Kidney Medicine Original Research

α-Klotho, Plasma Asymmetric Dimethylarginine, and Kidney Disease Progression

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Rationale & Objective: We aimed to explore the associated factors of endothelial injury in chronic kidney disease (CKD) and the relationship between endothelial dysfunction and CKD prognosis.

Study Design: A prospective observational cohort study.

Setting & Participants: 77 adults with CKD stages 1-5 were enrolled January 2010 to December 2010 and followed up until December 2015.

Exposure: Serum asymmetric dimethylarginine (ADMA) level at baseline, α-klotho, sodium-phosphorus synergistic transporter, and dimethylarginine-dimethylamine hydrolase expression in kidney biopsy samples.

Outcome: Initiation of kidney replacement therapy (KRT).

Analytical Approach: Kaplan-Meier analysis was used for evaluation of the incidence rate of KRT. All tests were 2 tailed, and statistical significance was defined as $P < 0.05$.

Results: Mean serum ADMA level of 77 patients was 64.3 ± 34.6 ng/mL. ADMA level increased with CKD stages ($P = 0.06$) and declining kidney function ($r = -0.267$; $P = 0.02$). The expression of α-klotho in kidney biopsy specimens also decreased. Median follow-up time was 56 (interquartile range, 50.5-62) months. Kaplan-Meier analyses showed that during a total follow-up of 6 years, the incidence of KRT initiation in the high-ADMA group was significantly higher than that in the low group (35.9% vs 13.2%; $P = 0.03$). ADMA level was negatively correlated with α-klotho ($r = -0.233$; $P = 0.04$) and positively correlated with phosphorus level ($r = 0.243$; $P = 0.04$). The expression of sodium-phosphorus synergistic transporter in kidney tubules, which promoted phosphorus reabsorption, and the expression of dimethylarginine-dimethylamine hydrolase isoform 1, which regulated ADMA, were decreased. Correlation analysis also showed that ADMA level decreased while age increased at baseline ($r = -0.292$; $P = 0.01$).

Limitations: Small sample size with limited longer-term follow-up.

Conclusions: Serum ADMA levels increased as kidney function declined, and high serum ADMA level was associated with incident kidney failure. Low tissue α-klotho and high levels of plasma phosphorus or tissue expression of type II sodium/phosphate cotransporter in the kidney are associated with higher circulating ADMA levels, suggesting that they may be involved in the pathogenesis of endothelial dysfunction in patients with CKD.

Visual Abstract included

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Impaired endothelial proliferation and endothelial-to-mesenchymal transition occur after ischemic reperfusion injury and it has been suggested that they play a role in microvascular rarefaction.[1,2] Recent evidence directly implicates kidney endothelial dysfunction in the progression of chronic kidney disease (CKD).[3] Damage to the kidney triggers an endothelial switch from a quiescent to an activated state, which can lead to a cascade of pathways that contribute to fibrosis.[1,2]

Asymmetric dimethylarginine (ADMA), an endogenous inhibitor of nitric oxide synthase, closely related to L-arginine, can block the active sites for nitric oxide synthesis and thereby decrease local nitric oxide synthesis, which then leads to endothelial dysfunction. ADMA can be degraded by dimethylarginine dimethylamine hydrolase (DDAH). Destruction of DDAH-rich kidney tissue will reduce ADMA degradation,[4,5] which plays a role in the pathophysiology of endothelial dysfunction.[6,7] Plasma ADMA accumulation has been found in early stages of kidney disease and has been proposed to relate to the progression of CKD.[8-11]

However, kidney endothelial dysfunction is a multifaceted process involving many factors. *KLOTHO*, a gene that has been associated with ageing, encodes the α-klotho protein. The tissue with by far the most abundant expression of α-klotho is the kidney, and specifically the distal tubule.[12] In our previous CKD cohort study, we found a significant and gradual decrease in circulating α-klotho level when comparing patients with CKD stages 1-2 with those with CKD stage 5 and a significant negative correlation between circulating α-klotho level and CKD stage.[13] Studies have reported that an endothelium-dependent vasodilatory response to acetylcholine is attenuated in α-klotho–deficient mice[14] and that nitric oxide production was increased by adenovirus-mediated α-klotho gene delivery to Otsuka Long-Evans Tokushima Fatty rats (an atherosclerotic rodent model).[15]

Moreover, low α-klotho levels increase levels of serum phosphorus,[16] another important regulator of endothelial function. High serum phosphorus levels impair endothelial function by increasing oxidative stress and decreasing nitric oxide production.[17] Abnormalities in phosphorus

PLAIN-LANGUAGE SUMMARY

This prospective observational cohort study explored the associated factors of endothelial injury in chronic kidney disease (CKD) and the relationship between endothelial dysfunction and CKD prognosis. Seventy-seven adults with CKD during January 2010 to December 2010 were divided into 2 groups by the median serum ADMA level at baseline and followed up until December 2015. The end point was initiation of kidney replacement therapy. Serum asymmetric dimethylarginine (ADMA) level increased with deterioration of kidney function and high ADMA level was associated with occurrence of kidney failure. Low α-klotho and high levels of plasma phosphorus or expression of type II sodium/phosphate cotransporter in the kidney are associated with increased circulating ADMA levels, which implies that they may be involved in the pathogenesis of endothelial dysfunction in patients with CKD.

metabolism are critical components of mineral and bone disorders in CKD[18] and the main cause of cardiovascular complications associated with CKD.[19]

Therefore, we investigated the relationship between ADMA level and kidney replacement treatment events and the role of indicators of endothelial function with ADMA levels in patients with CKD, particularly α-klotho and phosphorus, in CKD before initiation of kidney replacement therapy (KRT).

METHODS

Selection of Participants

Adult patients with CKD stages 1-5 according to the Kidney Disease Outcomes Quality Initiative (KDOQI) criteria published in January 2010 were eligible for inclusion. Exclusion criteria were as follows: (1) confirmed and/or suspected acute kidney injury (defined as an increase in serum creatinine > 50% within 1 week) in the 3 months before the study, (2) initiation of KRT, (3) planning for kidney transplantation, (4) diagnosis of tumor metastasis, (5) use of immunosuppressants, (6) enrollment in other studies, and (6) pregnancy.

This study was approved by the ethics committee of Huashan Hospital, Fudan University, China (2009-113). All patients provided written informed consent for participation, and the study was conducted according to the tenets of the Declaration of Helsinki.

Clinical Evaluation and Laboratory Procedures

At the time of study enrollment, we recorded baseline demographic data including age, sex, cause of kidney disease, and use of angiotensin-converting enzyme (ACE) inhibitors/angiotensin II receptor blockers (ARBs). We also obtained blood samples for laboratory measurements at the time of study enrollment. Sample tubes were kept at room temperature and centrifuged within 1 hour of drawing the blood. All blood samples were then stored at −80 °C until analysis.

Serum ADMA level was determined using ADMA enzyme-linked immunosorbent assay kits from BioVision Inc. The sensitivity threshold was <9.375 ng/mL. Intra-assay coefficient of variation was <8%, and interassay coefficient of variation was <10%. The assay has high sensitivity and excellent specificity for detection of ADMA. No significant cross-reactivity or interference between ADMA and analogues was observed.

Serum soluble α-klotho concentration was determined with enzyme-linked immunosorbent assay kits obtained from Immuno-Biological Laboratories Co, Ltd according to the manufacturer's instructions.

Immunohistochemical Staining

Twenty kidney puncture samples (immunoglobulin A [IgA] nephropathy, the main pathologic type of primary nephritis in China) from 77 patients with CKD and 5 normal kidney samples (normal kidney tissue from a nephrectomy secondary to kidney cancer without proteinuria) as control were examined histologically. Briefly, fresh tissues were fixed in 4% paraformaldehyde (Sigma-Aldrich) for 30 minutes at room temperature. The tissue was then dehydrated by gradient ethanol, embedded in paraffin, sectioned (2-μm thickness), and immersed in xylenes for dewaxing. Sections were blocked in an immunohistochemical blocking solution (Beyotime Biotechnology Co, Ltd) at 37 °C for 30 minutes. After the blocking solution was discarded, samples were washed 3 times in TBST (tris-buffered saline and Polysorbate 20) washing buffer (25 mmol/L of Tris-HCl, pH 8.0, 125 mmol/L of sodium chloride, and 0.1% Triton X-100) at room temperature for 5 minutes. Samples were incubated simultaneously with a polyclonal goat anti-human DDAH1 antibody (ab2231, 1:200; Abcam), a monoclonal rabbit anti-human α-klotho antibody (ab181373, 1:200; Abcam), and a monoclonal mouse anti-human sodium/phosphate (NaPi)-2α antibody (SLC34A1; NBP2-42216C, 1:200; Novus Biologicals) at 4 °C overnight. Samples were washed in TBST 3 times, 5 minutes each, at room temperature. The secondary antibodies Alexa Fluor 555 Donkey anti-Goat IgG (antigen is gamma Immunoglobins Heavy and Light chains, H+L) antibody, Alexa Fluor 647 Goat anti-Rabbit IgG (H+L) antibody, and Alexa Fluor 488 Goat anti-Mouse IgG (H+L) antibody (A0502, A0468, and A0428, respectively; Beyotime Biotechnology) were added and sections were incubated for 60 minutes at room temperature. Samples were then thoroughly washed with TBST and viewed through a fluorescence microscope (DMI3000; Leica).

Study Outcomes and Definitions

All patients underwent follow-up after enrollment and persisted until December 2015. Initiation of KRT was the primary outcome. Patients who were candidates for KRT but

either declined to undergo the procedure or had contraindications were also included in this outcome. Estimated glomerular filtration rate (eGFR) was calculated using the CKD Epidemiology Collaboration (CKD-EPI) equation.

Statistical Analyses

Statistical analyses were carried out using the SPSS statistical software program (version 17.0; SPSS). Normally distributed variables are expressed as mean ± standard deviation and were compared using 1-way analysis of variance or t test. Non-normally distributed variables are expressed as median value with interquartile range (IQR) and were compared using the rank sum test. Categorical variables are expressed as percentages and were compared using Pearson χ^2 test or Fisher exact test. Correlations among continuous data were analyzed using Pearson or Spearman correlation coefficients. Initiation of KRT between the high-ADMA group and the low-ADMA group (divided based on median ADMA value) was evaluated using Kaplan-Meier analysis and log-rank test. All tests were 2 tailed, and statistical significance was defined as $P < 0.05$.

RESULTS

Patient Demographics and Clinical Characteristics

Seventy-seven patients with a diagnosis of CKD entered our cohort study and completed up to 6 years of follow-up. Median follow-up time was 56 (IQR, 50.5-62) months. Mean age was 64.1 ± 11.1 years and 68.8% were men (53/77). On enrollment, median serum creatinine level was 1.79 ± 0.63 mg/dL; 14 (18.2%) patients had CKD stages 1-2, 44 (57.1%) had CKD stage 3, and 19 (24.7%) had CKD stages 4-5. The main causes of CKD were diabetic nephropathy/arteriolar nephrosclerosis in 40 (51.9%) patients and primary nephritis in 24 (31.2%) patients. There were 75.0% of patients (54/77) using an ACE inhibitor or ARB at time of enrollment.

Baseline demographic and biochemical characteristics of the patients are shown in Table 1. Patients were classified into 2 subgroups by median serum ADMA level. At baseline, the high-ADMA group was younger, with lower eGFRs and higher urinary albumin-creatinine ratios compared with the low-ADMA group. During 1.5 years of follow-up, serum ADMA levels increased from 63.4 ± 34.6 to 74.8 ± 47.4 pg/mL ($P < 0.001$, paired t test) and serum α-klotho levels also increased from 468.3 (IQR, 383.7-570.5) to 479.6 ± 207.5 pg/mL ($P = 0.02$, paired t test).

Endothelial Function and CKD Prognosis

Mean baseline serum ADMA level was 64.3 ± 34.6 ng/mL. ADMA concentrations were 48.4 ± 34.6, 62.3 ± 34.6, and 76.9 ± 30.9 ng/mL, respectively, with increasing CKD stage categories ($P = 0.06$, analysis of variance; Fig 1). Correlation analysis also showed that serum ADMA level

Table 1. Baseline Demographic and Clinical Data of Patients With CKD Stratified by Low and High ADMA Groups

CKD Stage	Low-ADMA Group	High-ADMA Group	Total	*P*
No. of patients	38	39	77	
Age, y	67.9 ± 9.4	60.5 ± 11.5	64.1 ± 11.1	0.003
Male sex	29 (76.3%)	24 (61.5%)	53 (68.8%)	0.16
Cause				
Diabetic nephropathy/arteriolar nephrosclerosis	22 (57.9%)	18 (46.2%)	40 (51.9%)	0.30
Primary nephritis	9 (23.7%)	15 (38.5%)	24 (31.2%)	0.05
ACEI/ARB	27 (71.1%)	27 (69.2%)	54 (70.1%)	0.68
Mean arterial pressure, mm Hg	95.0 ± 8.3	92.0 ± 8.4	93.5 ± 8.4	0.15
Hemoglobin, g/dL	13.0 ± 1.8	12.5 ± 1.8	12.7 ± 1.8	0.32
Serum creatinine, mg/dL	1.60 ± 0.53	1.98 ± 0.67	1.79 ± 0.63	0.009
eGFR, mL/min	46.2 ± 1.7	38.0 ± 1.6	42.1 ± 1.73	0.04
Calcium, mg/dL	2.36 ± 0.14	2.35 ± 0.12	2.36 ± 0.13	0.89
Phosphorus, mg/dL	1.11 ± 0.14	1.18 ± 0.16	1.14 ± 0.18	0.07
iPTH, pg/mL	61.7 ± 41.1	64.8 ± 48.5	63.3 ± 44.7	0.76
Albumin, g/dL	4.3 [4.2-4.6]	4.3 [4.0-4.5]	4.3 [4.2-4.5]	0.07
Cholesterol, mg/mL	4.8 ± 1.1	5.2 ± 1.0	5.00 ± 1.06	0.12
LDL-C, mg/dL	2.73 ± 0.80	2.70 ± 0.67	2.72 ± 0.73	0.88
Urinary albumin-creatinine ratio, mg/g	99.8 [18.7-365.0]	198.0 [36.7-653.0]	215.3 [24.1-796.4]	<0.001
ADMA at baseline, pg/mL	34.9 ± 16.6	91.1 ± 22.9	63.4 ± 34.6	<0.001
ADMA at 1.5-y follow-up, pg/mL	59.0 ± 26.4	90.1 ± 57.6	74.8 ± 47.4	0.003
α-Klotho at baseline, pg/mL	550.2 ± 294.5	461.5 ± 155.8	505.3 ± 237.4	0.10
α-Klotho at 1.5-y follow-up, pg/mL	523.4 ± 247.7	436.9 ± 150.4	479.6 ± 207.5	0.07

Note: Data were obtained at the time of enrollment in the study unless otherwise noted. Values expressed as mean ± standard deviation, number (percent), or median [interquartile range].
Abbreviations: ACEI, angiotensin-converting enzyme inhibitor; ADMA, asymmetric dimethylarginine; ARB, angiotensin receptor blocker; CKD, chronic kidney disease; eGFR, estimated glomerular filtration rate; iPTH, intact parathyroid hormone; LDL-C, low-density lipoprotein cholesterol.

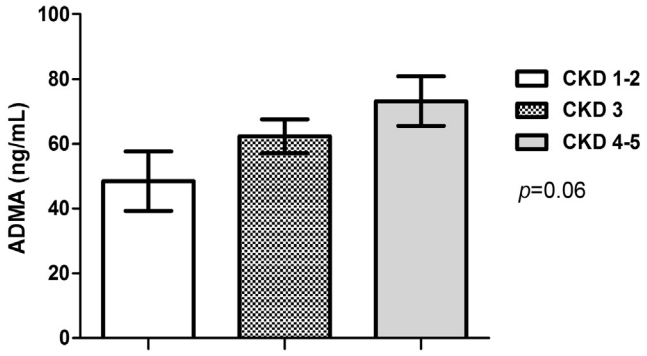

Figure 1. Asymmetric dimethylarginine (ADMA) levels in different chronic kidney disease (CKD) stages.

increased with declining kidney function ($r = -0.267$; $P = 0.02$; Fig 2) and that ADMA level at baseline correlated with eGFR at 1.5 years of follow-up ($r = -0.296$; $P = 0.009$).

Kidney biopsy specimens examined with immunofluorescence and light microscopy showed that DDAH1 expression was widely positive in normal kidney tissue, especially in the tubular epithelia, while its expression in kidney biopsy specimens from patients with CKD was essentially absent (Fig 3).

We further used the median serum ADMA level (59.33 ng/mL) as a cut-off point to divide the 77 patients with CKD into 2 subgroups: high-ADMA group (>59.33 ng/mL; n = 38) and low-ADMA group (<59.33 ng/mL; n = 37). There were no significant differences in sex, eGFRs, or urinary albumin-creatinine ratios between the 2 subgroups at baseline. All patients were treated with ACE inhibitors/ARBs according to the KDOQI guidelines.

Among the patients in the high-ADMA group, 35.9% (14/39) of patients started KRT compared with 13.2% (5/38) in the low-ADMA group. In Kaplan-Meier analyses,

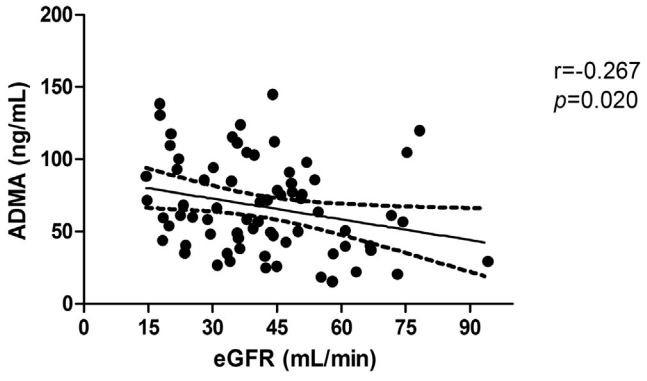

Figure 2. Correlation analysis between asymmetric dimethylarginine (ADMA) level and estimated glomerular filtration rate (eGFR). Correlation between ADMA and eGFR ($r = -0.267$; $P = 0.02$). Dotted lines indicate 95% CI.

survival of patients with high ADMA levels was significantly lower than for those with low ADMA levels. The incidence of KRT initiation at the 6-year follow-up was significantly higher in the group with high serum ADMA levels ($P = 0.03$ by log-rank test; Fig 4). However, Cox proportional hazards regression analyses showed no statistical significance in baseline ADMA level (hazard ratio, 1.126; $P = 0.84$) when adjusted by age, sex, eGFR, and urinary albumin-creatinine ratio at baseline.

Correlation Analysis With α-Klotho, Phosphorus, and ADMA

Correlation analysis showed that ADMA level increased while α-klotho level at 1.5 years of follow-up decreased ($r = 0.233$; $P = 0.04$; Fig 5) and phosphorus levels increased ($r = 0.243$; $P = 0.04$; Fig 6). No relationship was found between ADMA and α-klotho levels at the 1.5-year follow-up, and no correlation was found between ADMA and calcium levels or blood pressure.

The expression of α-klotho in normal compared with CKD kidney tissue showed a pattern similar to that of DDAH1. NaPi-2α staining was increased in the tissue of individuals with CKD compared with normal controls. Masson trichrome staining of specimens from participants with CKD showed an increased number of tubular and interstitial fibrosis lesions and glomerular segmental mesangial hyperplasia compared with normal controls acquired during a nephrectomy for kidney cancer (Fig 3).

No relationship was found between protein expression of DDAH1 and klotho in the kidneys. In addition, there was a negative correlation between α-klotho and phosphate levels at the 1.5-year follow-up ($r = -0.174$; $P = 0.03$) that was not found between α-klotho and phosphate levels at baseline.

Correlation Analysis With Age at Enrollment and ADMA

Correlation analysis also showed that ADMA levels decreased with increasing age at baseline ($r = -0.292$; $P = 0.01$). Mean age in the high-ADMA subgroup was younger than that in the low-ADMA subgroup (60.5 ± 11.5 vs 67.9 ± 9.4 years; $P = 0.003$). Although there was no significant difference in age at baseline among different CKD stages ($P = 0.21$), patients in the CKD stages 4-5 group were younger than those in the CKD stages 1-2 or CKD stage 3 groups (60.5 ± 10.8, 63.8 ± 11.9, and 66.0 ± 10.8 years, respectively). The age at baseline of patients with KRT was younger than for those without KRT (58.1 ± 12.1 vs 66.2 ± 10.1 years; $P = 0.005$).

DISCUSSION

ADMA levels are regulated by DDAH, which can degrade ADMA by hydrolyzing it to L-citrulline and dimethylamine.[4] It is estimated that humans generate ~300 μmol of ADMA per day, and 250 μmol of this is metabolized by

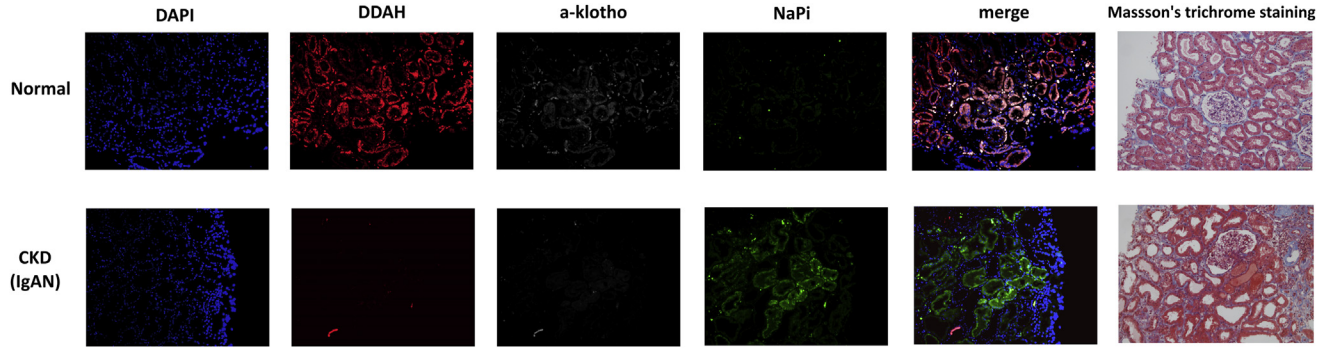

Figure 3. Expression of dimethylarginine dimethylamine hydrolase isoform 1 (DDAH1), α-klotho, and sodium-phosphorus cotransporter (NaPi) in normal (n = 5) and chronic kidney disease (CKD) kidney tissue (n = 20). Abbreviations: DAPI, 4′,6-diamidino-2-phenylindole; IgAN, immunoglobulin A nephropathy.

DDAH, with only a small amount excreted by the kidneys unchanged.[20] DDAH and nitric oxide synthesis are colocalized in endothelial cells within the glomerulus and kidney tubular cells, which supports the hypothesis that intracellular ADMA concentration is actively regulated in nitric oxide–generating endothelial cells within the kidney as well.[5] Thus, destruction of DDAH-rich kidney tissue can impair ADMA degradation. We found that DDAH1 expression in normal kidney tissue was widely positive, especially in tubules, but substantially decreased in kidney tissue from patients with CKD (Fig 3). These data may explain why decreased kidney function leads to ADMA accumulation because we observed a trend of increased serum ADMA levels in patients with CKD. It follows that as an endogenous nitric oxide synthase inhibitor, the high level of ADMA may increase endothelial dysfunction.

Together, the kidneys constitute about 0.4% of total body weight but receive 22% of cardiac output. This high flow is necessary for precise regulation of body fluid volumes. Normal functioning kidney endothelium is vital for kidneys to perform efficiently under physiologic conditions. Kidney endothelial cells are involved in a number of key processes such as regulation of vasomotor tone, thrombosis, and inflammation in addition to their physical barrier function.[21] Recent research has demonstrated that kidney endothelial cells are essential for maintenance of normal kidney hemodynamics because they are involved in regulating kidney vascular conductance, endothelium-derived vasodilatation, and autoregulatory mechanisms.[1]

In kidney transplant recipients, elevated plasma ADMA levels have been reported to be associated with increased morbidity, mortality, and deterioration in graft function.[22] Other studies have shown that ADMA is related to the incidence of kidney failure because kidney endothelial dysfunction is directly implicated in the progression of CKD.[10,11] In the present study, we found similar results in baseline measurements because a significant and gradual increase in circulating ADMA level was observed when comparing patients with CKD stages 1-2 with those with

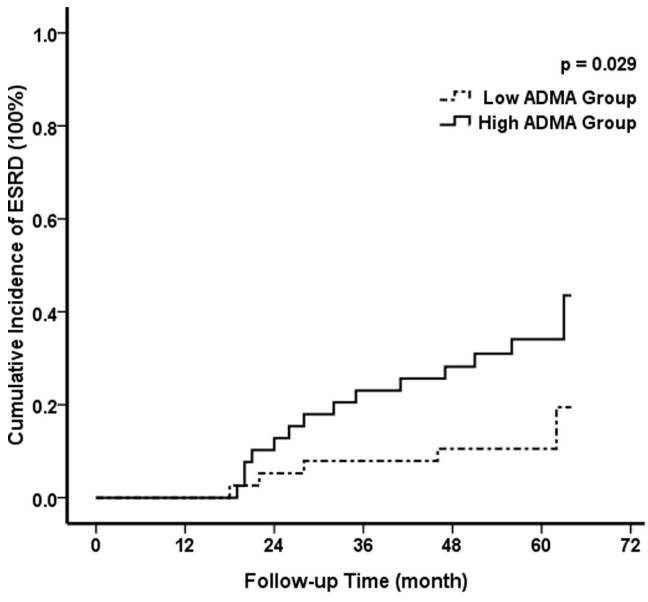

Figure 4. Kaplan-Meier analysis for end-stage renal disease (ESRD). $P = 0.03$. Abbreviation: ADMA, asymmetric dimethylarginine.

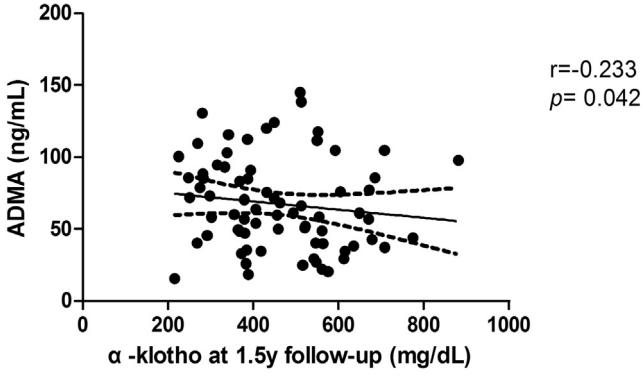

Figure 5. Correlation analysis between asymmetric dimethylarginine (ADMA) and α-klotho at the 1.5-year follow-up ($r = -0.233$; $P = 0.04$). Dotted lines indicate 95% CI.

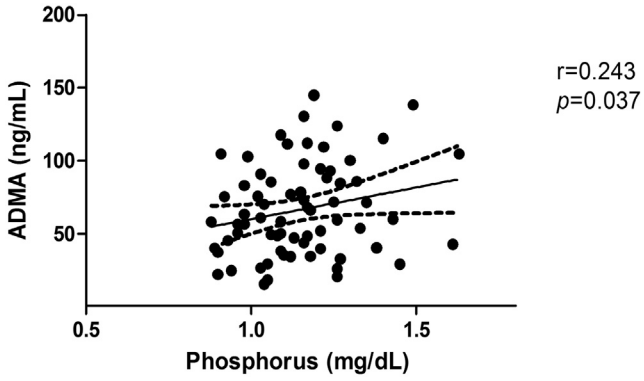

Figure 6. Correlation analysis between asymmetric dimethylarginine (ADMA) and phosphorus levels ($r = 0.243$; $P = 0.04$). Dotted lines indicate 95% CI.

CKD stages 4-5 (Fig 1). In addition, we found a significant negative correlation between circulating ADMA level and eGFR at 1.5 years of follow-up (Fig 2). Moreover, after 6 years of follow-up, we observed in our small cohort that baseline ADMA level was associated with KRT initiation, which supports the hypothesis that endothelial dysfunction plays an important role in CKD progression (Fig 4).

Several large CKD cohort studies have reported other factors with a predictive role in CKD progression and end-stage kidney disease outcomes. Our group has previously shown that α-klotho levels decrease in a manner that corresponds with damage to kidney function.[13] The tissue with the most abundant expression of α-klotho by far is the kidney.[12] Kidney α-klotho transcript and protein levels and serum α-klotho concentration have been shown to be reduced in CKD.[23] In addition to its role in arterial calcification, α-klotho is involved in the arterial remodeling process associated with CKD.[24] In vitro, α-klotho has been shown to decrease oxidative stress and apoptosis in both smooth muscle cells and endothelial cells and prevent μ-calpain overactivation in endothelial cells.[25] Therefore, α-klotho deficiency may damage endothelial function. Consistent with our hypothesis, the immunofluorescence results showed that α-klotho expression was essentially absent in the nephritis kidney biopsy tissues, similar to DDAH1, and in contrast to α-klotho expression in the normal kidney (Fig 3). In a correlation analysis, we found a negative correlation between circulating α-klotho and ADMA levels. ADMA level increased while α-klotho level decreased at 1.5 years of follow-up (Fig 5). α-Klotho deficiency mitigates nitric oxide production, which may affect the function of fibroblast growth factor 23 (FGF-23) under high phosphate conditions.[26,27]

High serum phosphorus level is the most important nontraditional risk factor related to uremia that is associated with vascular calcification in patients with CKD and in the general population.[28] Abnormalities in phosphorus metabolism are critical determinants of the cardiovascular complications associated with CKD.[18] Increased extracellular phosphate directly affects endothelial cells and leads to cell apoptosis and the transformation of vascular smooth muscle cells to an osteogenic phenotype.[29] It has been demonstrated that the type II Na/Pi cotransporter is a target for the physiologic and pathophysiologic regulation of proximal reabsorption of phosphate.[30,31] Our current study showed that serum phosphate level was higher in the CKD stages 4-5 group than in the CKD stages 1-2 group (Table 1), and Na/Pi cotransporter expression was upregulated in CKD kidney tissue compared with normal kidney tissue, as shown by immunostaining (Fig 3). Phosphate level was positively correlated with circulating ADMA level (Fig 6), while Na/Pi cotransporter and DDAH1 expression in the CKD kidney tissue showed an inverse relationship (Fig 3). The high Na/Pi cotransporter expression promotes increased reabsorption of phosphate, which leads to impaired endothelial cell function. Therefore, phosphate concentrations are related to that of ADMA in patients with CKD.

We did not identify any relationship between ADMA and intact parathyroid hormone levels. Intact parathyroid hormone levels in our patient cohort were higher than normal because of reduced kidney function but were still within the normal range for patients with CKD not treated by KRT. None of the patients in our cohort took vitamin D or its receptor activators before starting KRT.

Another unexpected result in this study is that patient age was negatively correlated to serum ADMA concentration. It has been reported in healthy individuals that age is correlated significantly with plasma ADMA levels[32] and animal studies have shown the same results.[33] However, in our CKD cohort, we found the reverse. Further analysis showed that the average age of patients with CKD stages 4-5 was younger than that of patients with CKD stages 1-3. Selection bias caused by small sample size and study design might account for the reverse finding. Notably, in a cohort study of patients 85 years and older without cardiovascular disease, ADMA was not associated with cardiovascular disease.[34]

In conclusion, we showed that high circulating ADMA levels were associated with decreased kidney function and with KRT initiation in patients with CKD. However, the hydrolyze enzyme of ADMA, DDAH1, was substantially decreased in kidney biopsy specimens from patients with CKD. Reduction of α-klotho or increased serum phosphate and kidney type II Na/Pi cotransporter expression were related to an increase in circulating ADMA levels, which implies that they may play an important role in endothelial dysfunction. Further studies with larger numbers of patients are required to validate our findings.

ARTICLE INFORMATION

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Authors' Contributions: Study design: LL, CH; cohort follow-up: LL, YG; data collection: MY, PC; measurement of ADMA and α-klotho: LL, JZ; immunohistochemical staining: SL; statistical analysis: JQ, LL. Each author contributed important intellectual content during manuscript drafting or revision and accepts accountability for the overall work by ensuring that questions pertaining to the accuracy or integrity of any portion of the work are appropriately investigated and resolved.

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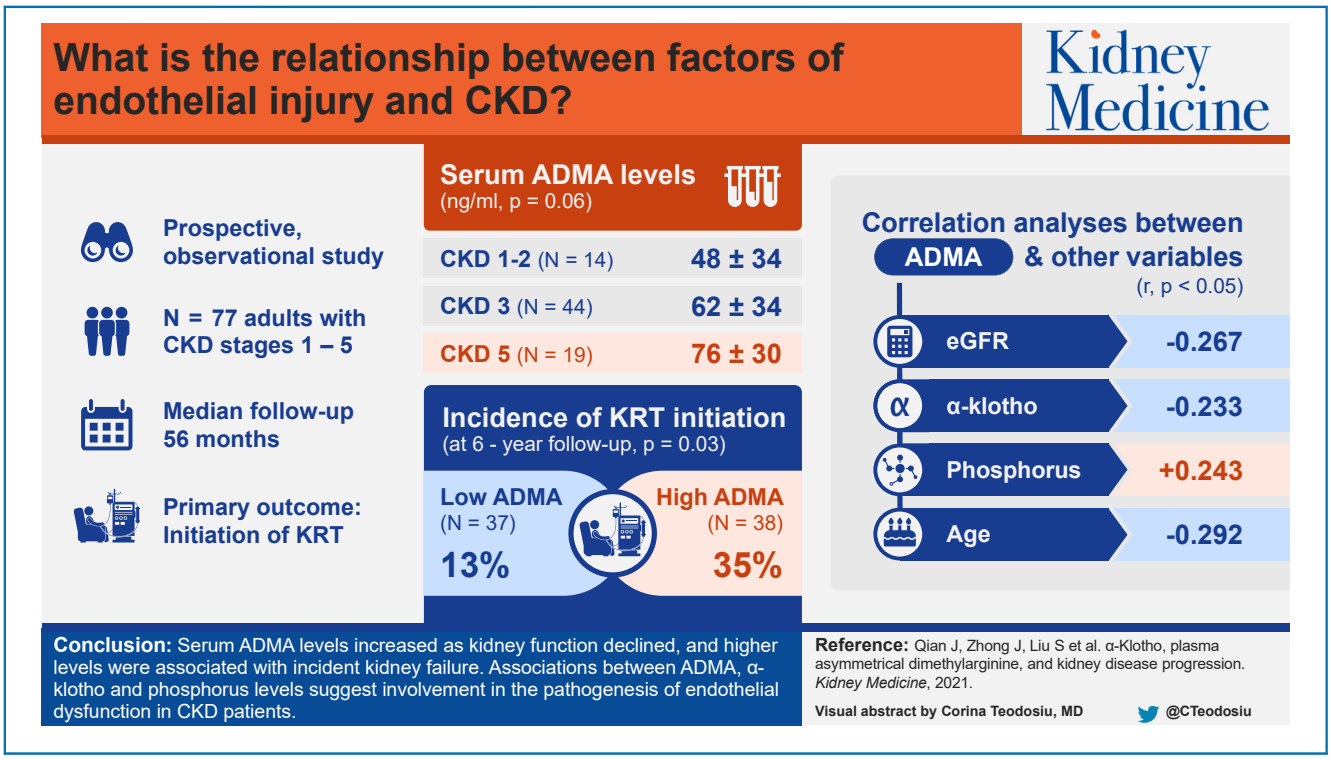
What is the relationship between factors of endothelial injury and CKD?
Kidney Medicine
Prospective, observational study
N = 77 adults with CKD stages 1 – 5
Median follow-up 56 months
Primary outcome: Initiation of KRT
Serum ADMA levels
(ng/ml, p = 0.06)
CKD 1-2 (N = 14) 48 ± 34
CKD 3 (N = 44) 62 ± 34
CKD 5 (N = 19) 76 ± 30
Incidence of KRT initiation
(at 6 - year follow-up, p = 0.03)
Low ADMA
(N = 37)
13%
High ADMA
(N = 38)
35%
Correlation analyses between ADMA & other variables
(r, p < 0.05)
eGFR -0.267
α-klotho -0.233
Phosphorus +0.243
Age -0.292
Conclusion: Serum ADMA levels increased as kidney function declined, and higher levels were associated with incident kidney failure. Associations between ADMA, α-klotho and phosphorus levels suggest involvement in the pathogenesis of endothelial dysfunction in CKD patients.
Reference: Qian J, Zhong J, Liu S et al. α-Klotho, plasma asymmetrical dimethylarginine, and kidney disease progression. Kidney Medicine, 2021.
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