Quantitative Analysis of Optic Disc Color

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Purpose: To evaluate the reproducibility of ImageJ software in analyzing the color of the optic disc.

Methods: One hundred twelve normal participants (56 males and 56 females) were enrolled in this study. The image of the optic disc was taken using Kowa digital disc photogaphy, and the gray scales of the nasal rim (NR), brightest cupping center (BCC) and largest inferior retinal vein (IRV) were calculated using histogram in ImageJ. Three different observers calculated the gray scales three separate times. Reproducibility was assessed using the interclass correlation coefficient (ICC).

Results: The mean age of the participants was 50.6 years old (range, 11 to 82 years). The mean gray scales of the nasal rim were 91.81, 94.91, and 93.24; those of the brightest cupping center were 174.84, 179.94, and 177.76; and those of the largest inferior retinal vein were 61.85, 53.48, and 56.73 for observers 1, 2, and 3, respectively. Inter-observer reproducibility for NR, BCC and IRV was considered good based upon ICC values of 0.944, 0.860, and 0.789 for observers 1, 2, and 3, respectively. Significant age-related differences between the values of the brightest cupping center were noted, and the gray scale score was decreased in the older participants ($p < 0.001$).

Conclusions: The gray scale of the brightest cupping center diminished with age. ImageJ can be a useful objective tool with high reproducibility in the analysis of optic disc color.

Key Words: Gray scale, ImageJ, Optic disc, Reproducibility

A change of color in the optic disc is one of the important parameters in diagnosing diseases such as toxic optic neuropathy, Leber’s hereditary optic neuropathy, compressive optic neuropathy, and traumatic optic neuropathy. In glaucoma, a disc color change precedes glaucomatous visual field defects, and the optic discs of ocular hypertensive eyes show an increase in pallor but not in cupping [1,2].

Although the color of the optic disc possesses several diagnostic significances, objective measurement of the color of the optic disc is very difficult and complicated to perform. A number of researchers have reported a method to objectively measure the color of the optic disc [3-5]. However, the previously reported method was so complicated that it was not easy to analyze the results and apply them to clinical practice.

ImageJ is a Java-based image-processing and analysis program that was created by the National Institute of Health. This program is already available as freeware and is widely used in various studies [6]. Examples of biologic image analysis using ImageJ include area calculation, particle counting, DNA length measurement, dot blot analysis, optic density calibration, and density histogram creation. While ImageJ is a well-known and useful tool in biology image analysis, few reports have used ImageJ for disc color analysis so far.

The purpose of this study was to introduce ImageJ as a new method to analyze the color of the optic disc and to evaluate its reproducibility as an objective tool in analyzing optic disc color.

Materials and Methods

Participants

Eligibility criteria included normal participants of 112 pairs of eyes (112 patients) recruited from patients referred due to glaucoma suspicion from March 2008 to August 2008 at an urban tertiary-care hospital. They had normal intraocular pressure (<20 mmHg), normal findings in retinal nerve fiber layer photography, which showed no focal wedge-
shaped defects or diffuse retinal nerve fiber layer loss, and normal and reliable results upon visual field testing. One eye from each patient was selected randomly to be analyzed in this study. Exclusion criteria included a best-corrected visual acuity worse than 20 / 25 in both eyes, previous medication for glaucoma, past history of optic neuropathy, previous intraocular surgery (such as cataract removal), refractive error greater than -6.00 diopters, and a dysmorphic optic disc. We also excluded patients with an optic nerve head with abnormal findings, such as notching, rim thinning, excavation, presence of hemorrhage, asymmetric cup/disc (C/D) ratios (>0.2 between eyes), large C/D ratio (>0.7), and noncompliance with the ISNT rule. This research study was reviewed and approved by the Institutional Review Board.

Optic disc photography and ImageJ analysis

All patients had instillation of tropicamide-phenylephrine hydrochloride (Mydrin-P; Santen Pharmaceutical, Osaka, Japan) when the study began. Optic disc photographs were then taken using a Kowa nonmyd7 fundus camera (Kowa, Tokyo, Japan) with a Nikon d70s digital camera (Nikon, Tokyo, Japan) after dilatation. All disc photographs were taken by one experienced examiner. Optic disc images were captured using an image capture program that was presented by Kowa. In the settings of the image capture program, the image format was JPEG, and the image resizing quality was set to medium. Optic disc color analysis using ImageJ was done by gray scale calculation of the optic disc. Three different observers calculated the gray scales at three different points for three separate times.

The three points selected by each observer were as follows: 1) nasal rim (NR), the point at three or nine o’clock from the optic disc center; 2) the brightest cupping center (BCC), nearly the center of cupping where the color is brightest; 3) the largest inferior retinal vein (IRV), the point at which the inferior retinal vein comes in contact with the margin (Fig. 1).

To select the site of the optic disc, we applied the Wand tool to the three selected points in the ImageJ program. This Wand tool automatically creates a selection by tracing objects of uniform color or thresholded objects. We used gray scale for the optic disc color analysis with ImageJ. The gray scale was implied to the intensity per pixel area in which it was calculated using brightness values. For color images, the histogram for gray scale was calculated by converting each RGB image into a gray scale value using the formula $V = 0.299R + 0.587G + 0.114B$. We analyzed the correlation between the gray scale of the three points and the patient’s age. The intra-observer and inter-observer correlations were also evaluated to determine the reproducibility of ImageJ.

Statistical analyses

The data were analyzed using the SPSS ver. 15.0 (SPSS Inc., Chicago, IL, USA). The Pearson’s correlation coefficient was calculated to evaluate the correlation between gray scales and age. An interclass correlation coefficient (ICC) was used to evaluate intra-observer reproducibility. We calculated the Pearson product-moment correlation to assess the correlation between the measurements. Differences were considered significant when the $p$-value was less than 0.05.

Results

One hundred and twelve eyes of 112 patients (56 male and 56 female) were enrolled in this study. The mean age of participants was 50.6 years old (range, 11 to 82 years). The mean gray scale scores of the NR were 91.81 ± 20.47, 94.91 ± 21.90, and 93.24 ± 20.45; the BCC results were 174.84 ± 31.63, 179.94 ± 31.59, and 177.76 ± 31.56; and the largest IRV calculations were 61.85 ± 14.09, 53.48 ± 12.32, and 56.73 ± 13.62 for observers 1, 2, and 3, respectively. Inter-observer reproducibility for the NR, BCC and largest IRV were

Table 1. Mean gray scale and inter-observer reproducibility of the nasal rim, brightest cupping center and largest inferior retinal vein

<table>
<thead>
<tr>
<th></th>
<th>Nasal rim</th>
<th>Brightest cupping center</th>
<th>Largest inferior retinal vein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Observer 1</td>
<td>91.81</td>
<td>174.83</td>
<td>61.85</td>
</tr>
<tr>
<td>Observer 2</td>
<td>94.91</td>
<td>179.94</td>
<td>53.48</td>
</tr>
<tr>
<td>Observer 3</td>
<td>93.24</td>
<td>177.76</td>
<td>56.73</td>
</tr>
<tr>
<td>Correlation*</td>
<td>0.944</td>
<td>0.860</td>
<td>0.789</td>
</tr>
<tr>
<td>$p$-value†</td>
<td>&lt;0.01</td>
<td>0.002</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

*Interclass correlation coefficient.  †Repeated measures analysis of variance.