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 intravisit 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

Retinal arteriolar central light reflection from the surface of retinal arterioles (Fig. 1), a sign of hypertensive retinopathy, has been described in several ways, such as blood vessel wall reflection, blood column surface, opacification, copper wiring, silver wiring, arteriolar light streak, and central arteriolar light reflex (CR). An overall increase in CR can be a consequence of wall thickening or hardening; and previous reports have suggested a link between CR and systemic vascular diseases, including hypertension and coronary artery disease. However, previous work involving CR estimation has relied on qualitative and manual assessment, which may introduce significant intra- and interobserver variability. In addition, current CR assessment methods are subjective, time-consuming, and exhausting.

Advances in retinal imaging techniques and image-processing methods have provided the opportunity to measure hypertensive retinopathy signs using quantitative methods. Recent studies have demonstrated that the quantitative measurement of retinal microvasculature from fundus photographs, for example, arteriolar caliber, tortuosity, and fractal dimension, are associated with high blood pressure and major systemic vascular diseases, including coronary heart disease, and diabetes mellitus and its complications. To date, however, no method has been proposed to quantify the CR from fundus photographs. The quantification of CR thickness and determination of its ratio with arteriolar wall thickness can provide a normalized and quantified value of CR for any image set that can then be used as a new biomarker for disease prediction. Furthermore, quantification provides a continuous value for CR, which can then be categorized as CR at many distinct levels, rather than just absent or present (the current form of distinction and analysis) and provide richer information for identifying any association with particular diseases, such as atherosclerosis or hypertension.

In this article, we describe a new methodology for CR quantification and determine its reliability and associations with systemic and ocular factors.
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 camera, 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 at least 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 of the edge points \((x_{m1}, y_{m1})\) 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 \((x_{e1}, y_{e2})\) 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...
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 ($x_c$, $y_c$) 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 $(x_{m1}, y_{m1})$, $(x_{m2}, y_{m2})$, and $(x_4, y_4)$ are obtained at $r_1$ normal distance from the center points $(x_m, y_m)$ 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 ($x_c$, $y_c$) 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 edge properties and select the potential vessel and central reflex edges. A Gaussian derivative operator is applied to extract the edges for vessel and central reflex. Although most edges can be detected 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.\(^\text{11}\) 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 method\(^\text{11}\) 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 or 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 grading, 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-
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 intragrader 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).
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 at risk 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. 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 arteriole and venular calibers to assess generalized narrowing\textsuperscript{19} 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.\textsuperscript{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.\textsuperscript{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 subjectiver 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:

$$\theta = \theta + \pi/2 \text{ if } x_{e1} < c_x \text{ and } x_{e2} < c_x$$

$$\theta = \theta + 3\pi/2 \text{ if } x_{e1} > c_x \text{ and } x_{e2} > c_x$$

$$\theta = \theta + \pi/2 \text{ if } y_{e1} > c_y \text{ and } y_{e2} > c_y \text{ and } \theta < 0$$

$$\theta = \theta + 3\pi/2 \text{ if } y_{e1} > c_y \text{ and } y_{e2} > c_y \text{ and } \theta > 0$$

$$\theta = \theta + \pi/2 \text{ if } y_{e1} < c_y \text{ and } y_{e2} < c_y$$

$$\theta = \theta + 3\pi/2 \text{ if } y_{e1} < c_y \text{ and } y_{e2} < c_y \text{ and } \theta < 0.$$  \hspace{1cm} (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 3 is obtained as follows:

$$x_{m2} = x_{m1} + r_2\cos(\theta)$$

$$y_{m2} = y_{m1} + r_2\sin(\theta).$$  \hspace{1cm} (2)

The coordinates of boundary points ($x_1$, $y_1$), ($x_2$, $y_2$), ($x_3$, $y_3$), and ($x_4$, $y_4$) and the midpoint of the imaginary axis ($x_0$, $y_0$) are calculated as follows: \textsuperscript{24}:

\begin{table}
\centering
\begin{tabular}{c|c|c|c}
\hline
$x$ & $y$ & $x$ & $y$ \\
\hline
1 & 2 & 3 & 4 \\
\hline
\end{tabular}
\end{table}
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\[ x_1 = x_{m1} + r_1 \cos(\theta_c + 3\pi/2) \]
\[ y_1 = y_{m1} - r_1 \sin(\theta_c + 3\pi/2) \]
\[ x_2 = x_{m1} + r_1 \cos(\theta_c + \pi/2) \]
\[ y_2 = y_{m1} - r_1 \sin(\theta_c + \pi/2) \]
\[ x_3 = x_{m2} + r_1 \cos(\theta_c + \pi/2) \]
\[ y_3 = y_{m2} - r_1 \sin(\theta_c + \pi/2) \]
\[ x_4 = x_{m2} + r_1 \cos(\theta_c + 3\pi/2) \]
\[ y_4 = y_{m2} - r_1 \sin(\theta_c + 3\pi/2) \]
\[ x_0 = x_{\min} + \frac{x_{\max} - x_{\min}}{2} \quad \text{and} \quad y_0 = y_{\min} + \frac{y_{\max} - y_{\min}}{2} \]

\[(3)\]

References