucomatous patients

being 20–78 years *P* Value (two-tailed) was found to be <0.001 which was statistically significant. Thus, glaucoma shows a positive correlation with age.

The mean IOP of the glaucoma patients and nonglaucomatous patients were 16.944 ± 4.768 mm Hg and 14.784 ± 2.888 mm Hg, respectively.

P Value (two-tailed) was found to be <0.05 which was statistically significant. Thus, glaucoma shows a correlation with IOP.

Thirty-eight female patients were included in the study, of which 26 were found to have glaucoma, whereas 12 were found to be nonglaucomatous. Fifty-three male patients were included in the study, of which 28 were found to have glaucoma, whereas 25 were found to be nonglaucomatous.

P = 0.135 which was statistically not significant. Thus gender was not found to be a determining factor for Glaucoma.

Tables 1-4 show the sensitivities, specificities, positive and negative LRs associated with the classification of SAP, SWAP, and SDOCT parameters to diagnose GON.

Table 1: Analysis of diagnostic parameters of SAP test results

Parameter	Estimate	95% confidence intervals
Sensitivity	42.59%	(30.33, 55.84)
Specificity	94.59%	(82.3, 98.5)
Positive predictive value	92%	(75.03, 97.78)
Negative predictive value	53.03%	(41.16, 64.57)
Diagnostic Accuracy	63.74%	(53.49, 72.87)
Positive Likelihood ratio	7.88	(2.636-23.55)
Negative Likelihood ratio	0.6069	(0.5679-0.6486)

Table 2: Analysis of diagnostic parameters of SWAP test results

Parameter	Estimate	95% Confidence Intervals
Sensitivity	51.85%	(38.85, 64.61)
Specificity	97.3%	(86.18, 99.52)
Positive predictive value	96.55%	(82.82, 99.39)
Negative predictive value	58.06%	(45.67, 69.52)
Diagnostic Accuracy	70.33%	(60.28, 78.74)
Positive Likelihood ratio	19.19	(2.532-145.3)
Negative Likelihood ratio	0.4949	(0.4582-0.5344)

Table 5 compares the diagnostic parameter results observed with all the diagnostic tests. Sensitivity of average RNFL thickness and average GCL thickness were statistically significantly greater than that of SAP and SWAP as shown by Table 6. Specificity and positive LR of SAP and SWAP were statistically significantly greater than those of all RNFL and GCL parameters of SDOCT. Negative LR of SAP was significantly inferior (greater) than that of RNFL thickness and GCL thickness. Among the RNFL parameters, inferior quadrant RNFL thickness had higher specificity and positive predictive value compared with superior and average RNFL thickness parameters. Positive and negative LRs of all RNFL parameters were comparable. Among the GCL parameters, sensitivity of average GCL thickness was better, whereas the specificity and positive predictive value were higher with inferior sector GCL thickness parameters. Positive LRs of inferior sector GCL thickness was the higher, whereas better values for Negative LR was seen with average GCL thickness. Comparing the diagnostic ability parameters of RNFL and GCL parameters of SDOCT, sensitivity and negative LR of average GCL thickness was better than those of all the RNFL parameters. Specificity, positive predictive value and positive LR were better with inferior sector GCL thickness than that of other GCL thickness parameters and all RNFL parameters.

Discussion

In this study to compare the diagnostic ability parameters of SDOCT with SAP and SWAP, we found that most of the SDOCT parameters had higher sensitivities but lower specificities compared with SAP and SWAP in picking up GON. To the best of our knowledge, there is no study comparing the diagnostic abilities of SDOCT with both SAP and SWAP. Comparing the diagnostic abilities of SDOCT, SAP, and SWAP, we found that SWAP had a very high specificity compared with most of the SDOCT parameters in differentiating GON from nonglaucomatous eyes. The corresponding value for SAP was slightly lower than that of SWAP. This would mean that the SWAP and SAP would classify more true normals as nonglaucomatous compared with SDOCT. However, the sensitivity of SAP and SWAP was significantly lower than that of the SDOCT parameters. This would mean that SAP and SWAP missed significantly more eyes with GON compared with SDOCT. These eyes are the ones with pre-perimetric glaucoma, and previous studies have also reported good diagnostic ability of SDOCT in pre-perimetric glaucoma.[12] In addition to sensitivity and specificity, diagnostic tests are also summarized in terms of LRs, which are higher than the previous measures in hierarchy, as they express the magnitude by which the probability of a diagnosis in a given patient is

Table 3: Analysis of diagnostic parameters of SD-OCT RNFL test results

Parameter	Avg. thickness	Superior Quadrant	Nasal Quadrant	Inferior Quadrant	Temporal Quadrant
Sensitivity	72.22%	62.96%	61.11%	64.81%	62.96%
Specificity	67.57%	72.97%	72.97%	75.68%	72.97%
Positive predictive value	76.47%	77.27%	76.74%	79.55%	77.27%
Negative predictive value	62.5%	57.45%	56.25%	59.57%	57.45%
Diagnostic Accuracy	70.33%	67.03%	65.93%	69.23%	67.03%
Positive Likelihood ratio	2.227	2.33	2.261	2.665	2.33
Negative Likelihood ratio	0.4111	0.5075	0.5329	0.4649	0.5075

Table 4: Analysis of diagnostic parameters of SD-OCT GCL test results

Parameter	Average thickness	Superior Hemisphere	Inferior Hemisphere
Sensitivity	79.63%	70.37%	74.07%
Specificity	81.08%	83.78%	86.49%
Positive Predictive Value	86%	86.36%	88.89%
Negative Predictive Value	73.17%	65.96%	69.57%
Diagnostic Accuracy	80.22%	75.82%	79.12%
Positive Likelihood ratio	4.209	4.34	5.481
Negative Likelihood ratio	0.2512	0.3536	0.2998

Table 5: Comparison of diagnostic parameters of GCL thickness analysis vs. RNFL thickness analysis vs. SWAP vs. SAP

Parameter	Average GCL thickness	Average RNFL thickness	SWAP	SAP
Sensitivity	79.63%	72.22%	51.85%	42.59%
Specificity	81.08%	67.57%	97.3%	94.59%
Positive Predictive Value	86%	76.47%	96.55%	92%
Negative Predictive Value	73.17%	62.5%	58.06%	53.03%
Diagnostic Accuracy	80.22%	70.33%	70.33%	63.74%
Positive Likelihood ratio	4.209	2.227	19.19	7.88
Negative Likelihood ratio	0.2512	0.4111	0.4949	0.6069

Table 6: Significance values of difference in diagnostic parameters of GCL thickness analysis as compared to RNFL thickness analysis, SWAP and SAP

P	GCL thickness analysis			
	Sensitivity	Specificity	Positive likelihood ratio	Negative likelihood ratio
RNFL thickness analysis	0.454	0.267	0.151	0.516
SWAP analysis	0.001	0.031	0.02	1.00
SAP analysis	<0.001	0.125	0.83	0.288

modified by the results of the test. In other words, the LR indicates how much a given diagnostic test result will raise or lower the pretest probability of the disease in question. We therefore evaluated the LRs associated with the diagnostic categorization of SDOCT parameters. Positive LR of SWAP, similar to specificity was significantly higher than that of the SDOCT parameters and was also higher than SAP. This would mean that likelihood of an eye being glaucomatous was significantly higher if the SWAP result was positive as compared with the OCT result being positive. However, the negative LRs of the SDOCT parameters especially GCL were significantly better (lower) than that of SAP and SWAP. This would mean that likelihood of an eye being nonglaucomatous was significantly higher if the OCT result was negative as compared with the SAP and SWAP result being negative. Analyzing the diagnostic abilities of the RNFL parameters of SDOCT, we found that the average RNFL thickness had the best sensitivity and negative LR, whereas the inferior RNFL thickness had the best specificity and positive LR. Among the GCL parameters, average GCL thickness had the best sensitivities and negative LRs, whereas inferior sector GCL thickness had high specificity and positive LR. Although the diagnostic ability parameters were comparable between the best RNFL and GCL parameters, GCL parameters seemed to have better specificities and positive LRs compared with the RNFL parameters. These results are also in agreement with previous studies comparing the diagnostic abilities of RNFL and GCL parameters of SDOCT in diagnosing glaucoma.[13,14] Additionally it has been found that although the diagnostic accuracy of GCL thickness analysis increases with more severe glaucomatous damage and higher signal strength values, it is not affected by increasing axial length, resulting in a more accurate discrimination of glaucomatous damage in myopic eyes or advanced circumpapillary retinal nerve fiber layer damage with respect to the traditional RNFL thickness.[15,16]

A concept that can be further used is SNOUT and SPIN, that is, a sensitive test when negative can rule out the disease and a Specific test when Positive can rule in the disease, respectively. So SD-OCT parameters can be used to rule the disease out in those testing negative by virtue of their high sensitivity rates. Similarly VF tests can be used to rule in the disease in those testing positive due to their high specificity rates.[17]

All subjects included in this study were the ones referred from general ophthalmologists to a tertiary care facility for a glaucoma opinion. Therefore, the pre-test probability of glaucoma in these subjects is likely to be high. The reference standard against which we compared the diagnostic abilities of SAP, SWAP, and SDOCT was the masked classification of optic discs by glaucoma experts. A limitation of the current study, therefore, is the possible misclassification of a few nonglaucomatous eyes as GON and vice versa. We, however,

believe that this was less likely; although the optic discs were found to be suspicious of glaucoma by the general ophthalmologists, two glaucoma experts could independently classify them into GON and nonglaucomatous groups. There was, however, no ambiguity in their classification by the glaucoma experts. Those discs where a classification into the above 2 groups was not possible by either of the experts were called "true suspects" and excluded from the current study. Therefore in true sense, optic discs included in the nonglaucomatous group were not true suspects but were mostly large optic discs with large physiological cups (disc size was significantly larger in nonglaucomatous compared with glaucomatous group) that caused a diagnostic uncertainty among general ophthalmologists. True glaucoma suspect eyes, based on optic disc appearance, would require a longitudinal study to definitively classify them into glaucoma or nonglaucoma groups.

Another limitation of using the structural changes on disc evaluation as the gold standard while comparing SAP and SDOCT is the advantage inherently offered towards OCT because of its evaluation of structural changes, similar to the gold standard. It is understandable that a test evaluating structural change is more likely to agree with a test evaluating structural change than with a test evaluating functional change. However, this is related to the lack of a nonstructural and nonfunctional reference standard for diagnosing glaucoma at this point in time.

It is important to note that the results of our study are dependent on the abnormality criteria chosen with SAP, SWAP and SDOCT. With the SAP and SWAP, abnormality was based on 2 of the 3 criteria proposed by Anderson and Patella (PSD depressed to $P < 5\%$ and glaucoma hemifield test result outside normal limits). Similarly, for the SDOCT classification, we had clubbed the "borderline" result with the "outside normal limits" result to create a dichotomous classification. Clubbing the "borderline" result with the "within normal limits" result would have led to different results. Any result on SAP or SDOCT therefore needs to be interpreted along with the total clinical picture before accepting it or discarding it as a false result.

Conclusion

In conclusion, we found that most of the SDOCT parameters especially the GCL thickness analysis had better sensitivities and negative LRs to diagnose GON compared with SAP and SWAP. The specificities and positive LRs of SDOCT parameters to diagnose GON were, however, inferior to that of SWAP and SAP. The diagnostic accuracy, which is an average of the sensitivity and specificity for the said test, is found to be highest for the GCL thickness analysis amongst all the tests, thus identifying it as the single most useful test for diagnosis of glaucoma. In addition it had the highest value for sensitivity as compared to the other tests thus giving it additional value in early detection of glaucoma.

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Conflicts of interest

There are no conflicts of interest.

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