

# Peripheral nerve morphology and intraneural blood flow in chronic kidney disease with and without diabetes

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## Abstract

**Introduction/Aims:** Sonographic alterations of peripheral nerves in pre-dialytic kidney disease are yet to be determined. We aimed to assess peripheral nerve cross-sectional area (CSA) and intraneural blood flow in patients with pre-dialytic chronic kidney disease (CKD) and diabetic kidney disease (DKD).

**Methods:** Subjects with CKD ( $n = 20$ ) or DKD ( $n = 20$ ) underwent ultrasound to assess CSA of the median and tibial nerves as well as intraneural blood flow of the median nerve. Blood flow was quantified using maximum perfusion intensity. Neuropathy was assessed using the Total Neuropathy Score. A 6-m timed walk test was also performed. Healthy controls ( $n = 28$ ) were recruited for comparison.

**Results:** The DKD group had more severe neuropathy ( $p = .024$ ), larger tibial nerve CSA ( $p = .002$ ) and greater median nerve blood flow than the CKD group ( $p = .023$ ). Blood flow correlated with serum potassium in disease groups ( $r = 0.652$ ,  $p = .022$ ). Disease groups had larger tibial nerve CSA than controls ( $p < .05$ ). No blood flow was detected in controls. Tibial nerve enlargement was associated with slower maximal walking speeds in disease groups ( $r = -0.389$ ,  $p = .021$ ).

**Discussion:** Subjects with DKD demonstrated enlarged tibial nerve CSA and increased median nerve blood flow compared to those with CKD. Elevations in serum potassium were associated with increased blood flow. Sonographic alterations were detectable in pre-dialytic kidney disease compared to controls, highlighting the utility of ultrasound in the assessment of nerve pathology in these patient groups.

## KEYWORDS

chronic kidney disease, diabetic kidney disease, intraneural blood flow, nerve ultrasound, uremic neuropathy

## 1 | INTRODUCTION

Peripheral neuropathy is a common complication of chronic kidney disease (CKD) and affects 70% of pre-dialysis patients.<sup>1</sup> In patients with CKD caused by diabetes (diabetic kidney disease, DKD), the overlap of these two conditions contributes to an earlier onset of nerve injury and results in a clinical phenotype of neuropathy that is more severe than either disease alone.<sup>2,3</sup>

Nerve ultrasound has emerged as a useful technique to assess nerve morphology and blood flow.<sup>4,5</sup> In diabetic neuropathy, nerve and fascicle enlargement, loss of fascicle pattern, and in severe cases, intraneural blood flow have been observed.<sup>5,6</sup> In the context of neuropathy in kidney disease, ultrasound studies have been limited to dialysis patients but have demonstrated peripheral nerve enlargement, which was associated with serum potassium, and intraneural blood flow.<sup>7-9</sup> The assessment of intraneural blood flow in neuropathy is an emerging area and the lack of data regarding the validity and reliability of technique should be noted.

The purpose of the current study was to investigate if there are alterations in peripheral nerve structure and intraneural blood flow in pre-dialytic kidney disease with and without diabetes. A secondary

**Abbreviations:** BMI, body mass index; CSA, cross-sectional area; CKD, chronic kidney disease; DKD, diabetic kidney disease; eGFR, estimated glomerular filtration rate; HbA<sub>1c</sub>, glycated haemoglobin A<sub>1c</sub>.

aim was to determine the association between ultrasound measures and walking speed, which is a clinically relevant assessment of physical function in these patient groups.<sup>1</sup>

## 2 | METHODS

### 2.1 | Study participants

Ethics approval was provided by South Eastern Sydney Local Health District. All subjects provided informed consent. Inclusion criteria were CKD or DKD (type 2 diabetes only) and an estimated glomerular filtration rate (eGFR) between 15 and 59 mL/min/1.73 m<sup>2</sup>. Patients with either CKD or DKD were recruited from diabetes and renal outpatient clinics at the Prince of Wales Hospital in Sydney, Australia.<sup>10</sup> A cohort of healthy controls was recruited from the University of New South Wales for comparison with the disease groups. Exclusion criteria for all participants appear in the Appendix S1.

### 2.2 | Neuropathy and walking assessments

Neuropathy severity was evaluated using the Total Neuropathy Score, which is validated for CKD and DKD.<sup>11</sup> Total neuropathy scores range from 0 to 32 and a higher score is indicative of more severe neuropathy while a score of zero indicates an absence of neuropathy. Sural, tibial, and median nerve conduction studies were done according to established protocols.<sup>12</sup> Maximal walking speed was assessed over a 6-m distance from a standing start.<sup>1</sup>

### 2.3 | Nerve ultrasound: Cross-sectional area and intraneural blood flow

Participants underwent ultrasound of the median and tibial nerves in the transverse plane at non-entrapment sites using a MyLabOne system with a 10–18 MHz linear probe (Esaote, Genoa, Italy).<sup>6,7</sup> The median nerve was imaged at the junction of the middle and distal third of the forearm and the tibial nerve was imaged 5 cm proximal to the medial malleolus under the “Musculoskeletal” preset (acoustic power 100%, line density at medium, dynamic range at 14, and persistence set at 1).<sup>6,7</sup> The cross-sectional area (CSA) of each nerve was measured (in mm<sup>2</sup>) by outlining the inner margin of the epineurium. To improve reliability, each measurement was completed by two observers and then averaged. Median nerve sonograms were analysed for intraneural blood flow in the cross-sectional and transverse plane, with frequency set at 14 MHz, pulse repetition at 600 Hz, power doppler gain at 70%–80% of maximum and medium persistence.<sup>8</sup> Doppler signals present for at least 3 cardiac cycles were considered to indicate pulsatile blood flow and were automatically quantified using PixelFlux<sup>®</sup> Scientific software (Chameleon Software, Munster,

Germany), validated in obtaining maximum perfusion intensity from the intensity of the colour hues of the pixels (Figure S1).

### 2.4 | Statistical analyses

Data were analysed using SPSS Version 25.0 for Windows (IBM, Armonk, NY, USA). Details regarding statistical analyses are in the Supplemental Methods. Statistical significance was considered when  $p \leq .05$ .

## 3 | RESULTS

### 3.1 | Participant demographics

A total of 40 patients (CKD = 20, DKD = 20) and 28 controls were recruited. All groups were similar in age and sex distribution (Table 1). The CKD and DKD groups were not significantly different in eGFR, systolic blood pressure, serum potassium, urea and creatinine. Glycated haemoglobin A<sub>1c</sub> (HbA<sub>1c</sub>) of the DKD group was indicative of suboptimal glycaemic control.

### 3.2 | Nerve ultrasound

Ultrasound findings are summarised in Table 2. The DKD group demonstrated significant enlargement in tibial nerve CSA compared to the CKD cohort (Figure S2). Both disease groups had larger tibial nerves compared to controls (DKD,  $p < .001$ ; CKD,  $p = .034$ ). No differences were observed in median nerve CSA between disease groups. Intraneural blood flow in the median nerve, detectable in a subgroup of patients, was higher in the DKD group compared to CKD participants. No intraneural blood flow was observed in controls.

### 3.3 | Neuropathy and walking assessments

Patients with DKD had significantly higher total neuropathy scores and slower maximal walking speed compared to those with CKD alone (Table 2).

### 3.4 | Correlation analysis

Correlation analysis was undertaken in the patient groups as a whole (Table 3). Tibial nerve CSA was associated with maximal walking speed ( $r = -0.389$ ,  $p = .021$ ). Maximum perfusion intensity correlated with serum potassium ( $r = 0.652$ ,  $p = .022$ ) and median nerve CSA ( $0.415$ ,  $p = .018$ ). No association was found between CSA or maximum perfusion intensity and age, sex, body mass index (BMI), systolic blood pressure, HbA<sub>1c</sub>, diabetes duration, insulin usage, eGFR, urea

**TABLE 1** Participant demographics

	CKD (n = 20)	DKD (n = 20)	Control (n = 28)	CKD versus DKD	Overall
Age (years)	64 ± 13	67 ± 11	62 ± 7.5	0.653	0.262
Sex (% male)	55	60	47	0.749	0.634
BMI (kg/m <sup>2</sup> )	27 (23–30)	32 (29–36)	25 (23–27)	<b>0.003</b>	<b>&lt;0.001</b>
SBP (mmHg)	133 ± 15	135 ± 20	–	0.726	–
HbA <sub>1c</sub> (%)	–	8.3 ± 1.7	–	–	–
Diabetes duration (years)	–	16 ± 7	–	–	–
Patients on insulin (%)	–	85	–	–	–
eGFR (mL/min/1.73 m <sup>2</sup> )	36 ± 10	40 ± 9	–	0.167	–
Potassium (mmol/L)	4.5 ± 0.4	4.6 ± 0.5	–	0.349	–
Urea (mg/dL)	30.8 (28.0–36.4)	30.8 (22.4–36.4)	–	0.405	–
Creatinine (mg/dL)	1.8 (1.7–1.9)	1.6 (1.4–1.8)	–	0.209	–

Note: Normally distributed data is expressed as mean ± SD while non-normally distributed data is expressed as median and quartile 1 to quartile 3. Abbreviations: BMI, body mass index; eGFR, estimated glomerular filtration rate; HbA<sub>1c</sub>, glycated haemoglobin A<sub>1c</sub>; SBP, systolic blood pressure.

**TABLE 2** Participant results

	CKD (n = 20)	DKD (n = 20)	Control (n = 28)	CKD versus DKD	Overall
Total neuropathy score	2 (0–9)	7 (4–11)	0	<b>0.024</b>	<b>&lt;0.001</b>
Tibial CMAP amplitude (mV)	10.0 (4.3–14.7)	3.7 (0.3–8.6)	10.3 (8.5–15.0)	<b>0.023</b>	<b>0.003</b>
Sural SNAP amplitude (μV)	10.5 ± 10.0	7.5 ± 6.6	19.7 ± 13.4	0.281	<b>0.002</b>
Median CMAP amplitude (mV)	8.6 (6.3–13.0)	5.7 (4.3–7.6)	9.8 (8.1–13.4)	0.060	<b>0.001</b>
Tibial nerve CSA (mm <sup>2</sup> )	14.7 ± 4.7	22.2 ± 8.0	12.3 ± 3.1	<b>0.002</b>	<b>&lt;0.001</b>
Median nerve CSA (mm <sup>2</sup> )	7.8 ± 1.8	8.3 ± 1.9	7.0 ± 1.5	0.308	<b>0.05</b>
Median nerve INBF (cm/s)	0.32 ± 0.15 (n = 12)	0.56 ± 0.32 (n = 14)	0	<b>0.023</b>	–
Maximal walking speed (m/s)	1.5 ± 0.4	1.3 ± 0.3	1.6 ± 0.3	<b>0.049</b>	<b>0.024</b>

Note: Normally distributed data is expressed as mean ± SD while non-normally distributed data is expressed as median and quartile 1 to quartile 3. Abbreviations: CMAP, compound muscle action potential; CSA, cross-sectional area; INBF: intraneural blood flow; SNAP, sensory nerve action potential.

**TABLE 3** Correlation analyses

	Total neuropathy score	Tibial nerve CMAP amplitude	Median nerve CMAP amplitude
Tibial nerve CSA	<b>0.552***</b>	<b>–0.579***</b>	–0.132
Median nerve CSA	0.196	–0.015	<b>–0.353*</b>
Median nerve INBF	0.139	–0.167	<b>–0.352*</b>

Note: Depending on the distribution of the data, Pearson (normally distributed) or Spearman (non-normally distributed) correlations were applied.

Abbreviations: CSA, cross-sectional area; CMAP: compound muscle action potential; INBF: intraneural blood flow.

Note:\*\*\*  $p < .001$ , \*\*  $p < .01$  \*  $p \leq .05$ .

and creatinine. No correlation was identified between CSA and serum potassium.

## 4 | DISCUSSION

The current study determined that sonographic alterations occur in pre-dialytic kidney disease. Tibial nerve CSA was significantly enlarged in both disease groups compared to controls. It was also found that the DKD group had a larger tibial nerve CSA than the CKD group. The finding that subjects with DKD had a larger tibial but not median

nerve CSA than those with CKD is consistent with the length-dependent nature of peripheral neuropathy. The altered osmotic gradients that occur in diabetes, as a result of the conversion of glucose to sorbitol and sodium-potassium pump dysfunction, may also contribute to the greater enlargement in the DKD group via intracellular sodium retention.<sup>13,14</sup> However, the reasons for nerve swelling in diabetes are yet to be determined and the ultrasound appearance in Supplementary Figure S2 does not particularly demonstrate features of oedema.<sup>15</sup> While an association between serum potassium and nerve CSA was not observed in this study, our participants were not hyperkalaemic and had less severe kidney

dysfunction than those recruited in a previous study of end-stage kidney disease patients.<sup>7</sup> Presumably, this resulted in less pronounced effects on nerve structure than would occur in patients on dialysis. The correlation between larger tibial CSA and slower maximal walking speed is relevant given that walking speed is an important marker of physical function in pre-dialytic kidney disease cohorts.<sup>1</sup>

In addition to larger tibial CSA, greater intraneural blood flow was observed in the median nerve in the DKD cohort compared to the CKD group. In diabetes, intraneural blood flow has been attributed to hypervascularity of the epineural vessels in response to endoneurial ischaemia secondary to microangiopathy.<sup>6</sup> Thus, the association between serum potassium and intraneural blood flow observed in the current study may suggest a similar process of nerve hypoxia however the underlying mechanism requires further exploration. Alternatively, studies indicate that nerve injury in kidney disease occurs due to potential neurotoxic effects of potassium, which may accumulate underneath the myelin sheath.<sup>2,16,17</sup> An accumulation of potassium in the submyelinic space, which could occur in pre-dialytic kidney disease due to elevated total body potassium, may cause a release of inflammatory mediators resulting in an increase of intraneural blood flow. However, it should be noted that a previous study in dialysis patients did not report an association between serum potassium and maximal perfusion intensity.<sup>8</sup> Taken together, the combined effects of diabetes and kidney disease as well the greater neuropathy severity may explain why the DKD group had greater intraneural blood flow compared to the CKD group.

With respect to the median nerve ultrasound findings, patients in the current study had smaller CSA and less intraneural blood flow compared to previously reported values in dialysis patients suggesting these measures worsen with kidney disease progression.<sup>7,8</sup> Intraneural blood flow was not detectable in healthy nerves, providing further evidence that detectable blood flow is pathological.<sup>5</sup>

Limitations of this study include the sample size, the absence of nerve conduction studies to exclude carpal tunnel syndrome, the difference in BMI between disease groups, the lack of sural nerve imaging and other ultrasound parameters such as echogenicity as well as the absence of dialysis groups for comparison. Future studies could examine associations between ultrasound and clinical variables in larger DKD and CKD cohorts and examine specific effects of diabetic and uremic neuropathy.

The present study demonstrates that nerve enlargement and increased intraneural blood flow occur in pre-dialytic kidney disease and are more severe in DKD than CKD. Intraneural blood flow correlated with serum potassium, providing further evidence that potassium is implicated in the pathophysiology of peripheral neuropathy in kidney disease, both with and without diabetes.

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## CONFLICT OF INTEREST

None of the authors has any conflict of interest to disclose.

## AUTHOR CONTRIBUTIONS

TI, SW, and AVK were involved in study design. TI and SW acquired data. All authors were involved in data analysis, data interpretation and revision of the manuscript.

## ETHICS STATEMENT

We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

## DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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#### SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

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## Autoantibody profile in myasthenia gravis patients with a refractory phase

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#### Abstract

**Introduction/Aims:** A subgroup of myasthenia gravis (MG) patients fail to respond adequately to recommended treatments, a condition referred to as refractory MG. During the refractory phase, patients experience persistent debilitating symptoms with potential life-threatening events or inability to reduce immunosuppressant dosages and minimize long-term toxicities.

**Methods:** We conducted a retrospective, single-center study of 113 MG patients to investigate the autoantibody profile and clinical characteristics of refractory MG patients, compared with nonrefractory patients, based on predefined criteria.

**Results:** Fifteen patients (13.3%) were classified as refractory. Double-seronegative MG (DSNMG), without detectable nicotinic acetylcholine receptor (AChR) or muscle-specific tyrosine kinase (MuSK) antibodies, was identified in six refractory patients, significantly higher than those with nonrefractory MG (40% vs 16.3%;  $P = .031$ ). None of the refractory patients had MuSK antibodies. Patients in the refractory group more frequently had an earlier disease onset, thymic pathology, and thymectomy ( $P \leq .03$  for all).

**Discussion:** In this study, patients with refractory MG were more likely than those with nonrefractory MG to be DSN; and refractory DSNMG patients had worse MGFA classes in their recent visit compared with anti-AChR positive refractory