Relationship of Retinal Vascular Tortuosity with the Neuroretinal Rim: The Singapore Malay Eye Study

Victor Koh,1 Carol Yim-lui Cheung,1 Yingfeng Zheng,1 Tien Yin Wong,1,2,5 Wanling Wong,1 and Tin Aung1,2

PURPOSE. To describe the association of retinal vascular tortuosity, measured quantitatively, with the neuroretinal rim.

METHODS. A population-based, cross-sectional study was conducted in Malay persons aged 40 to 80 years residing in Singapore. Retinal vascular tortuosity was quantified by using a semiautomated, computer-assisted program assessing retinal fundus photographs, according to a standardized protocol. Optic disc measurements including disc area, rim area, and rim-to-disc area (RDA) ratio were obtained with a confocal scanning laser ophthalmoscope.

RESULTS. In analyses adjusting for age, sex, spherical equivalent, and retinal vascular caliber, reduced arteriolar and venular tortuosity were associated with a decrease in global RDA ratio ($P = 0.006$ and $P = 0.001$, respectively). When compared with the arterioles, retinal venular tortuosity demonstrated a stronger association with RDA ratio. The temporal-inferior region of the neuroretinal rim was most strongly associated with retinal vascular tortuosity.

CONCLUSIONS. Straighter retinal vessels were significantly associated with a thinner neuroretinal rim. These findings may provide additional insights into the geometric retinal vascular changes seen in early glaucomatous optic neuropathy. (Invest Ophthalmol Vis Sci. 2010;51:3736–3741) DOI:10.1167/iovs.09-5008

The retinal vasculature is unique, as it allows the observer to examine the vessels in vivo noninvasively.1,2 Various retinal vascular changes (e.g., narrower retinal vascular caliber) have been shown to be significantly associated with cardiovascular diseases such as hypertension,1–3 ischemic heart disease,4 stroke,5 diabetes mellitus,6,7 and migraine,8 suggesting that such retinal changes may reflect early pathophysiology in the systemic microvasculature.

Glaucoma is a chronic progressive optic neuropathy and a leading cause of blindness worldwide.9 New studies report that narrower retinal vascular caliber is related to glaucomatous optic neuropathy.11–13 Smaller optic disc size,14,15 and thinner retinal nerve fiber layer (RNFL) thickness.16,17 There is also evidence that alteration in ocular blood flow or intraocular perfusion pressure may play a role in the pathogenesis of glaucomatous optic neuropathy.18–20 These findings lend support to a vascular etiology for glaucoma.9,21

Tortuosity, or curvature, of the retinal vessels is a key parameter describing the geometric pattern of the retinal vasculature and is indicative of the optimality of the state of the microcirculation and the level of ocular perfusion.22 Studies have linked less tortuous (or straighter) retinal vessels with ischemic heart disease, higher blood pressure, body mass index, and cigarette smoking,2 and more tortuous vessels with diabetes,23 anemia,24 and prematurity in preterm children.25 No studies have evaluated a possible association between retinal vascular tortuosity with optic nerve head (ONH) changes that may represent early structural changes of glaucomatous optic neuropathy.

The purpose of this study was to determine the relationship of retinal vascular tortuosity, measured quantitatively by computer software from fundus photographs, with neuroretinal rim area, measured by confocal scanning laser ophthalmoscopy, in a population-based study of normal Asian Malay adults.

METHODS

Study Population

The Singapore Malay Eye Study (SiMES) examined 3280 (78.7% response) persons aged 40 to 80 years of Malay ethnicity between August 2004 and June 2006, as described in detail elsewhere.26 Ethics approval was obtained from the Institutional Review Board of the Singapore Eye Research Institute, Singapore, and the study was conducted in accordance with the World Medical Association’s Declaration of Helsinki. Informed written consent was obtained from each participant.

Examination

All patients underwent a standardized and complete ophthalmic examination at the Singapore Eye Research Institute, as described in detail elsewhere.26 Intraocular pressure (IOP) was measured with the Goldmann applanation tonometer (Hagg-Streit, Koniz, Switzerland) before pupil dilation. A single reading was taken from each eye. If the IOP was greater than 21 mm Hg, then a second reading was taken and used as the final measurement. Subsequently, the pupil was dilated and the optic disc visualized with a +78-D lens at ×16 magnification.

Neuroretinal Rim Measurements

Confocal scanning laser ophthalmoscopy was performed (Heidelberg Retinal Tomograph II; [HRT II]; Heidelberg Engineering, Heidelberg Germany) for imaging of the optic nerve head ONH and analyzed with the system software (HRT II, version 1.4.1.0; Heidelberg Engineering). Each subject underwent ONH scanning after pupil dilation in a dim room. HRT II cylindrical lenses were adapted for subjects with astig-
matsism greater than or equal to 1.0 D. All examinations were performed by trained operators. Each image was coupled with a standard deviation to reflect image quality; a standard deviation higher than 50 μm was used as the exclusion criterion. The optic disc margin was manually drawn by a single trained ophthalmologist and was defined as the inner edge of Elschning’s ring. The system software then calculated multiple optic disc parameters (e.g., neuroretinal rim area, cup area, rim-to-disc area [RDA] ratio, and cup-to-disc area ratio) automatically using a standard reference plane that was defined at 50 μm posterior to the mean retinal height between 350° and 356° along the contour line.

In this study, we focused on the neuroretinal rim measurements and used the RDA ratio as the main outcome parameter. Both global and sectoral RDA ratio measurements were analyzed. Sectors (right eye) were classified into temporal (240 –300°), temporal-inferior (180 –240°), nasal-superior (0 – 60°), nasal-inferior (120 –180°), and nasal regions (60 –120°).

**Retinal Vascular Tortuosity Measurement**

Digital fundus photography was taken using a 45° digital retinal camera (model CR-DGi with a 10D SLR digital camera backing; Canon, Tokyo, Japan) after pupil dilatation. We used a semiautomated, computer-based program (Singapore I Vessel Assessment [SIVA], ver. 1.0, an in-house program developed at the National University of Singapore) to quantitatively measure a range of retinal vascular parameters from digital retinal images, including retinal vascular tortuosity and retinal vascular caliber. SIVA automatically identifies the optic disc, places a grid with reference to the center of the optic disc, identifies vessel type, and measures retinal vascular tortuosity. Trained graders are responsible for the visual evaluation of SIVA automated measurements and perform manual intervention if necessary, according to a standardized grading protocol.

Retinal vascular tortuosity is defined as the integral of the curvature square along the path of the vessel, normalized by the total path length. All vessels with a width larger than 40 μm coursing through a zone between 0.5 and 2.0 disc diameters away from the optic disc margin were measured (Fig. 1). Inter- and intragrader reliability was assessed in 50 randomly selected retinal photographs. The intraclass correlation coefficient (ICC) ranged from 0.755 to 0.897.

**Statistical Analysis**

Demographic characteristics were compared between included and excluded subjects by independent t-tests or χ² tests. Linear regression models were constructed to examine the association of retinal vascular tortuosity and neuroretinal rim with RDA ratio as the dependent variable and retinal vascular tortuosity as the independent variable. The mean differences in RDA ratios were estimated for each standard deviation change in retinal arteriolar tortuosity (SD, 0.0091) and retinal venular tortuosity (SD, 0.0094). We performed these analyses, initially adjusting for age and sex, and multivariate models for global and sectoral RDA ratios were additionally adjusted for disc area, spherical equivalent refraction, systolic blood pressure, and retinal vascular caliber (CRAE, CRVE) which accounted for potential confounding from retinal vascular caliber (Cheung CY, unpublished data, 2010). An additional multivariate analysis of cup-to-disc area ratio and global optic disc area with retinal vascular tortuosity was performed with adjustment for age, sex, systolic blood pressure, and retinal vascular caliber (all analyses performed with SPSS, ver. 17.0; SPSS, Chicago, IL).

**RESULTS**

Of the 3280 persons in the study, we excluded 224 persons who did not undergo HRT examination (for logistics reasons in the first few weeks of field work), 195 who had HRT tests with results of unacceptable quality, and 105 who had retinal photographs that could not be graded for tortuosity (without an adequate area for measurement). Of the remaining, we further excluded 115 subjects who had glaucoma. Thus, 2641 (80.5% of the 3280 participants) normal subjects were included in the study. Of the included participants, the range and mean (SD) of arteriolar tortuosity were 0.00 to 0.09 and 0.025 (0.009). The corresponding values for venular tortuosity were 0.01 to 0.09 and 0.028 (0.009).

Table 1 shows the characteristics of the participants included and excluded from the study. Compared with those who were excluded, the subjects who were included were younger, were less likely to have cataracts or diabetes mellitus, and had lower systolic blood pressure and body mass index.

Table 2 shows the regression analysis between retinal vascular tortuosity and global RDA ratio. The normality distribution for retinal vascular tortuosity was checked and confirmed with histograms and a Q–Q normality plot (not shown) that shows that most of the data followed a straight line. It showed that for every standard deviation decrease in retinal arteriolar tortuosity, there was a corresponding decrease in global RDA ratio by 7.79 × 10⁻⁶ (P = 0.006) in both the univariate and multivariate models. On stratification by sex, the men (P ≤ 0.035) showed a more significant relationship between retinal vascular tortuosity and RDA ratio than did the women (all P ≤ 0.074). The relationship was also similar for retinal venular tortuosity in all the subjects (all P ≤ 0.001) and for the men (all P < 0.001). A strong increasing linear association between RDA ratio and retinal vascular tortuosity is also demonstrated in Figure 2, where the mean RDA ratio decreased with each decreasing vascular tortuosity quintile (P trend < 0.001).

Table 3 shows the regression analysis between retinal arteriolar and venular tortuosity with different sectoral RDA ratios. The analysis showed that a decrease in vessel tortuosity was associated with a significant reduction of RDA ratio in all four sectors.
sectors of the ONH. The temporal-inferior sector was the most affected by both retinal arteriolar ($-12.27 \times 10^{-6}, P < 0.002$) and venular ($-12.45 \times 10^{-6}; P = 0.001$) tortuosity.

In the supplementary analysis shown in Table 4, a smaller optic disc area was significantly associated with increased retinal vascular tortuosity after adjustment for age, sex, systolic blood pressure, and retinal vascular caliber. For every standard deviation decrease in retinal arteriolar tortuosity, there was an associated increase in optic disc area of 29.57 $\times 10^{-6} \text{ mm}^2 (P = 0.002)$. Similarly, for every standard deviation decrease in venular tortuosity, the optic disc area was increased by 19.11 $\times 10^{-6} \text{ mm}^2 (P = 0.041)$.

**DISCUSSION**

We examined the relationship between quantitatively measured retinal vascular tortuosity and optic nerve parameters in a large population-based, non-glaucomatous cohort. Less tortuous (or straighter) retinal vessels were associated with a thinner retinal neuroretinal rim area. Although our study included only normal subjects, these findings may provide additional insight into the vascular pattern changes in early glaucomatous optic neuropathy, in which structural changes have been shown to precede visual field defect.31–32 Nevertheless, these relationships may also reflect physiological thinning of the neuroretinal rim.

We have reported that another retinal vascular parameter (narrower retinal arteriolar caliber) is associated with thinner RNFL as measured by laser scanning ophthalmoscopy16 and optical coherence tomography.17 We have also shown that thinner rim area of the ONH is also associated with narrower retinal vascular caliber. The results of these studies suggest that retinal vessel caliber is closely associated with the ocular structures that it nourishes. To date, however, there are few studies that have explored the relationship of optic disc changes with geometric patterns of the retinal vasculature, which, together with vessel caliber, may more comprehensively indicate the optimality of the microcirculation.22

In our study, retinal vascular tortuosity was significantly related to the area of the neuroretinal rim. Such an association could be attributable to a metabolic relationship such as vascular endothelial dysfunction,33–34 which has been shown to play a role in the pathogenesis of glaucomatous optic nerve damage.35–38 Vasculature tortuosity is associated with tissue hypoxia as a complex response mechanism that is mediated by secretions from vascular endothelial cells. The endothelial cells that lined the vessel wall play an important role in autoregulating blood flow by secreting mediators such as nitric oxide39 and endothelin.40 These chemicals are thought to stimulate angiogenesis and thus increase tortuosity, which subsequently promotes better tissue perfusion.41–42 Chronic ischemia in the neuroretinal rim, which is metabolically hyperactive, causes it to thin with time.

Alternatively, the relationships could be purely anatomic in nature. Because of the higher rigidity of the arterial wall, the retinal veins may be more prone to compression forces and caliber reduction at the lamina cribrosa. This results in limitation of vascular outflow and an increase in the distal venular caliber. This increase in the intravascular pressure and tortuosity is consistent with that found in previous studies.39–42 Similarly, we also demonstrated that smaller optic disc sizes are

**TABLE 1. Characteristics of Participants and Nonparticipants**

<table>
<thead>
<tr>
<th></th>
<th>Included (n = 2641)</th>
<th>Excluded (n = 639)</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>57.38 (10.58)</td>
<td>65.55 (10.82)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Men, %</td>
<td>1279 (48.4)</td>
<td>289 (45.3)</td>
<td>0.162</td>
</tr>
<tr>
<td>Presence of cataract, %</td>
<td>1106 (42.8)</td>
<td>370 (76.1)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Total cholesterol, mM</td>
<td>5.61 (1.15)</td>
<td>5.70 (1.23)</td>
<td>0.116</td>
</tr>
<tr>
<td>Presence of diabetes, %</td>
<td>618 (22.5)</td>
<td>147 (27.8)</td>
<td>0.008</td>
</tr>
<tr>
<td>Serum glucose, mg/dL</td>
<td>6.76 (3.61)</td>
<td>7.00 (4.01)</td>
<td>0.216</td>
</tr>
<tr>
<td>Glycosylated hemoglobin, %</td>
<td>6.43 (1.54)</td>
<td>6.57 (1.61)</td>
<td>0.081</td>
</tr>
<tr>
<td>Systolic blood pressure, mm Hg</td>
<td>145.4 (25.2)</td>
<td>155.9 (24.7)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Diastolic blood pressure, mm Hg</td>
<td>79.6 (11.1)</td>
<td>80.2 (11.6)</td>
<td>0.255</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>26.4 (5.04)</td>
<td>25.9 (5.47)</td>
<td>0.045</td>
</tr>
<tr>
<td>Nonsmokers, %</td>
<td>1676 (61.0)</td>
<td>336 (64.0)</td>
<td>0.191</td>
</tr>
<tr>
<td>Presence of cardiovascular disease, %†</td>
<td>296 (10.8)</td>
<td>72 (13.8)</td>
<td>0.050</td>
</tr>
</tbody>
</table>

Data are expressed as the mean (SD) or number (%).
* Independent t-tests or chi square tests based on difference in means or proportions.
† Includes angina pectoris, myocardial infarction and cerebrovascular events.

**TABLE 2. Relationship of Retinal Vascular Tortuosity with Global Optic RDA Ratio**

<table>
<thead>
<tr>
<th>Arteriolar tortuosity (per SD decrease)</th>
<th>n</th>
<th>Age- and Sex-Adjusted (95% CI)</th>
<th>P</th>
<th>Multivariable-Adjusted (95% CI)*</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>All persons</td>
<td>2641</td>
<td>-7.79 (−13.29 to −2.28)</td>
<td>0.006</td>
<td>-7.79 (−13.31 to −2.21)</td>
<td>0.006</td>
</tr>
<tr>
<td>Men</td>
<td>1279</td>
<td>-8.83 (−17.00 to −0.66)</td>
<td>0.034</td>
<td>-8.74 (−16.99 to −0.49)</td>
<td>0.038</td>
</tr>
<tr>
<td>Women</td>
<td>1362</td>
<td>-6.82 (−14.26 to 0.61)</td>
<td>0.072</td>
<td>-6.85 (−14.34 to 0.63)</td>
<td>0.075</td>
</tr>
<tr>
<td>Venular tortuosity (per SD decrease)</td>
<td>n</td>
<td>Age- and Sex-Adjusted (95% CI)</td>
<td>P</td>
<td>Multivariable-Adjusted (95% CI)*</td>
<td>P</td>
</tr>
<tr>
<td>All persons</td>
<td>2641</td>
<td>-10.26 (−15.61 to −4.90)</td>
<td>&lt;0.001</td>
<td>-9.43 (−14.85 to −4.02)</td>
<td>0.001</td>
</tr>
<tr>
<td>Men</td>
<td>1279</td>
<td>-15.86 (−23.67 to −8.05)</td>
<td>&lt;0.001</td>
<td>-14.90 (−22.84 to −6.96)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Women</td>
<td>1362</td>
<td>-5.13 (−12.47 to 2.22)</td>
<td>0.171</td>
<td>-4.41 (−11.82 to 2.99)</td>
<td>0.243</td>
</tr>
</tbody>
</table>

Data are the difference in global RDA ratio ($\times 10^{-6}$), 95% CI, 95% confidence interval.
* Adjusted for age, sex, spherical equivalent, systolic blood pressure, retinal arteriolar caliber (except for the models for venular tortuosity), and retinal venular caliber (except for the models for arteriolar tortuosity).
Table 4. Relationship of Retinal Vascular Tortuosity with Global Optic Cup-to-Disc Area Ratio and Disc Area in the Singapore Malay Eye Study

<table>
<thead>
<tr>
<th>Arteriolar tortuosity (per SD decrease)</th>
<th>Regression Coefficient (95% CI)</th>
<th>P</th>
<th>Multivariate-Adjusted (95% CI)*</th>
<th>P</th>
<th>Difference in Global Cup-to-Disc Area Ratio (×10⁻⁵)</th>
<th>P</th>
<th>Difference in Disc Area (μm²)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>All persons</td>
<td>2641</td>
<td>7.76 (2.21–13.31)</td>
<td>0.006</td>
<td>29.57 (10.80–48.34)</td>
<td>0.002</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Men</td>
<td>1279</td>
<td>8.74 (0.49–16.99)</td>
<td>0.038</td>
<td>35.57 (6.09–61.05)</td>
<td>0.017</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Women</td>
<td>1362</td>
<td>6.85 (~0.63–14.34)</td>
<td>0.075</td>
<td>25.99 (0.30–51.68)</td>
<td>0.047</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Venular tortuosity (per SD decrease)</td>
<td>2641</td>
<td>9.43 (4.02–14.85)</td>
<td>0.001</td>
<td>19.11 (0.74–37.48)</td>
<td>0.041</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All persons</td>
<td>1279</td>
<td>14.90 (6.96–22.84)</td>
<td>&lt;0.001</td>
<td>32.10 (5.48–58.73)</td>
<td>0.018</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Women</td>
<td>1362</td>
<td>4.41 (~2.99–11.82)</td>
<td>0.243</td>
<td>6.57 (18.87–32.01)</td>
<td>0.612</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Adjusted for age, sex, spherical equivalent, systolic blood pressure and retinal arteriolar caliber (except for the models for venular tortuosity) and retinal venular caliber (except for the models for arteriolar tortuosity).
ated with the RDA ratio. Studies of retinal vascular caliber had shown that arteries and veins are affected to a different extent by hypertension (stronger relationship with arterioles)\(^{50}\) and diabetes mellitus (stronger associations with venules).\(^{5,51}\) Such relationships could be due to the complex interaction between the different mediators for vasodilation and vasoconstriction. Similarly, arteriolar and venular tortuosity could also be influenced differently, depending on the prevailing disease conditions in our population. Alternatively, the difference in relationships between arterioles/venules could be anatomic: The arteriolar wall is thicker and less compliant because of the tunica media and thus is subject to less contortion than is the venular wall.

The strengths of our study include a large sample size, a common Asian ethnicity, a community-based population, and a reliable semiautomatic system that can quantify retinal vasculature parameters with high reliability, as shown by the ICC mentioned earlier. This system was consistent with other previous grading systems used to quantify retinal vasculature parameters.\(^{52,53}\) However, there are also limitations to consider. First, as this was a population-based, cross-sectional study, we could only establish an association between vessel tortuosity with RDA ratio. A prospective study would be needed to find out more about the causative relationship between the parameters in question. Second, the HRT parameters are operator dependent as the contours of the optic discs have to be drawn before the HRT software can begin its analysis. Thus, it was important that interobserver variability be kept to a minimum, that contours be drawn with high accuracy and that the observer be masked to the vessel tortuosity. As such, to minimize errors, all the optic disc contours were drawn by a single, masked, experienced ophthalmologist. Last, there was an inherent scaling error in the HRT II software that affected the area and volume measurements of both the ONH and the RNFL thickness. The HRT II software based its measurement on the drawn contour lines, which were affected by image scaling.\(^{54,55}\)

In conclusion, we report that less tortuous or straighter retinal vessels were significantly associated with a thinner neuroretinal rim in a sample of Asian persons without glaucoma. When compared to arteriolar tortuosity, venular tortuosity exhibited a stronger correlation with RDA ratio. The current analysis may improve our understanding of the relationships between geometric changes in the retinal vasculature and the optic nerve.

References


