Scanning the macula for detecting glaucoma

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Background: With the advent of spectral domain optical coherence tomography (SDOCT), there has been a renewed interest in macular region for detection of glaucoma. However, most macular SDOCT parameters currently are thickness parameters which evaluate thinning of the macular layers but do not quantify the extent of area over which the thinning has occurred. We therefore calculated a new macular parameter, “ganglion cell complex surface abnormality ratio (GCC SAR)” that represented the surface area over which the macular thickness was decreased. Purpose: To evaluate the ability of SAR in detecting perimetric and preperimetric glaucoma. Design: Retrospective image analysis. Materials and Methods: 68 eyes with perimetric glaucoma, 62 eyes with preperimetric glaucoma and 165 control eyes underwent GCC imaging with SDOCT. SAR was calculated as the ratio of the abnormal to total area on the GCC significance map. Statistical Analysis: Diagnostic ability of SAR in glaucoma was compared against that of the standard parameters generated by the SDOCT software using area under receiver operating characteristic curves (AUC) and sensitivities at fixed specificities. Results: AUC of SAR (0.91) was statistically significantly better than that of GCC average thickness (0.86, P = 0.001) and GCC global loss volume (GLV; 0.88, P = 0.01) in differentiating perimetric glaucoma from control eyes. In differentiating preperimetric glaucoma from control eyes, AUC of SAR (0.72) was comparable to that of GCC average thickness (0.70, P > 0.05) and GLV (0.72, P > 0.05). Sensitivities at specificities of 80% and 95% of SAR were comparable (P > 0.05 for all comparisons) to that of GCC average thickness and GLV in diagnosing perimetric and preperimetric glaucoma. Conclusion: GCC SAR had a better ability to diagnose perimetric glaucoma compared to the SDOCT software provided global GCC parameters. However, in diagnosing preperimetric glaucoma, the ability of SAR was similar to that of software provided global GCC parameters.

Key words: Ganglion cell complex, spectral domain optical coherence tomography, surface abnormality ratio

Glaucoma is a leading cause of irreversible blindness in the world,[1] in which the pathological loss of retinal ganglion cells is associated with clinically recognizable alterations in the retinal nerve fibers layer (RNFL) and optic nerve head (ONH). In addition to the changes that occur in ONH and RNFL, another retinal region which has been proposed to manifest changes in glaucoma is the macula.[2] This is because of the fact that more than 50% of the ganglion cells in the retina are located at macula and the ganglion cell layer is more than one cell layer thick at the macula.[2,3] Earlier imaging technologies in glaucoma predominantly focused on the RNFL and ONH for evaluating the structural damage in glaucoma. With the advent of spectral domain optical coherence tomography (SDOCT), there has been a renewed interest in scanning the macular region for detection of glaucomatous changes. Macular scanning on SDOCT evaluates the thickness of the inner layers of the retina that essentially comprise of the ganglion cell bodies and their appendages.[4,5] Recent studies have reported that the macular thickness parameters of SDOCT were as good as the RNFL parameters in diagnosing glaucoma.[6-10] However, most macular parameters of SDOCT currently, are thickness parameters which evaluate the thinning of the macular layers but do not quantify the extent of area over which the thinning has occurred.

We therefore calculated a new macular parameter, “surface abnormality ratio SAR)” that represented the surface area over which the macular thickness was decreased. The purpose of this study was to evaluate the ability of SAR to differentiate perimetric and preperimetric glaucoma eyes from control eyes.

Materials and Methods

This was a retrospective image analysis performed on the data from a cross-sectional study of subjects referred by general ophthalmologists to a tertiary eye care facility between September 2010 and November 2012 for glaucoma evaluation. Informed consent was obtained from all subjects and the Ethics Committee of L V Prasad Eye Institute 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, best corrected visual acuity of 20/40 or better and refractive error within ± 5 D sphere and ± 3 D cylinder. Exclusion criteria were presence of any media opacities that prevented good quality optic disc photographs and SDOCT imaging and any retinal (including macular) disease other than glaucoma which could confound the evaluations. All participants underwent a comprehensive ocular examination which included a detailed medical history, best corrected visual acuity measurement, slit-lamp biomicroscopy, Goldmann applanation tonometry, gonioscopy, dilated fundus examination, visual field (VF) examination,
digital optic disc photography and SDOCT imaging with RTVue (Optovue Inc, Fremont, CA).

VF examination was performed using a Humphrey Field analyzer, model 750i (Zeiss Humphrey Systems, Dublin, CA), with the Swedish interactive threshold algorithm (SITA) standard 24-2 algorithm. Reliability criteria were fixation losses, false positive and false negative response rates of less than 20%. All VFs were graded by a single observer masked to the optic disc classification, SDOCT findings and the other eye status. VFs were classified as “glaucomatous” if the pattern standard deviation (PSD) had a ‘p’ value of less than 5% and the glaucoma hemifield test result was outside normal limits.[11] VFs were classified as “normal” otherwise. The observer also noted the VF classification as “repeatable” if the VF classification was similar for the 2 most recent VFs of an eye.

Digital optic disc photographs were obtained by trained technicians (FF 450plus with VISUPAC 4.2.2, Carl Zeiss Meditec Systems GmbH, Pirmasens, Germany). Photographs consisted of a 50 degree image centered on the optic disc, a similar image centered on the macula, a 30 degree image centered on the optic disc and a 20 degree image centered on the disc. All these images also consisted of one colored and one red-free image each. Each optic disc photograph was evaluated independently by two of the 3 glaucoma experts (with at least 5 years’ experience of working as glaucoma specialists), who were masked to the clinical examination results of the subjects and also the VF, SDOCT and other eye examination results. They classified the optic discs into glaucomatous and non-glaucomatous (control) 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 two experts were resolved by consensus. Eyes, where a classification to either glaucoma or control group was not possible by either of the experts, were labeled as “suspects” and excluded from the analysis.

SDOCT examination was performed with the RTVue (software version 5.1.0.90). The protocol used for imaging the macula was GCC protocol. This protocol and the parameters generated by the protocol have been explained earlier.[9] GCC protocol is designed to measure the inner retinal thickness which includes the nerve fiber layer, ganglion cell layer and the inner plexiform layer, collectively called the GCC. GCC thickness over the scan area is compared with the internal normative database within the software to provide a GCC significance map. Significance map consists of three diagnostic categorizations, which are color coded. “Outside normal result” categorization, coded in red, indicates that the GCC thickness value is lesser than the lower 99% confidence limit of the healthy, age-matched population. “Borderline” result, coded in yellow, indicates that the value is between the 95% and 99% confidence limits, and a “within-normal-limits”, coded in green indicates that the value is within the 95% confidence limits. In addition to the GCC thickness, the GCC protocol provides three other parameters called GLV (global loss volume), FLV (focal loss volume) and RMS (root mean square). These parameters have been explained previously.[10] Only well-centered images with a signal strength index (SSI) of ≥30 were used for analysis. All patients had SDOCT, disc photography and one of the VF examination performed on a single day.

GCC significance map was used to calculate the SAR as follows. The symmetry printout of the SDOCT was opened as a JPEG image using Photoshop CS5 (Adobe Systems Inc, San Jose, CA) and the GCC significance map of preferred eye was cropped. First the area in yellow was selected using a “magic wand” tool. This is an automated tool which selects all contiguous areas in yellow. Neighboring yellow areas, not selected initially, were selected manually using the “similar” option [Fig. 1]. Once selected, the entire area in yellow is automatically given in pixel values. A similar procedure was used to select the red area separately. In addition to these, the whole area of the GCC scan was also calculated in pixel values using the same steps. The gray area in the GCC map, representing the fovea, was excluded from all measurements. All these measurements were done by a single observer, who was masked to the optic disc and VF classification. Following this, SAR was calculated for each eye using 2 criteria separately. First was a sensitive criterion, where both red and yellow areas were considered abnormal and SAR was calculated as the combined area of red and yellow divided by the entire scan area. Second was a specific criterion, whereby SAR was calculated as the area of red divided by the entire scan area. The same observer, in a masked manner, repeated the exercise of calculating the SAR in a random sample of 30 eyes (10 in each of the three groups) to evaluate the variability of SAR measurement. Variability was calculated as the difference in the two SAR measurements in these 30 eyes.

Eyes with both the optic disc and VF classification as “glaucomatous” formed the perimetric glaucoma group. Eyes with both the classification as “normal” formed the control group. Eyes with optic disc classification as “glaucomatous” and VF classification as “normal” formed the preperimetric glaucoma group.

Statistical analysis

Descriptive statistics included mean and standard deviation for normally distributed variables and median and inter-quartile range (IQR) for non-normally distributed variables. Receiver operating characteristic (ROC) curves were used to describe the ability of the SDOCT software-provided parameters to discriminate glaucomatous eyes from control eyes. To obtain

![Figure 1: The selection of the color-coded area from the ganglion cell complex surface abnormality ratio in glaucoma](http://example.com/figure1.png)
confidence intervals for area under the ROC curves (AUC), a bootstrap re-sampling procedure was used ($n = 1000$ re-samples). Sensitivities at fixed specificities of 80% and 95% for all parameters were also obtained from the ROC curves.

Statistical analyses were performed using commercial software (Stata ver. 11.2; StataCorp, College Station, TX). A $P < 0.05$ was considered statically significant.

**Results**

Six hundred and seventy eight eyes of 382 consecutive subjects referred for glaucoma evaluation to our center were analyzed. Forty two eyes with unreliable VF s and 7 eyes with poor quality disc photographs were excluded. Further, 12 eyes with segmentation algorithm failure on SDOCT, 6 eyes with SSI <30 on GCC scans were excluded. Of the remaining, we randomly selected 68 eyes with perimetric glaucoma, 62 eyes with preperimetric glaucoma and 165 control eyes for this study. Table 1 shows the age, VF and GCC parameters of the different groups of subjects. Age was comparable between the 3 groups. VF and GCC parameters were significantly different between normal and perimetric glaucoma eyes. VF and GCC parameters were also significantly different between preperimetric and perimetric glaucoma eyes. VF parameters were similar but the GCC parameters were significantly different between the normal and the preperimetric glaucoma groups. SSI was significantly better in normal and preperimetric glaucoma groups compared to the perimetric glaucoma group. ROC curves of the parameters were therefore adjusted for differences in SSI values between the perimetric glaucoma and control group using the method of covariate-adjustment, as proposed by Pepe. Variability of SSI was significantly better than GCC average thickness, GLV and the 2 criteria of SAR to diagnose perimetric (a) and preperimetric glaucoma (b).

**Discussion**

Evaluation of the inner layers of retina at the macular region for detection of glaucomatous changes has seen a renewed interest with the advent of SDOCT. This scanning protocol initially available on the RTVue SDOCT is now available with other SDOCT devices. Unlike the GCC protocol of RTVue SDOCT which provides the thickness of the inner 3 layers of the retina together, current algorithms with other SDOCT devices segment out the RNFL layer at the macula from the thickness of the ganglion cell layer and the inner plexiform layers. Studies evaluating the inner retinal layers at macula using the RTVue SDOCT for detection of glaucoma have reported diagnostic abilities similar to that of the peripapillary RNFL. The current protocols available with the SDOCT devices predominantly measure the thickness of the inner retinal layers paying little attention to the extent of macular region over which the inner retinal layer thinning has occurred. GLV and FLV are additional parameters provided by the software that measure the GCC volume changes in comparison with the normative database. These parameters

**Table 1: Demographic, visual field and spectral domain optical coherence tomography features of the study participants. All values represent median (with interquartile range in brackets) unless otherwise specified**

|                          | Control group (n=165 eyes) | Preperimetric glaucoma (n=62 eyes) | Glaucoma (n=68 eyes) | $P$ value 1 | $P$ value 2 | $P$ value 3 |
|--------------------------|---------------------------|-------------------------------------|----------------------|-------------|------------|-------------|
| Age (years)              | 54 (41, 63)               | 54 (41, 62)                         | 56 (48, 61)          | 0.20        | 0.86       | 0.37        |
| Mean deviation (dB)      | -1.8 (-3.3, -0.8)         | -2.3 (-3.9, -0.9)                  | -9.1 (-14.8, -4.8)   | <0.001      | 0.10       | <0.001      |
| Pattern standard deviation (dB) | 1.7 (1.5, 2.0)            | 1.8 (1.5, 2.2)                     | 8.2 (3.7, 10.5)      | <0.001      | 0.10       | <0.001      |
| Signal strength index*   | 62±10                     | 61±11                               | 55±9                 | <0.001      | 0.33       | <0.001      |
| Average GCC thickness (µm) | 94 (88, 99)               | 87 (79, 93)                         | 77 (70, 85)          | <0.001      | <0.001     | <0.001      |
| Superior average GCC thickness (µm) | 94 (89, 99)          | 89 (79, 95)                         | 81 (70, 88)          | <0.001      | <0.001     | <0.001      |
| Inferior average GCC thickness (µm) | 95 (88, 99)          | 86 (78, 96)                         | 74 (68, 83)          | <0.001      | <0.001     | <0.001      |
| GCC Focal loss volume    | 1.1 (0.2, 2.2)            | 3.8 (1.4, 6.3)                      | 7.1 (4.2, 10.5)      | <0.001      | <0.001     | <0.001      |
| GCC Global loss volume   | 4.7 (2.2, 9.5)            | 12.5 (5.9, 19.8)                    | 21.6 (14.3, 28.5)    | <0.001      | <0.001     | <0.001      |
| GCC Root mean square     | 0.1 (0.1, 0.1)            | 0.1 (0.1, 0.2)                      | 0.2 (0.1, 0.2)       | <0.001      | <0.001     | <0.001      |
| GCC SAR (sensitive criteria) | 4% (0.15)                | 23% (5, 46)                         | 57% (38, 84)         | <0.001      | <0.001     | <0.001      |
| GCC SAR (specific criteria) | 0% (0, 3)               | 8% (1, 25)                          | 36% (17, 64)         | <0.001      | <0.001     | <0.001      |

dB: decibel; GCC: ganglion cell complex; SAR: surface abnormality ratio; * mean and standard deviation. $P$ value 1 represents the $P$ value associated with the comparison between control and glaucoma group; $P$ value 2 represents the $P$ value associated with the comparison between control and preperimetric glaucoma group; $P$ value 3 represents the $P$ value associated with the comparison between glaucoma and preperimetric glaucoma group.
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are similar to the global indices, mean deviation and PSD of the VF respectively and measure the loss of GCC volume over the entire scan area. We therefore developed a new parameter which evaluated the extent over which the inner retinal layer thinning is present and evaluated the ability of this new parameter to diagnose perimetric and preperimetric glaucoma in a clinical setting. Our results showed that the new parameter was statistically significantly better than the other two global GCC parameters provided by the software (GCC average thickness and GLV) in diagnosing perimetric glaucoma. The actual differences in AUCs however were small and the 95% CIs of AUCs were overlapping. In diagnosing preperimetric glaucoma, the AUC of the new parameter however was statistically similar to that of the GCC average and GLV parameters. We also evaluated two criteria of the new parameter, specific criteria which was the abnormality coded in red (abnormality at a P < 1%) and sensitive criteria which was the abnormality coded in red and yellow (abnormality at a P < 5%). The AUCs and sensitivities at fixed specificities however were similar with both the criteria.

The diagnostic ability parameters like AUCs and sensitivities at fixed specificities of the software provided GCC parameters found in our study were lower than those reported by previous studies in both perimetric [4,5,7,15] and preperimetric glaucoma[4,15-18] groups. One of the most likely reasons for this is the nature of the control group in our study. The control group used in the above studies consisted of subjects with no suspicious findings of glaucoma. The control group in our study was selected from the group of subjects referred as glaucoma suspects based on their optic disc appearance by general ophthalmologists. These subjects were however diagnosed as normal based on the optic disc evaluation by glaucoma experts and the normal VFs. Therefore in true sense, eyes included in the control group though were referred as suspects for glaucoma, were not true suspects but were the ones that caused a diagnostic uncertainty among general ophthalmologists. More explanation about this group is provided in our earlier reports.[19,20] We believe that including a control group which is

**Table 2: Area under the receiver operating characteristic curves and sensitivities at fixed specificities of the spectral domain optical coherence tomograph parameters to diagnose glaucoma and preperimetric glaucoma. Values in brackets represent 95% confidence intervals**

|                     | Glaucoma | Preperimetric glaucoma |
|---------------------|----------|------------------------|
|                     | AUC      | Sensitivity at 95% specificity | Sensitivity at 80% specificity | AUC      | Sensitivity at 95% specificity | Sensitivity at 80% specificity |
| Average GCC thickness (μm) | 0.86 (0.78-0.92) | 63% (39-81) | 78% (64-89) | 0.70 (0.61-0.78) | 27% (6-46) | 50% (37-64) |
| Superior average GCC thickness (μm) | 0.80 (0.71-0.87) | 46% (26-65) | 68% (54-83) | 0.68 (0.58-0.76) | 29% (13-47) | 44% (30-59) |
| Inferior average GCC thickness (μm) | 0.88 (0.80-0.94) | 57% (37-75) | 82% (71-93) | 0.71 (0.62-0.79) | 24% (10-44) | 55% (41-71) |
| GCC Focal loss volume | 0.89 (0.83-0.94) | 57% (8-75) | 90% (81-98) | 0.75 (0.66-0.83) | 24% (2-43) | 56% (41-74) |
| GCC Global loss volume | 0.88 (0.81-0.93) | 60% (38-76) | 78% (63-88) | 0.72 (0.62-0.80) | 29% (13-48) | 52% (37-66) |
| GCC Root mean square | 0.87 (0.80-0.92) | 46% (3-73) | 82% (64-94) | 0.73 (0.65-0.81) | 29% (2-47) | 52% (36-69) |
| GCC SAR (sensitive criteria) | 0.91 (0.86-0.96) | 68% (46-86) | 87% (76-96) | 0.72 (0.63-0.81) | 27% (13-50) | 53% (40-71) |
| GCC SAR (specific criteria) | 0.91 (0.84-0.95) | 65% (35-83) | 91% (83-99) | 0.70 (0.60-0.79) | 24% (6-46) | 53% (39-70) |

GCC: Ganglion cell complex; SAR: Surface abnormality ratio

**Figure 2: Receiver operating characteristic curves of the global parameters of ganglion cell complex (GCC) protocol in diagnosing perimetric glaucoma (a) and preperimetric glaucoma (b). SAR: surface abnormality ratio**
likely to cause some amount of diagnostic uncertainty is more meaningful and mimics the real-life clinical situation than a control group with no suspicious findings of the disease. We have earlier reported the effect of such a control group on the diagnostic ability of the GCC parameters of SD-OCT in both perimetric and preperimetric glaucoma.[19,20] However, a possible limitation of our study is the inclusion of a few preperimetric glaucoma eyes into the control group and vice versa, in spite of two glaucoma experts independently agreeing on the classification. Though a possibility, this is less likely as optic discs that were unable to be classified into glaucoma or non-glaucoma group (by one or both of the experts), were excluded from the analysis. Such true optic disc suspects would require a longitudinal study to look for progressive structural changes and to definitively classify them into glaucoma or non-glaucoma groups.[21]

Another limitation of the present study is the inclusion of a limited sample from the larger data that was available for the analysis. The image analysis on the whole available data might have provided more robust results. However, we feel that with no validated method to calculate a sample size for diagnostic accuracy studies, the chosen sample was adequate enough for the current exploratory analysis. We also chose the sample randomly to avoid any selection bias. The digital optic disc photographs used in this study were two-dimensional. Though simultaneous stereoscopic optic disc photographs are considered better than two-dimensional photographs in evaluating subtle features like excavation of the neuroretinal rim, earlier studies have shown similar agreement between experts under both two-dimensional and stereoscopic conditions both in parameter estimation like cup to disc ratio and in classifying optic discs as glaucomatous.[22,23]

In conclusion, we described a new macular GCC parameter that evaluated the extent of the macula over which the GCC thinning was present and found that this parameter had a better diagnostic ability in perimetric glaucoma but had similar diagnostic ability in preperimetric glaucoma compared to the SD-OCT software provided GCC parameters. We believe that new parameter provides additional information on the extent of glaucomatous damage at macular region which the other two dimensional measures like GCC thickness or the three dimensional measure like GLV do not. Therefore GCC SAR may be used with other software provided measures to develop more efficient parameters for analyzing GCC changes in glaucoma. Future studies should explore developing such parameters and evaluating their diagnostic abilities in early and preperimetric stages of glaucoma.

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