ORIGINAL ARTICLE

Ann Acad Med Singap 2022;52:125-34
https://doi.org/10.47102/annals-acadmedsg.2022350

Association between lower phase angle and chronic kidney disease progression in type 2 diabetes patients

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ABSTRACT

Introduction: Phase angle (PhA), derived from bioelectrical impedance analysis (BIA), is the angle of vector determined by the body's resistance and reactance. It indicates cellular integrity and hydration status. Though extracellular volume excess was associated with chronic kidney disease (CKD) progression, the association between PhA and CKD progression is unknown. Matrix metalloproteinase-2 (MMP-2) is a member of zinc-dependent endopeptidase family and promotes renal interstitial fibrosis. We investigated association between PhA and CKD progression, and whether the association was through MMP-2 in patients with type 2 diabetes mellitus (T2DM).

Method: We conducted a prospective study on 1,078 patients with T2DM (mean age 58.9±9.1 years). PhA was measured using BIA. CKD progression was defined as ≥25% decrease in estimated glomerular filtration rate (eGFR) from baseline with deterioration across eGFR categories. Multiplex immunoassay was used to quantitate MMP-2. We examined association between PhA and CKD progression using Cox proportional hazards model, adjusting for demographics, clinical parameters and medications.

Results: Over 8.6 years of follow-up, 43.7% of participants had CKD progression. Compared to tertile 3 PhA (higher level), tertiles 1 and 2 PhA were associated with higher hazards of CKD progression, with corresponding unadjusted hazard ratios (HRs) of 2.27 (95% confidence interval [CI] 1.80–2.87, $P<0.001$) and 1.57 (95% CI 1.24–2.01, $P<0.001$). The positive association between tertiles 1 and 2 PhA with CKD progression persisted in the fully adjusted model with corresponding HRs of 1.71 (95% CI 1.30–2.26, $P<0.001$) and 1.46 (95% CI 1.13–1.88, $P=0.004$). MMP-2 accounted for 14.7% of association between tertile 1 PhA and CKD progression.

Conclusion: Our findings revealed a previously unobserved association between BIA-derived lower PhA and CKD progression through MMP-2 in patients with T2DM.

Ann Acad Med Singap 2022;52:125-34

Keywords: Bio-impedance analysis, chronic kidney disease, diabetes, matrix metalloproteinase, phase angle

INTRODUCTION

Chronic kidney disease (CKD) is a global public health problem with an estimated prevalence of 13.4%.[1] One of the key drivers of the global increase in CKD is the rising prevalence of diabetes mellitus (DM).[1] CKD affects about 25–40% of patients with type 2 diabetes mellitus (T2DM).[2] A few studies also reported that the prevalence of CKD among patients with T2DM was about 53% in Singapore.[3,4] The rising prevalence of DM in Singapore[5] will likely produce a ripple effect on the growing healthcare and economic burden of CKD among patients with T2DM.[6]

Despite optimal regulation of blood pressure with renin-angiotensin system (RAS) blockage (e.g. angiotensin-converting enzyme inhibitors or angiotensin-receptor blockers,) and the emergence of new anti-diabetic medications (e.g. sodium-glucose cotransporter-2 inhibitors and glucagon-like peptide-1 receptor agonists)

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CLINICAL IMPACT

What is New

- To our knowledge, this is the first study to show a longitudinal association between low phase angle (PhA) and chronic kidney disease (CKD) progression in type 2 diabetes mellitus (T2DM).
- Matrix metalloproteinase-2 mediated the association between low PhA and CKD progression in T2DM.

Clinical Implications

- Low PhA may serve as a risk marker of CKD progression in T2DM.
- There is potential for therapeutic interventions to target the underlying pathophysiological mechanisms leading to lowered PhA.

to prevent or slow down diabetic complications, a residual risk of renal outcome remains.[7] Hence, it is necessary to understand the pathophysiological mechanisms and risk factors of CKD development and progression. A diabetic milieu can result in structural alterations in the kidney, which include renal hypertrophy, glomerular sclerosis, inflammation and fibrosis of the tubule-interstitial tissue.[8] Traditional metabolic risk markers such as DM duration, sub-optimal glycaemic control, hyperlipidaemia and hypertension are well-established risk factors of CKD in T2DM.[9] Some of these risk factors have been incorporated in predictive models developed to predict the risk of CKD progression.[10] However, there is an increasing need to move beyond these conventional risk factors to explore markers that may also shed light on the pathophysiological mechanism of CKD in T2DM.

Of note, there is accumulating interest in the phase angle (PhA) of bioelectrical impedance analysis (BIA), which is the angle of vector determined by resistance and reactance in the body.[11] BIA determines impedance of the electric current in the body. The impedance comprises resistance (which describes the opposition to alternating electric current of body fluids and reflects hydration of tissue), and reactance (which describes the opposition to alternating electric current of cell membrane and is produced by capacitance of the cell membrane).[12-14] Reactance leads to a lag of the current behind the voltage, thus resulting in a phase shift expressed geometrically as PhA.[14] PhA is the angular transformation of the reactance to resistance ratio, and reflects cellular health with higher levels indicative of healthier cell membrane.[11,13,14] Numerous studies have shown that PhA may act as a prognostic indicator of nutritional status for DM, kidney disease, liver cirrhosis and malignancy.[13] PhA was also found to be associated with fasting blood glucose and haemoglobin A1c (HbA1c) in patients with T2DM.[13] This suggested that PhA may be a useful indicator of clinical outcomes and severity of T2DM.[13]

Extracellular volume excess may explain the possible deleterious effects of low PhA on renal function in T2DM. In T2DM, hyperglycaemia may exert an osmotic force that shifts water from the intracellular compartment to extracellular compartment, thereby leading to higher resistance value.[14,15] As PhA is derived from the ratio of reactance of cell membranes to resistance of body water, it is possible that low PhA is attributed to changes in body water distribution.[14] On the other hand, disruption in cellular integrity, which characterises low PhA,[13] results in accumulation of extracellular water and leads to overhydration.[16] Hence, PhA also reflects tissue hydration and is inversely associated with extracellular water.[13] Studies have shown that an increase in extracellular to total body water (ECW/TBW) ratio, which indicates extracellular volume excess, is associated with renal function decline.[17,18] Extracellular volume excess leads to increase in efferent arterial pressure and decrease in renal blood flow, thereby resulting in renal function decline.[19] Furthermore, extracellular volume excess may lead to translocation of bacterial and endotoxins via the congested intestinal walls into the systemic circulation, thereby resulting in inflammation, which is a risk factor for CKD progression.[20,21] Hitherto, the association between PhA and CKD progression remains unknown.

Interestingly, PhA is also a marker of oxidative stress which is increased in T2DM.[22,23] Oxidative stress can in turn activate matrix metalloproteinase-2 (MMP-2),[24] which is a member of the zinc-dependent endopeptidase family. MMP-2 has been implicated in CKD progression as it plays a pivotal role in the pathogenesis of renal interstitial fibrosis.[25] The interplay of MMP-2, PhA and CKD progression has not been studied.

We aimed to examine the association between PhA and CKD progression in T2DM. We hypothesised that lower PhA was associated with CKD progression. A secondary objective was to investigate whether MMP-2 accounted for the association between PhA and CKD progression.

METHOD

Population

This was a prospective cohort study on patients with T2DM. The patients were on follow-up for routine management of T2DM at Diabetes Centre in Admiralty Medical Centre, and primary care polyclinics, in Singapore. They were first recruited between January 2011 and March 2014, and were followed up for their renal function until March 2020. The following exclusion criteria were applied: active malignancy, active inflammation, oral intake of steroids with dose of more than 7.5mg per day, and/or intake of non-steroidal anti-inflammatory medications on the day of research assessment. For the purpose of this analysis, patients were further excluded if they had fewer than 2 estimated glomerular filtration rate (eGFR) readings, eGFR less than 15mL/min/1.75m^2 at baseline, and shorter than 1 year of follow-up. A total of 1,078 out of 1,732 participants were eligible for the analysis. Fig. 1 shows the flowchart of patients. All the participants provided written informed consent, and the study was granted ethics approval.

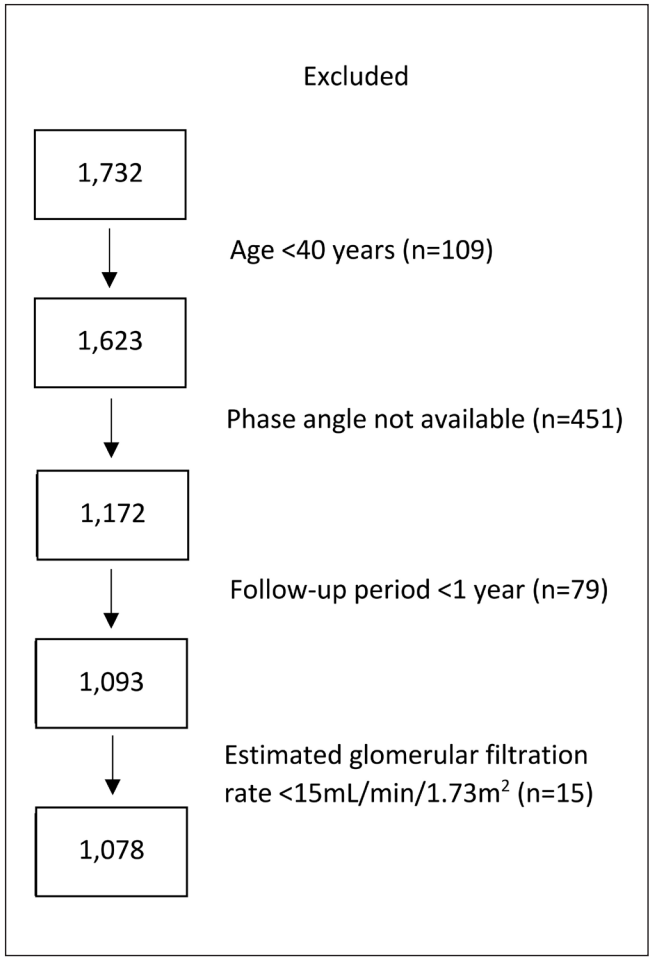

Fig. 1. Flowchart of recruited patients.

Data collection

Our trained research nurses collected data on demographics, medical history and medications using a standard questionnaire administered to the patients. Body mass index (BMI) was calculated based on weight and height, which were measured using a standard weighing scale and stadiometer, respectively. The research nurses also took blood pressure measurement using a standard automated blood pressure monitor (HEM-C7011-C1, Omron Corp, Kyoto, Japan) following a rest period of 10 to 15 minutes in a seated position.

A tetra-polar multifrequency BIA method (InBody-S10, InBody Co Ltd, South Korea) was used to measure PhA at study baseline. In BIA, electric currents flow through the body, allowing calculation of impedance and reactance of the currents. Measurements were taken at various frequencies ranging from 5–1000kHz. The PhA is the angle of the time lag between voltage waveform at 50kHz and current waveform.[11] It can be calculated as arc tangent (reactance/resistance) $\times$ 180°/π.[13]

Fasting blood and urine samples were collected at baseline and measured at the hospital diagnostic laboratory accredited by the College of American Pathologists for the following parameters: HbA1c using Tina-quant HA1c Gen.3 (Roche, Mannheim, Germany); low-density lipoprotein cholesterol (LDL-C) and triglycerides using enzymatic colorimetric test; and urinary albumin using immunoturbidimetric assay (cobas c 501 analyser, Roche, Mannheim, Germany). Serum creatinine was measured using enzymatic colorimetric test (cobas c 501 analyser, Roche, Mannheim, Germany). Serial serum creatinine readings were collected during follow-ups and used to calculate eGFR based on the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation.[26] We measured plasma MMP-2 with multiplex immunoassay on Luminex 200 platform (Thermo Fisher Scientific, Santa Clara, US).

The outcome was CKD progression—defined as at least 25% drop in eGFR—coupled with deterioration across eGFR categories as follows: stage 1 (≥90mL/min/1.73m^2), stage 2 (60–89mL/min/1.73m^2), stage 3a (45–59mL/min/1.73m^2), stage 3b (30–44mL/min/1.73m^2) and stage 4 (15–29mL/min/1.73m^2) according to the Kidney Disease: Improving Global Outcomes (KDIGO) Clinical Practice Guidelines for the Evaluation and Management of Chronic Kidney Disease.[27]

Statistical analysis

We presented baseline characteristics as mean ± standard deviation (SD) or median with interquartile range (IQR)

for continuous variables, and number with percentages for categorical variables. As there is no current cut-off point for PhA,[28] we analysed PhA as a continuous variable and in tertiles. One-way analysis of variance or Kruskal-Wallis test was used to compare continuous variables across PhA in tertiles depending on the distribution of variables. Student's t-test or Wilcoxon rank-sum test was used to compare continuous variables by CKD progression depending on the distribution of variables. Chi-square test was used to compare categorical variables across PhA in tertiles, and between CKD progression and non-CKD progression.

We examined the association between PhA and CKD progression with Cox proportional hazards model. Model 1 was adjusted for age, sex and ethnicity. Model 2 was additionally adjusted for DM duration, BMI, systolic blood pressure (SBP), LDL-C, triglycerides, eGFR, urinary albumin-to-creatinine ratio (uACR), use of insulin and use of RAS antagonist. These adjusting covariates were chosen as the *P* value of the association with CKD progression if univariate analysis was less than 0.1 or they were known risk factors of renal decline or CKD progression.[29,30] There was no violation of the assumption of proportional hazard in the Cox regression model according to the Schoenfeld residual test. We repeated the analysis stratified by sex.

Binary mediation analysis, based on the Baron and Kenny Framework,[31] was performed to examine the mediating role of MMP-2 (mediator) in the relationship between low PhA (tertile 1 PhA) (independent variable) and CKD progression (dependent variable). The following criteria for mediation were required: (1) association between low PhA and MMP-2 in pathway a; (2) association between MMP-2 and CKD progression in pathway b; (3) association between low PhA and CKD progression in pathway c; and (4) weakening of the association between low PhA and CKD progression when MMP-2 was included in the model in pathway c'.

Statistical analysis was conducted with STATA version 14.0 (STATA Corp, College Station, US). A *P* value less than 0.05 was considered statistically significant.

RESULTS

The baseline characteristics of the 1,078 patients are presented in Table 1. The mean age was 58.9±9.1 years, with a slight male predominance (51.0%). The distribution of participants by ethnicity was 53.0% Chinese, 19.5% Malays and 24.9% Indians. The mean DM duration was 12.1±9.4 years. The mean PhA was 5.5±0.9°. The median rate of eGFR change was -1.3 mL/min/1.73m^2/year (IQR -3.6 to 0.0). When stratified by PhA in tertiles, the age, DM duration, SBP and uACR of patients decreased while the proportion of males and BMI increased with increasing PhA tertiles. The patients with tertiles 2 and 3 PhA also had higher eGFR and lower proportions of insulin and RAS antagonist use compared to those with tertile 1 PhA. The proportions of lower baseline eGFR category (Stage 3a to 4) were higher in patients with tertile 1 PhA compared to those with tertiles 2 and 3 PhA (*P*=0.005). Females had lower PhA than males (5.2±0.8° versus 5.8±0.9°; *P*<0.001).

Over a follow-up period of up to 8.6 (median 5.0, IQR 3.2–6.9) years, there were 471 patients (43.7%) who experienced CKD progression. The proportions of lower last follow-up eGFR category (Stage 3b to 5) decreased with increasing PhA tertiles (*P*<0.001). Fig. 2 shows the Kaplan-Meier survival curve for CKD progression by PhA in tertiles. The tertile 1 PhA group had the shortest time to CKD progression, followed by tertile 2 PhA group and tertile 3 PhA group (log-rank test 50.3, *P*<0.001).

Table 2 shows that patients with CKD progression tended to be older and of Malay ethnicity. They also had longer DM duration, higher proportions of insulin and RAS antagonist use, and poorer metabolic profile in terms of BMI, SBP, HbA1c, eGFR and uACR compared to those without CKD progression. The PhA was also lower in patients with CKD progression.

In Table 3, every SD increase in baseline PhA was associated with lower hazards of CKD progression in unadjusted analysis, and Models 1 and 2 with corresponding hazard ratios (HRs) 0.70 (95% CI 0.63–0.78, *P*<0.001), 0.73 (95% CI 0.65–0.82, *P*<0.001) and 0.84 (95% CI 0.75–0.95, *P*=0.004).

Tertiles 1 and 2 PhA were associated with higher hazards of CKD progression, compared to tertile 3 PhA with corresponding unadjusted HRs 2.27 (95% CI 1.80–2.87, *P*<0.001) and 1.57 (95% CI 1.24–2.01, *P*<0.001). The positive association between tertiles 1 and 2 PhA with CKD progression persisted in Models 1 and 2. In the fully adjusted Model 2, tertiles 1 and 2 PhA were associated with 71% and 46% respective increases in hazards of CKD progression, with corresponding HRs 1.71 (95% CI 1.30–2.26, *P*<0.001) and 1.46 (95% CI 1.13–1.88, *P*=0.004).

There was a statistically significant interaction in terms of sex and PhA tertile groups (*P*=0.041). Table 3 shows the association between PhA and CKD progression stratified by sex. Among females, every SD increase in baseline PhA was associated with lower hazards of CKD progression, with HRs 0.55 (95% CI 0.46–0.66,

Table 1. Baseline characteristics of patients stratified by phase angle in tertiles.

	All	T1	T2	T3	*P* value
No. of patients	1,078	360	359	359	
Age, mean ± SD, years	58.9 ± 9.1	63.0 ± 9.3	58.5 ± 7.8	55.1 ± 8.5	<0.001
Male, no. (%)	550 (51.0)	109 (30.3)	178 (49.6)	263 (73.3)	<0.001
Ethnicity, no. (%)					0.646
Chinese	571 (53.0)	193 (53.6)	197 (54.9)	181 (50.4)	
Malay	199 (18.5)	70 (19.4)	64 (17.8)	65 (18.1)	
Indian	268 (24.9)	85 (23.6)	88 (24.5)	95 (26.5)	
Others	40 (3.7)	12 (3.3)	10 (2.8)	18 (5.0)	
DM duration, mean ± SD, years	12.1 ± 9.4	14.6 ± 10.6	12.1 ± 9.1	9.5 ± 7.7	<0.001
BMI, mean ± SD, kg/m^2	27.5 ± 4.9	27.0 ± 5.1	27.4 ± 4.7	28.1 ± 4.9	0.015
SBP, mean ± SD, mmHg	139.1 ± 17.7	144.4 ± 19.1	137.6 ± 16.9	135.2 ± 15.5	<0.001
HbA1c, mean ± SD, %	7.8 ± 1.3	7.8 ± 1.3	7.9 ± 1.2	7.7 ± 1.2	0.096
LDL-C, mean ± SD, mmol/L	2.7 ± 0.8	2.7 ± 0.9	2.7 ± 0.7	2.8 ± 0.8	0.374
TG, median (IQR), mmol/L	1.4 (1.0–1.9)	1.3 (1.0–1.9)	1.4 (1.0–1.9)	1.4 (1.1–2.0)	0.568
eGFR, mean ± SD, mL/min/1.73m^2	85.1 ± 24.7	80.9 ± 26.6	87.3 ± 24.1	87.1 ± 22.6	<0.001
Rate of eGFR change, median (IQR), mL/min/1.73m^2/year	-1.3 (-3.6 to 0.0)	-1.5 (-4.0 to -0.1)	-1.3 (-3.4 to 0.0)	-1.0 (-3.2 to 0.1)	0.051
Baseline eGFR category, no. (%)					0.005
G1, ≥90mL/min/1.73m^2	568 (52.7)	163 (45.3)	206 (57.4)	199 (55.4)	
G2, 60–89mL/min/1.73m^2	320 (29.7)	110 (30.6)	97 (27.0)	113 (31.5)	
G3a, 45–59mL/min/1.73m^2	90 (8.4)	39 (10.8)	29 (8.1)	22 (6.1)	
G3b, 30–44mL/min/1.73m^2	62 (5.8)	29 (8.1)	18 (5.0)	15 (4.2)	
G4, 15–29mL/min/1.73m^2	38 (3.5)	19 (5.3)	9 (2.5)	10 (2.8)	
Last follow-up eGFR category, no. (%)					<0.001
G1, ≥90mL/min/1.73m^2	358 (33.2)	85 (23.6)	128 (35.7)	145 (40.4)	
G2, 60–89mL/min/1.73m^2	339 (31.5)	109 (30.3)	106 (29.5)	124 (34.5)	
G3a, 45–59mL/min/1.73m^2	140 (13.0)	48 (13.3)	52 (14.5)	40 (11.1)	
G3b, 30–44mL/min/1.73m^2	92 (8.5)	42 (11.7)	33 (9.2)	17 (4.7)	
G4, 15–29mL/min/1.73m^2	77 (7.1)	40 (11.1)	21 (5.9)	16 (4.5)	
G5, <15mL/min/1.73m^2	72 (6.7)	36 (10.0)	19 (5.3)	17 (4.7)	
uACR, median (IQR), mg/g	21.7 (7.3–84.5)	35.0 (10.0–156.4)	20.0 (7.0–88.3)	17.3 (5.3–53.0)	<0.001
Use of insulin, no. (%)	312 (29.0)	111 (30.8)	119 (33.4)	82 (22.8)	0.005
Use of RAS antagonist, no. (%)	673 (62.5)	248 (68.9)	214 (59.8)	211 (58.8)	0.009
Phase angle, mean ± SD, °	5.5 ± 0.9	4.5 ± 0.4	5.4 ± 0.2	6.5 ± 0.7	<0.001

BMI: body mass index; DM: diabetes mellitus; eGFR: estimated glomerular filtration rate; HbA1c: haemoglobin A1c; IQR: interquartile range; LDL-C: low-density lipoprotein cholesterol; RAS: renin-angiotensin system; SBP: systolic blood pressure; SD: standard deviation; TG: triglycerides; uACR: urinary albumin-to-creatinine ratio

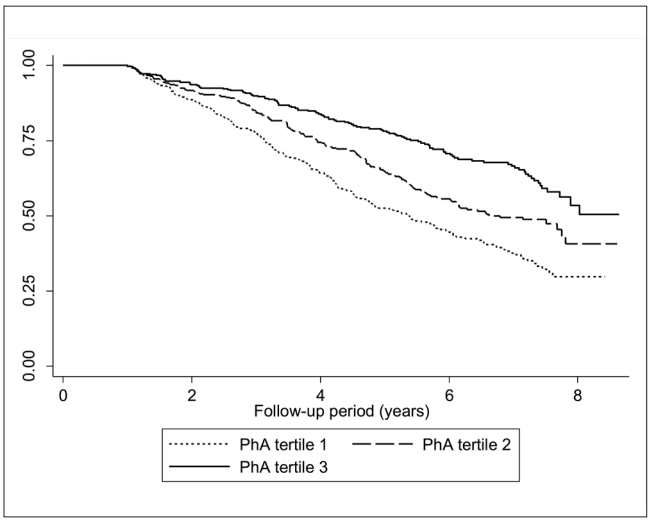

Fig. 2. Kaplan-Meier survival curve for chronic kidney disease progression by phase angle in tertiles.

P<0.001) and 0.67 (95% CI 0.55–0.81, *P*<0.001) in unadjusted analysis and Model 2, respectively. Although there was an association between per SD increase in baseline PhA and CKD progression in unadjusted analysis and Model 1 among males, the association lost statistical significance in Model 2 (*P*=0.956). Similar findings were noted when PhA was analysed in tertiles.

The mediation analysis in Fig. 3 showed that: (1) low PhA was positively associated with MMP-2 with β for pathway a=5.51, *P*<0.001; (2) MMP-2 was positively associated with CKD progression with β for pathway b=0.01, *P*=0.021; (3) low PhA was positively associated with CKD progression with β for pathway c=0.40, *P*=0.022; and (4) the association between low PhA and CKD progression was weakened upon inclusion of MMP-2 in the model with β for pathway c'=0.35, *P*=0.048. MMP-2 accounted for 14.7% of the association between low PhA and CKD progression (*P*=0.037).

DISCUSSION

Our study showed that lower PhA was associated with higher hazards of CKD progression in patients with T2DM. The association was independent of traditional cardio-metabolic risk factors. Hence, low PhA may play a potential role in the pathogenesis of CKD progression in T2DM. The association between low PhA and CKD progression was observed in females but not in males in the fully adjusted analyses. Furthermore, MMP-2 accounted for the association between lower PhA and CKD progression.

The study by Han et al. showed that PhA was higher in patients on peritoneal dialysis than those with non-dialysis CKD Stage 5.[32] PhA was also associated with protein-energy wasting that was prevalent in patients with end-stage renal disease.[32] Hence, PhA appeared as a manifestation of advanced CKD rather than a risk factor of CKD progression. Although an earlier study revealed an association between low PhA and diabetic CKD Stage 5, the study design was cross-sectional and did not establish a causal relationship between PhA and CKD.[11] To the best of our knowledge, there was no study that reported the relationship between PhA and CKD progression. Our study revealed a previously unobserved longitudinal association between low PhA and CKD progression in T2DM, and provided a fresh perspective on the contribution of PhA to renal decline.

The association between low PhA and CKD progression was observed in females but not in males in our study. In our cohort, females had lower baseline PhA than males. It was noted that reduction in oestrogen level during menopausal transition in females could result in accelerated loss of muscle mass.[33,34] As there is a correlation between low muscle mass and high resistance,[13] it is plausible that low PhA, which is influenced by high resistance, is associated with low muscle mass in females.

Our current study demonstrated that lower PhA was characterised by older age, longer DM duration, higher SBP and uACR, and lower eGFR. Our results corroborate the findings from another study that showed an inverse relationship between PhA and DM duration in patients with T2DM.[14] The same study also demonstrated that patients with T2DM had lower PhA than control subjects without DM.[14] These findings suggested that the cardio-metabolic burden may be high in patients with low PhA. Low PhA indicates reduced body cell mass. Body cell mass comprises mainly the cellular components of viscera and muscles. As DM affects these tissues, it is plausible that low PhA indicates catabolism in DM.[14]

A novel finding of this study was that lower PhA was associated with CKD progression through MMP-2 in patients with T2DM. A mediator is considered to be the mechanism via which the independent variable is able to influence the dependent variable.[31] In this case, low PhA (an independent variable) is associated with CKD progression (a dependent variable) through MMP-2 (a mediator). In T2DM, there is increased oxidative stress whereby reactive oxygen species (ROS) are produced in excess.[23] The ROS promote cellular damage and trigger inflammatory signaling.[35] Oxidative stress and inflammation can damage cellular structure and cause apoptosis.[22] PhA reflects cellular integrity, with higher levels indicating more intact cell membranes

Table 2. Baseline characteristics of patients stratified by chronic kidney disease progression.

	CKD progression		
	No	**Yes**	***P* value**
No. of patients	607	471	
Age, mean ± SD, years	57.5 ± 8.9	60.6 ± 9.2	<0.001
Male, no. (%)	305 (50.3)	245 (52.0)	0.564
Ethnicity, no. (%)			<0.001
Chinese	331 (54.5)	240 (51.0)	
Malay	73 (12.0)	126 (26.8)	
Indian	182 (30.0)	86 (18.3)	
Others	21 (3.5)	19 (4.0)	
DM duration, mean ± SD, years	10.5 ± 8.7	14.1 ± 10.0	<0.001
BMI, mean ± SD, kg/m^2	27.1 ± 4.9	27.9 ± 5.0	0.006
SBP, mean ± SD, mmHg	134.9 ± 15.5	144.4 ± 18.9	<0.001
HbA1c, mean ± SD, %	7.6 ± 1.2	8.0 ± 1.3	<0.001
LDL-C, mean ± SD, mmol/L	2.7 ± 0.8	2.7 ± 0.8	0.774
TG, SD, mmol/L	1.3 (1.0–1.8)	1.5 (1.1–2.0)	<0.001
eGFR, mean ± SD, mL/min/1.73m^2	91.8 ± 20.9	76.4 ± 26.4	<0.001
Rate of eGFR change, median (IQR), mL/min/1.73m^2/year	-0.8 (-2.0 to 0.3)	-2.7 (-5.3 to -0.6)	<0.001
Baseline eGFR category, no. (%)			<0.001
G1, ≥90mL/min/1.73m^2	388 (63.9)	180 (38.2)	
G2, 60–89mL/min/1.73m^2	160 (26.4)	160 (34.0)	
G3a, 45–59mL/min/1.73m^2	30 (4.9)	60 (12.7)	
G3b, 30–44mL/min/1.73m^2	20 (3.3)	42 (8.9)	
G4, 15–29mL/min/1.73m^2	9 (1.5)	29 (6.2)	
Last follow-up eGFR category, no. (%)			<0.001
G1, ≥90mL/min/1.73m^2	321 (52.9)	37 (7.9)	
G2, 60–89mL/min/1.73m^2	214 (35.3)	125 (26.5)	
G3a, 45–59mL/min/1.73m^2	42 (6.9)	98 (20.8)	
G3b, 30–44mL/min/1.73m^2	23 (3.8)	69 (14.7)	
G4, 15–29mL/min/1.73m^2	4 (0.7)	73 (15.5)	
G5, <15mL/min/1.73m^2	3 (0.5)	69 (14.7)	
uACR, median (IQR), mg/g	14.0 (5.3–36.5)	59.0 (16.0–279.0)	<0.001
Use of insulin, no. (%)	140 (23.1)	172 (36.7)	<0.001
Use of RAS antagonist, no. (%)	325 (53.6)	348 (73.9)	<0.001
Phase angle, mean ± SD, °	5.6 ± 0.9	5.3 ± 1.0	<0.001
Phase angle, no. (%), °			<0.001
T1	160 (26.4)	200 (42.5)	
T2	199 (32.8)	160 (34.0)	
T3	248 (40.9)	111 (23.6)	

BMI: body mass index; CKD: chronic kidney disease; DM: diabetes mellitus; eGFR: estimated glomerular filtration rate; HbA1c: haemoglobin A1c; IQR: interquartile range; LDL-C: low-density lipoprotein cholesterol; RAS: renin-angiotensin system; SBP: systolic blood pressure; SD: standard deviation; TG: triglycerides; uACR: urinary albumin-to-creatinine ratio

Table 3. Association between phase angle and chronic kidney disease progression.

	Hazard ratio (95% confidence interval) *P* value		
	Unadjusted	**Model 1[a]**	**Model 2[b]**
Phase angle (per SD increase)	0.70 (0.63–0.78) *P*<0.001	0.73 (0.65–0.82) *P*<0.001	0.84 (0.75–0.95) *P*=0.004
Phase angle in tertiles			
T1	2.27 (1.80–2.87) *P*<0.001	2.19 (1.68–2.85) *P*<0.001	1.71 (1.30–2.26) *P*<0.001
T2	1.57 (1.24–2.01) *P*<0.001	1.60 (1.24–2.05) *P*<0.001	1.46 (1.13–1.88) *P*=0.004
T3	Reference	Reference	Reference
Female			
Phase angle (per SD increase)	0.55 (0.46–0.66) *P*<0.001	0.60 (0.49–0.72) *P*<0.001	0.67 (0.55–0.81) *P*<0.001
Phase angle in tertiles			
T1	2.93 (1.88–4.55) *P*<0.001	2.70 (1.71–4.26) *P*<0.001	2.56 (1.59–4.14) *P*<0.001
T2	1.55 (0.96–2.50) *P*=0.071	1.64 (1.01–2.64) *P*=0.044	1.87 (1.13–3.09) *P*=0.014
T3	Reference	Reference	Reference
Male			
Phase angle (per SD increase)	0.75 (0.65–0.86) *P*<0.001	0.83 (0.71–0.96) *P*=0.016	1.00 (0.85–1.16) *P*=0.956
Phase angle in tertiles			
T1	2.18 (1.57–3.03) *P*<0.001	1.83 (1.27–2.62) *P*=0.001	1.34 (0.90–1.98) *P*=0.146
T2	1.85 (1.39–2.48) *P*<0.001	1.70 (1.25–2.31) *P*=0.001	1.36 (0.99–1.86) *P*=0.055
T3	Reference	Reference	Reference

SD: standard deviation
[a] Model 1: Age, sex and ethnicity (age is not included in sex-stratified analysis).
[b] Model 2: Model 1 + diabetes duration, body mass index, haemoglobin A1c, systolic blood pressure, low-density lipoprotein cholesterol, log-transformed triglyceride, estimated glomerular filtration rate, log-transformed urinary albumin-to-creatinine ratio, use of insulin and use of renin-angiotensin system antagonist (age is not included in sex-stratified analysis).

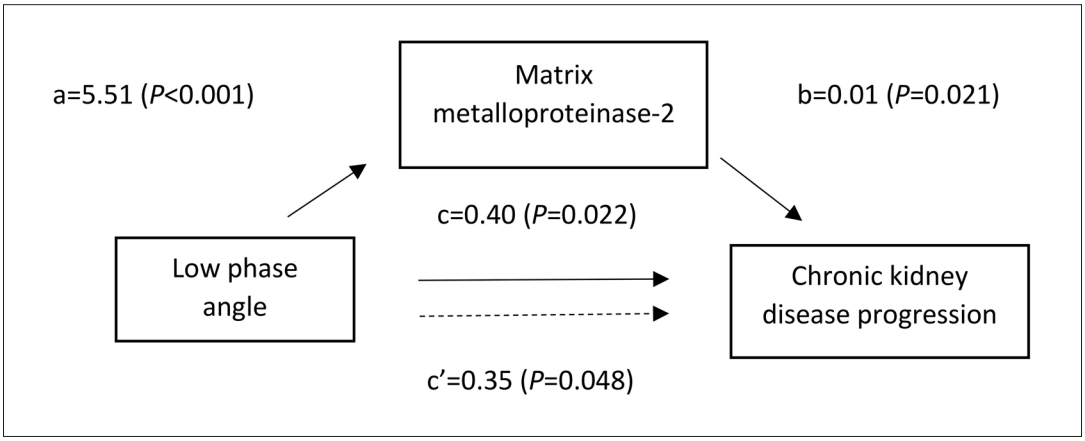

Fig. 3. Mediation of the association between phase angle and chronic kidney disease progression. The binary mediation model decomposes the total effect quantified by path c (solid arrow) of phase angle on the chronic kidney disease progression into indirect effect quantified by the product of a and b, and direct effect with the effect of matrix metalloproteinase-2 removed and quantified by path c' (dotted arrow).

Adjusted for age, sex, ethnicity, diabetes duration, body mass index, haemoglobin A1c, systolic blood pressure, low-density lipoprotein cholesterol, log-transformed triglyceride, estimated glomerular filtration rate categories, log-transformed urinary albumin-to-creatinine ratio, use of insulin and use of renin-angiotensin system antagonist.

and lower levels indicating reduced cellular integrity or cellular dysfunction.[11,13] It is plausible that oxidative stress, as reflected by low PhA,[22] activates MMP-2, which in turn initiates a cascade of events resulting in infiltration of macrophages, deposition of extracellular matrix, blockade of renal interstitial vasculature and promotion of renal hypoxia.[25] These changes lead to renal interstitial fibrosis that characterises CKD.[25] As MMP-2 was only measured at baseline, further prospective studies measuring MMP-2 at baseline and follow-ups are needed to confirm the role of MMP-2 accounting for the association between low PhA and CKD progression.

Several clinical implications emerged in our current study. Firstly, low PhA may serve as a risk marker of CKD progression in T2DM. Hence, healthcare providers may consider proactively monitoring renal function in patients with T2DM and low PhA. Secondly, it is possible to monitor PhA in routine DM management since BIA is a convenient, non-invasive, inexpensive and reliable method to measure body composition.[36] Thirdly, PhA may be considered a useful indicator for monitoring therapies targeted at slowing down CKD progression in patients with T2DM. It has been reported that high-dose vitamin D supplementation and nutritional counselling, combined with whey proteins isolate supplementation, improve PhA in patients with cancer.[37,38] Nevertheless, data on therapeutic interventions targeting the underlying pathophysiological mechanisms leading to lowered PhA remain sparse.

Our study results have provided new insights into the increased susceptibility of T2DM patients with low PhA to CKD progression. The novel interplay involving low PhA, MMP-2 and CKD progression suggests a causal pathway underlying the association between low PhA and CKD progression, although future studies are needed to confirm the finding. The other strengths of our study include a relatively large sample size, long duration of follow-up, and adjustment for a wide range of demographic and clinical variables.

We also acknowledge some limitations in our study. Firstly, we did not collect data on nutrition, which may affect the level of PhA.[39] Secondly, we were unable to generalise the results to the general population as our study population comprised only patients with T2DM.

CONCLUSION

Low PhA, mediated by MMP-2, was independently associated with CKD progression in patients with T2DM. PhA may be potentially considered as a safe, inexpensive and simple marker to predict CKD progression, especially in female patients with T2DM.

Funding

This study was supported by the Singapore Ministry of Health's (MOH) National Medical Research Council (NMRC) under its Clinician Scientist-Individual Research Grant (MOH-000066). The corresponding author is supported by NMRC under its Clinician Scientist Award (NMRC/CSA-INV/0020/2017). The first author is supported by NMRC under its Research Training Fellowship (NMRC/MOH000226).

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