The Microvasculature in Chronic Kidney Disease

Qi Lun Ooi,* Foong Kien Newk-Fon Hey Tow,* Raj Deva,* Mohamad Afzal Alias,* Ryo Kawasaki,* Tien Y. Wong,* Nor Mohamad,* Deb Colville,* Anastasia Hutchinson,* and Judy Savige*

Summary
Background and objectives Individuals with chronic kidney disease (CKD) stages 3 to 5 have an increased risk of cardiac and other vascular disease. Here we examined the association of CKD 3 to 5 with small vessel caliber.

Design, setting, participants, & measurements This was a cross-sectional observational study of 126 patients with CKD stages 3 to 5 (estimated GFR [eGFR] <60 ml/min per 1.73 m²) and 126 age- and gender-matched hospital patients with CKD 1 or 2. Retinal vessel diameters were measured from digital fundus images by a trained grader using a computer-assisted method and summarized as the central retinal artery equivalent (CRAE) and central retinal vein equivalent (CRVE).

Results Patients with CKD 3 to 5 had a smaller mean CRAE and CRVE than hospital controls (139.4 ± 17.8 μm versus 148.5 ± 16.0 μm, P < 0.001; and 205.0 ± 30.7 μm versus 217.4 ± 25.8 μm, respectively; P = 0.001). CRAE and CRVE decreased progressively with each stage of renal failure CKD1–2 to 5 (P for trend = 0.08 and 0.04, respectively). CKD and hypertension were independent determinants of arteriolar narrowing after adjusting for age, gender, diabetes, dyslipidemia, and smoking history. Patients with CKD 5 and diabetes had a larger mean CRAE and CRVE than nondiabetics (141.4 ± 14.9 μm versus 132.9 ± 14.2 μm; 211.1 ± 34.4 μm versus 194.8 ± 23.8 μm).

Conclusions The microvasculature is narrowed in patients with reduced eGFR.


Introduction
Individuals with renal impairment (chronic kidney disease [CKD] stages 3 to 5) have an increased risk of macrovascular disease, with cardiac mortality 20 times greater than in the nonrenal population (1) and an increased likelihood of cerebrovascular, aortic, and peripheral vascular disease (2–4). Even patients with CKD stages 3 and 4 have an increased risk of vascular disease (5), which in these early stages is associated with the traditional risk factors of age, gender, hypertension, diabetes, smoking, dyslipidemia, and family history. The mechanisms are less well understood in late disease but include “nontraditional” risk factors such as systemic inflammation, oxidative stress, vascular calcification, endothelial dysfunction, prothrombotic tendency, and anemia (6).

Microvascular disease is also common in CKD but has been described as the “Cinderella of uremic heart disease," important but neglected (7). Cardiac microvascular disease results from increased extravascular resistance from left ventricular failure and interstitial fibrosis and from an abnormal arteriole and capillary structure (7). Patients with CKD have an increased risk of lacunar infarcts, and up to 70% of dialysis patients aged over 55 have some degree of cognitive impairment that is probably microvascular in origin (8–10). Even progressive renal failure may be due in part to small vessel disease (11,12).

The microvasculature can be visualized directly in the retina. Focal abnormalities include localized vessel narrowing, arteriovenous nicking, hemorrhage, microaneurysms, and soft exudates (13,14). Generalized retinal arteriolar narrowing typically reflects permanent or persistent damage usually from atherosclerosis or long-standing hypertension (15). Recent advances in the quantification of retinal vascular caliber provide an accurate and reproducible measure of arteriolar narrowing (16–18), and caliber changes closely parallel microvascular disease elsewhere in the body (19). Population-based studies suggest that retinal microvascular disease predicts cardiovascular events in other high-risk populations including those with diabetes or dyslipidemia (20–23). This study determined the association between CKD 3 to 5 and retinal vascular caliber, and the role of the major determinants of caliber.

Materials and Methods
Study Population
The patients in this study were part of a cohort whose retinal changes have been described in reference (39). This was a cross-sectional observational study of 151 hospital patients with CKD 3 to 5 (estimated GFR [eGFR] <60 ml/min per 1.73 m² for at least 3 months)
from any cause (24). Their eGFR was calculated from the serum creatinine concentration in mmol/L using the Modification of Diet in Renal Disease equation (25). Proteinuria was not taken into account. Consecutive patients were invited to participate during a weekly renal clinic at each of two metropolitan teaching hospitals over a period of 18 months, and from the general medical ward at one of these sites on certain days over a 3-month period. Patients who had a renal transplant were excluded because of a possible effect on retinal caliber. The participation rate in the primary study was greater than 80%. However, the retinal photographs in only 126 patients (83.4%) were suitable for microvascular grading and are described here.

Controls were patients with CKD 1 to 2 (eGFR ≥60 ml/min per 1.73 m²) recruited from the wards and clinic at the same time as patients with CKD 3 to 5, but age- and gender-matched. Further medical details and the presence of any retinal disease were not known at the time of recruitment for any of the patients. This study was approved by the Human Research Ethics Committee of Northern Health and Austin Health in accordance with the Principles of Helsinki, and all participants provided signed, informed consent.

Study Assessments
Participants completed a structured questionnaire that included their medical details, traditional macrovascular risk factors, and current medications. Hospital records were used to confirm details and obtain laboratory test results. Hypertension was diagnosed with a systolic BP of ≥90 mmHg or diastolic pressure of ≥60 mmHg; diabetes with a random glucose measurement of ≥11.0 mmol/L; and dyslipidemia with serum cholesterol >5.0 mmol/L or HDL <2.0 mmol/L. These diseases were also diagnosed if self-reported as physician diagnosed or treated.

Retinal Imaging and Caliber Measurements
Patients underwent color retinal photography of both eyes after dark adaptation or dilation with 1% tropicamide using a KOWA 7 (Optimed) or Canon CR5–45NM (Canon) nonmydriatic digital retinal camera. Standard 45° images were taken of both eyes, with at least one view centered on the macula and another on the optic disc. A trained grader at the Centre for Eye Research Australia (Melbourne, Australia), who was masked to subject identity, CKD stage, and other clinical details, evaluated the digital retinal images using a standardized protocol. Retinal vessel width was measured from the images using a method described previously (16,26). Briefly, all vessels coursing through a zone 0.5 to 1.0 disc diameters from the disc margin were measured using a computer imaging program (University of Wisconsin). Summary measures on the basis of the six largest vessels referred to as the central retinal artery equivalent (CRAE) and the central retinal vein equivalent (CRVE) were calculated using the formulae proposed by Knudtson et al. (27). The reproducibility of retinal vascular measurements was very high, with intra-class correlation coefficients of 0.99 (95% confidence interval [CI] 0.98 to 0.99) for the CRAE and 0.94 (95% CI 0.92 to 0.96) for the CRVE.

Statistical Analyses
The categorical data were recorded in contingency tables and reported as frequencies and percentages. Mean and SD or median and range were used for continuous data. Fisher’s two-tailed test and the unpaired t test were used to compare the baseline characteristics of patients with CKD 3 to 5 and controls with CKD 1 to 2. Differences between the mean CRAE and CRVE values for patients and controls were compared using the unpaired t test.

CRAE and CRVE were then categorized into quartiles, and the difference in values for the first and fourth quartiles calculated. Finally, the determinants of CRAE and CRVE were examined in a linear regression model after adjusting for various risk factors. All statistical analyses were performed using STATA version 10 software (StataCorp, College Station, TX).

Results
Clinical Features and Vascular Risk Factors in Patients with CKD 3 to 5 (Table 1)
The 126 patients with CKD stages 3 to 5 included 82 men (65%) and 44 women (35%), with a median age of 61 years (range, 20 to 84) (Table 1). They had CKD stage 3 (n = 44, 35%), CKD 4 (n = 31, 25%), or CKD5 (n = 51, 40%), and their mean eGFR was 24.3 ml/min per 1.73 m² (range

<p>| Table 1. Characteristics of patients with CKD 3 to 5 or CKD 1 to 2 |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|</p>
<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Patients with CKD 3 to 5 (n = 126)</th>
<th>Hospital Controls with CKD 1 to 2 (n = 126)</th>
<th>Odds Ratio</th>
<th>95% Confidence Interval</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (median, range)</td>
<td>61 (20 to 84)</td>
<td>60.5 (20 to 84)</td>
<td>1.00</td>
<td>0.60 to 1.68</td>
<td>0.97</td>
</tr>
<tr>
<td>Gender (male, %)</td>
<td>82 (65.1%)</td>
<td>82 (65.1%)</td>
<td>1.00</td>
<td>0.98 to 0.99</td>
<td>0.31</td>
</tr>
<tr>
<td>eGFR, (mean, SD, ml/min per 1.73 m²)</td>
<td>24.3 (16.4)</td>
<td>24.3 (16.4)</td>
<td>1.00</td>
<td>0.98 to 0.99</td>
<td>0.31</td>
</tr>
<tr>
<td>Hypertension (%)</td>
<td>101 (80.2%)</td>
<td>56 (44.4%)</td>
<td>5.05</td>
<td>2.88 to 8.85</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Number of antihypertensives (mean, SD)</td>
<td>2.3 (1.4)</td>
<td>0.94 (1.2)</td>
<td>0.94</td>
<td>0.92 to 0.96</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Diabetes (%)</td>
<td>46 (36.0%)</td>
<td>29 (23.0%)</td>
<td>1.92</td>
<td>1.11 to 3.34</td>
<td>0.03</td>
</tr>
<tr>
<td>Dyslipidemia (%)</td>
<td>86/124 (69.4%)</td>
<td>52/122 (46.2%)</td>
<td>3.05</td>
<td>1.80 to 5.14</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Smoking history (%)</td>
<td>67 (53.2%)</td>
<td>84 (66.7%)</td>
<td>0.57</td>
<td>0.34 to 0.95</td>
<td>0.04</td>
</tr>
</tbody>
</table>

eGFR, estimated GFR; CKD, chronic kidney disease.
Renal disease was due to diabetes (37, 29%), glomerulonephritis (35, 28%), hypertension/renovascular disease (22, 17%), reflux nephropathy and other structural malformations (6, 5%), polycystic kidney disease (5, 4%), or miscellaneous causes (cancer, trauma, nephrotoxic agents, or unknown; 21, 17%).

Patients with CKD 3 to 5 were more likely to have hypertension (P < 0.001), require more antihypertensives (P < 0.001) and have diabetes (P = 0.03) or dyslipidemia more often (P < 0.001) than age- and gender-matched patients with CKD 1 to 2. However, patients with renal failure were less likely to have smoked (P = 0.04). HbA1C levels were not different between diabetics with CKD 3 to 5 and CKD 1 to 2 (7.6 ± 2.2 and 8.16 ± 2.31, respectively, mean difference −0.56, 95% CI −1.46 to 0.38, P = 0.25).

Retinal Vessel Caliber Decreases with Lower eGFR

Patients with CKD 3 to 5 had a smaller CRAE and CRVE than those with CKD 1 to 2 (P < 0.001 and P = 0.001) (Table 2). There was a significant trend for CRAE to decrease between the groups with CKD 3 and CKD 5 (P for trend = 0.08), and CRVE was less in CKD 5 than in CKD 3 (P = 0.04) (Table 2).

Retinal Vessel Caliber Is Increased in CKD 5 in Diabetics Compared with Nondiabetics

CRAE and CRVE were larger in patients with CKD 5 and diabetes (n = 17, 141.4 ± 14.9 μm; 211.1 ± 34.4 μm) than in nondiabetics (n = 34, 132.9 ± 14.2 μm, P = 0.05; 194.8 ± 23.8 μm, P = 0.05).

Retinal Arteriolar Caliber in CKD 3 to 5 Depends on eGFR and Hypertension

When patients with CKD 3 to 5 or with CKD 1 to 2 were studied together, those with a CRAE in the first or lowest quartile had a lower eGFR (43.9 compared with 66.6 ml/min per 1.73 m², P < 0.01) and were 3.5 times more likely to have CKD 3 to 5 than those with a CRAE in the fourth or highest quartile (63.5% compared with 33.3%, odds ratio [OR] 2.31, 95% CI 1.29 to 4.33; P = 0.001) (Table 3). This was significant after adjusting for age, gender, hypertension, diabetes, dyslipidemia, and smoking history (adjusted OR 2.84, 95% CI 1.25 to 6.46; P = 0.013) (Table 4). CRAE did not depend on HbA1C in diabetic patients with CKD 3 to 5 after adjusting for age, gender, hypertension, and eGFR (OR 0.79, 95% CI −0.79 to 2.37, P = 0.32).

Patients with CRAE in the first quartile were also more likely to have hypertension (P = 0.001) and to require more antihypertensive medications (P < 0.001; Table 3). This was significant after adjusting for the other variables (adjusted OR 3.94, 95% CI 1.52 to 10.2, P < 0.001).

Retinal Venular Caliber in CKD 3 to 5 Depends on eGFR

When patients with CKD 3 to 5 or with CKD 1 to 2 were studied together, those with a CRVE in the first quartile had a lower eGFR (37.3 compared with 60.8 ml/min per 1.73 m², P < 0.0001) and were four times more likely to have CKD 3 to 5 than those with a CRVE in the fourth quartile (76.2% compared with 44.4%, OR 4.00, 95% CI 1.86 to 8.58; P < 0.001) (Table 5). This association was significant after adjusting for age, gender, hypertension, diabetes, dyslipidemia, and smoking history (adjusted OR 4.75, 95% CI 2.00 to 11.3, P < 0.001) (Table 6). CRVE did not depend on HbA1C in diabetic patients with CKD 3 to 5 after adjusting for age, gender, hypertension, and eGFR (OR 1.29, 95% CI −1.76 to 4.33, P = 0.40).

Discussion

This study demonstrated that retinal arterioles and venules are narrowed in renal failure and that the narrowing worsens as kidney function declines. Renal failure and hypertension are independent determinants of microvascular narrowing even after adjusting for the traditional vascular risk factors of age, gender, diabetes, dyslipidemia, and smoking. Furthermore, the relative vasodilatation described in diabetics with normal renal function persists in CKD 5 when the narrowing associated with kidney failure is otherwise maximal.

Retinal microvascular changes have been studied previously in different populations with renal failure. In two epidemiologic series, focal arteriolar narrowing, arteriovenous nicking, and hemorrhage were increased (28,29). However, interpretation of these signs is subjective, and our study instead used computer-assisted measures of vessel caliber that were quantitative, standardized, and highly reproducible.

Retinal vascular caliber has also been examined previously in patients with renal disease using large population-based cohorts and in type 1 diabetics. However, the results have been contradictory. In one of the epidemiologic studies, retinal arterioles were narrowed in individuals with...
impaired renal function or albuminuria (30), but renal impairment was mild, and the median eGFR for each CRAE quartile was within the normal range. In patients with type 1 diabetes, the presence of proteinuria was associated with smaller vessels (23), and renal impairment with larger caliber vessels (31). Our study was the first to examine retinal vessel caliber in hospital-based patients with renal failure, and the effect of renal failure on vessel size was obvious. This was true even though nearly 40% of our patients had diabetes that typically produces relative dilation.

Our study found that retinal arterioles were narrowed in renal impairment. Arteriolar narrowing is usually due to hypertension, atherosclerosis, and, rarely, retinal edema or ischemia after, for example, panretinal photocoagulation (32). Retinal arteriolar narrowing in CKD depended on hypertension and CKD but not the other traditional vascular risk factors. Vascular narrowing worsened as renal function deteriorated, suggesting that microvascular disease in the kidney itself might contribute to progressive kidney failure or that there was a common pathogenesis. Retinal veins were also narrowed in renal impairment and became narrower as renal function declined. Retinal venular caliber typically parallels arteriolar caliber. CRVE has been studied previously in renal failure, but in all cases venules were dilated (23,30,33). Venular dilation also occurs in hypertriglyceridemia (33), diabetes (34), obesity (35), smoking (36), and systemic inflammation (37). Venular dilation in renal failure in the previously-reported series may have reflected the prevalence of diabetes and the use of albuminuria rather than eGFR to identify renal impairment.

Our study suggested that although arterioles and venules became smaller with progressive renal impairment, the vessels in end-stage renal failure were still relatively dilated in diabetics. Vasodilation of small vessels in diabetes is thought to be inflammatory in nature, and vasodilatation in end-stage renal disease is consistent with the ongoing consequences of diabetes in addition to the effects of hypertension and atherosclerosis.

The major strength of this study was that it examined a large population of well characterized but typical hospital patients with CKD 3 to 5 and demonstrated a narrowed microvasculature compared with hospital patients with CKD 1 to 2. Patient selection itself was unbiased because

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Odds Ratio</th>
<th>95% Confidence Interval</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (median, range, years)</td>
<td>1.02</td>
<td>0.98 to 1.06</td>
<td>0.41</td>
</tr>
<tr>
<td>Male gender</td>
<td>0.73</td>
<td>0.28 to 1.89</td>
<td>0.51</td>
</tr>
<tr>
<td>CKD 3–5</td>
<td>2.84</td>
<td>1.25 to 6.46</td>
<td>0.01</td>
</tr>
<tr>
<td>Hypertension</td>
<td>3.94</td>
<td>1.52 to 10.2</td>
<td>0.01</td>
</tr>
<tr>
<td>Diabetes</td>
<td>0.60</td>
<td>0.24 to 1.47</td>
<td>0.26</td>
</tr>
<tr>
<td>Dyslipidemia</td>
<td>1.10</td>
<td>0.49 to 2.47</td>
<td>0.83</td>
</tr>
<tr>
<td>Smoking history</td>
<td>1.13</td>
<td>0.47 to 2.73</td>
<td>0.79</td>
</tr>
</tbody>
</table>

Log-likelihood = −74.17. Probability > χ² = 0.002. CRAE, central retinal artery equivalent.
consecutive patients were approached to participate in the study by a team member who was not aware of their medical histories or retinal appearance. There is no reason to believe that the 25 patients recruited to the larger study whose retinal photographs were ungradable had any features that might have biased the results. In almost all cases, their optic fundi could not be visualized because of cataracts.

Although the population with CKD 3 to 5 was not homogeneous, but rather comprised individuals with well- or poorly-controlled BP, and both diabetics and nondiabetics, the study still demonstrated that the microvasculature was narrowed compared with patients with CKD 1 to 2. The choice of antihypertensive medication was at the discretion of the individual clinicians, but regardless has little effect on microvascular caliber (38). Furthermore, we were able to adjust for these variables in the multivariate analysis.

Another strength was that this study measured retinal vessel caliber using a quantitative, precise, and highly reproducible method.

A major limitation of this study was that it was unable to demonstrate a causal relationship between CKD 3 to 5 and microvascular narrowing. This requires a longitudinal study. A further limitation is that it has not addressed whether retinal microvascular narrowing predicts cardiac events in CKD, but we will examine cardiac events in this cohort over the next 2 years. Despite these limitations, this study still demonstrates clearly that systemic microvascular disease is more common in patients with CKD 3 to 5 than in other hospital patients.

Are these results generalizable to other cohorts of patients with CKD 3 to 5? Our patients were mainly male, aged 61 years, with a mean eGFR of 24 ml/min per 1.73 m². 80% had hypertension, 36.5% had diabetes, 69% had dyslipidemia, and 53% were former smokers. Clinicians must determine how closely these features are characteristic of their own CKD populations.

## Conclusions

Microvascular disease is a neglected aspect of the increased cardiac risk in patients with renal failure. This study demonstrated that microvascular disease is increased in hospital patients with CKD 3 to 5 compared with other patients. In addition, it suggests that any studies of retinal vessel caliber must control for renal impairment.

### Table 5. Characteristics of patients according to CRVE quartile

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Quartile 1 (n=63)</th>
<th>Quartile 2 (n=63)</th>
<th>Quartile 3 (n=63)</th>
<th>Quartile 4 (n=63)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (median, range)</td>
<td>62 (20 to 83)</td>
<td>61 (20 to 84)</td>
<td>65 (35 to 84)</td>
<td>60 (29 to 81)</td>
</tr>
<tr>
<td>Gender (male, %)</td>
<td>41 (65.1%)</td>
<td>35 (55.6%)</td>
<td>44 (68.3%)</td>
<td>45 (71.4%)</td>
</tr>
<tr>
<td>eGFR (mean, SD, ml/min per 1.73 m²)</td>
<td>43 (25.5)</td>
<td>47 (26.4)</td>
<td>40 (29.5)</td>
<td>47 (26.1)</td>
</tr>
<tr>
<td>CKD 3 to 5</td>
<td>48 (76.2%)</td>
<td>36 (56.6%)</td>
<td>39 (59.5%)</td>
<td>45 (71.4%)</td>
</tr>
<tr>
<td>Hypertension</td>
<td>45 (71.4%)</td>
<td>42 (66.7%)</td>
<td>34 (54.0%)</td>
<td>45 (71.4%)</td>
</tr>
<tr>
<td>Diabetes</td>
<td>34 (54.0%)</td>
<td>34 (54.0%)</td>
<td>35 (53.6%)</td>
<td>45 (71.4%)</td>
</tr>
<tr>
<td>Dyslipidemia</td>
<td>39 (61.9%)</td>
<td>36 (56.6%)</td>
<td>36 (56.6%)</td>
<td>36 (56.6%)</td>
</tr>
<tr>
<td>Smoking history</td>
<td>36 (57.1%)</td>
<td>36 (57.1%)</td>
<td>36 (57.1%)</td>
<td>36 (57.1%)</td>
</tr>
</tbody>
</table>

The P values, odds ratios, and 95% Confidence intervals were compared between individuals in quartiles 1 and 4. CRVE, central retinal vein equivalent; eGFR, estimated GFR; CKD, chronic kidney disease.

### Table 6. Determinants of CRVE in patients with CKD

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Odds ratio</th>
<th>95% Confidence Interval</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>1.02</td>
<td>0.99 to 1.06</td>
<td>0.17</td>
</tr>
<tr>
<td>Male gender</td>
<td>0.60</td>
<td>0.24 to 1.48</td>
<td>0.27</td>
</tr>
<tr>
<td>CKD 3–5</td>
<td>4.75</td>
<td>2.00 to 11.3</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Hypertension</td>
<td>1.63</td>
<td>0.64 to 4.15</td>
<td>0.30</td>
</tr>
<tr>
<td>Diabetes</td>
<td>0.50</td>
<td>0.21 to 1.22</td>
<td>0.13</td>
</tr>
<tr>
<td>Dyslipidemia</td>
<td>0.84</td>
<td>0.37 to 1.93</td>
<td>0.69</td>
</tr>
<tr>
<td>Smoking history</td>
<td>1.00</td>
<td>0.44 to 2.26</td>
<td>0.99</td>
</tr>
</tbody>
</table>

Log-likelihood = −72.91. Probability > χ² = 0.003. CRVE, central retinal vein equivalent; CKD, chronic kidney disease.
Acknowledgments

We would like to thank the many patients who participated in these studies. This work was undertaken by medical students as part of their B Medical Science degrees at the University of Melbourne. The work was presented as a poster at the American Society of Nephrology Annual Meeting, Denver, CO, November 18-21, 2010.

Disclosures

None.

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Received: November 22, 2010 Accepted: April 22, 2011

Published online ahead of print. Publication date available at www.cjasn.org.