Glaucoma

Novel Heidelberg Retina Tomograph–Based Morphological Parameters Derived from Optic Disc Cupping Surface Processing

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PURPOSE. To explore new features of the optic nerve head morphology using the Heidelberg retina tomograph (HRT) and to assess their discriminating power between glaucomatous patients and normal subjects.

METHODS. HRT reports, exported as TIFF images, from 97 normal subjects and 97 primary open-angle glaucoma (POAG) patients were used. For each image the contour of the dominant region of the optic disc cupping surface (dROCS) was transformed into a data series by calculating the distance of each contour pixel from the centroid. The length of contour (LC) and SD of contour (SDC) along with the dROCS area divided by the disc area (DA) HRT parameter were examined as novel parameters.

RESULTS. The means of LC and SDC, after adjustment for cup area (CA) and DA HRT parameters, and dROCS/DA, after adjustment for CA, presented statistically significant differences (ANCOVA, P < 0.001) between the two groups. Using LC and SDC together in discriminant analysis with leave-one-out cross-validation, 75.3% of cases were correctly classified. Using dROCS/DA together with SDC, the correct classification percentage was 80.6%. The area under the ROC curve was 0.782 for LC, 0.725 for SDC, 0.861 for dROCS/DA, and 0.879 for the linear discrimination function that combines dROCS/DA and SDC.

CONCLUSIONS. These findings suggest that LC, SDC, and dROCS/DA can be exploited to the discrimination between glaucomatous and normal subjects. LC and SDC seem to arise from the difference in the shape of the contour of dROCS between the groups, suggesting bigger deviations and irregularities in the POAG group. (Invest Ophthalmol Vis Sci. 2011; 52:947–951) DOI:10.1167/iovs.10-6298

Glaucoma is the leading cause of irreversible blindness that could be prevented.1 Morphologic changes at the optic nerve head (ONH) are a key feature of glaucoma. Qualitative and quantitative assessments of the ONH are established methods of detecting the disease2–5 and identifying progression.6 With the emergence of newer optical imaging techniques, assessment of optic disc morphology has become more objective and quantitative. Confocal scanning laser ophthalmoscopy provides a topographic, three-dimensional image of the ONH and has become an important investigation for the detection of glaucomatous changes of the optic disc.

The purpose of this work is to define and to investigate novel Heidelberg Retina Tomograph (HRT; Heidelberg Engineering, Heidelberg, Germany) parameters based on optic disc cupping surface morphology. More precisely our work focuses on the analysis of the morphology of the optic disc cup contour and whether this can reveal new information with discriminatory power in the classification of glaucoma.

METHODS

Subjects

In this study the dataset was derived from a retrospective analysis of HRT report images from 97 primary open-angle glaucoma (POAG) patients and 97 normal subjects, acquired during two studies conducted at the Laboratory of Research and Clinical Application in Ophthalmology of the Aristotle University of Thessaloniki. All study procedures adhered to the principles outlined in the Declaration of Helsinki for research involving human subjects, and all participants gave written informed consent before their participation. The first subset of 50 normal subjects and 40 POAG patients were from the Pythagoras Study, a case-control study evaluating phenotypes of glaucoma and the contribution of laser imaging technologies in early diagnosis and phenotypic classification (Pappas T. et al. IOVS 2009;50:ARVO EAbstract 5265). The second subset of 47 normal subjects and 57 POAG patients was from the Thessaloniki Eye Study, a population-based study of glaucoma in the Greek population.” All subjects were classified by glaucoma experts after a standardized eye examination with visual acuity measurement, a visual field test with Humphrey automated perimetry, applanation tonometry, gonioscopy, slit-lamp anterior segment examination, and dilated fundus slit-lamp biomicroscopy.

The criteria used to define glaucoma were those used in the Thessaloniki Eye Study.7 Subjects were classified as POAG if they had glaucoma and an open, normal-appearing anterior chamber angle and absence of other secondary causes of open-angle glaucoma (OAG) in either eye.

For a subject to be classified as normal there should be no glaucomatous damage in the optic disc and visual field. In addition intraocular pressure should be <21 mm HG.

HRT Image Acquisition

Images were acquired with the HRT, using a laser beam of a 670-nm diode laser, which scans 32 sequential 256 × 256 pixel slices. Using a 10° field of view for the HRT, three consecutive single image series of
each eye were taken under the same experimental conditions for all subjects. Before image acquisition, refractive error and keratometry readings were obtained, and they were entered into the HRT software to help correct for magnification errors. A mean topography was calculated from these three single images for each eye and used for further analysis. The standard reference plane was used in all images. Quality assessment of the mean images and contour line drawing at the inner edge of the scleral ring of Elschnig was performed by a single trained observer. When the quality indication of the mean HRT images was good (< 30, HRT software v. 2.0.2), meaning good reproducibility of the three single-image acquisitions, then the optic disc measurements were performed on a mean topographic image. When quality indication was greater than 30, showing poor quality of the mean image, the best single image was used.

The reports of HRT analysis was a set of stereometric parameters, along with an image depicting the cup of the optic nerve head in red and the rim area in blue and green.

Reports of one eye per subject were selected for analysis: randomly for normal subjects, whereas for POAG patients the eye with glaucomatous damage or randomly if damage was found in both eyes. Based on these criteria 158 reports were based on mean images (86 normal, 72 glaucoma), and 36 reports were based on single images (11 normal, 25 glaucoma). All 194 reports selected to be included in the analysis conformed to the above criteria and to the criteria suggested by the analysis as described later.

**Image Analysis and Contour Time Series**

Using HRT software, the reports of HRT analysis on the topography images for the 194 subjects were exported as TIFF image files accompanied by text files having the calculated stereometric parameters in HRT proprietary format.

The files were named after subject's study ID, eye, and expert's classification, for discrimination purposes.

An application was developed in a numerical computing environment (MATLAB; MathWorks, Natick, MA) to perform the analysis tasks. The application accepts as input the list of the TIFF images' file names and creates as output a spreadsheet file having the analysis results. The same process was performed for every subject regardless of classification.

The analysis steps performed in each TIFF image are described as follows.

1. The application locates the optic disc area by use of the HRT pseudo-coloring, where the cup of the optic nerve head is displayed in red and the rim is displayed in blue and green.
2. The larger connected red region of the HRT report is defined as the dominant region of the optic disc cupping surface (dROCS). Small isolated red regions were not considered as being part of the dROCS.
3. The geometric center (centroid) of the dROCS is calculated as follows: $C(x_c, y_c)$ are the coordinates of each pixel of the dROCS, the centroid is $C(\text{mean } x, \text{mean } y)$.
4. The application continues with the tracing of the contour of dROCS pixels starting always from the temporal side of dROCS, and the procedure is performed with respect to the eye of origin. In left-eye images, the rightmost pixel of the dROCS in the vertical level of the centroid is selected ($x_{\text{contour max}}, y_{\text{centroid}}$), followed by the other adjacent pixels of the contour in a counterclockwise direction. In right-eye images the leftmost pixel in the vertical level of the centroid is selected ($x_{\text{contour max}}, y_{\text{centroid}}$) to start, and it is followed by the other boundary pixels in clockwise direction.
5. Having the coordinates of the centroid of the dROCS and the positions of the contour pixels, the distance in pixels between each pixel of the contour and the centroid is calculated following the same sequence as described above in the contour tracing. This step resulted in a one-dimensional data series for each separate HRT report, which represents the shape of the contour.

The steps described above are further illustrated in Figure 1

**Contour Features**

We defined the parameter length of contour (LC) to be the number of pixels of the contour of the dROCS of a given HRT report.

We defined the standard deviation of contour (SDC) of a given HRT report as the SD of the mean distance in pixels from the contour line to the centroid. This is calculated as follows:

\[
\text{LC (pixels)} = \sum_{i=1}^{n} x_{i} \quad \text{SDC (pixels)} = \sqrt{\frac{1}{n} \sum_{i=1}^{n} (x_{i} - \mu)^2} \quad \text{dROCS/DA (pixels/mm²)} = \frac{\sum_{i=1}^{n} x_{i}}{\text{Area (mm²)}}
\]

### Table 1. Group Statistics of LC, SDC, and dROCS/DA

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group</th>
<th>N</th>
<th>Mean</th>
<th>SD</th>
<th>SE Mean</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>LC (pixels)</td>
<td>Normal</td>
<td>97</td>
<td>320.06</td>
<td>93.451</td>
<td>9.489</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Glaucoma</td>
<td>97</td>
<td>459.23</td>
<td>125.532</td>
<td>12.746</td>
<td></td>
</tr>
<tr>
<td>SDC (pixels)</td>
<td>Normal</td>
<td>97</td>
<td>5.729</td>
<td>2.1192</td>
<td>0.21517</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Glaucoma</td>
<td>97</td>
<td>7.719</td>
<td>2.7835</td>
<td>0.28262</td>
<td></td>
</tr>
<tr>
<td>dROCS/DA (pixels/mm²)</td>
<td>Normal</td>
<td>97</td>
<td>2449.1</td>
<td>1163.9</td>
<td>118.18</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Glaucoma</td>
<td>97</td>
<td>4636.8</td>
<td>1792.7</td>
<td>182.03</td>
<td></td>
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</tbody>
</table>
Table 2. Adjusted Means per Group LC, SDC, and dROCS/DA

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group</th>
<th>Mean</th>
<th>SE</th>
<th>Lower Bound</th>
<th>Upper Bound</th>
</tr>
</thead>
<tbody>
<tr>
<td>LC (pixels)</td>
<td>Normal</td>
<td>338.8*</td>
<td>9.602</td>
<td>319.8</td>
<td>357.7</td>
</tr>
<tr>
<td></td>
<td>Glaucoma</td>
<td>420.5*</td>
<td>9.602</td>
<td>401.6</td>
<td>439.5</td>
</tr>
<tr>
<td>SDC (pixels)</td>
<td>Normal</td>
<td>5.758*</td>
<td>.2580</td>
<td>5.249</td>
<td>6.267</td>
</tr>
<tr>
<td></td>
<td>Glaucoma</td>
<td>7.691†</td>
<td>.2580</td>
<td>7.182</td>
<td>8.200</td>
</tr>
<tr>
<td>dROCS/DA (pixels/mm²)</td>
<td>Normal</td>
<td>2644.5†</td>
<td>139.94</td>
<td>2368.5</td>
<td>2920.5</td>
</tr>
<tr>
<td></td>
<td>Glaucoma</td>
<td>4441.5†</td>
<td>139.94</td>
<td>4165.5</td>
<td>4717.6</td>
</tr>
</tbody>
</table>

* Adjustment for HRT CA and HRT DA stereometric parameters.
† Adjustment for HRT CA stereometric parameter.

\[
SDC = \sqrt{\frac{1}{|LC|} \sum_{i=1}^{|LC|} (j_i - \bar{j})^2}
\]  

where \(j_i\) is the one-dimensional data series representing the contour.

We defined the parameter dROCS/DA as the ratio of the area of the dROCS in pixels versus the disc area (DA) HRT parameter in mm².

As previously mentioned, additional exclusion criteria suggested by the analysis were used based on the properties and limitations of the data under investigation. Specifically, reports found to have LC < 200 pixels were excluded (8% of the reports of normal subjects, <1% of reports of POAG patients). This was necessary, because in too small of a dROCS the calculated parameters and spotted irregularities on the contour may arise from low resolution rather than structural differences. Additionally, images were excluded from the study if presenting a seriously fragmented cup area (CA) and lacking a dominant cup region (i.e., presenting two or more large regions and the larger, in terms of area in pixels, is not at least four times bigger than the smaller, <0.5% of all reports).

Statistical Tools

Statistical analyses were performed using commercial statistical software (SPSS, v. 16.0; SPSS, Chicago, IL). The tests used to check the statistical significance of the difference of the parameters means between the two groups were one-sample Kolmogorov-Smirnoff to test normality, Levene's test of equality of error variances to test homogeneity of variances, and an independent sample t-test (ANOVA) and analysis of covariances (ANCOVA) to adjust the results for DA and CA where needed. To test the discrimination power of the parameters, ROC curves and discriminant analysis were used. Discriminant analysis was performed using the leave-one-out cross-validation method to assess how the results would generalize to an independent dataset.

RESULTS

The age of the subjects was normally distributed for each group, varying from 53 to 91 years. The mean age for patients with POAG was 72 years (SD, ±6.8), and the mean age of normal subjects was 70 years (SD, ±5.4), presenting no significant difference between the two groups (\(P = 0.180\)).

The mean, SD, and SEM for the two groups for three novel parameters are presented in Table 1. The three calculated parameters (LC, SDC, dROCS/DA) of the 194 eligible reports have a normal distribution in both groups as verified by the one-sample Kolmogorov-Smirnoff test. There was statistically significant difference (\(P < 0.001\)) for the means of LC, SDC,
and dROCS/DA between glaucomatous and normal subjects (ANOVA).

ANOVA presented statistically significant differences ($P < 0.001$) in the means between normal and POAG groups for LC after the adjustment for CA and DA. The normal group had decreased adjusted mean LC, 338.8 pixels (95% confidence interval [CI], 319.8–357.7), compared with the POAG group (420.5 pixels, 95% CI, 401.6–439.5). The SDC parameter mean difference remained statistically significant after the same adjustment ($P < 0.001$), with the adjusted mean for the normal group at 5.758 pixels (95% CI, 5.249–6.267) and for the POAG group at 7.691 pixels (95% CI, 7.182–8.200). The difference in mean of dROCS/DA between the two groups remained statistically significant ($P < 0.001$) after adjustment for CA. The adjusted mean of the POAG group was higher at 4442 pixels/mm$^2$ (95% CI, 4165–4395) while for normal patients it was 2644 pixels/mm$^2$ (95% CI, 2368–2920). The estimated adjusted means are presented in Table 2.

To test the discrimination power of LC, SDC, and dROCS/DA, the ROC curve was plotted (Fig. 2), and the area under the curve (AUC) was calculated (Table 3). The AUC for dROCS/DA was 0.861, for LC, 0.782, and for SDC, 0.725. The ROC curves and AUC are also shown in Figure 2 and Table 4, respectively.

Using the parameters LC and SDC in discriminant analysis with leave-one-out-classification, 75.3% of cross-validated grouped cases were correctly classified. Performing the same type of discriminant analysis with dROCS/DA and SDC resulted in 80.4% of cross-validated grouped cases being correctly classified. Using HRT-based CA and cup-to-disc ratio together, 62.4% of cross-validated grouped cases were correctly classified. Furthermore, stepwise discriminant analysis method (using Wilk’s lambda) was applied, starting with all five parameters previously used (LC, SDC, dROCS/DA, CA, and cup-to-disc ratio) and iteratively selecting the best out of the five parameters. The most dominant parameters finally used in the classification were dROCS/DA and SDC, resulting in the same percentage of correctly classified cases as mentioned above (80.4%). The linear discrimination function (LDF) that corresponds to the above results is the following:

$$ LDF_{(dROCS/DA), SDC} = 0.154 \times SDC + 5.54 \times 10^{-4} 
\times (dROCS/DA) - 3.068 $$

The classification results using this function are shown in detail in Table 4. The ROC curve of the LDF combining SDC and dROCS/DA is presented in Figure 3, and the AUC for the ROC of the LDF combining SDC and dROCS/DA parameters is 0.879.

**DISCUSSION**

The aim of this study was to seek for new parameters that can be extracted from the two-dimensional image of surface of the ONH as this is captured by the HRT. Furthermore the power of these parameters in discriminating glaucomatous patients from normal subjects was assessed. The rationale of searching new parameters based on this image was that the existing stereometric parameters fully ignore the morphology of the contour of the surface of the optic cupping surface.

The novel LC and SDC parameters apart from their relation to the measured size of the dROCS and disc seem to present different levels of irregularity in the contour of the optic cup surface between glaucomatous and nonglaucomatous subjects.

The LC is a parameter that represents not only the size of the dROCS but also the irregularities on the contour that lead to a variety of distances from the centroid that may sum to different LC values for equally sized dROCSs.

The SDC parameter represents the variability in the distance of the dROCS contour pixels from the centroid. Increased variability may arise from optic nerve fiber loss.

Several studies use cup-to-disc area or area ratio to assess morphologic changes in the ONH in correlation with various diseases. Also, for normal patients the cup-to-disc area ratio has been found to remain relatively constant throughout adulthood. Often in HRT reports, apart from the main region of the cup, additional smaller disconnected regions are marked by HRT as parts of the cup area (colored red). These regions were omitted in all calculations of this study.

Studies have shown that the cup-to-disc area ratio and the rim area are the most powerful HRT stereometric parameters that can be calculated from the two-dimensional image of the ONH surface. In the tests performed with our data sample the AUC of the newly defined dROCS/DA parameter is increased compared with the AUC of the cup-to-disc ratio calculated from the HRT. Although it is already known that the cup

**Table 3. Area under the Curve for LC, SDC, and dROCS/DA new parameters, CA and Cup-to-Disc Ratio HRT Stereometric Parameters, and LDF Combining dROCS/DA and SDC**

<table>
<thead>
<tr>
<th>Test Result Variable(s)</th>
<th>Area under Curve</th>
</tr>
</thead>
<tbody>
<tr>
<td>LC</td>
<td>0.782</td>
</tr>
<tr>
<td>SDC</td>
<td>0.725</td>
</tr>
<tr>
<td>dROCS/DA</td>
<td>0.861</td>
</tr>
<tr>
<td>CA (HRT)</td>
<td>0.684</td>
</tr>
<tr>
<td>Cup-to-disc ratio (HRT)</td>
<td>0.670</td>
</tr>
</tbody>
</table>

**Table 4. Classification Results of the LDF Combining SDC and dROCS/DA Parameters**

<table>
<thead>
<tr>
<th>Group</th>
<th>Original</th>
<th>Cross-validated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Count</td>
<td>Normal</td>
<td>Glaucoma</td>
</tr>
<tr>
<td></td>
<td>84</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>75</td>
</tr>
<tr>
<td></td>
<td>86.6</td>
<td>13.4</td>
</tr>
<tr>
<td></td>
<td>24.7</td>
<td>75.3</td>
</tr>
<tr>
<td>Percent</td>
<td>Normal</td>
<td>Glaucoma</td>
</tr>
<tr>
<td></td>
<td>84</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>72</td>
</tr>
<tr>
<td></td>
<td>86.6</td>
<td>13.4</td>
</tr>
<tr>
<td></td>
<td>25.8</td>
<td>74.2</td>
</tr>
</tbody>
</table>

Cross-validation is done for the cases included in the analysis. In cross-validation, each case is classified by the functions derived from all cases other than that case. Here 80.9% of original grouped cases are correctly classified, and 80.4% of cross-validated grouped cases are correctly classified.
area of glaucomatous eyes is larger than normal, because of the loss of retinal ganglion cells as the disease progresses, the findings of this work suggest that there is a difference in the contour of the optic disc cupping surface as this is represented by the novel parameters LC and SDC. Higher mean SDC values found on glaucomatous subjects may correlate to loss of optic nerve fibers. Therefore, these findings not only can lead to a better screening/classification between the normal and glaucoma population, but also may add a new pathway of investigation into optic disc glaucomatous structural changes.

Further research is needed to investigate the discrimination power provided by the combination of these novel parameters along with the HRT stereometric parameters, which are based on the three-dimensional structure of the optic disc.

Acknowledgments

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References