Original Paper

Circulating α -Klotho is Related to Plasma Aldosterone and Its Follow-Up Change Predicts CKD Progression

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Key Words

A-klotho • Aldosterone • Chronic kidney disease • Renal replacement treatment

Abstract

Background/Aims: We aimed to determine if soluble α -klotho level was an indicator of chronic kidney disease (CKD) progression and whether α -klotho interacted with aldosterone during the course of further renal damage. **Methods:** 112 adults with stages 1–5 CKD were enrolled into our cohort study. All of the patients were followed up for 6 years (from January 2010 to December 2015). Serum soluble α -klotho and aldosterone were measured at baseline and at 1.5-years follow-up. The primary outcome was the initiation of renal replacement therapy (RRT) and the secondary outcome was the occurrence of cardio-cerebrovascular events. Long-term progression to RRT and cardio-cerebrovascular events in patients was analyzed with a risk-adjusted Cox proportional hazards regression model. Adjustment included age, gender, eGFR, mean arterial pressure, 24-h protein excretion and the change in α -klotho level from baseline at 1.5-years follow-up. **Results:** Baseline circulating α -klotho levels were positively associated with baseline estimated glomerular filtration rate (eGFR; $r = 0.224$, $p = 0.017$), but not age, calcium, phosphate, or parathyroid hormone levels. The change in α -klotho level from baseline at 1.5-years follow-up ($p = 0.002$) was independently associated with renal replacement treatment (RRT) initiation after adjustment for age, gender, eGFR, mean arterial pressure, and 24-h protein excretion in Cox regression analysis. Aldosterone levels were positively associated with CKD stage, and were inversely correlated with circulating α -klotho levels. **Conclusion:** The change in concentration of soluble α -klotho during the 1.5-years follow-up was an indicator of CKD progression. Renal damage associated with a reduction of α -klotho may involve the upregulation of plasma aldosterone. Future studies are needed to validate our findings, and

to investigate the underlying mechanism by which α -klotho and aldosterone may cause renal damage.

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Introduction

Chronic kidney disease (CKD) is a major public health problem with increasing incidence and prevalence worldwide. Numerous studies have been conducted to identify new biomarkers to predict progression of CKD and adverse clinical outcomes such as cardio-cerebrovascular events. With the advent of the sensitive and specific enzyme-linked immunosorbent assay (ELISA) technique [1], animal studies have shown that soluble α -klotho plays an important role not only in the progression of CKD but also in the development of acute kidney injury (AKI) [2-4].

KLOTHO, a gene that has been associated with ageing, encodes the α -klotho protein. α -klotho mRNA is expressed in multiple tissues, including the heart, aorta, colon, pituitary gland, thyroid gland, pancreas, and gonads. The tissue with the most abundant expression by far is the kidney; specifically the distal tubule [5-7]. Renal α -klotho transcript and protein levels [4, 8-14] and serum α -klotho concentration [4, 10] were demonstrated to be reduced in rodents with CKD caused by nephron reduction surgery, ischemia-reperfusion injury, immune complex glomerulonephritis, polygenic or hormonal hypertension, metabolic syndrome, and diabetes [4, 8-14]. α -klotho deficiency has also been documented in AKI in both rodents and humans [2]. α -klotho is a transmembrane protein [15] that exists in two forms: membrane-bound and soluble. The soluble form of α -klotho can be found in blood, urine, and cerebrospinal fluid, but unlike membrane-bound α -klotho, it exerts its biologic effects independently of fibroblast growth factor (FGF)-23 and fibroblast growth factor receptor [16].

Several large CKD cohort studies have reported on the predictive role of soluble α -klotho levels in CKD progression and end-stage renal disease (ESRD) outcomes. However, many of them yielded contradictory results. In one such study (Seiler's study), a large cohort of 312 patients with stages 2-4 CKD were followed for an average of 2.2 years and it was found that the level of soluble α -klotho was not associated with the glomerular filtration rate (GFR) nor was it a predictor of adverse outcomes in patients with CKD [17]. Kim and colleagues reported contrasting results, however, when they found that the baseline soluble α -klotho level was a biomarker for CKD progression in a study of 243 patients with stages 1-5 CKD who were followed for an average of 5 years [18]. There is therefore a clear need to further investigate how this protein is associated with CKD.

Although some studies have suggested that α -klotho deficiency may promote renal damage [2, 19], the mechanism underlying α -klotho's effect on kidneys is not yet well characterized. The role of the renin-angiotensin-aldosterone system (RAAS) in kidney and cardio-cerebrovascular disease is well established, and, more recently, a greater understanding of the final element in that pathway, aldosterone, has emerged. New data suggest that α -klotho may interact with aldosterone, which has been reported to be involved in kidney damage in CKD. Further, a study published last year showed that silencing of the *KLOTHO* gene upregulated CYP11B2 expression in cultured human adrenocortical cells. CYP11B2 is a key rate-limiting enzyme in aldosterone synthesis [20].

The current study therefore explored whether aldosterone is involved in further renal damage caused by α -klotho deficiency in CKD patients. We conducted a prospective study in adults with CKD to determine if the baseline soluble α -klotho level was an indicator of CKD progression and whether α -klotho interacted with aldosterone in the course of further renal damage. We found that at 1.5-years follow-up, the change in concentration of soluble α -klotho from the baseline ($\Delta\alpha$ -klotho), instead of the baseline α -klotho level, was associated with prognosis. Multivariate Cox regression analysis showed that $\Delta\alpha$ -klotho was an independent predictor of renal replace therapy (RRT) initiation in CKD patients. Further, aldosterone

and α -klotho appeared to have a related effect on the progression of renal damage. We also detected an association between α -klotho and cardio-cerebrovascular events in CKD. Baseline plasma aldosterone levels were higher and serum α -klotho levels were lower in those who had cardio-cerebrovascular events, although these results were not statistically significant.

Materials and Methods

Selection of Participants

Adult patients were screened if they had CKD stages 1–5 according to the Kidney Disease Outcomes Quality Initiative (KDOQI) criteria published in January 2010. The exclusion criteria were as follows: (1) confirmed and/or suspected AKI (defined as an increase in serum creatinine of >50% within 1 week) in the 3 months prior to the study, (2) initiation of RRT, (3) planning for renal transplantation, (4) diagnosis of tumor metastasis, (5) use of immunosuppressants, (6) enrolment in other studies, and (6) pregnancy. According to the KDOQI criteria, patients were classified into five stages based on their estimated GFR (eGFR). eGFR was determined by the CKD Epidemiology Collaboration creatinine equation. A total of 115 adults were screened, of whom 3 were excluded as they had been diagnosed with AKI. Finally, 112 patients were enrolled and underwent a 6-year follow-up.

This study was approved by the ethics committee of Huashan Hospital, Fudan University, China. All of the 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

Baseline demographic data, including age and gender, were recorded at the time of enrolment. Blood samples for laboratory measurements were obtained at the time of enrolment and at a 1.5-years follow-up. The sample tubes were kept at room temperature and centrifuged within 1 h of drawing the blood. All blood samples were stored at -80°C until analysis.

The concentration of serum soluble α -klotho was determined with ELISA kits obtained from Immuno-Biological Laboratories Co. Ltd. Japan according to the manufacturer's instructions. This kit is the most common-used commercially available assay to detect the exact levels of both forms of circulating klotho protein [17, 19]. It uses a purified monoclonal antibody described by Yamazaki which binds the tertiary structure of the human α -klotho extracellular domain, aKl, with high selectivity [1]. The α -klotho level remains stable under different conditions when freshly collected samples are stored at -80°C [1].

The concentration of serum aldosterone was performed using a chemiluminescent immunoassay obtained from Shen New Industries Biomedical Engineering Co. Ltd. China. iPTH was measured with Electro-chemiluminescence Immunoassay (Siemens Healthcare Diagnostics Products Co. Ltd. China). Other laboratory items were determined in the clinical laboratory.

Study Outcomes and Definition

All of the patients were followed up for 6 years (from January 2010 to December 2015). The primary outcome was initiation of RRT. Patients who were suitable for RRT but either refused to undergo the procedure or had contraindications were also included in this outcome.

The secondary outcome was occurrence of cardio-cerebrovascular events (acute coronary syndrome and cerebral apoplexy). Acute coronary syndrome was diagnosed by cardiologists according to myocardial markers and electrocardiogram, and included three clinical manifestations: ST elevation myocardial infarction (STEMI), non ST elevation myocardial infarction (NSTEMI), and unstable angina. Cerebral apoplexy was defined as new onset of ischemic and hemorrhagic stroke, diagnosed by neurologists according to magnetic resonance imaging (MRI).

The eGFR was calculated by the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation:

$GFR = 141 \times \min(Scr/\kappa, 1)^\alpha \times \max(Scr/\kappa, 1)^{-1.209} \times 0.993^{Age} \times 1.018$ [if female] $\times 1.159$ [if black], where Scr is serum creatinine, κ is 0.7 for females and 0.9 for males, α is -0.329 for females and -0.411 for males, min indicates the minimum of Scr/ κ or 1, and max indicates the maximum of Scr/ κ or 1.

$\Delta\alpha$ -klotho, Δ aldosterone, and Δ eGFR were defined as the differences in the concentration of α -klotho, aldosterone, and eGFR, respectively, between measurements at baseline and at the 1.5-year follow-up (Δ value = value at the 1.5-year follow-up – value at the baseline).

Statistical Analyses

Normally distributed variables are expressed as mean $\pm$ standard deviation (SD) values and were compared using one-way analysis of variance (ANOVA) or Student's *t*-test. Non-normally distributed variables are expressed as median values with interquartile ranges and were compared using the rank-sum test. Categorical variables are expressed as percentages and were compared using the Pearson chi-squared test or Fisher's exact test. Correlations among continuous data were analyzed using Pearson correlation coefficients. In multivariate analysis, Cox proportional hazards regression was used to identify independent predictors of RRT and cardio-cerebrovascular events in CKD patients. The covariates age, gender, eGFR, mean arterial pressure (MAP), baseline cholesterol, phosphorus, and 24-h protein excretion were used for stepwise adjustment. Events were defined as initiation of RRT and occurrence of cardio-cerebrovascular events. Cases lost to follow-up were censored at their last observation. All tests were two-tailed, and statistical significance was defined at $p < 0.05$. The SPSS statistical software program (version 17.0, SPSS) was used for all analyses.

Results

Patient Demographics and Clinical Characteristics

The cohort comprised 112 patients (77 men and 35 women) with a mean age of 64.5 ± 12.7 years. Upon enrolment, the median serum creatinine level was 1.83 mg/dL (1.46–2.55 mg/dL). The two main causes of CKD were chronic glomerular nephritis (36.6%) and diabetic nephropathy (19.6%). Of the 112 patients, 71.4% were having angiotensin-converting enzyme inhibitor (ACEI) or angiotensin receptor blocker (ARB) treatment. None of the patients required treatment with active vitamin D or its analogues during the follow-up period, as their parathyroid hormone (iPTH) and serum phosphate levels were stable.

Table 1 shows the baseline demographic and biochemical characteristics of the patients. The patients were classified into four subgroups by CKD stage (stage 1–2, stage 3, stage 4, and stage 5) based on their eGFR: 15 (13.4%) patients had stage 1–2 disease, 55 (49.1%) had stage 3, 31 (27.7%) had stage 4, and 11 (9.8%) had stage 5. The mean ages of the stage 1–2, stage 3, stage 4, and stage 5 patients were 61.1 ± 14.8 , 66.8 ± 11.8 , 60.6 ± 13.3 , and 69.2 ± 9.0 years ($p = 0.062$, ANOVA), respectively, and the percentages of males were 80.0%, 80.0%, 51.6%, and 45.5% ($p = 0.011$, ANOVA), respectively. Hemoglobin levels were lower in patients with worse renal function, while phosphorus and iPTH levels were higher ($p < 0.001$). There were no significant differences in MAP, serum albumin, calcium, C-reactive protein (CRP), 24-h protein excretion, or ACEI/ARB use among the four subgroups (Table 1).

At 1.5-years follow-up (median, 538.5 days), eGFR had decreased in 63 (56.2%) CKD patients and increased in 49 (43.8%). The patients in whom eGFR decreased were younger and had greater 24-h protein excretion values ($p < 0.05$, *t*-test). No significant differences were observed in gender, MAP, or serum albumin between these two subgroups (Table 2).

Table 1. Baseline Demographic and Clinical Data of CKD Patients Stratified by Disease Stage. *Statistical significance. Data were obtained at the time of enrolment. Abbreviations: ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker; CKD, chronic kidney disease; CRP, C-reactive protein; iPTH, parathyroid hormone; LDL-C, low-density lipoprotein C; MAP, mean artery pressure

Variable	Stage 1–2	Stage 3	CKD Stage Stage 4	Stage 5	Total	P value
No. of patients	15	55	31	11	112	
Age (y)	61.1 ± 14.8	66.8 ± 11.8	60.6 ± 13.3	69.2 ± 9.0	64.5 ± 12.7	0.062
Gender (Male (%))	12 (80.0%)	44 (80.0%)	16 (51.6%)	5 (45.5%)	77 (68.8%)	0.011*
MAP (mmHg)	95.4 ± 7.6	92.7 ± 8.8	94.3 ± 10.1	97.8 ± 13.0	93.9 ± 9.3	0.530
Hemoglobin (g/dL)	14.0 ± 1.1	13.0 ± 1.7	11.6 ± 1.5	10.7 ± 1.8	12.5 ± 1.8	<0.001*
Serum creatinine (mg/dL)	1.07 ± 0.16	1.65 ± 0.27	2.65 ± 0.54	4.29 ± 1.14	1.83 (1.46–2.55)	<0.001*
Calcium (mg/dL)	2.37 ± 0.18	2.34 (2.28–2.44)	2.32 ± 0.14	2.32 ± 0.15	2.34 (2.25–2.43)	0.792
Phosphorus (mg/dL)	1.04 ± 0.23	1.13 ± 0.15	1.20 ± 0.17	1.38 ± 0.16	1.16 ± 0.19	<0.001*
iPTH (pg/mL)	47.7 ± 31.5	58.5 ± 39.9	95.3 ± 60.7	143.0 ± 101.1	54.8 (35.8–95.7)	<0.001*
CRP (mg/L)	3.29 ± 2.07	2.97 (2.97–3.19)	3.19 (2.97–3.36)	2.34 ± 1.41	2.97 (2.97–3.19)	0.102
24-h protein excretion (g)	1.04 ± 1.62	0.5 (0.09–1.38)	1.26 ± 1.13	3.18 ± 2.00	0.69 (0.14–1.71)	0.188
Albumin (g/dL)	4.4 ± 0.3	4.3 (4.2–4.5)	4.3 ± 0.3	4.2 ± 0.3	4.3 (4.1–4.5)	0.493
Cholesterol (mg/mL)	4.70 ± 1.01	5.03 ± 1.02	4.90 ± 1.07	5.52 ± 0.72	5.00 ± 1.02	0.253
LDL-C (mg/dL)	2.79 ± 0.70	2.82 ± 0.71	2.49 ± 0.76	3.15 ± 0.81	2.76 ± 0.75	0.083
ACEI/ARB (%)	10 (66.7%)	38 (69.1%)	28 (90.3%)	8 (72.7%)	84 (75.0%)	0.139
Aldosterone (pg/mL)	223.0 ± 99.2	173.6 ± 78.1	242.3 ± 115.6	315.2 ± 83.6	213.1 ± 102.5	<0.001*
α-klotho (pg/mL)	661.5 ± 371.3	524.8 ± 179.1	473.4 ± 157.4	467.0 ± 126.3	523.4 ± 211.4	0.029*

Table 2. Demographic and Clinical Data of CKD Patients at 1.5-years Follow-Up According to eGFR Changes. *Statistical significance. #Data obtained at 1.5-years follow-up. Data were obtained at the time of enrolment, unless otherwise noted. Abbreviations: CKD, chronic kidney disease; eGFR, estimated glomerular filtration rate; MAP, mean artery pressure; RRT, renal replacement therapy

Variable	Total	ΔeGFR > 0	ΔeGFR < 0	P value
No. of patients	112	49	63	
Age (y)	64.5 ± 12.7	70.7 ± 8.0	59.7 ± 13.6	<0.001*
No. of males (%)	77 (68.8%)	32 (65.3%)	45 (71.4%)	0.541
MAP (mmHg)	93.9 ± 9.3	93.1 ± 10.4	94.3 ± 8.6	0.536
MAP (mmHg)#	94.3 ± 9.8	92.6 ± 9.5	95.8 ± 9.8	0.108
Serum albumin (g/dL)	4.3 (4.1–4.5)	4.3 ± 0.5	4.2 ± 0.3	0.426
Serum albumin (g/dL)#	4.3 ± 0.3	4.3 ± 0.3	4.3 ± 0.3	0.671
24-h protein excretion (g/L)	0.69 (0.14–1.71)	0.83 ± 1.01	1.84 ± 2.33	0.030*
24-h protein excretion (g/L)#	0.77 (0.30–1.83)	0.72 ± 0.85	1.89 ± 2.13	0.013*
eGFR (ml/min)	37.5 ± 1.9	32.6 ± 1.6	41.3 ± 1.9	0.013*
eGFR (ml/min)#	33.8 (21.7–46.8)	37.1 (26.6–45.3)	33.7 ± 1.9	0.05
Serum creatinine (mg/dL)	1.83 (1.46–2.55)	1.87 (1.60–2.84)	1.97 ± 0.85	0.095
Serum creatinine (mg/dL)#	1.83 (1.44–2.64)	1.80 ± 0.70	2.22 (1.44–3.00)	<0.001*
Aldosterone (pg/mL)	213.1 ± 102.4	216.6 ± 110.2	210.5 ± 96.9	0.758
Aldosterone (pg/mL)#	203.5 (128.8–312.1)	224.4 ± 166.2	263.3 ± 136.2	0.178
ΔAldosterone (pg/mL)	34.2 ± 130.2	7.8 ± 117.8	55.1 ± 136.6	0.057
α-klotho (pg/mL)	523.4 ± 192.4	508.1 ± 143.2	535.3 ± 252.6	0.502
α-klotho (pg/mL)#	504.1 ± 192.4	501.5 ± 148.1	506.1 ± 222.4	0.901
Δα-klotho (pg/mL)	-10.9 (-69.2 to 37.4)	-6.6 ± 99.7	-37.2 ± 132.2	0.180
RRT (%)	44 (39.3%)	13 (26.5%)	31 (49.2%)	0.015*

Circulating α-Klotho Levels

The baseline circulating α-klotho levels were highest in patients with stage 1–2 disease and lowest in those with stage 5 disease ($p = 0.029$, ANOVA) (Table 1). α-klotho levels showed a significant positive correlation with eGFR ($r = 0.224$, $p = 0.017$) (Fig. 1). However, there were no significant correlations between α-klotho levels and the levels of calcium, phosphorous, or iPTH.

α-Klotho Level as a Predictor of Renal Replacement Therapy

Of the 112 CKD patients, RRT was initiated in 44 (39.3%) during the 6-year follow-up. Cox proportional hazards regression was used to identify independent predictors of RRT in these patients. The covariates gender, age, baseline eGFR, baseline MAP, baseline 24-h protein excretion, and baseline Δα-klotho were used for stepwise adjustment. The results showed that Δα-klotho was an independent predictor of RRT in CKD patients ($p = 0.002$) (Table 3).

Cardio-Cerebrovascular Events

Cardio-cerebrovascular events before RRT initiation occurred in 14 of the 112 CKD patients (12.5%) during follow-up. The incidence of cardio-cerebrovascular events was 31.8%, 14.3%, and 4.9% respectively in CKD patients with diabetic nephropathy, arteriosclerosis, and chronic nephritis. No patients with polycystic kidney disease or urological diseases suffered cardio-cerebrovascular accidents.

In univariate analyses, baseline plasma aldosterone levels were higher and serum α -klotho levels were lower in those who suffered cardio-cerebrovascular accidents, but the differences were not statistically significant ($p > 0.05$).

Cox proportional hazards regression was used to identify independent predictors of cardio-cerebrovascular events (Table 4). The covariates gender, age, diabetic nephropathy, baseline eGFR, baseline 24-h protein excretion, baseline cholesterol, baseline aldosterone, and baseline α -klotho were used for stepwise adjustment. The results showed that 24-h protein excretion was an independent predictor of cardio-cerebrovascular events ($p = 0.048$). α -klotho and aldosterone levels could not independently predict cardio-cerebrovascular events with statistical significance ($p > 0.05$).

Circulating α -Klotho and Plasma Aldosterone Level Relationships

The baseline circulating aldosterone levels were lower in patients with stage 1–2 CKD and higher at more advanced stages ($p < 0.001$, ANOVA) (Table 1). The aldosterone level was negatively correlated with eGFR ($r = -0.220$, $p = 0.020$) (Fig. 2a). Phosphorous and iPTH levels were positively associated with the baseline circulating aldosterone level. Negative correlations were also found between Δ aldosterone and Δ eGFR ($r = -0.319$, $p = 0.001$), and between Δ aldosterone and baseline eGFR ($r = -0.201$, $p = 0.035$) (Fig. 2b and c). Moreover, circulating α -klotho levels were negatively correlated with Δ aldosterone levels (Fig. 2d).

Fig. 1. Correlation Analysis of α -Klotho with eGFR at Baseline. $r = 0.224$, $p = 0.017$. eGFR, estimated glomerular filtration rate

Table 3. Multivariate Cox Regression Analysis to Identify Predictors of RRT in CKD patients. *Statistical significance. Data were obtained at the time of enrolment. Abbreviations: CKD, chronic kidney disease; eGFR, estimated glomerular filtration rate; HR, hazard ratio; MAP, mean artery pressure

Variable	HR	p value
Gender	1.060	0.914
Age (y)	0.933	0.001*
eGFR (mL/min)	0.925	<0.001*
MAP (mmHg)	1.070	0.026*
24-h protein excretion (g)	1.140	0.215
$\Delta\alpha$ -klotho (10 pg/mL)	0.909	0.002*

Table 4. Multivariate Cox Regression Analysis to Identify Predictors of Cardio-Cerebrovascular Events in CKD patients. *Statistical significance. Data were obtained at the time of enrolment. Abbreviations: CKD, chronic kidney disease; eGFR, estimated glomerular filtration rate; HR, hazard ratio

Variable	HR	p value
Gender	0.880	0.924
Age (y)	1.043	0.510
Diabetic nephritis	14.416	0.142
eGFR (mL/min)	0.920	0.144
24-h protein excretion	1.507	0.048*
Cholesterol (mg/mL)	1.732	0.130
Phosphorus (mg/dL)	0.530	0.081
Aldosterone (pg/mL)	1.292	0.710
α -klotho (100 pg/mL)	1.673	0.231

Fig. 2. Correlation Analysis of Aldosterone, Δ Aldosterone, eGFR, Δ eGFR and α -Klotho. (a) Correlation between aldosterone and eGFR at the baseline ($r = -0.220$, $p = 0.020$). (b) Correlation between Δ aldosterone and Δ eGFR ($r = -0.319$, $p = 0.001$). (c) Correlation between Δ aldosterone and baseline eGFR ($r = -0.201$, $p = 0.035$). (d) Correlation between Δ aldosterone and baseline α -klotho ($r = -0.199$, $p = 0.037$). eGFR, estimated glomerular filtration rate.

Discussion

Animal studies have identified various physiological roles of α -klotho in the progression of CKD, and experimental studies have shown that extra α -klotho given to animal models of CKD reduces their kidney damage [2, 19]. However, its role as a biomarker for the prognosis of ESRD in CKD patients is controversial. To shed some light on this, in the present study, we measured the circulating α -klotho level at baseline and 1.5 years into the follow-up period in CKD patients. The goal was to explore the role of circulating α -klotho as a biomarker for CKD progression and its possible relationship with aldosterone.

The serum α -klotho level of the CKD patients in our study was lower than that of a healthy population in a previous report that used the same ELISA kit [1, 21]. A significant and gradual drop in the baseline circulating α -klotho level was noticed from stage 1–2 patients to stage 5 patients. There was a significant negative correlation between baseline circulating α -klotho level and CKD stage, although the baseline level was not predictive of RRT initiation. However, the change in circulating α -klotho levels at 1.5 years was predictive of RRT initiation. This indicated that the change in α -klotho level was a better biomarker for CKD progression than baseline level. No difference in the α -klotho level was found according to different causes of CKD.

Although our study showed that the baseline circulating α -klotho level was lower in patients with lower eGFR, similar to the results of Shimamura et al. [22] and Kim et al. [18], there was no evidence that the serum α -klotho level could be used to predict ESRD outcomes. However, our data do show that a change in circulating α -klotho level at 1.5 years is an independent predictor of RRT initiation. In our study, we recruited patients with CKD stages 1–5 (before RRT), and followed them for 6 years. However, Seiler's study only included CKD patients with stage 2–4 disease and followed them for an average of 2.2 years. A total of 19 (5.9%) patients reached ESRD and underwent RRT in Seiler's study compared with 44 (39.3%) in our study. The short follow-up period and exclusion of CKD stage 1 might explain why this observation was not made in the Seiler study.

As previous studies have demonstrated the role of aldosterone in renal damage and the relationship between α -klotho and aldosterone, we investigated aldosterone changes in the patients enrolled in our study. We found that the plasma aldosterone level was significantly elevated with the decline of renal function, especially in CKD stage 4–5 patients, which is consistent with published studies [23]. Importantly, the present study showed that a decrease in circulating α -klotho was correlated with an increase in the plasma aldosterone level. This finding further supports the notion that α -klotho and aldosterone play a linked role in the pathogenesis of the progressive decline of renal function in CKD. That is to say, α -klotho may interact with aldosterone in the case of extremely pleiotropic effects and physiologic processes related to renal damage. However, the mechanism by which α -klotho affects CYP11B2 and aldosterone synthesis is not clear. The α -klotho deficiency-induced increase in aldosterone is not caused by over activity of the renin-angiotensin system [20]. Moreover, it has been reported that blockade of aldosterone receptors almost abolished klotho deficiency-induced kidney damage in *Kl^(+/−)* mice [20]. Further studies are required to characterize the interaction between α -klotho and aldosterone, and its role in the progression of CKD.

In this study we also investigated cardio-cerebrovascular events as the secondary outcome. We showed that 24 h proteinuria was an independent predictor of cardio-cerebrovascular events, which is consistent with previous reports [24–27]. Higher aldosterone and lower α -klotho levels tended to be accompanied by a higher incidence of cardio-cerebrovascular events, but this was not statistically significant. Previous work has reported vascular calcification in *Klotho* knockout mice [4], and aldosterone and mineralocorticoid receptor activation have been shown to play an important role in the pathogenesis of cardiovascular, renal, and metabolic disease. In a key longitudinal investigation, aldosterone was shown to predict future hypertension [28]. However, few studies have shown that klotho or aldosterone alone can predict cardio-cerebrovascular events independently, the present study included. It is presumed that klotho or aldosterone may interact with other traditional and CKD-related risk factors to increase the morbidity of cardio-cerebrovascular events rather than acting directly. Furthermore, CKD itself is an independent risk factor for the development of coronary artery disease and stroke, with most events occurring in patients with ESRD [29, 30]. Therefore, further investigation of the relationships between klotho or aldosterone and cardio-cerebrovascular events needs to focus on patients having RRT.

The *KLOTHO* gene has gained attention as a powerful regulator of the aging process [15]. In humans, serum levels of α -klotho decrease with age after the age of 40 years [31]. In our study, the soluble α -klotho level in serum did not decrease with age, as in Seiler's study [17]. The average age of the patients in our study was 64.2 ± 12.7 years, and that in Seiler's study was 65.5 ± 12.1 years [17]. However, in Kim's study, the patients were much younger, with an average age of 45.7 ± 15.7 year, and correlation analyses revealed that the log α -klotho values were positively correlated with age [18]. Based on these findings, we hypothesize that α -klotho renal synthesis level in CKD patients older than 60 years is affected more by renal injury than age.

We did not evaluate FGF-23 because many studies have found that an increase in FGF-23 is not associated with loss of renal α -klotho production [32, 16]. Our patients' iPTH levels were higher than normal on account of the deterioration of renal function, but they were still

within the normal range for CKD patients before RRT. Therefore, none of the patients in our cohort took vitamin D or its receptor activators before RRT. We did not find any relationship between serum α -klotho and serum phosphate, calcium, or iPTH levels. These results were similar to those reported by some other studies [33]. Therefore, the complex regulatory interactions between α -klotho and numerous other players in CKD-related mineral and bone disorder should be further explored.

Conclusion

We showed that low levels of circulating α -klotho were associated with decreased kidney function. In addition, reduction in the serum α -klotho level after 1.5 years was seen to be predictive of RRT initiation in patients with CKD. The data indicate that changes in the serum α -klotho level, especially over more than a year after blood pressure control, nephrotoxic medicine avoiding and blood glucose, uric acid control, et al, may serve as a useful clinical biomarker for progression of CKD. In addition, α -klotho and aldosterone appear to play a related role in renal damage. Further studies with larger numbers of patients are required to validate our findings.

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LYL and CMH designed the study and participated in revising the manuscript. LYL and YG followed up the cohort. MHY, PC, and HZS obtained blood samples and recorded clinical data. LYL and JYZ carried out the measurement of α -klotho. JQ and LYL performed the statistical analysis and drafted the manuscript. All authors read and approved of the final manuscript.

Disclosure Statement

The authors declare that they have no competing financial interests.

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