Received: 2 November 2020 | Revised: 2 December 2020 | Accepted: 3 December 2020

DOI: 10.1111/jch.14138

ORIGINAL PAPER

WILEY

Microvascular changes at different stages of chronic kidney disease

Dennis Kannenkeril MD[1,2] | Shaun Frost PhD[3,4] | Janis M. Nolde MD[1] | Márcio Galindo Kiuchi MD[1] | Revathy Carnagarin MD, PhD[1] | Leslie Marisol Lugo-Gavidia MD, MSc[1] | Justine Chan MSc[1,5] | Anu Joyson MSc[1,5] | Vance B. Matthews PhD[1] | Lakshini Y. Herat PhD[1] | Omar Azzam MD[1] | Maryam Mehdizadeh MSc[3,4] | Janardhan Vignarajan PGDip, BCompSc[3,4] | Yogesan Kanagasingam PhD[3,4] | Markus P. Schlaich MD[1,5,6]

[1]Dobney Hypertension Centre, School of Medicine - Royal Perth Hospital Unit / Medical Research Foundation, University of Western Australia, Perth, WA, Australia

[2]Department of Nephrology and Hypertension, University Hospital Erlangen, Friedrich-Alexander-University Erlangen-Nürnberg (FAU), Erlangen, Germany

[3]Commonwealth Scientific and Industrial Research Organisation (CSIRO), Perth, WA, Australia

[4]Australian e-Health Research Centre, Perth, WA, Australia

[5]Departments of Cardiology and Nephrology, Royal Perth Hospital, Perth, WA, Australia

[6]Neurovascular Hypertension & Kidney Disease Laboratory, Baker Heart and Diabetes Institute, Melbourne, Vic., Australia

Correspondence
Markus P. Schlaich, Dobney Hypertension Centre, School of Medicine - Royal Perth Hospital Unit, Faculty of Medicine, Dentistry & Health Sciences, The University of Western Australia, Level 3, MRF Building, Rear 50 Murray St, PERTH WA 6000, Australia.
Email: markus.schlaich@uwa.edu.au

Funding information
Department of Health, Western Australia

Abstract

Patients with progressing chronic kidney disease (CKD) are more likely to experience cardio- and cerebrovascular events than progressing to end-stage renal disease. The authors explored whether retinal microvascular calibers differed with the degree of renal impairment and between the standard and extended optic disk and may serve as a simple additional tool for risk stratification in this highly vulnerable patient cohort. The authors analyzed central retinal arteriolar and venular equivalent calibers (CRAE, CRVE) at different retinal zones (zone B&C) using digital retinal imaging in hypertensive patients with stage 2 (n = 66) or stage 3 CKD (n = 30). Results were adjusted for age, sex, HbA1c, and 24-hour diastolic blood pressure. Mean eGFR was 77.7 ± 8.9 and 48.8 ± 7.9 ml/min/1.73 m^2 for stage 2 and 3 CKD, respectively. CRAE and CRVE in zones B and C were significantly lower in patients with stage 3 CKD compared to patients with stage 2 CKD (CRAE-B:141.1 ± 21.4 vs. 130.5 ± 18.9 µm, p = .030; CRAE-C:137.4 ± 19.4 vs 129.2 ± 18.2 µm, p = .049; CRVE-B:220.8 ± 33.0 vs. 206.0 ± 28.4 µm, p = .004; and CRVE-C:215.9 ± 33.0 vs. 201.2 ± 25.1µm, p = .003). In patients with stage 2 CKD, CRAE-B was higher than CRAE-C (141.1 ± 21.4 vs. 137.4 ± 19.4µm, p < .001). In contrast,

Abbreviations: ACR, Albumin/creatinine ratio; BMI, body mass index; BP, blood pressure; CHS, cardiovascular health study; CKD, chronic kidney disease; CONACYT, Consejo Nacional de Ciencia y Tecnología; CRAE, central retinal arteriolar equivalent caliber; CRVE, central retinal venular equivalent caliber; CSIRO, Commonwealth Scientific and Industrial Research Organisation; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate; HR, heart rate; SBP, systolic blood pressure; SD, standard deviation; WESDR, Wisconsin Epidemiologic Study of Diabetic Retinopathy.

Dennis Kannenkeril and Shaun Frost equally contributing first authors.

such a difference was not found in patients with stage 3 CKD. CRAE of both retinal zones correlated with eGFR for the entire cohort. In patients with stage 3 CKD, retinal narrowing is more pronounced compared to patients with stage 2 CKD. Whether the novel observation of difference in arteriolar caliber between zones B and C in stage 2 CKD could serve as an early marker of CKD progression warrants further investigation.

1 | INTRODUCTION

Chronic kidney disease (CKD) is a global health burden [1] and is estimated to affect up to 10 to 16 percent of the adult population.[2,3] The prevalence of CKD is probably underestimated since the disease is often asymptomatic at its early stages. It is estimated that over 90% of diagnosed cases have already reached stage 3 CKD.[4] Early detection and therapeutic intervention may slow down or halt progression of the disease to end-stage renal disease and vascular complications.[5]

CKD is associated with increased risks of cerebrovascular and cardiovascular morbidity and mortality and decreased quality of life in all stages of the disease.[6-8] It has been reported that CKD increases the risk of stroke by a factor of 3.7 with end-stage kidney disease being associated with the highest risk.[9] Patients with progressing CKD are more likely to experience cardiovascular and cerebrovascular events rather than progressing to end-stage renal disease.

Renal microvascular pathology is thought to play an important role in the development of CKD.[10] Several studies have demonstrated correlations between retinal and renal microvascular changes in various diseases.[11,12] Moreover, the microvascular changes that can be observed in the retina resemble those in the brain.[13,14] Thus, investigation of the retinal vasculature is likely to also reflect renal and cerebral microcirculatory changes. Advances in technology have enabled noninvasive, in-depth assessment of microcirculation using retinal photography and computerized image analysis.

Analysis of retinal microvascular calibers is emerging as an important marker of many vascular conditions.[15,16] Previous studies have looked at the relationship between retinal calibers and CKD, but findings were mixed. In this study, we explored whether retinal microvascular calibers differed with the early degree of renal impairment in patients with primary hypertension and may serve as a simple additional tool for risk stratification in this highly vulnerable patient cohort. The novelty of this study is that we also explored the differences in retinal microvascular calibers between the standard and extended optic disks.

2 | METHODS

2.1 | Study design

We performed a prospective, cross-sectional study in patients with arterial hypertension from February 2016 to October 2019. The study was conducted at the Dobney Hypertension Centre, Royal Perth Hospital, Perth, Australia. The study was approved by the East Metropolitan Health Service Ethics and Governance Committees (EC00270, REG Number: RGS1040). The study was performed in accordance with the Declaration of Helsinki and the principles of Good Clinical Practice guidelines. All patients provided written informed consent prior to inclusion in the study. In all patients, digital retinal imaging was performed. Patients with retinal disease (glaucoma, age-related macular degeneration) or previous retinal surgery were excluded. The glomerular filtration rate (eGFR) was calculated using the CKD-EPI formula. Patients with eGFR between 60 and 89 ml/min/1.73 m^2 were categorized to have stage 2 CKD and patients with eGFR between 30 and 59 ml/min/1.73 m^2 to have stage 3 CKD.

2.2 | Study cohort

Patients were referred to our tertiary hospital-based hypertension clinic at the Royal Perth Hospital, Perth, Australia, for management of hypertension predominantly from primary care physicians. Following a thorough medical history, physical examination, and collection of anthropometric data, a series of relevant tests was performed routinely in all patients, which includes simultaneous office blood pressure (BP) measurement on both arms (WatchBP®; Microlife AG Swiss Corporation, Widnau, Switzerland). On the arm with higher BP, 3 unattended consecutive automated office BPs were measured (HEM 907 Automatic Blood Pressure Monitor®; Omron Healthcare Co., Kyoto, Japan). The average of three measurements was recorded. All patients had routine blood samples and midstream urine within 4 weeks prior to retinal imaging for biochemistry testing including serum creatinine, spot urine albumin, ACR (albumin/creatinine ratio in mg/mmol creatinine), and tests relevant to screen for potential secondary causes of hypertension. Patients with identified secondary hypertension were not included in this study. On a separate day, patients attend the clinic for extended phenotypic testing, which includes retinal imaging obtained to assess the retinal vasculature. All participants had an ambulatory BP monitor (Spacelabs 90 207, Spacelabs Healthcare, Snoqualmie, Washington, United States) in a standardized fashion according to the guideline recommendations.[17]

2.3 | Digital retinal imaging

Patients attended the Dobney Hypertension Centre (before 12:00 h), having fasted for 12 hours and abstained from strenuous

exercise, caffeine, and alcohol for 24 hours. All participants were seated quietly in semidark room for 5 minutes. Patients were asked to take their usual BP medications if any after retinal imaging was performed. Digital retinal color photographs (disk-centered, 45O field) were collected from both eyes with a Canon CR-2 fundus camera (Canon CR-2® camera , Ōta, Tokyo, Japan, www.canon.com).

2.4 | Retinal image analysis

The analysis of the retinal images has previously been described in detail.[18] Briefly, retinal images were analyzed with CSIRO semi-automated software (Vessel AnalysiS Platform). The right eye was used mostly for analysis. When images of the right eye were of insufficient quality and images of high quality were available for the left eye, then these were utilized for the analysis. The measured retinal zones of interest for the vessel width measurements were 0.5-1.0 disk diameters away from the disk margin (zone B) or 1.0-2.0 disk diameters away from the disk margin (zone C) (Figure 1). Vascular calibers were calculated for the six largest arterioles and six largest venules. Summary measures of vascular equivalent caliber were calculated (central retinal arteriolar (CRAE) and venular (CRVE) equivalent caliber), based on the improved Knudtson-Parr-Hubbard formula.[19,20] CRAE and CRVE represent the equivalent single-vessel parent caliber for the six arterioles and venules, respectively.

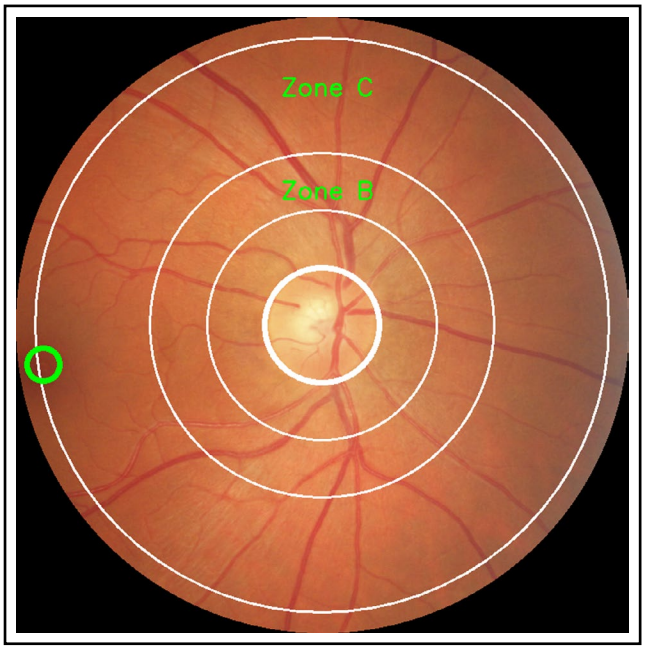

FIGURE 1 Retinal image showing zones of interest for assessment of retinal vessel calibers. Zone B 0.5-1.0 and Zone C 1.0-2.0 disk diameter distance from the disk margin. Green circle at left indicates the fovea

2.5 | Statistical analyses

Normal distribution of data was confirmed by the Kolmogorov-Smirnov tests and histograms before further analysis. Patients with missing data were not included in the analysis. Data are presented as means with standard deviation (SD) and percentages, respectively. Demographic comparisons were performed using a chi-square test for categorical variables (sex). Data were compared by paired and unpaired Student's *t* test as appropriate. The covariance analysis was performed using univariate linear analysis. Adjustment was made for clinical variables including age, sex, HbA1c, and 24-hour diastolic BP. Since clinical characteristics were similar between the groups, no further adjustments were made. Bivariate correlation analyses were performed using Pearson's test. Two-tailed values of $p < .05$ were considered statistically significant. Statistical analysis was performed using IBM SPSS Statistics 25.0.0.1, USA.

3 | RESULTS

3.1 | Study population

The clinical characteristics of the patients with stage 2 and 3 CKD who enrolled are presented in Table 1. Sixty-six patients with stage 2 CKD and thirty patients with stage 3 CKD were included in the study. Patients with stage 2 and stage 3 CKD were of similar age (63.5 ± 12.1 vs. 63.3 ± 13.1 years). The mean eGFR of patients with stage 2 CKD was 77.7 ± 8.9 ml/min/1.73 m^2 and that of patients with stage 3 CKD was 48.8 ± 7.9 ml/min/1.73 m^2. Urinary ACR was significantly higher in patients with stage 3 CKD (2.4 ± 4.1 mg/mmol vs. 46.8 ± 78.4 mg/mmol, $p = .008$). There was no difference in systolic and diastolic BP and 24-hour ambulatory BP between the groups. Twenty-two patients with stage 2 CKD and thirteen patients with stage 3 CKD had type 2 diabetes mellitus without any significant difference in HbA1c between the groups. All patients with either stage 2 or stage 3 CKD were on antihypertensive drugs. The most common drug types prescribed in both groups were calcium channel blockers (stage 2/3 CKD: 62.1% / 63.3%), angiotensin receptor blockers (stage 2/3 CKD: 48.5% / 53.3%), β-blockers (stage 2/3 CKD: 39.4% / 50.0%), and angiotensin-converting enzyme inhibitors (stage 2/3 CKD: 15.2% / 26.7%).

3.2 | Retinal arteriolar caliber

CRAE in zone B was significantly lower in patients with stage 3 CKD compared to patients with stage 2 CKD (CRAE-B: 141.1 ± 21.4 vs. 130.5 ± 18.9 µm, adjusted $p = .030$; Figure 2). Similar results were found with respect to CRAE in zone C (CRAE-C: 137.4 ± 19.4 vs. 129.2 ± 18.2 µm, adjusted $p = .049$; Figure 2). For patients with stage 2 CKD, the arteriolar caliber in zone B was larger than that in zone C (CRAE-B vs. CRAE-C: 141.1 ± 21.4 vs. 137.4 ± 19.4 µm, $p < .001$; Figure 2). Such a difference

TABLE 1 Clinical characteristics of study population

Parameter	Stage 2 CKD patients (n = 66) mean ± SD	Stage 3 CKD patients (n = 30) mean ± SD	*p* value
Age, years	63.5 ± 12.1	63.3 ± 13.1	.938
Sex, % male	53	46.7	.838
Body weight, kg	86.1 ± 19.9	86.3 ± 20.9	.974
BMI, kg/m^2	30.5 ± 5.8	30.9 ± 7.0	.743
Office SBP, mmHg	143.3 ± 18.8	143.3 ± 27.0	.998
Office DBP, mmHg	80.7 ± 14.1	78.8 ± 18.9	.615
Office HR, bpm	69.8 ± 11.6	68.2 ± 13.6	.581
24-h SBP, mmHg	140.5 ± 18.0	139.6 ± 19.1	.840
24-h DBP, mmHg	80.9 ± 11.9	76.8 ± 13.4	.146
24-h HR, bpm	72.5 ± 11.1	68.3 ± 12.3	.105
Fasting glucose, mmol/l	6.5 ± 2.2	6.9 ± 2.4	.520
HbA1c, %	6.1 ± 1.2	6.6 ± 1.3	.144
eGFR, ml/min/1.73m^2	77.7 ± 8.9	48.8 ± 7.9	<.001
UACR, mg/mmol creatinine	2.4 ± 4.1	46.8 ± 78.4	.008

Note: Data are given as mean ± SD.

Abbreviations: BMI, body mass index; bpm, beat per minute; CKD, chronic kidney disease; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate; HR, heart rate; SBP, systolic blood pressure; SD, standard deviation; UACR, urinary albumin/creatinine ratio.

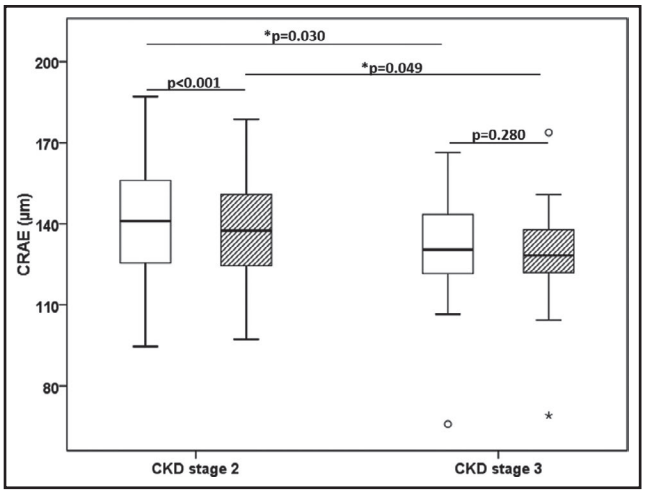

FIGURE 2 Comparison of retinal arteriolar caliber between stage 2 and stage 3 CKD and between 2 different zones (zone B and zone C) in each group. The first and second boxplot represent CRAE-B and CRAE-C of patients with stage 2 CKD. The third and fourth boxplot represent CRAE-B and CRAE-C of patients with stage 3 CKD. CRAE—central retinal arteriolar equivalent caliber (µm), followed by -B for zone B or -C for zone C, *p*—*p* value, **p*—*p* value adjusted for age, sex, HbA1c, and 24-hour diastolic BP

between zones was not evident in patients with stage 3 CKD (CRAE-B vs. CRAE-C: 130.5 ± 18.9 vs. 129.2 ± 18.2 µm, *p* = .280; Figure 2). A correlation was found between CRAE in both retinal zones and eGFR (CRAE-B: r = 0.230, *p* = .024; CRAE-C: r = 0.208, *p* = .042; Figure 3). No correlation was observed between CRAE of either zone and urinary ACR (CRAE-B: r=−0.137, *p* = .212; CRAE-C: r=−0.159, *p* = .146).

3.3 | Retinal venular caliber

CRVE in zone B was significantly lower in patients with stage 3 CKD compared to patients with stage 2 CKD (CRVE-B: 220.8 ± 33.0 vs. 206.0 ± 28.4 µm, adjusted *p* = .004; Figure 4). Lower CRVE was also found in zone C in patients with stage 3 CKD (CRVE-C: 215.9 ± 33.0 vs. 201.2 ± 25.1 µm, adjusted *p* = .003; Figure 4). CRVE was significantly different between zones B and C in both CKD stages (CRVE-B vs. CRVE-C [stage 2 CKD]: 220.8 ± 33.0 vs. 215.9 ± 33.0 µm, *p* < .001; CRVE-B vs. CRVE-C [stage 3 CKD]: 206.0 ± 28.4 vs. 201.2 ± 25.1 µm, *p* = .015; Figure 4). No correlation was observed between CRVE of both retinal zones, eGFR (CRVE-B: r = 0.196, *p* = .061; CRVE-C: r = 0.186, *p* = .070) and urinary ACR (CRVE-B: r=−0.120, *p* = .275; CRVE-C: r=−0.098, *p* = .372).

Both arteriolar and venular retinal calibers of patients with CKD are presented in Table 2.

4 | DISCUSSION

The principal finding of our study is that both retinal arteriolar and venular calibers are reduced in patients with stage 3 CKD compared to patients with stage 2 CKD. Moreover, we found an association between eGFR and arteriolar caliber. Our findings suggest that retinal microvascular abnormalities may reflect subclinical renal microvascular abnormalities involved in the development of CKD. Whether the observed difference in arteriolar caliber between zones B and C in stage 2 CKD may be used as an early marker of progression warrants further investigation.

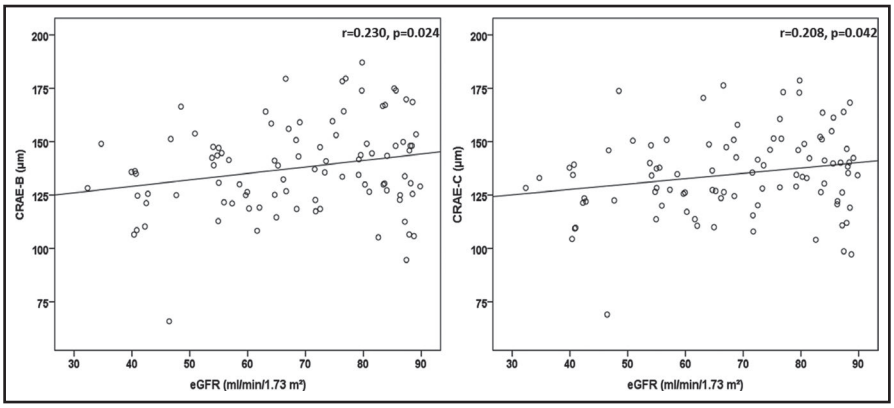

FIGURE 3 Illustration of the relationship between eGFR and retinal arteriolar caliber. CRAE—central retinal arteriolar equivalent caliber (µm), followed by -B for zone B or -C for zone C, eGFR—estimated glomerular filtration rate calculated by CKD-EPI formula, r—correlation coefficient, *p—p* value

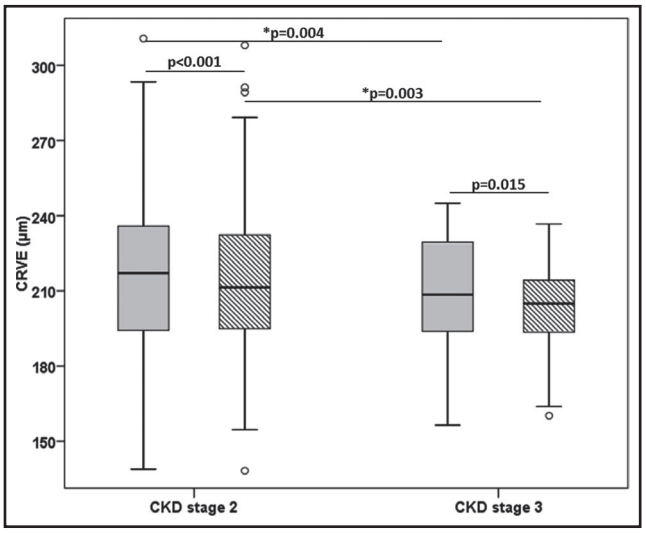

FIGURE 4 Comparison of retinal venular caliber between stage 2 and stage 3 CKD and between 2 different zones (zone B and zone C) in each group. The first and second boxplot represent CRVE-B and CRVE-C of patients with stage 2 CKD. The third and fourth boxplot represent CRVE-B and CRVE-C of patients with stage 3 CKD. CRVE-central retinal venular equivalent caliber (µm), followed by -B for zone B or -C for zone C, *p—p* value, **p—p* value adjusted for age, sex, HbA1c, and 24-hour diastolic BP

TABLE 2 Retinal arteriole and venule width parameters in different zones of the retina

Retinal parameters (µm)	Stage 2 CKD patients (n = 66)	Stage 3-CKD patients (n = 30)
CRAE-B	141.1 ± 21.4	130.5 ± 18.9
CRAE-C	137.4 ± 19.4	129.2 ± 18.2
CRVE-B	220.8 ± 33.0	206.0 ± 28.4
CRVE-C	215.9 ± 33.0	201.2 ± 25.1

Abbreviations: CKD, chronic kidney disease; CRAE, central retinal arteriolar equivalent caliber, followed by -B for zone B or -C for zone C; CRVE, central retinal venular equivalent caliber, followed by -B for zone B or -C for zone C.

The association between CKD and retinopathy is well established.[10,21] However, only a few previous studies have analyzed the association between retinal microvascular parameters and CKD.[11,22-25] In strong support to our results, Sabanayagam et al found an association of both hypertension and retinal arteriolar narrowing with a threefold increased likelihood of having CKD.[23] Wong et al demonstrated in middle-aged people that narrowed arteriolar vessels are related to a more pronounced decline in renal function over a 6-year period.[11] Contrastingly, another study found retinal microvascular caliber in the general population was not associated with incident stage 3 CKD.[24] However, in ethnicity-stratified analysis, narrower retinal arterioles in Caucasians were associated with a higher risk of developing stage 3 CKD, providing evidence that microvascular disease precedes the development of CKD. Furthermore, in Caucasians without diabetes and hypertension, narrower arterioles were associated with approximately threefold higher risk of stage 3 CKD. This suggests that the risk of stage 3 CKD may be related to microvascular mechanisms beyond the effects of diabetes and hypertension. Similar findings were described in Asian populations.[22] In our study, all patients had arterial hypertension and were predominantly of Caucasian background. In contradiction to our findings, the Cardiovascular Health Study (CHS) found no association between retinal arteriolar caliber and declining renal function, this was however in an elderly population.[25] The absence of an association was attributed to a possible selection and survival bias in this elderly population.

Another important finding of our study was the pronounced narrowing of CRVE in patients with stage 3 CKD. Wong et al also demonstrated that narrowed CRVE is associated with greater 6-year change in renal function in a population-based investigation in four US communities.[11] On the other hand, in patients with type 2 diabetes, larger CRVE was associated with a higher incidence of CKD.[26,27] Similarly, in a prospective, population-based study of individuals with type 1 diabetes, larger CRVE was associated with an increased risk of developing diabetic nephropathy.[28]

The Wisconsin Epidemiologic Study of Diabetic Retinopathy (WESDR) demonstrated that narrower CRAE was cross-sectionally associated with proteinuria.[27] However, the same correlation was not demonstrated in a 16-year follow-up of the WESDR.[28] In another study, arteriolar narrowing in Danish patients with type 1 diabetes and proteinuria was related to nephropathy and proliferative diabetic retinopathy compared to those without proteinuria.[29] In the Multi-Ethnic Study of Atherosclerosis, a U-shaped association between albuminuria and retinal arteriolar caliber was observed.[30] In our study, comprised of patients with arterial hypertension, we found no association between retinal vessel caliber and urinary ACR. The fact that both in WESDR [28] and in the Danish study [29] the population consisted only of patients with diabetes may account for some of these discrepancies.

In general, the primary site of narrowing appears to be the precapillary arteriole; therefore, changes are most prominent in second- and third-order arterioles, and less common in arterioles closer to the disk.[31] Interestingly, in our patients with stage 3 CKD the arteriolar caliber at two different zones (B and C) of the retina was similar, of which zone B was within 0.5-1.0 and zone C within 1.0-2.0 disk diameter from the disk margin. In contrast, in patients with stage 2 CKD, both arteriolar and venular calibers differed between the two zones. This difference between stage 2 and stage 3 CKD might be explained by the difference in anatomical features at different zones of the retina and difference in sympathetic activity at different stages of the disease.[32,33] The microvessels in the macular area lack smooth muscle and are largely controlled by pericytes, which are predominantly responsive to $\alpha 2$ adrenergic receptors.[32,33] In contrast, the relatively large vessels in the peripapillary area have $\alpha 1$ adrenergic receptors.[34] In support of this view is the finding that topical sympathetic agents reduced the retinal vessel density within the peripapillary area, but not within the macular area.[35]

Alternatively, given the absence of a difference in arteriolar calibers between zones B and C in stage 3 CKD patients with more advanced disease, the observed difference between the two zones in less pronounced kidney disease (stage 2 CKD) may contain information relevant to the progression of CKD, a proposition that warrants further investigation in larger cohorts.

Animal experiments demonstrated that vascular changes in the retinal and renal microcirculation are highly correlated in hypertensive rats.[36] Different pathophysiological mechanisms might underlie retinal arteriolar narrowing in CKD. It has been found that retinal arteriolar narrowing is morphologically related to extracellular matrix accumulation in renal specimens of patients with type 1 diabetes, thereby leading to declining renal function.[37] Endothelial dysfunction is likely to be another underlying mechanism involved in narrowing of retinal microvascular caliber, which also plays a key role in CKD.[38,39] In addition, retinal arteriolar caliber is also associated with inflammatory markers and with the triglyceride and high-density lipoprotein cholesterol changes associated with inflammation.[40,41] Sympathetic overdrive may be another mechanism explaining the narrowing of retinal vessels in our study. It is a phenomenon detectable already in early phases of CKD, and the magnitude of sympathetic drive increases with deterioration of renal function.[42] A significant association between retinal vessel caliber and autonomic nervous stimulation has been demonstrated previously.[18,43,44] Lanigan et al induced a significant retinal arteriolar constriction of 8.6% and venule constriction of 4.8% using sympathetic agonists.[43] Similar response of the retinal vessels has been induced by hand-grip exercise. Harazny et al observed that application of a parasympatholytic agent reduced retinal capillary flow.[44] Previously, we observed a reduction in retinal arteriolar and venular widths with instillation of a parasympatholytic agent.[18] However, after adjustment for image magnification, no change in vessel width parameters was registered. Heart rate, sometimes used a surrogate marker of sympathetic drive, was not found to be elevated in our patients with stage 3 CKD. However, concomitant medication may account for this. The autoregulation of the retinal circulation by means of myogenic response may be an explanation for narrowed vessels in the CKD 3 population. Some studies reported an increase in retinal arteriolar caliber following reduction in retinal perfusion pressure.[45] However, in our study, neither office nor ambulatory BP was different between the CKD groups. Furthermore, in sympathectomized eyes of cats, acute elevation of the BP caused overperfusion of the retina.[46] This was confirmed by preventing retinal overperfusion by sympathetic stimulation under conditions of acute arterial hypertension.[47]

The association between retinal microvascular alterations and cerebrovascular diseases is well established.[14] Retinal microvascular caliber has been found to be associated with enlarged perivascular space in the brain, which is a marker for microvascular brain damage.[48] Population-based studies using retinal imaging found that alterations in retinal microvascular structure are related to ischemic strokes.[49,50] Narrow retinal arterioles were more frequent among patients with lacunar stroke compared with those with cortical ischemic stroke.[51] In our study, we did not obtain any vascular parameters of the cerebrovascular system, which would have been helpful to explore potential associations. We plan, however, to analyze in future longitudinal studies if retinal microvascular caliber changes are associated with increased risk of cerebrovascular accidents in CKD patients at different stages of the disease.

Our study has strengths and limitations to be discussed. Our study population had a thorough clinical workup including ambulatory BP monitoring, and secondary causes of hypertension were excluded. Moreover, analysis of the retinal images was performed in a blinded fashion by an operator with no access to other relevant clinical data of the study population. One of the limitations of this study is that renal function in our population was estimated using the CKD-EPI formula and not measured by inulin clearance, but it has been shown that estimated GFR is accurate, precise, and less-biased.[52,53] Furthermore, our results are only valid for patients with stage 3 and stage 2 CKD and cannot be extrapolated to other stages of CKD. Finally, we have not assessed sympathetic nerve activity, which might have provided a better insight into the mechanisms behind retinal caliber changes in CKD patients.

5 | CONCLUSIONS

In summary, this study demonstrated that patients with stage 3 CKD had greater CRAE and CRVE narrowing compared with stage 2 CKD patients. Moreover, that a narrowed CRAE was associated with lower eGFR in patients with CKD, and that there was a difference in the retinal arteriolar calibers between the standard and extended optic zones of stage 2 CKD patients compared to patients with stage 3 CKD. Further research may be useful in determining the mechanisms of microvascular changes in CKD, which might explain the higher incidence of cerebrovascular accidents in this population. Measurement of arteriolar and venular calibers in population studies may allow evaluation of the independent contribution of arteriolar disease to various ischemic diseases. Whether the novel observation of difference in arteriolar calibers between zone B and C in stage 2 CKD could serve as an early marker of progression of CKD warrants further investigation.

ACKNOWLEDGEMENTS

We gratefully acknowledge all the participants of the study and the expert administrative and technical assistance of Mrs Derrin Brockman and Mrs Ancy Elisabeth Jose.

CONFLICT OF INTEREST

DK and other authors declare that they have no conflict of interest with respect to this study. LMLG has received a scholarship from the National Council on Science and Technology, Mexico (CONACYT). RC is receiving a scholarship from the National Heart Foundation. MPS is supported by an NHMRC Research Fellowship and has received consulting fees, and/or travel and research support from Medtronic, Abbott, and Boehringer Ingelheim.

AUTHOR CONTRIBUTIONS

DK designed the study, analyzed all data, and wrote manuscript. SF analyzed retinal data, contributed to discussion and methods, and reviewed manuscript. JMN contributed to acquisition of data, contributed to discussion, and reviewed the data/manuscript. MGK, RC, LMLG, JC, AJ, VBM, LYH, and OA contributed to acquisition of data, contributed to discussion, and reviewed the manuscript. MM and JV contributed to discussion, reviewed the manuscript, and developed analysis software. YK contributed to study conception and discussion, and reviewed the manuscript. MPS designed the study, contributed to discussion, and reviewed the data/manuscript.

FUNDING INFORMATION

This study was funded by the Department of Health, Western Australia.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Written informed consent was obtained from each patient before study inclusion. The study protocol of each trial was approved by the East Metropolitan Health Service Ethics and Governance Committees (EC00270, REG Number: RGS1040), and the studies were conducted in accordance with the Declaration of Helsinki and the principles of good clinical practice guidelines.

CONSENT FOR PUBLICATION

All authors gave full consent for publication.

DATA AVAILABILITY STATEMENT

The data underlying this article cannot be shared publicly due to the privacy of individuals that participated in the study.

ORCID

Janis M. Nolde https://orcid.org/0000-0001-6864-8514
Markus P. Schlaich https://orcid.org/0000-0002-1765-0195

REFERENCES

1. K/doqi clinical practice guidelines for chronic kidney disease. Evaluation, classification, and stratification. *Am J Kidney Dis*. 2002;39:S1-266.
2. Coresh J, Selvin E, Stevens LA, et al. Prevalence of chronic kidney disease in the United States. *JAMA*. 2007;298:2038-2047.
3. Chadban SJ, Briganti EM, Kerr PG, et al. Prevalence of kidney damage in Australian adults: The AusDiab kidney study. *J Am Soc Nephrol*. 2003;14:S131-138.
4. Simon de Lusignan CT, Harris K, van Vlymen J, Gallagher H. Uk prevalence of chronic kidney disease for the adult population is 6.76% based on two creatinine readings. *Nephron Clin Pract*. 2012;120:107.
5. Ruggenenti P, Schieppati A, Remuzzi G. Progression, remission, regression of chronic renal diseases. *Lancet*. 2001;357:1601-1608.
6. Toyoda K, Ninomiya T. Stroke and cerebrovascular diseases in patients with chronic kidney disease. *Lancet Neurol*. 2014;13:823-833.
7. Go AS, Chertow GM, Fan D, McCulloch CE, Hsu CY. Chronic kidney disease and the risks of death, cardiovascular events, and hospitalization. *N Engl J Med*. 2004;351:1296-1305.
8. Perlman RL, Finkelstein FO, Liu L, et al. Quality of life in chronic kidney disease (ckd): A cross-sectional analysis in the renal research institute-ckd study. *Am J Kidney Dis*. 2005;45:658-666.
9. Usrds 2006 annual data report: Atlas of end-stage renal disease in the United States. *U S. Renal Data System*. 2006.
10. Skyler JS. Microvascular complications. Retinopathy and nephropathy. *Endocrinol Metab Clin North Am*. 2001;30:833-856.
11. Wong TY, Coresh J, Klein R, et al. Retinal microvascular abnormalities and renal dysfunction: The atherosclerosis risk in communities study. *J Am Soc Nephrol*. 2004;15:2469-2476.
12. Kotlarsky P, Bolotin A, Dorfman K, Knyazer B, Lifshitz T, Levy J. Link between retinopathy and nephropathy caused by complications of diabetes mellitus type 2. *Int Ophthalmol*. 2015;35:59-66.
13. London A, Benhar I, Schwartz M. The retina as a window to the brain-from eye research to cns disorders. *Nat Rev Neurol*. 2013;9:44-53.
14. Cabrera DeBuc D, Somfai GM, Koller A. Retinal microvascular network alterations: Potential biomarkers of cerebrovascular and neural diseases. *Am J Physiol Heart Circ Physiol*. 2017;312:H201-h212.
15. McGeechan K, Liew G, Macaskill P, et al. Meta-analysis: Retinal vessel caliber and risk for coronary heart disease. *Ann Intern Med*. 2009;151:404-413.
16. Nguyen TT, Wang JJ, Islam FM, et al. Retinal arteriolar narrowing predicts incidence of diabetes: The Australian diabetes, obesity and lifestyle (ausdiab) study. *Diabetes*. 2008;57:536-539.
17. Mancia G, Fagard R, Narkiewicz K, et al. 2013 esh/esc practice guidelines for the management of arterial hypertension. *Blood Press*. 2014;23:3-16.

18. Frost S, Gregory C, Robinson L, et al. Effect of pupil dilation with tropicamide on retinal vascular caliber. *Ophthalmic Epidemiol*. 2019;26:400-407.
19. Knudtson MD, Lee KE, Hubbard LD, Wong TY, Klein R, Klein BE. Revised formulas for summarizing retinal vessel diameters. *Curr Eye Res*. 2003;27:143-149.
20. Hubbard LD, Brothers RJ, King WN, et al. Methods for evaluation of retinal microvascular abnormalities associated with hypertension/sclerosis in the atherosclerosis risk in communities study. *Ophthalmology*. 1999;106:2269-2280.
21. Chavers BM, Mauer SM, Ramsay RC, Steffes MW. Relationship between retinal and glomerular lesions in IDDM patients. *Diabetes*. 1994;43:441-446.
22. Sabanayagam C, Shankar A, Koh D, et al. Retinal microvascular caliber and chronic kidney disease in an Asian population. *Am J Epidemiol*. 2009;169:625-632.
23. Sabanayagam C, Tai ES, Shankar A, Lee J, Sun C, Wong TY. Retinal arteriolar narrowing increases the likelihood of chronic kidney disease in hypertension. *J Hypertens*. 2009;27:2209-2217.
24. Yau JW, Xie J, Kawasaki R, et al. Retinal arteriolar narrowing and subsequent development of ckd stage 3: The multi-ethnic study of atherosclerosis (mesa). *Am J Kidney Dis*. 2011;58:39-46.
25. Edwards MS, Wilson DB, Craven TE, et al. Associations between retinal microvascular abnormalities and declining renal function in the elderly population: The cardiovascular health study. *Am J Kidney Dis*. 2005;46:214-224.
26. Klein R, Klein BE, Moss SE, Wong TY. Retinal vessel caliber and microvascular and macrovascular disease in type 2 diabetes: Xxi: The Wisconsin epidemiologic study of diabetic retinopathy. *Ophthalmology*. 2007;114:1884-1892.
27. Klein R, Klein BE, Moss SE, et al. Retinal vascular abnormalities in persons with type 1 diabetes: The Wisconsin epidemiologic study of diabetic retinopathy: Xviii. *Ophthalmology*. 2003;110:2118-2125.
28. Wong TY, Shankar A, Klein R, Klein BE. Retinal vessel diameters and the incidence of gross proteinuria and renal insufficiency in people with type 1 diabetes. *Diabetes*. 2004;53:179-184.
29. Grauslund J, Hodgson L, Kawasaki R, Green A, Sjølie AK, Wong TY. Retinal vessel calibre and micro- and macrovascular complications in type 1 diabetes. *Diabetologia*. 2009;52:2213-2217.
30. Awua-Larbi S, Wong TY, Cotch MF, et al. Retinal arteriolar caliber and urine albumin excretion: The multi-ethnic study of atherosclerosis. *Nephrol Dial Transplant*. 2011;26:3523-3528.
31. Garner A, Ashton N, Tripathi R, Kohner EM, Bulpitt CJ, Dollery CT. Pathogenesis of hypertensive retinopathy. An experimental study in the monkey. *Br J Ophthalmol*. 1975;59:3-44.
32. Michael J, Hogan JAA, Weddell JE. *Histology of the Human Eye : An Atlas and Textbook*. Philadelphia, PA: Saunders; 1971.
33. Ferrari-Dileo G, Davis EB, Anderson DR. Effects of cholinergic and adrenergic agonists on adenylate cyclase activity of retinal microvascular pericytes in culture. *Invest Ophthalmol Vis Sci*. 1992;33:42-47.
34. Ferrari-Dileo G, Davis EB, Anderson DR. Response of retinal vasculature to phenylephrine. *Invest Ophthalmol Vis Sci*. 1990;31:1181-1182.
35. Cheng J, Yu J, Jiang C, Sun X. Phenylephrine affects peripapillary retinal vasculature-an optic coherence tomography angiography study. *Front Physiol*. 2017;8:996.
36. Nag S, Robertson DM, Dinsdale HB. Morphological changes in spontaneously hypertensive rats. *Acta Neuropathol*. 1980;52:27-34.
37. Klein R, Knudtson MD, Klein BE, et al. The relationship of retinal vessel diameter to changes in diabetic nephropathy structural variables in patients with type 1 diabetes. *Diabetologia*. 2010;53:1638-1646.
38. Sun C, Wang JJ, Mackey DA, Wong TY. Retinal vascular caliber: Systemic, environmental, and genetic associations. *Surv Ophthalmol*. 2009;54:74-95.
39. Zoccali C. Endothelial dysfunction and the kidney: Emerging risk factors for renal insufficiency and cardiovascular outcomes in essential hypertension. *J Am Soc Nephrol*. 2006;17:S61-63.
40. Klein R, Sharrett AR, Klein BE, et al. Are retinal arteriolar abnormalities related to atherosclerosis?: The atherosclerosis risk in communities study. *Arterioscler Thromb Vasc Biol*. 2000;20:1644-1650.
41. Jabs DA, Van Natta ML, Trang G, et al. Association of systemic inflammation with retinal vascular caliber in patients with aids. *Invest Ophthalmol Vis Sci*. 2019;60:2218-2225.
42. Grassi G, Quarti-Trevano F, Seravalle G, et al. Early sympathetic activation in the initial clinical stages of chronic renal failure. *Hypertension*. 2011;57:846-851.
43. Lanigan LP, Clark CV, Hill DW. Retinal circulation responses to systemic autonomic nerve stimulation. *Eye (Lond)*. 1988;2(Pt 4):412-417.
44. Harazny JM, Schmieder RE, Welzenbach J, Michelson G. Local application of tropicamide 0.5% reduces retinal capillary blood flow. *Blood Press*. 2013;22:371-376.
45. Russell RW. Evidence for autoregulation in human retinal circulation. *Lancet*. 1973;2:1048-1050.
46. Kaskel D, Meetais P, Bill A. The effects of arterial blood pressure increments on the regional blood flow in the eye and brain of cats. *Bibl Anat*. 1977;36-41.
47. Bill A, Linder M, Linder J. The protective role of ocular sympathetic vasomotor nerves in acute arterial hypertension. *Bibl Anat*. 1977;30-35.
48. Groeschel S, Chong WK, Surtees R, Hanefeld F. Virchow-robin spaces on magnetic resonance images: Normative data, their dilatation, and a review of the literature. *Neuroradiology*. 2006;48:745-754.
49. Wong TY. Is retinal photography useful in the measurement of stroke risk? *Lancet Neurol*. 2004;3:179-183.
50. Wong TY, Klein R, Couper DJ, et al. Retinal microvascular abnormalities and incident stroke: The atherosclerosis risk in communities study. *Lancet*. 2001;358:1134-1140.
51. Lindley RI, Wang JJ, Wong MC, et al. Retinal microvasculature in acute lacunar stroke: A cross-sectional study. *Lancet Neurol*. 2009;8:628-634.
52. Lamb EJ, Stevens PE. Estimating and measuring glomerular filtration rate: Methods of measurement and markers for estimation. *Curr Opin Nephrol Hypertens*. 2014;23:258-266.
53. Levey AS, Stevens LA, Schmid CH, et al. A new equation to estimate glomerular filtration rate. *Ann Intern Med*. 2009;150:604-612.

How to cite this article: Kannenkeril D, Frost S, Nolde JM, et al. Microvascular changes at different stages of chronic kidney disease. *J Clin Hypertens*. 2021;23:309–316. https://doi.org/10.1111/jch.14138