Development and Reliability of Retinal Arteriolar Central Light Reflex Quantification System: A New Approach for Severity Grading

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PURPOSE. To describe the methodology and assess the reliability of novel computer-based semiautomated software that quantifies retinal arteriolar central light reflex (CR) from digital retinal photographs.

METHODS. A total of 150 optic disc–centered digital color retinal photographs were selected from a population-based cross-sectional study of persons aged 40 to 80 years (the Singapore Malay Eye Study [SiMES]). Computer-assisted software was developed to quantify retinal arteriolar CR by selecting vessel edge points semiautomatically. This software then automatically computes the CR, vessel diameter, and the CR-to-vessel diameter ratio (CRR). Reliability was assessed using the intraclass correlation coefficient (ICC) and Bland–Altman plots. Multiple linear regression analyses were performed to assess the associations between CRR and systemic and ocular factors, to further validate the novel software.

RESULTS. The ICCs for the intergrader and intragrader CRR measurement were 0.76 (95% confidence interval [CI] 0.53–0.89) and 0.86 (95% CI 0.67–0.94), respectively. The ICC for intravist repeatability was 0.87 (95% CI 0.71–0.95). In the multivariate model, a higher CRR was associated with elevated mean arterial blood pressure (per 10 mm Hg increase) (β = 0.017, P < 0.001).

CONCLUSIONS. Quantitative assessment of retinal arteriolar wall opacification is a reliable method using a new computer-assisted system. This new CRR measurement system is a potentially useful tool to study retinal arteriolar abnormalities with systemic diseases.

Keywords: retinal arteriolar central light reflex, retinal color photograph, hypertension, image processing, grading
MATERIALS AND METHODS

Retinal Image Data

A total of 150 retinal photographs were selected from the baseline phase of the Singapore Malay Eye Study (SiMES) and were assessed using the proposed method. The selected images represent the top 5% of good-quality images in the SiMES study. The retinal photographs were taken using a Canon D-60 digital fundus Tokyo, Japan. Image resolution was 3072 × 2048 pixels, with each pixel representing 5.11 μm. The disc-centered images were selected by independent graders at the Australian E-Health Research Centre (AEHRC), Commonwealth Scientific and Industrial Research Organisation, Perth, Australia.

Image Processing and CR Quantification System

The new CR method requires the grader’s input to determine the vessel edge points. The software then computes the edges based on a region growing process, and measures the vessel and central reflex width and their ratio. The vessel detection method relies on the optic disc (OD) center for determining vessel direction and orientation, which are used to identify the vessel region for mapping. The OD center is also used to map the zone B area, the boundary of the region for central reflex quantification (Fig. 1).

Optic Disc Center Detection.

We proposed a method for OD detection and center computation that uses OD size, shape, and color information to detect the OD in the image. First, the method automatically determines the threshold intensity level from the image histogram. Second, using the threshold value, the potential OD area is selected from the image. The Region Growing Technique is then applied in the thresholded image to identify the potential OD regions. Finally, the Hough circle detection algorithm is applied in the gradient image based on the potential OD regions, to identify the OD and compute its center and radius.

The reflectivity of the OD has an effect on the light intensity and brightness in the vessels around it. This also affects the CR in the artery. This is why we selected an artery-area that is a certain distance from the OD: for consistency we use the zone B area. Zone B in an annular fundus area centered on the OD, with an inner radius of 1 disc diameter and an outer radius of 1.5 disc diameter. The zone B area is computed using the OD center and radius.

Vessel Area Mapping.

The boundaries of the area to be cropped for further analysis are defined by edge points. Assume that the two edge points of the vessel are selected, the method obtains the vessel region by cropping from the image. To achieve this, the region boundary points are computed as follows. Assume that the two edge points selected by the user are \((x_{e1}, y_{e1})\) and \((x_{e2}, y_{e2})\). From these two points, the boundary points \((x_1, y_1), (x_2, y_2), (x_3, y_3)\) and \((x_4, y_4)\) are mapped as shown in Figure 2. This is achieved by calculating the midpoint \((x_{m1}, y_{m1})\) of the edge points where \(x_{m1} = (x_{e1} + x_{e2})/2\) and \(y_{m1} = (y_{e1} + y_{e2})/2\). This is then used to map the other points as center points of the cropped image and can be approximated as two centerline points of the vessel within the zone B area. The second center point \((x_{m2}, y_{m2})\) is obtained using the slope of the two edge points \((x_{e1}, y_{e1})\) and \((x_{e2}, y_{e2})\), and projected orthogonal to the first center point \((x_{m1}, y_{m1})\). The direction of the second center point is computed based on the OD center \((c_x, c_y)\) (i.e., the same direction from the OD to

FIGURE 1. Grading interface for central reflex quantification that shows the zone B area and graded arterial central reflex (red and green color).
the first center point). The image quadrant must also be
defined to determine the directional information for the vessel
in the image, relative to the OD. The OD center \((c_x, c_y)\)

\[ \begin{align*}
(x_{11}, y_{11}) & = \text{first mid point} \\
(x_{21}, y_{21}) & = \text{second mid point} \\
(x_{31}, y_{31}) & = \text{third mid point} \\
(x_{41}, y_{41}) & = \text{fourth mid point}
\end{align*} \]

is computed automatically and used to compute the actual
position or real slope of the line between the edge points
(details in the Appendix).

Once the two mid or center points are identified, they are
used to determine the line end points and define the four
boundary points of the region of interest. The boundary points
\((x_1, y_1), (x_2, y_2), (x_3, y_3), \) and \((x_4, y_4)\) are obtained at \(r_1\) normal
distance from the center points \((x_{m1}, y_{m1})\) and \((x_{m2}, y_{m2})\). The
distance \(r_1\) is set as 25 pixels, based on observations made to
obtain most of the target vessel. Once the coordinates of
the cropped image are determined, the image is processed with an
affine translation so that the vessel is aligned horizontally in the
center of the image. This provides greater ability to obtain the
vessel edges with confidence. To achieve this, the coordinates
are translated with respect to an imaginary axis \((x_m, y_m)\)
through the center of the cropped image. The coordinates
of the origin are defined using Equation 5 in the Appendix. The
image is then rotated to align the vessel horizontally. Finally,
the region for vessel detection is defined from the new position
in the translated and rotated image.

**Vessel and Cr Edge-Detection and Caliber Measurement.** Once the desired image region and orientation are
obtained, edge detection and profiling are applied to assess the
effectiveness of the vessel and central reflex edges. A Gaussian derivative operation is applied to
equalize the effect of the vessel and central reflex. Although most
detection by the Canny edge-detection method, it is
not appropriate for CR edge detection for a number of
reasons. Typically, CR edges change abruptly, and compared
with the vessel boundary occasionally have a very low intensity
gradient, which Canny edge detection fails to detect within a
certain threshold value. Unfortunately, if a very low-gradient
magnitude is selected in the Canny method, the level of noise
is extremely high. To avoid this limitation, we chose to apply
gradient pattern detection for computing the edges, described
below.

The Gaussian first derivative operator returns a bell-shaped
distribution in the intensity profile (i.e., gradient magnitude)
along the edge vertical cross-section.11 For edge start-point
selection, this pattern is considered while traversing through a
column of pixels from low to high row numbers. We check if
any pixel’s gradient magnitude is higher or equal to its vertical
neighbors. This follows the second pixel above and below in
the row of the current pixel. The region growing method13 is
then applied, traversing toward the higher column number and

selecting the pixels that have a value greater than or equal to
the current pixel. If all the values are lower than the current
pixel, the closest one is selected.

Once all the potential edges are detected, the edges are
selected that satisfy the first and second edge profiles. This is
performed by checking the pixel intensity level on both sides of
the edge; the first edge has consistent low to high change
(e.g., 80% pixel intensity level) if traveling toward a higher row
number. Following this, the method selects the two edges that
are closest to the selected points. The middle edge(s) are
considered central reflex edges, which should also have the
profile property. After identifying vessel and central reflex
edges (Fig. 3), cross-sectional caliber is measured through
mapping edge pixel pairs from both edges. Finally, the mean
width of the vessel and central reflex for the vessel segment are
computed, and the ratio of vessel reflex width to vessel width
is considered as the CRR. We selected the largest CRR for our
study.

There is occasional need to correct incorrectly computed
vessel and CR widths. In this scenario, the grader has the
option to remove the entire vessel and CR measured widths,
or part of the vessel and CR widths. To allow correction of the
measurements, the method creates a list of the widths
represented in the grading interface (Fig. 1). The widths are
sorted according to their positions (i.e., distance from the OD).
In the grading interface, the grader can activate the correction
and determine if the entire vessel or only the beginning or the
ending of the widths (from the list) are to be removed. The
grader can perform the correction as many times as required
until at least two vessel and CR widths exist.

**Statistical Analysis**

Statistical analyses were conducted using SPSS Statistics
software (IBM SPSS Statistics, IBM Corporation, Chicago, IL,
USA). Three approaches were taken for reliability assessment
using intraclass correlation coefficients (ICC): intragrader
reliability, intergrader reliability, and intravisit repeatability for
CRR measures. For intragrader reliability assessment of the
system, the same images were graded by two individual
graders. For intergrader reliability grading, the same image was
graded by the same grader at two different sessions. For
intravisit repeatability; two images taken at baseline and follow-
up visits were graded by the same grader. The intra- and

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**FIGURE 2.** Mapping points for cropping vessel region.

**FIGURE 3.** Image analysis steps showing edge start points (top left), detected edges (top right), filtered edges (bottom left), and central reflex edges (bottom right).
intergrader reliability of CRR measurement were assessed using 22 retinal photographs and the intravisit repeatability of CRR measurement was assessed using 21 paired retinal photographs. Careful attention was given by graders to measure the same vessel locations. Reliability was assessed by ICC and Bland–Altman plots.

A total of 150 SiMES participants were included for the final analysis of CRR and mean arteriolar blood pressure. Univariate and multiple linear regression models were created to examine the relationship of CRR with systemic factors. Factors significant at $P < 0.1$ with CRR from univariate analysis and potential confounders (age and sex) were included in the multiple regression model.

**RESULTS**

The mean (SD) age of participants was 58.71 (7.14) years, the mean systolic blood pressure was 147.72 (30.74) mm Hg, and the diastolic blood pressure was 81.52 (13.77) mm Hg. The prevalence rate of hypertension (systolic blood pressure $> 140$ mm Hg, the diastolic blood pressure $> 90$ mm Hg) in these subjects was 36.7% (Table 1).

Table 2 shows the intragrader and intergrader reliability analysis for CRR computation. The ICC was high for intergrader reliability, intragrader reliability, and intravisit repeatability, at 0.76, 0.86, and 0.87, respectively. Figure 4 shows the Bland–Altman plot depicting the agreement between the first and second graders, and for intragrader agreement, and intravisit agreement for CRR.

Table 3 shows the univariate analysis between systemic and ocular factors with CRR measurement. Systolic blood pressure, diastolic blood pressure, mean arterial blood pressure, body mass index (BMI), C-reactive protein level, and presence of opacification were significantly associated with CRR. In the multiple regression model adjusting for age, sex, BMI, blood glucose, C-reactive protein, and spherical equivalent, elevated mean arterial blood pressure was associated with higher CRR (Table 4; Fig. 5).

| TABLE 1. Demographics of the Study Population |
|-------------------------------|---|---|
| Age, y                        | 58.71 | 7.14 |
| Systolic blood pressure, mm Hg | 147.72 | 30.74 |
| Diastolic blood pressure, mm Hg | 81.52 | 13.77 |
| Mean arterial blood pressure, mm Hg | 103.59 | 18.30 |
| BMI, kg/m²                     | 26.29 | 4.86 |
| Blood glucose, mM              | 7.62  | 4.62 |
| Total cholesterol, mM          | 5.77  | 1.21 |
| Low-density lipoprotein cholesterol, mM | 3.62 | 1.00 |
| High-density lipoprotein cholesterol, mM | 1.36 | 0.35 |
| C-reactive protein, mg/L       | 4.10  | 7.46 |
| Spherical equivalent, diopters | 0.55  | 1.60 |
| IOP, mm Hg                     | 15.64 | 3.39 |
| n                             | 150  | 100 |
| Male                          | 67   | 44.7 |
| Hypertension                  | 55   | 36.7 |
| Diabetes                      | 45   | 30  |
| Presence of opacification      | 54   | 36  |

| TABLE 2. Reliability Estimates of Quantitative CRR Measurements Using a Computer-Assisted Program in a Subset of Eyes from the SiMES |
|-------------------------------|---|---|
| ICC 95% Confidence Interval   |   |
| Intragrader reliability, n = 22 | 0.86 | 0.67–0.94 |
| Intergarder reliability, n = 22 | 0.76 | 0.53–0.89 |
| Intravisit repeatability, n = 21 | 0.87 | 0.71–0.95 |

| FIGURE 4. Bland-Altman plot showing the agreement between (a) the first and second graders, (b) intragrader agreement, (c) and intravisit agreement for CRR. |
Table 3.

<table>
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<th>95% Confidence Interval</th>
<th>Standardized β</th>
<th>P Value</th>
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<tr>
<td>CRR, per SD increase</td>
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<td>3.09</td>
<td>8.59</td>
<td>0.317</td>
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<tr>
<td>Age</td>
<td>0.20</td>
<td>-0.21</td>
<td>0.62</td>
<td>0.76</td>
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<td>Sex</td>
<td>1.16</td>
<td>-4.38</td>
<td>6.69</td>
<td>0.031</td>
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<tr>
<td>BMI, per SD increase</td>
<td>5.18</td>
<td>2.21</td>
<td>8.15</td>
<td>0.271</td>
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<tr>
<td>Blood glucose, per SD increase</td>
<td>1.76</td>
<td>-0.98</td>
<td>4.50</td>
<td>0.094</td>
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<tr>
<td>C-reactive protein, per SD increase</td>
<td>1.90</td>
<td>-0.84</td>
<td>4.63</td>
<td>0.103</td>
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<tr>
<td>Spherical equivalent, per SD increase</td>
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<td>-6.19</td>
<td>-0.48</td>
<td>-0.180</td>
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<tr>
<td>R²</td>
<td>0.343</td>
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Discussion

In this article, we described a new method for quantifying the retinal arteriolar CR, a marker for hypertensive retinopathy, and examined its reliability and validity. We demonstrated that our method for quantifying the CR has good reliability. We also found that elevated blood pressure is significantly associated with higher CRR, which may further validate the method for assessing the link between retinal vascular changes and hypertension.

Previous studies of CR grading performed qualitative assessments, with reproducibility a major limitation. We were unable to find any reports on the reproducibility of manual or qualitative assessment of CR measures. However, previous studies reported that the graders' subjective assessment for the reproducibility for artery–vein nicking was only 0.4.15,16 We anticipate a similar reproducibility for qualitative CR assessment. To the best of our knowledge, this is the first attempt to achieve a computer-based quantification of retinal arteriolar CR values.

An advantage of this study is the efficiency of the CRR computation. The system requires only a second to compute the CRR value and visualize the measured widths of CR and vessel in the grading window. If it requires any correction, the grader can select the point from which the incorrect measurements can be removed. Grading time for one image on average is 10 to 15 seconds. In comparison, manual qualitative assessment is subjective and relies on comparison with reference images, which often requires several minutes.

Hence, the proposed method is ideally suited to large-scale population-based studies for its accuracy, efficiency, and repeatability of CRR measurement.

A major challenge to extracting quantitative central reflex caliber is the fuzziness of the vessel edge and the accuracy of its detection for caliber measurement. For this reason, this semiautomatic system was developed to allow identification of all the possible edge combinations within the region of interest.

We have also developed an automated vessel segmentation method that outperforms most of the state-of-the-art vessel segmentation methods. However, like all vessel segmentation methods, it suppresses the central reflex region and aims to detect vessel pixels accurately. We have also developed a highly accurate and reliable method for vessel width measurement, but due to the CR edge properties, the method does not work effectively. For this reason, we proposed the current method and developed the CRR quantification system, which weighs on local-region-based vessel and CR width measurements with forcing to find CR edges, considering that CR exists. The grading interface also allows the grader to edit the detected artery and CR caliber to minimize the SD in the measured widths. Our method can provide high reliability and repeatability of retinal arteriolar central reflex measurement, which should provide enhanced information to find a higher confidence level or probability of association between CR and systemic diseases.

Visible broadening of the light reflex from retinal arterioles also has been found to be associated with atherosclerosis. Our proposed CR quantification system, with improved grading accuracy compared with qualitative assessment, can benefit studies such as enhanced arteriolar light reflex signs and systemic vascular diseases, including hypertension and coronary artery disease. This quantified CRR also could establish the CR as a new biomarker for clinical practice, with risk stratification and identification of individuals for vascular diseases.

Our proposed method for CR measurement may provide another option to quantify retinal microvascular abnormalities, in particular to quantify hypertensive retinopathy signs, in addition to current retinal vascular parameters (e.g., retinal vessel diameter), for assessment of retinal microcirculation. We note that currently available cardiovascular disease (CVD) risk prediction tools (e.g., Framingham risk equations) can detect CVD cases with only 50% accuracy.18 It is clear that there is still a need in clinical practice for further markers for risk stratification and identification of persons at risk of hypertension and CVD. Recent studies have investigated quantification...
of arteriolar and venular calibers to assess generalized narrowing\(^2\) and its association with hypertension and CVD. Studies also have been performed to quantify the wall-to-lumen ratio of retinal vessels using scanning laser Doppler flowmetry, and in finding associations with blood flow and cerebrovascular damage.\(^12,20,21\) Central reflex measurement is a potential marker for assessing CVD risk, as hypertensive retinopathy has long been regarded as a risk indicator for systemic morbidity and mortality.\(^22,23\) Prospective studies are needed to establish the sequential effect of quantified CR value with blood pressure and CVD.

The strengths of this study include standardized assessment of retinal images and quantitative measurement of CR and CRR with a computer-assisted program. However, the study has a number of limitations. First, despite the standardized protocols used, the CR and CRR measurement includes measurement errors related to subjective grader input (both intra- and intergrader), variability in image quality (e.g., image contrast or brightness) and other unknown issues (e.g., pulse cycle) that may lead to misclassification or less precision of the measurement. Second, although the retinal camera setting was standardized during photography with the SIMES participants, CRR measurement by the proposed program may vary with different retinal camera settings.

Further studies are required to more extensively validate the proposed software. For example, the robustness of the software should be tested in specific clinical cohorts, such as those with hypertension. Moreover, the effect of image-disturbing artifacts, such as low contrast, background noise, shadows, and lighting conditions, on the software performance also should be evaluated to further validate the software.

In summary, we proposed a new method to quantify the retinal central light reflex and demonstrated its reliability and validity and agreement against the current gold standard manual method. Our findings show that a quantitative and reliable assessment of retinal arteriolar wall opacification is possible, which can potentially help to improve our understanding of the role of the microvasculature and systemic diseases such as hypertension.

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**APPENDIX**

The optic disc center \((c_x, c_y)\) is computed automatically and used to compute the actual position or real slope of the line between edge points, which is as follows:

\[
\begin{align*}
\theta = \theta + \pi/2 & \quad \text{if} \quad x_{e1} \leq c_x \quad \text{and} \quad x_{e2} \leq c_x \\
\theta = \theta + 3\pi/2 & \quad \text{if} \quad x_{e1} > c_x \quad \text{and} \quad x_{e2} > c_x \\
\theta = \theta + \pi/2 & \quad \text{if} \quad y_{e1} > c_y \quad \text{and} \quad y_{e2} > c_y \quad \theta < 0 \\
\theta = \theta + 3\pi/2 & \quad \text{if} \quad y_{e1} > c_y \quad \text{and} \quad y_{e2} > c_y \quad \theta > 0 \\
\theta = \theta + \pi/2 & \quad \text{if} \quad y_{e1} < c_y \quad \text{and} \quad y_{e2} < c_y \quad \theta > 0 \\
\theta = \theta + 3\pi/2 & \quad \text{if} \quad y_{e1} < c_y \quad \text{and} \quad y_{e2} < c_y \quad \theta < 0.
\end{align*}
\]  \(1\)

Following this, the slope of the centerline is computed as \(= \theta = \theta + \pi/2\) (i.e., perpendicular to the line between the edge points). Then the second midpoint \((x_{m2}, y_{m2})\) in Figure 5 is obtained as follows:

\[
\begin{align*}
x_{m2} &= x_{m1} + r_2 \cos(\theta) \\
y_{m2} &= y_{m1} + r_2 \sin(\theta).
\end{align*}
\]  \(2\)

The coordinates of boundary points \((x_1, y_1), (x_2, y_2), (x_3, y_3), (x_4, y_4)\) and the midpoint of the imaginary axis \((x_0, y_0)\) are calculated as follows\(^2\):
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\[ x_1 = x_{m1} + r_1 \cos(\theta_e + \pi/2); \]
\[ y_1 = y_{m1} - r_1 \sin(\theta_e + \pi/2) \]
\[ x_2 = x_{m1} + r_1 \cos(\theta_e + \pi/2); \]
\[ y_2 = y_{m1} - r_1 \sin(\theta_e + \pi/2) \]
\[ x_3 = x_{m2} + r_1 \cos(\theta_e + \pi/2); \]
\[ y_3 = y_{m2} - r_1 \sin(\theta_e + \pi/2) \]
\[ x_4 = x_{m2} + r_1 \cos(\theta_e + \pi/2); \]
\[ y_4 = y_{m2} - r_1 \sin(\theta_e + \pi/2) \]
\[ x_0 = x_{\min} + \frac{x_{\max} - x_{\min}}{2} \quad \text{and} \quad y_0 = y_{\min} + \frac{y_{\max} - y_{\min}}{2}. \]

References