Original Contribution

Retinal Microvascular Caliber and Chronic Kidney Disease in an Asian Population

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Retinal arteriolar narrowing is a marker of microvascular damage from elevated blood pressure. Between August 2004 and June 2006, the authors examined the association between retinal vascular diameter and chronic kidney disease in a population-based cohort of 3,280 community-dwelling adults of Malay ethnicity aged 40–80 years living in Singapore. Chronic kidney disease was defined as 1) an estimated glomerular filtration rate (eGFR) of <60 mL/minute/1.73 m² from serum creatinine or 2) the presence of micro/macroalbuminuria defined as urinary albumin:creatinine ratios of ≥17 mg/g for men and ≥25 mg/g for women. Retinal arteriolar and venular diameters were measured and summarized as central retinal arteriolar equivalent (CRAE) and central retinal venular equivalent (CRVE). Individuals with reduced CRAE were more likely to have chronic kidney disease than those with increased CRAE. After controlling for age, gender, education, smoking, diabetes, hypertension, body mass index, and total and high density lipoprotein cholesterol, the authors found the odds ratio comparing the smallest with the largest CRAE quartile to be 1.42 (95% confidence interval: 1.03, 1.96; \( P_{\text{trend}} = 0.02 \)) for eGFR of <60 mL/minute/1.73 m² and 1.80 (95% confidence interval: 1.11, 2.91; \( P_{\text{trend}} = 0.01 \)) for micro/macroalbuminuria. Retinopathy was also found to be positively associated with both eGFR and micro/macroalbuminuria. Retinal venular diameter was not associated with chronic kidney disease. These data suggest that retinal arteriolar narrowing is associated with chronic kidney disease, independent of diabetes and hypertension.

Microvascular disease has been hypothesized to contribute to the development of chronic kidney disease (1, 2), particularly among persons with known microvascular risk factors, such as diabetes (3) and hypertension (4). The role and contribution of microvascular disease to kidney damage in the general population, however, are less clear (5, 6).

The retinal microvasculature represented by retinal arterioles and venules allows for noninvasive visualization of the systemic microcirculation (7) and, thus, retinal vessel changes may serve as markers of preclinical stages of systemic microvascular diseases (8). Retinal vessel diameters have been previously associated with risk factors of microvascular disease, such as hypertension (9–11) and diabetes (12–14). Three recent studies conducted in the United States have suggested a possible association between retinal vessel diameter and chronic kidney disease (15–17). However, it is not clear whether it is the narrowing of retinal arterioles or venules or both that is linked to kidney disease. Second, the association between retinal vessel diameter and chronic kidney disease has never been studied in Asian populations, where the prevalence of diabetes (18) and hypertension (19) has increased substantially in the last 2 decades. Moreover, in Asian populations, the relative contribution of some of the known risk factors of kidney disease, such as high blood pressure (20) and overweight/obesity (21, 22), is shown to be different from that of Caucasians. In this context, we examined the association between retinal vessel diameters and chronic kidney disease in an Asian population in Singapore.

Abbreviations: CI, confidence interval; CRAE, central retinal arteriolar equivalent; CRVE, central retinal venular equivalent; eGFR, estimated glomerular filtration rate; ETDRS, Early Treatment for Diabetic Retinopathy Study; HbA1C, glycosylated hemoglobin.
MATERIALS AND METHODS

Study population

The Singapore Malay Eye Study is a population-based study designed to study the prevalence and risk factors for eye and related conditions affecting urban people living in Asia. Details of the study participants and methods have been described elsewhere (23). In brief, of 4,168 eligible individuals aged 40–80 years selected by an age-stratified random sampling method, 3,280 participated in the study (78.7% response rate) (24).

Informed, written consent was obtained from all participants, and ethical approval was obtained from the Institutional Review Board of the Singapore Eye Research Institute.

Digital fundus photography was performed for 3,264 (99.5% of the total study population) participants, of which 3,019 participants had gradable fundus photographs for retinal vascular measurements (25). We further excluded participants with a history of prevalent cardiovascular disease, defined as self-reported myocardial infarction, angina, or stroke (n = 317), and participants with missing information on serum creatinine (n = 116) or other relevant covariates (n = 5), leaving 2,581 for the analysis of estimated glomerular filtration rate (eGFR) of <60 mL/minute/1.73 m². Similarly for the micro/macroalbuminuria analysis, we included 792 participants who had information on the urinary albumin:creatinine ratio and other relevant covariates. Compared with participants included for both analyses, those excluded were older and less educated and had a higher prevalence of diabetes mellitus and hypertension (P < 0.01).

Retinal vessel diameter measurement

Color retinal photographs of both eyes were taken after dilating the pupils with 1% tropicamide and 2.5% phenylephrine hydrochloride, using a digital nonmydriatic retinal camera (CR-DGi with a 10D SLR backing; Canon, Tokyo, Japan). Two retinal images of each eye were obtained, one centered at Early Treatment for Diabetic Retinopathy Study (ETDRS) standard field 1 (the optic disc) and another centered on the ETDRS standard field 2 (the fovea). Images were sent to the Retinal Vascular Imaging Centre, Centre for Eye Research Australia, University of Melbourne, for measurement of retinal vascular caliber (25).

For each participant, the image with the best quality for 1 eye was graded for retinal vessel measurements by using computer-assisted software (IVAN; University of Wisconsin, Madison, Wisconsin) (26, 27) by a trained grader, who was masked to participant characteristics. All arterioles and venules coursing through a specified area from 0.5- to 1-disc diameter from the optic disc margin were measured and combined into summary measures (referred to as “central retinal arteriolar equivalent” (CRAE) and “central retinal venular equivalent” (CRVE)) by using the improved Parr and Hubbard formulas later modified by Knudtson et al. (27, 28). The reproducibility of retinal vascular measurements was very high, with intragraded intraclass correlation coefficients of 0.99 (95% confidence interval (CI): 0.98, 0.99) for CRAE and 0.94 (95% CI: 0.92, 0.96) for CRVE. In addition to retinal vascular diameters, retinopathy changes were also assessed among the study participants. Retinopathy was considered present if any characteristic lesion as defined by the ETDRS severity scale was present: microaneurysms, hemorrhages, cotton wool spots, intraretinal microvascular abnormalities, hard exudates, venous beading, and new vessels (29).

Measurement of chronic kidney disease

Chronic kidney disease was defined as 1) eGFR of <60 mL/minute/1.73 m², an outcome related to kidney function based on the US National Kidney Foundation Kidney Disease Outcome Quality Initiative Working Group definition (30), and 2) the presence of micro/macroalbuminuria defined as a urinary albumin:creatinine ratio of ≥17 mg/g for men and ≥25 mg/g for women, an outcome related to kidney damage (30).

The glomerular filtration rate was estimated from the serum creatinine concentration by using the modification-of-diet-in-renal-disease equation (31) defined as follows: eGFR = 186.3 × (serum creatinine (mg/dL))⁻¹.154 × age⁻₀.₂₀₃ × (0.742 for women). The serum creatinine measurement was carried out at the National University Hospital Reference Laboratory and was reported as μmol/L. Spot untimed urine samples were collected for measurement of albumin and creatinine. Albumin was measured in mg/L and creatinine in mmol/L. The concentration ratio of urinary albumin to creatinine expressed in μg/mg was used to estimate the total daily albumin excretion.

Measurement of other variables

Information on participants’ demographic characteristics, educational attainment, cigarette smoking, alcohol consumption, and medical history was obtained by using a standardized questionnaire administered by trained personnel. Education was categorized into 1) primary or lower (<6 years), 2) secondary (7–10 years), and 3) postsecondary (≥11 years, including university education). Blood pressure was measured with a digital automatic blood pressure monitor (Dinamap model Pro Series DP110X-RW, 100V2; GE Medical Systems Information Technologies, Inc., Wauwatosa, Wisconsin) after the participants were seated for at least 5 minutes. For each participant, the average of the last 2 out of a total of 3 measurements was used as the systolic and diastolic blood pressure value. Hypertension was defined as systolic blood pressure of ≥140 mm Hg, diastolic pressure of ≥90 mm Hg, or self-reported previously diagnosed hypertension. Hypertension was also categorized according to the Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure (32). Diabetes mellitus was defined as a casual plasma glucose measurement of ≥200 mg/dL (11.1 mmol/L), self-reported physician-diagnosed diabetes, use of glucose-lowering medication, or glycosylated hemoglobin (HbA1C) of ≥7% (2 standard deviations above the normal mean) (33). Body mass index was calculated as weight (kg)/height (m)². Cigarette smoking was categorized into current smoker, former smoker, or nonsmoker, and alcohol consumption was categorized into drinkers

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Statistical analysis

All statistical analyses were performed by using SAS, version 9.1, software (SAS Institute, Inc., Cary, North Carolina). CRAE and CRVE measurements were categorized into quartiles. We compared selected baseline characteristics of the cohort by quartiles of CRAE, using the chi-square test or analysis of variance, as appropriate. We performed the following prespecified analyses to test the association among CRAE, CRVE, and chronic kidney disease. We calculated the odds ratio and 95% confidence interval associated with quartiles of CRAE and CRVE, with the largest quartile (quartile 4) as the reference for the 2 chronic kidney disease-related outcomes: 1) eGFR of <60 mL/minute/1.73 m² and 2) micro/macrolembulminuria in 2 multivariable logistic regression models. In the first model, we adjusted for covariates known to be associated with chronic kidney disease chosen a priori, such as current smoking (absent, present), diabetes status (absent, present), hypertension (normal, prehypertension, stage 1 hypertension, stage 2 hypertension) (32), body mass index (kg/m²), total cholesterol (mmol/L), and high density lipoprotein cholesterol (mmol/L). Education was not included in the multivariable model as there was no substantial association between education and chronic kidney disease (Table 1). Tests for trend were performed by modeling CRAE and CRVE quartiles as an ordinal variable in the corresponding multivariable logistic regression models. We also analyzed CRAE and CRVE as a continuous variable (per standard deviation change). We performed subgroup analyses stratified by gender, body mass index, diabetes mellitus, and hypertension status to determine whether our results were consistent across categories of possible confounders. Finally, we analyzed the association between retinopathy and the 2 chronic kidney disease-related outcomes using multivariable model 1. In a supplementary analysis, to examine if the observed association between CRAE and chronic kidney disease is confounded by fellow vessel diameter, we additionally adjusted for fellow vessel diameter; that is, CRVE was included as an independent variable in the multivariable model. In a second supplementary analysis, we additionally adjusted for HbA1c and mean arteriolar pressure in addition to CRVE in the multivariable model.

RESULTS

Selected baseline characteristics of the study population included for the analysis of estimated glomerular filtration rate by quartiles of CRAE are shown in Table 1. Compared with individuals in the largest quartile (quartile 4) of CRAE, those in the smallest quartile (quartile 1) were more likely to be males and older, had higher total cholesterol and systolic and diastolic blood pressure measurements, and had a lower estimated glomerular filtration rate.

Table 2 presents the association among CRAE, CRVE, and eGFR of <60 mL/minute/1.73 m². Reduced CRAE was positively associated with eGFR of <60 mL/minute/1.73 m².
The association was persistent in the age- and sex-adjusted model and in the multivariable model. In contrast, there was no clear association between CRVE and eGFR of < 60 mL/minute/1.73 m².

The association among CRAE, CRVE, and micro/macroalbuminuria is shown in Table 3. Similar to the results for eGFR as the outcome, reduced CRAE was positively associated with micro/macroalbuminuria. In contrast, there was no clear association between CRVE and micro/macroalbuminuria. In a related subgroup analysis stratified by gender, body mass index, diabetes, and hypertension, the positive association observed among reduced CRAE, eGFR of < 60 mL/minute/1.73 m², and micro/macroalbuminuria persisted within these stratified subgroups also (data not presented).

The association among retinopathy, eGFR of < 60 mL/minute/1.73 m², and micro/macroalbuminuria is shown in Table 4. Retinopathy was found to be positively associated with both eGFR of < 60 mL/minute/1.73 m² and micro/macroalbuminuria. This positive association was also observed among those with diabetes or hypertension.

We conducted several sets of supplementary analyses. We repeated the main analysis for CRAE presented in Tables 2 and 3 while additionally adjusting for CRVE, and the results were essentially similar. For eGFR of < 60 mL/minute/1.73 m² as the outcome, compared with quartile 4 of CRAE (referent), the multivariable odds ratio for eGFR of < 60 mL/minute/1.73 m² was 1.13 (95% CI: 0.81, 1.59) in quartile 3, 1.29 (95% CI: 0.92, 1.81) in quartile 2, and 1.43 (95% CI: 1.00, 2.04) in quartile 1 (P_trend = 0.003). Similarly, for micro/macroalbuminuria as the outcome, compared with quartile 4 of CRAE (referent), the multivariable odds ratio was 1.06 (95% CI: 0.64, 1.75) in quartile 3, 1.30 (95% CI: 0.77, 2.18) in quartile 2, and 2.04 (95% CI: 1.19, 3.50) in quartile 1 (P_trend < 0.0001).

In a second supplementary analysis, we additionally adjusted for HbA1c and the mean arteriolar pressure in addition to CRVE in the multivariable model. As expected, this attenuated the association among CRAE, eGFR of < 60 mL/minute/1.73 m², and micro/macroalbuminuria, although the pattern remained the same. For example, for eGFR of < 60 mL/minute/1.73 m² as the outcome, compared with quartile 4 of CRAE (referent), the multivariable odds ratio was 1.11 (95% CI: 0.80, 1.56) in quartile 3, 1.25 (95% CI: 0.89, 1.76) in quartile 2, and 1.35 (95% CI: 0.94, 1.94) in quartile 1 (P_trend = 0.005). Similarly, for micro/macroalbuminuria as the outcome, compared with quartile 4 of CRAE, the multivariable odds ratio was 0.93 (95% CI: 0.56, 1.57) in quartile 3, 1.24 (95% CI: 0.73, 2.11) in quartile 2, and 1.64 (95% CI: 0.93, 2.91) in quartile 1 (P_trend = 0.002).

**DISCUSSION**

In a population-based study, reduced CRAE, representing retinal arteriolar narrowing, was associated with chronic kidney disease, as defined by eGFR of < 60 mL/minute/1.73 m²; Singapore Malay Eye Study, August 2004–June 2006.
1.73 m$^2$ or micro/macroalbuminuria, independent of age, gender, smoking, diabetes mellitus, hypertension, body mass index, and total and high density lipoprotein cholesterol. The association between reduced CRAE and eGFR of <60 mL/minute/1.73 m$^2$ or micro/macroalbuminuria was consistently present when CRAE was analyzed as a continuous variable and in analyses stratified by gender, body mass index, diabetes, and hypertension. CRVE was not associated with any of the kidney endpoints.

The glomerular filtration rate is the best measure of kidney function in health and disease, and microalbuminuria is a sensitive marker of kidney damage that precedes the decline in glomerular filtration rate and indicates earlier stages of chronic kidney disease (30). The retinal microvasculature provides a unique window for noninvasive visualization of the human circulation in vivo (34, 35). Early retinal microvascular caliber changes have been shown to predict the development of a range of cardio-metabolic conditions, such as diabetes (12, 14), hypertension (36–38), cardiovascular disease (39–41), and chronic kidney disease (15, 16), in large population-based studies. This is supported by animal studies that have demonstrated retinal vascular changes specific to systemic diseases in rat models (42).

Results from our study suggest that retinal arteriolar narrowing is associated with chronic kidney disease. This association was consistently present for both a measure of kidney function (eGFR of <60 mL/minute/1.73 m$^2$) and a marker of kidney damage (micro/macroalbuminuria). Further, retinal microvascular abnormalities, indicative of retinopathy, were also associated with chronic kidney disease. Few previous studies have examined the association between retinal vessel diameter and renal function with results inconclusive. In the Wisconsin Epidemiologic Study of Diabetic Retinopathy, retinal arteriolar narrowing was associated with proteinuria among individuals with type 1 diabetes in the cross-sectional analysis (43). However, in the prospective analysis, this association was not evident; instead, retinal venular dilation was associated with gross proteinuria and renal insufficiency. The authors concluded that this inconsistent association could possibly be due to selective mortality of those with retinal arteriolar narrowing and renal disease (16). In the Cardiovascular Health Study, retinal vascular caliber changes were not associated with renal function decline; again, selection and survival biases may have obscured these associations in elderly Americans (17). In the Atherosclerosis Risk in Communities Study, both retinal arteriolar and venular narrowing were associated with a 6-year change in serum creatinine among middle-aged people (15). Our study is the first to examine the association between retinal vessel diameter and chronic kidney disease in an Asian population. Unlike authors of previous articles, we were able to perform a detailed analysis looking at retinal arteriolar and venular diameters separately. Our data suggest that retinal arteriolar but not venular narrowing is associated with kidney disease.

### Table 3. Association Among Central Retinal Arteriolar Equivalent, Central Retinal Venular Equivalent, and Micro/macroalbuminuria, Singapore Malay Eye Study, August 2004–June 2006

<table>
<thead>
<tr>
<th>No. at Risk</th>
<th>No. of Cases</th>
<th>Micro/macroalbuminuria, %</th>
<th>Age- and Sex-adjusted Model</th>
<th>Multivariable Model</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Odds Ratio</td>
<td>95% Confidence Interval</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Odds Ratio$^a$</td>
<td>95% Confidence Interval</td>
</tr>
<tr>
<td>CRAE, $\mu$m</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Quartile 1 (89.7–128.5)</td>
<td>197 84</td>
<td>42.6</td>
<td>1.88</td>
<td>1.22, 2.89</td>
</tr>
<tr>
<td>Quartile 2 (128.6–137.0)</td>
<td>199 70</td>
<td>35.2</td>
<td>1.37</td>
<td>0.89, 2.12</td>
</tr>
<tr>
<td>Quartile 3 (137.1–146.4)</td>
<td>198 56</td>
<td>28.3</td>
<td>1.01</td>
<td>0.65, 1.59</td>
</tr>
<tr>
<td>Quartile 4 (146.5–196.4)</td>
<td>198 56</td>
<td>28.3</td>
<td>1.00</td>
<td>Referent</td>
</tr>
<tr>
<td>$P_{\text{trend}}$</td>
<td></td>
<td></td>
<td>0.002</td>
<td>0.01</td>
</tr>
<tr>
<td>1-SD (15.1-$\mu$m) decrease</td>
<td>792 266</td>
<td>33.6</td>
<td>1.24</td>
<td>1.06, 1.44</td>
</tr>
<tr>
<td>CRVE, $\mu$m</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Quartile 1 (154.6–207.7)</td>
<td>197 61</td>
<td>31.0</td>
<td>0.93</td>
<td>0.60, 1.45</td>
</tr>
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<td>Quartile 2 (207.8–220.7)</td>
<td>198 83</td>
<td>40.9</td>
<td>1.48</td>
<td>0.97, 2.27</td>
</tr>
<tr>
<td>Quartile 3 (220.8–235.4)</td>
<td>199 63</td>
<td>31.7</td>
<td>1.02</td>
<td>0.66, 1.57</td>
</tr>
<tr>
<td>Quartile 4 (235.5–286.3)</td>
<td>198 61</td>
<td>30.8</td>
<td>1.00</td>
<td>Referent</td>
</tr>
<tr>
<td>$P_{\text{trend}}$</td>
<td></td>
<td></td>
<td>0.78</td>
<td>0.54</td>
</tr>
<tr>
<td>1-SD (21.4-$\mu$m) decrease</td>
<td>792 266</td>
<td>33.6</td>
<td>1.00</td>
<td>0.86, 1.16</td>
</tr>
</tbody>
</table>

Abbreviations: CRAE, central retinal arteriolar equivalent; CRVE, central retinal venular equivalent; SD, standard deviation.

$^a$ Adjusted for age (years), gender (male, female), current smoking (absent, present), diabetes status (absent, present), hypertension (normal, stage 1 hypertension, stage 2 hypertension), body mass index (kg/m$^2$), total cholesterol (mmol/L), and high density lipoprotein cholesterol (mmol/L).
It is plausible that retinal arteriolar narrowing may affect kidney function through small-vessel damage resulting from advancing age, diabetes mellitus, hypertension, inflammation, and other related conditions (7, 44). Similarly, an association between diabetic retinopathy and nephropathy has been well documented (45, 46). Animal studies have shown that pathologic changes in the retinal and renal microcirculation are highly correlated in spontaneously hypertensive rats (47). Retinal arteriolar narrowing resulting from hypertension, cigarette smoking, inflammation, and other processes may provide a common pathophysiologic link for the development of diabetes and chronic kidney disease (12, 14). Furthermore, systemic markers of inflammation and endothelial dysfunction (44) associated with retinal vascular abnormalities could contribute to the development of chronic kidney disease.

The strengths of our study include its population-based sample, quantitative and masked evaluation of retinal vessel diameters, standardized measurement of renal function, and the availability of information on potential confounding factors. Several study limitations need to be considered while interpreting our results. First, the cross-sectional nature of the study limits making causal inferences. Second, micro/macrolbuminuria was defined on the basis of a single spot urinary albumin:creatinine ratio measurement, which could have either overestimated or underestimated the prevalence of micro/macrolalbuminuria. Third, exclusion of several eligible participants from the micro/macrolalbuminuria analysis because of missing data on the urinary albumin:creatinine ratio may have introduced a selection bias. However, the results were essentially similar when eGFR of <60 mL/minute/1.73 m² was used as an outcome. Fourth, because retinal arteriolar diameters are closely related to hypertension and high blood pressure, statistical adjustment for hypertension/high blood pressure in the multivariable model may be viewed as an “overadjustment” strategy; the true odds ratio is likely to be slightly higher than the values presented in the final multivariable model.

In conclusion, the results from these population-based data from an Asian population suggest a positive association between retinal arteriolar narrowing and eGFR of <60 mL/minute/1.73 m² or micro/macrolalbuminuria. As a corollary, the observed association between retinal arteriolar narrowing and chronic kidney disease may partly explain the excess cardiovascular mortality associated with retinal arteriolar narrowing among middle-aged persons (48). If supported by future prospective studies and replicated in other populations, our findings have clinical implications to evaluate kidney function in individuals with retinal arteriolar narrowing and to decide appropriate intervention strategies to reduce the burden of kidney disease.

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