Plasma s-Klotho is related to kidney function and predicts adverse renal outcomes in patients with advanced chronic kidney disease

Qi-feng Liu,[1] Jian-ming Ye,[1] Li-xia Yu,[1] Ao-lin He,[2] Qiang Sun,[1] Da-wei He,[2] Sha-sha Li[2]

[1]Department of Nephrology, Kunshan First People's Hospital Affiliated to Jiangsu University, Kunshan, Jiangsu, China
[2]Clinical and Lab Research Centre, Kunshan First People's Hospital Affiliated to Jiangsu University, Kunshan, Jiangsu, China

Correspondence to
Dr Sha-sha Li, Clinical & Lab Research Centre, Kunshan First People's Hospital, Affiliated to Jiangsu University, Kunshan 215300, Jiangsu, China; whitelss@163.com

Accepted 24 September 2017
Published Online First 23 October 2017

ABSTRACT

To investigate whether the soluble Klotho (s-Klotho) level in patients with chronic kidney disease (CKD) is related to kidney function and whether a low s-Klotho level can predict adverse renal outcomes or CKD progression in patients with advanced CKD. 112 patients with CKD stages 3–5 and 30 healthy volunteers were enrolled. Blood samples were collected to measure serum creatinine, calcium, phosphorus, intact parathyroid hormone, and hemoglobin. s-Klotho and fibroblast growth factor 23 (FGF23) were determined by ELISA. We first conducted a cross-sectional study to investigate correlations between s-Klotho and estimated glomerular filtration rate (eGFR) and other parameters. Patients were then followed prospectively for 20.1±10.1 months according to s-Klotho median level until serum creatinine doubled, or initiation of renal replacement therapy, or death. s-Klotho levels inpatients with CKD were significantly lower than that in the control group. For patients with CKD, there were no differences in age distribution among subgroups. However, s-Klotho level differed significantly across CKD stages, and it was lower in the advanced CKD group compared with the moderate CKD group. Correlation analysis revealed that s-Klotho was positively associated with eGFR, but inversely associated with FGF23. During the follow-up of 20.1±10.1 months, patients with higher s-Klotho levels showed a reduced risk of kidney adverse outcomes, including serum creatinine doubling and initiation of renal replacement therapy. Cox regression analysis revealed that low s-Klotho was an independent risk factor for CKD progression. s-Klotho level was closely correlated with kidney function, further, low s-Klotho level could predict adverse kidney disease outcomes in patients with progressive CKD.

Significance of this study

What is already known about this subject?

- Klotho is a novel found protein that exerts its diverse renoprotective effects.
- The kidney is the main organ which produces and metabolizes Klotho, thus chronic kidney disease (CKD) reduces its expression (both s-Klotho and membranous Klotho (m-Klotho)).
- It has been previously shown that the level of s-Klotho is related to the status of kidney function. However, the relationship between level of s-Klotho in plasma and prognosis in patients with CKD, especially in patients with advanced CKD has not been extensively studied.

What are the new findings?

- We demonstrated that progressive CKD decreased the level of s-Klotho significantly in younger patients with CKD after adjusting for age.
- We first investigated the possibility that lowered s-Klotho level can predict the adverse outcomes in patients with advanced CKD stages (stage 3–5).
- Our findings revealed that reduced s-Klotho level in plasma could predict the adverse outcomes and indicated that s-Klotho level may serve as a biomarker to predict the prognosis in patients with advanced CKD.

How might these results change the focus of research or clinical practice?

- CKD, which affects almost 10% of population, is being recognized as a major public health problem. Until now, there are no accurate available biomarkers in predicting adverse renal outcomes or CKD progression. Our findings provide valuable information that lowered s-Klotho may serve as a promising candidate marker to predict adverse outcomes in patients with advanced CKD. Furthermore, monitoring the s-Klotho level may be helpful for early intervention and prevention for CKD progression.

INTRODUCTION

Alpha Klotho is the protein product of the antiaging *klotho* gene and this protein exists in two forms: soluble Klotho (s-Klotho) and membranous Klotho (m-Klotho).[1] m-Klotho is a single transmembrane protein that acts as a coreceptor for fibroblast growth factor 23 (FGF23) in calcium-phosphate metabolism. s-Klotho is generated from the cleavage of

To cite: Liu Q, Ye J, Yu L, *et al*. *J Investig Med* 2018;**66**:669–675.

the extracellular domain of m-Klotho, or from alternative splicing of the *klotho* gene, then released into the blood, urine, and cerebrospinal fluid.[2] s-Klotho exerts pleiotropic actions such as anti-oxidative stress, anti-inflammatory effects, and inhibition of cell apoptosis and organ fibrosis independent of FGF23.[3 4] It has been reported that s-Klotho could exhibit beneficial effects against renal injuries resulting from ischemia, hypoxia, inflammation, and ureteral obstruction in experimental animal models.[5 6] Consistent with these findings, we recently observed that s-Klotho inhibited endoplasmic reticulum stress-induced apoptosis in obstructive nephropathy[7] and epithelial mesenchymal transition (EMT)-induced renal fibrosis in cyclosporin nephropathy.[8] These results indicate that s-Klotho is involved in various kidney diseases, and it has been proposed as a novel renal protective protein with therapeutic potential.[9] s-Klotho is located predominantly in the kidney, parathyroid gland, and choroid plexus. Increasing evidences suggest that kidney is the principal organ that produces, regulates, and metabolizes Klotho.[10] It is therefore not surprising that kidney disease influences the level of Klotho in plasma or tissue. Expression of Klotho has been reported to be decreased in plasma, urinary and kidney tissues in animal models with chronic kidney disease (CKD)[11 12] and acute kidney injury (AKI).[5] In patients with AKI, s-Klotho was decreased, but its expression was restored when kidney function recovered.[13 14] In patients with CKD, a decline of s-Klotho was detected in the early stages, and the decline increased in severity with loss of kidney function.[15 16] These findings suggest that s-Klotho is associated with kidney injury, and it may serve as an early biomarker and therapeutic target. However, some studies also reported that s-Klotho has not been decreased, even increased in patients with CKD, and no significant relationship was found between s-Klotho with kidney function or CKD progression.[17–19] Available evidences from cross-sectional studies concerning the relevance between s-Klotho and CKD were controversial,[15–17 19–22] and data from prospective studies were lacking and confusing.[18 23] Thus, this prospective cohort study aims to investigate whether s-Klotho levels are related to kidney function and whether s-Klotho deficiency precedes CKD progression in patients with advanced CKD.

MATERIALS AND METHODS

Patients

CKD stage was classified according to estimated glomerular filtration rate (eGFR): CKD stage 1, ≥90 mL/min/1.73 m^2; CKD stage 2, 60–89 mL/min/1.73 m^2; CKD stage 3, 30–59 mL/min/1.73 m^2; CKD stage 4, 15–29 mL/min/1.73 m^2; and CKD stage 5, <15 mL/min/1.73 m^2.[24] Inclusion criteria were: patients aged 20–80 years; >6-month history of CKD; eGFR <60 mL/min/1.73 m^2 (patients with eGFR <5 mL/min/1.73 m^2 were not included because renal replacement therapy (RRT) is estimated to be initiated within 3 months). Exclusion criteria were: evidence of acute illness (including AKI and infectious diseases), history of RRT or organ transplant, history of tumors or cancers, AKI on CKD or immunosuppressant use within 3 months prior to the study. We randomly screened 210 patients with CKD and 112 patients with CKD stage 3–5 were considered eligible and eventually included in the study (figure 1). Thirty age-matched volunteers, without acute or chronic underlying illness, served as the control group. Enrollment took place in our hospital between January 2013 and December 2014.

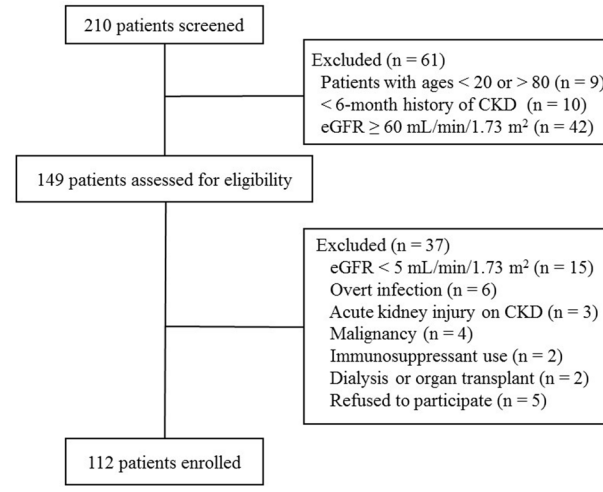

Figure 1 Flow chart of patients' recruitment and exclusion in the study. CKD, chronic kidney disease; eGFR, estimated glomerular filtration rate.

Study protocol

A cross-sectional study was conducted to analyze the relationship between s-Klotho level and kidney function, and then a prospective cohort study was conducted to examine whether a reduced s-Klotho level could predict CKD progression. Patients were divided into two groups based on above or below the overall median s-Klotho level and followed for 30 months. Moreover, they were contacted every 1 or 2 months for clinical assessment and blood test until serum creatinine (Scr) doubled, or initiation of RRT, or death. During the follow-up period, all patients received conservative treatment. Primary outcomes were the composite end point events, which were Scr doubling, initiation of RRT, or death. If participants were lost to follow-up, their clinical information recorded at the last visit were used for further analysis. The study used CKD-EPI equation for eGFR and we classified patients with CKD according to eGFR.[25] Age, sex, body weight, history and causes of CKD were recorded. Scr, blood urea nitrogen (BUN), blood calcium (Ca), phosphorus (P), intact parathyroid hormone (iPTH), and hemoglobin (HGB) were measured at our hospital using standard methods.

The levels of s-Klotho and FGF23 were determined by using a sandwich ELISA kit (Santa Cruz Biotechnology) with two affinity purified specific antibodies,[26] according to manufacturer's instructions.

Statistical analysis

Continuous data are reported as mean±SD and were compared using t-tests or one-way analysis of variance. Categorical variables are expressed as percentages and were compared using the χ^2 test. s-Klotho and FGF23 levels are reported as median and IQR and were compared using Kruskal-Wallis tests or Mann-Whitney U tests due to

Table 1 Comparison of biochemical data between control group and chronic kidney disease group

Variables	Control group (n=30)	CKD group (n=112)	p Value
Ages	45.3±12.9	50.1±14.0	0.082
Scr (μmol/L)	82.9±16.4	336.5±152.3	<0.001
BUN (mmol/L)	5.7±1.4	16.5±6.1	<0.001
HGB (g/L)	145.4±12.9	104.3±23.1	<0.001
Ca (mmol/L)	2.45±0.19	2.08±0.16	0.031
P (mmol/L)	0.96±0.43	1.47±0.29	0.012
Alb (g/L)	45.9±1.9	34.6±5.0	<0.001
Klotho (ng/mL)	9.81 (7.45, 12.2)	2.44 (1.68, 3.13)	<0.001
FGF23 (pg/mL)	298.95 (257.81, 364.51)	503.2 (377.7, 683.7)	<0.001

Data are presented as mean±SD for normally distributed variables, otherwise median with 25th–75th percentile.
Alb, albumin; BUN, blood urea nitrogen; Ca, calcium; FGF23, fibroblast growth factor 23; HGB, hemoglobin; P, phosphorus; Scr, serum creatinine.

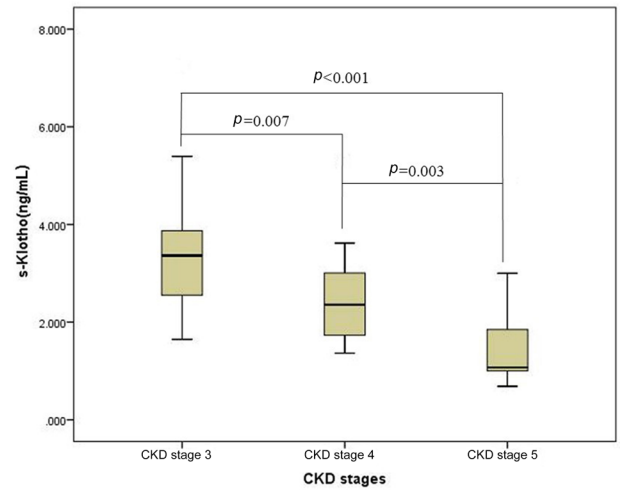

Figure 2 s-Klotho levels in different chronic kidney disease (CKD) groups.

skewed distribution. s-Klotho, iPTH, and FGF23 levels were log-transformed because of skewed distributions. Pearson's correlation and multiple regression analyses were performed to clarify the association between potential factors and eGFR. Kaplan-Meier curves were adopted to compare the time-to-event analysis of combined end points between groups. A Cox proportional hazards model was used to assess the independent variables associated with outcomes. Data analysis was performed using SPSS V.22.0 software. All tests were two-sides and a $p<0.05$ was considered to be statistically significant.

RESULTS

Patients' baseline characteristics

The baseline characteristics of participants in the control and CKD groups are listed in table 1. The primary causes of CKD was chronic glomerulonephritis (39.2%, 44/112), followed by diabetic nephropathy (21.4%, 24/112), hypertensive nephrosclerosis (9.8%, 11/112), hyperuricemic nephropathy (7.1%, 8/112), and others (22.3%, 25/112) (table 2). Not surprisingly, compared with the control group, patients in the CKD group had higher Scr, BUN, P, and FGF23 levels, but lower Ca, HGB, and s-Klotho levels. There was no significant difference in age between the two groups (table 1).

Relationship between s-Klotho level and eGFR

Median s-Klotho level was 2.44 ng/mL (IQR: 1.68–3.13 pg/mL) and median FGF23 level was 503.2 pg/mL (IQR: 377.7–683.7 pg/mL) in patients with CKD. Both s-Klotho and FGF23 levels differed significantly across CKD stages. Along with declining kidney function, s-Klotho levels dramatically decreased ($p<0.001$) (table 2 and figure 2), whereas FGF23 levels dramatically increased ($p<0.001$) (table 2). The differences of age in three subgroups were similar ($p=0.502$). Univariate analysis revealed that log-transformed s-Klotho positively correlated with eGFR ($r=0.593$, $p<0.001$) and Ca level ($r=0.302$, $p=0.029$), whereas it negatively correlated with age ($r=-0.326$, $p=0.016$), log-transformed iPTH ($r=-0.542$, $p<0.001$), and log-transformed FGF23 ($r=-0.702$, $p<0.001$). No significant association was found between plasma s-Klotho

Table 2 Comparison of biochemical and demographic data among CKD groups

Variables	CKD stage 3 (n=33)	CKD stage 4 (n=48)	CKD stage 5 (n=31)	p Value
Age	52.2±13.4	49.8±13.5	53.3±12.7	0.502
Male	20 (60.6%)	26 (54.2%)	16 (51.6%)	0.752
Diabetes	9 (27.3%)	10 (20.8%)	6 (19.4%)	0.710
Hypertension	14 (42.4%)	22 (45.8%)	23 (74.2%)	0.026
Vitamin D supplement	18 (54.5%)	32 (66.7%)	19 (61.3%)	0.544
eGFR (mL/min/1.73 m^2)	38.2±7.3	22.1±6.3	10.8±2.2	<0.001
Ca (mmol/L)	2.21±0.15	2.09±0.14	2.04±0.15	0.002
P (mmol/L)	1.23±0.21	1.46±0.31	1.48±0.34	0.008
HGB (g/L)	118.4±22.1	103.3±24.4	94.5±12.0	0.031
iPTH (pg/mL)	143.5 (67.8, 195.3)	298.4 (204.5, 376.5)	488.6 (400.5, 659.6)	<0.001
Klotho (ng/mL)	3.62 (1.24, 7.01)	2.35 (1.59, 2.98)	1.01 (1.00, 1.65)	<0.001
FGF23 (pg/mL)	387.4 (320.3, 454.7)	512.7 (441.3, 610.3)	760.4 (618.5, 874.2)	<0.001

Data are presented as mean±SD for normally distributed variables, otherwise median with 25th–75th percentile.
Ca, calcium; CKD, chronic kidney disease; eGFR, estimated glomerular filtration rate; FGF23, fibroblast growth factor 23; HGB, hemoglobin; iPTH, intact parathyroid hormone; Scr, serum creatinine.

Table 3 Association analysis between s-Klotho and clinical parameters or biochemical parameters in univariate or multivariate linear regression model

Parameters	Coefficient (r) or β	p Value
Univariate coefficient (r)		
Age (years)	−0.326	0.016
eGFR (mL/min/1.73 m^2)	0.593	<0.001
HGB (g/L)	0.248	0.072
Ca (mmol/L)	0.302	0.029
Phosphorus (mmol/L)	−0.207	0.113
Albumin (g/L)	0.124	0.312
iPTH (pg/mL)	−0.542	<0.001
FGF23 (pg/mL)	−0.702	<0.001
Multivariate β		
Age (years)	−0.164	0.105
eGFR (mL/min/1.73 m^2)	0.374	0.021
Ca (mmol/L)	0.101	0.257
iPTH (pg/mL)	0.038	0.768
FGF23 (pg/mL)	−0.448	<0.001

Data for Klotho, FGF23 and iPTH were log transformed to approximate normal distribution.
Ca, calcium; eGFR, estimated glomerular filtration rate; FGF23, fibroblast growth factor 23; HGB, hemoglobin; iPTH, intact parathyroid hormone.

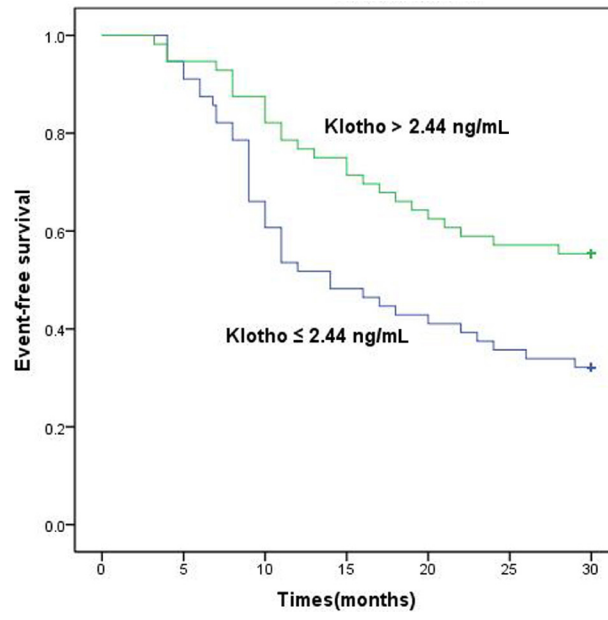

Figure 3 Kaplan-Meier curves of composite outcome according to median Klotho value (log-rank test, p=0.007).

level and HGB (r=0.248, p=0.072), P (r=−0.207, p=0.113), and albumin (r=0.124, p=0.312) (table 3). Multivariable linear regression analysis revealed that log-transformed s-Klotho positively correlated with eGFR (β=0.374, p<0.021) and negatively correlated with log-transformed FGF23 (β=−0.448, p<0.001) (table 3) and not correlated with age (β=−0.164, p=0.105), Ca (β=0.101, p=0.257), and iPTH (β=0.038, p=0.768) (table 3).

A lowered s-Klotho level predicts renal function loss or kidney disease outcome

Patients were followed for 20.1±10.1 months, with follow-up intervals ranging between 3.2 and 30.0 months. No patients lost to follow-up. Patients were divided into two groups based on median s-Klotho value (>2.44 or ≤2.44 ng/mL) to evaluate the prognostic role of s-Klotho, and the composite end points were compared between the two groups. Of the 56 patients with s-Klotho ≤2.44 ng/mL, 19 patients had Scr doubling, 17 patients underwent RRT, and 2 patients died. Of the 56 patients with s-Klotho > 2.44 ng/mL, 13 patients had Scr doubling, 10 patients underwent RRT, and 2 patient died. Thirty-eight patients with s-Klotho ≤2.44 ng/mL reached the composite end points compared with 25 patients with s-Klotho >2.44 ng/mL (p=0.013) (table 4). Kaplan-Meier curve analysis revealed a significant decrease in the occurrence of the composite outcomes in patients with s-Klotho > 2.44 ng/mL (log-rank test: p=0.007) (figure 3).

Table 4 Clinical outcomes based on median Klotho value

Outcomes	Klotho ≤2.44 ng/mL n(%)	Klotho >2.44 ng/mL n(%)	p Value
Scr doubling	19 (34.0)	13 (23.2)	0.209
RRT	17 (30.4)	10 (17.9)	0.122
Death	2 (3.6)	2 (3.6)	1.00
Composite	38 (67.9)	25 (44.6)	0.013

RRT, renal replacement therapy; Scr, serum creatinine.

As correlation analysis showed that s-Klotho value significantly correlated with FGF23 level, we also assessed the role of FGF23 in predicting adverse outcomes. Kaplan-Meier analysis showed that event-free survival was dramatically higher in patients with FGF23 ≤503.2 pg/mL (log-rank test: p=0.043) (figure 4). In univariate and multivariate Cox analyses, patients in the high s-Klotho group experienced fewer short-term end points; therefore, a lower s-Klotho level was determined as an independent predictor of adverse outcome (HR 3.291, 95% CI 1.056 to 9.823, p=0.048) (table 5). Interestingly, a higher FGF23 level also predicted outcomes with univariate Cox analysis (HR 0.391, 95% CI 0.341 to 0.915, p=0.029) (table 5), but not with multivariate Cox analysis (HR 1.316, 95% CI 0.472 to 3.779, p=0.707) (table 5).

DISCUSSION

We found that s-Klotho level was reduced in patients with CKD with severely decreased kidney function, and it independently correlated with eGFR and inversely associated with FGF23 and P levels after adjustment for age in our cross-sectional study. A lowered s-Klotho level could predict deterioration of renal function or CKD progression such as Scr doubling, or initiation of RRT in advanced patients with CKD in our prospective study. These findings suggest that s-Klotho may be a novel biomarker for predicting CKD progression.

s-Klotho has been increasingly highlighted as a renoprotective hormone with pleiotropic actions. In addition to modulation of mineral metabolism, we recently reported that s-Klotho therapy ameliorated renal tubular cell apoptosis via inhibition of endoplasmic reticulum stress[7] and

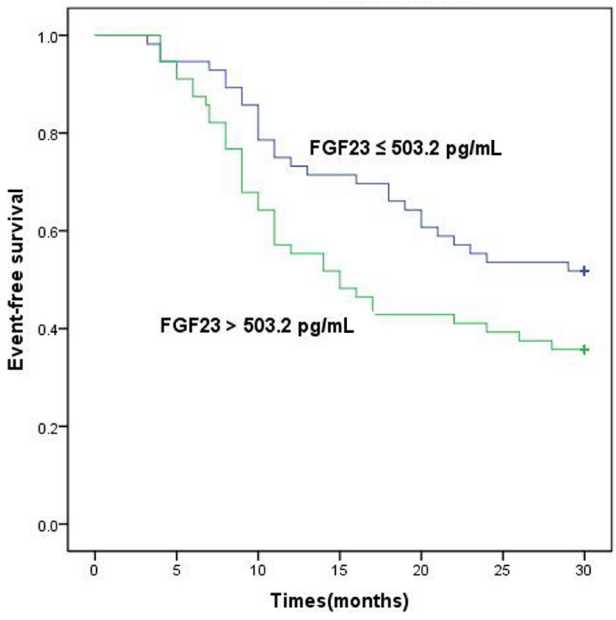

Figure 4 Kaplan-Meier curves of composite outcome according to median FGF23 value (log-rank test, p=0.043).

inhibited renal fibrosis by regulating renal EMT process.[8] Other researchers confirmed s-Klotho could suppress renal fibrogenesis through targeting Wnt/-catenin[27] and transforming growth factor-1 signaling pathways.[28] s-Klotho also functions as a modulator in oxidative stress and inflammation by enhancing antioxidant defenses[29] or inhibiting inflammatory mediators.[30] s-Klotho is generated in several organs, with the highest level seen in the kidney, suggesting the kidney as the primary organ producing s-Klotho. The underlying mechanism of deficient s-Klotho in patients with CKD is unclear. It is thought that the diseased kidney, with progressive tubular atrophy and interstitial fibrosis, reduces the synthesis and secretion of s-Klotho. Uremic toxins such as indoxyl sulfate directly decrease s-Klotho production by increasing *klotho* gene hypermethylation.[31] In many experimental animal models of disease, s-Klotho expression is severely decreased and the deficiency of s-Klotho further worsens kidney function. In human studies, Seo *et al* reported that the expression of renal Klotho decreased significantly according to the severity of AKI, meanwhile, a low s-Klotho level predicted a poorer outcome.[13] Shimamura *et al* observed that s-Klotho levels were reduced in the early stages of CKD, and it continued to fall as CKD progressed.[15] Similar results were seen in our own cross-sectional analysis. In addition, we found that patients in our study were younger than that in other studies,[15 18 22 32] and no age differences were observed among CKD groups. Surprisingly, CKD still decreased the level of s-Klotho and the trend was more apparent with deterioration of kidney function after balancing age distribution among groups.

Table 5 Univariate and multivariate Cox analysis for composite events

Parameters	HR (95% CI)	p Value
Univariate analysis		
Age	0.976 (0.952 to 1.034)	0.857
Hypertension (yes/no)	0.815 (0.480 to 1.702)	0.516
Diabetes (yes/no)	0.624 (0.261 to 1.664)	0.410
Vitamin D supplement (yes/no)	0.382 (0.414 to 1.852)	0.273
FGF23 level (>503.2 or ≤503.2 pg/mL)	0.391 (0.341 to 0.915)	0.029
s-Klotho level (>2.44 or ≤2.44 ng/mL)	3.152 (1.446 to 7.807)	0.013
Ca (mmol/L)	0.153 (0.026 to 1.322)	0.082
Phosphorus (mmol/L)	4.329 (1.083 to 16.733)	0.040
iPTH (mmol/L)	1.141 (1.083 to 1.226)	0.268
Albumin (g/L)	0.954 (0.885 to 1.101)	0.057
Multivariate analysis		
Phosphorus (mmol/L)	3.159 (0.672 to 14.826)	0.203
FGF23 level (>503.2 or ≤503.2 pg/mL)	1.316 (0.472 to 3.779)	0.707
s-Klotho level (>2.44 or ≤2.44 ng/mL)	3.291 (1.056 to 9.823)	0.048

Adjusted variables included in the model were: age, hypertension, vitamin D supplement, diabetes, FGF23 and Klotho levels, Ca, phosphorus, iPTH, and albumin.
Ca, calcium; FGF23, fibroblast growth factor 23; iPTH, intact parathyroid hormone.

Although increasing evidence suggests that s-Klotho level reflects the severity of kidney injury, its relationship with CKD progression or adverse kidney disease outcomes aroused controversy. Kim *et al* reported that more patients with CKD with lower s-Klotho levels reached adverse outcomes (death, Scr doubling, end-stage renal disease) over a follow-up of 29.7 months in a study of 243 patients with CKD.[23] After adjusting for eGFR, proteinuria, and iPTH, a higher s-Klotho level was consistently associated with reduced risk for adverse outcomes, indicating that s-Klotho concentration independently predicted adverse outcomes in kidney disease. A recent study also demonstrated the increased Klotho level was associated with reduced risks of decline in kidney function in patients with no CKD.[33] Aligning with these results, we also observed that the odds of reaching the combined end points in patients with a s-Klotho level greater than the median value was much lower compared with patients with a s-Klotho level less than the median value. Furthermore, renal event-free survival for patients with a higher s-Klotho level was significantly higher in Kaplan-Meier analysis. Cox regression analysis revealed that a low s-Klotho level was an independent risk factor for CKD outcome. These results indicated that s-Klotho deficiency accelerated the progression of kidney disease. However, the mechanism behind this process remains unclear.

Intriguingly, restoration of Klotho ameliorated kidney injury and improved kidney function,[34] demonstrating that Klotho is an effective renal protective protein with intervention potential. Because of loss of s-Klotho in the diseased kidney, the renal protective ability of s-Klotho is largely removed, and this in turn partly aggravates kidney function. This may be a possible mechanism connecting s-Klotho loss and kidney function deterioration or CKD progression. These results, together with our findings, suggest that a reduced s-Klotho level can predict adverse outcomes, and reduced s-Klotho may have a prognostic role in patients with advanced CKD.

A cohort study by Seiler *et al* of 321 patients with CKD reported that the s-Klotho level was independently associated with age, but not eGFR.[18] Patients were stratified into three groups according to s-Klotho level, and no difference in event-free survival among groups, which was observed during the follow-up period of 2.2±0.8 years. Interestingly, patients with the highest FGF23 level suffered the worst outcomes. Patients enrolled in that study were older than patients enrolled in the current study, and ages in the subgroups were significantly different. *klotho* was originally discovered as an antiaging gene; therefore, differences in age may attenuate the connection between s-Klotho and eGFR. Furthermore, that study excluded patients with advanced CKD stages, and this inevitably eliminated the relationship. Finally, different ELISA kits for determining s-Klotho levels may be partially responsible for the conflicting results.[35] Other conflicting results have also been observed in other studies,[20 21] but those studies recruited patients only in early CKD stages, not in advanced CKD stages,[15 18 21] or with significant differences in age distribution.[15 16 18 21] Therefore, conclusions drawn in those studies cannot be generalized to the CKD population.

Contrary to our observations on s-Klotho levels, we found that the FGF23 level was higher in patients with CKD and inversely correlated with eGFR and s-Klotho. FGF23 promotes urinary phosphate excretion[36] to reduce blood phosphorus. s-Klotho deficiency or decreases in eGFR lead to phosphorus retention, which in turn stimulates FGF23 synthesis and secretion. s-Klotho and FGF23 work together to regulate the metabolism of Ca and P. Many studies have reported that FGF23 levels are elevated in the early stages of CKD or patients with advanced CKD, and increases in FGF23 level even precede increases in iPTH and phosphate levels. Elevated FGF23 predicted an adverse clinical outcome and CKD progression in patients with CKD.[37] In the current study, we found that higher FGF23 levels were associated with higher risk for reaching kidney disease adverse outcomes, findings consistent with other studies.[38] Therefore, FGF23 level may be another predictive factor of adverse outcomes, similar to s-Klotho.

This study had some limitations. First, the number of enrolled patients was relatively small and the sample size may weaken the reliability of the results. Second, m-Klotho or urinary s-Klotho were not measured. Available data suggest that the s-Klotho level is closely associated with m-Klotho or urinary s-Klotho,[39] but we cannot exclude discrepancies in the expression of s-Klotho among tissues, urine, and plasma. Third, the level of s-Klotho detected was higher than that reported in other studies, but was in agreement with a recent study.[17] To date, validation of s-Klotho ELISA kit has not been standardized and a reference value across different people has not been achieved. Although we repeated identical measurements in the study and still obtained similar results, we cannot rule out the possibility of test errors.

In conclusion, we found that a decreased s-Klotho level closely correlated with reduced kidney function in younger patients with CKD after age adjustment, and a low s-Klotho level predicted kidney adverse outcomes in patients with advanced CKD. These results suggest that s-Klotho may be a predictive marker for the progression of CKD. Because of the limitations of the study, the findings cannot be generalized to all patients with CKD. Further studies are required to obtain precise information about the role of s-Klotho in the pathogenesis and progression of CKD.

Acknowledgements This study was supported by grants from the Social Development Foundation of Kunshan, Jiangsu Province, China (KS1534, KS1649).

Contributors Methodology: QL, JMY, LY. Project administration: AH, QS, DH, SL. Writing—original draft and editing: QL, SL.

Competing interests None declared.

Patient consent Obtained.

Ethics approval The ethics committee of Kunshan First People's Hospital Affiliated to Jiangsu University, China, and was performed in accordance with the principles contained within the Declaration of Helsinki.

Provenance and peer review Not commissioned; externally peer reviewed.

REFERENCES

1 Kuro-o M, Matsumura Y, Aizawa H, *et al*. Mutation of the mouse klotho gene leads to a syndrome resembling ageing. *Nature* 1997;390:45–51.
2 Kim JH, Hwang KH, Park KS, *et al*. Biological role of anti-aging protein klotho. *J Lifestyle Med* 2015;5:1–6.
3 Hu MC, Kuro-o M, Moe OW. Renal and extrarenal actions of Klotho. *Semin Nephrol* 2013;33:118–29.
4 Oh HJ, Nam BY, Lee MJ, *et al*. Decreased circulating klotho levels in patients undergoing dialysis and relationship to oxidative stress and inflammation. *Perit Dial Int* 2015;35:43–51.
5 Hu MC, Shi M, Zhang J, *et al*. Klotho deficiency is an early biomarker of renal ischemia-reperfusion injury and its replacement is protective. *Kidney Int* 2010;78:1240–51.
6 Sugiura H, Yoshida T, Shiohira S, *et al*. Reduced Klotho expression level in kidney aggravates renal interstitial fibrosis. *Am J Physiol Renal Physiol* 2012;302:F1252–64.
7 Liu QF, Ye JM, Deng ZY, *et al*. Ameliorating effect of Klotho on endoplasmic reticulum stress and renal fibrosis induced by unilateral ureteral obstruction. *Iran J Kidney Dis* 2015;9:291–7.
8 Liu QF, Ye JM, Yu LX, *et al*. Klotho mitigates cyclosporine A (CsA)-induced epithelial-mesenchymal transition (EMT) and renal fibrosis in rats. *Int Urol Nephrol* 2017;49:345–52.
9 Neyra JA, Hu MC. Potential application of klotho in human chronic kidney disease. *Bone* 2017;100:41–9.
10 Hu MC, Shi M, Zhang J, *et al*. Renal production, uptake, and handling of circulating αKlotho. *J Am Soc Nephrol* 2016;27:79–90.
11 Yu J, Deng M, Zhao J, *et al*. Decreased expression of klotho gene in uremic atherosclerosis in apolipoprotein E-deficient mice. *Biochem Biophys Res Commun* 2010;391:261–6.
12 Aizawa H, Saito Y, Nakamura T, *et al*. Downregulation of the Klotho gene in the kidney under sustained circulatory stress in rats. *Biochem Biophys Res Commun* 1998;249:865–71.
13 Seo MY, Yang J, Lee JY, *et al*. Renal Klotho expression in patients with acute kidney injury is associated with the severity of the injury. *Korean J Intern Med* 2015;30:489–95.
14 Liu YJ, Sun HD, Chen J, *et al*. Klotho: a novel and early biomarker of acute kidney injury after cardiac valve replacement surgery in adults. *Int J Clin Exp Med* 2015;8:7351–8.
15 Shimamura Y, Hamada K, Inoue K, *et al*. Serum levels of soluble secreted α-Klotho are decreased in the early stages of chronic kidney disease, making it a probable novel biomarker for early diagnosis. *Clin Exp Nephrol* 2012;16:722–9.
16 Sakan H, Nakatani K, Asai O, *et al*. Reduced renal α-Klotho expression in CKD patients and its effect on renal phosphate handling and vitamin D metabolism. *PLoS One* 2014;9:e86301.
17 Scholze A, Liu Y, Pedersen L, *et al*. Soluble α-klotho and its relation to kidney function and fibroblast growth factor-23. *J Clin Endocrinol Metab* 2014;99:E855–61.
18 Seiler S, Wen M, Roth HJ, *et al*. Plasma Klotho is not related to kidney function and does not predict adverse outcome in patients with chronic kidney disease. *Kidney Int* 2013;83:121–8.

19 Inci A, Sari F, Olmaz R, *et al*. Soluble Klotho levels in diabetic nephropathy: relationship with arterial stiffness. *Eur Rev Med Pharmacol Sci* 2016;20:3230–7.
20 Devaraj S, Syed B, Chien A, *et al*. Validation of an immunoassay for soluble Klotho protein: decreased levels in diabetes and increased levels in chronic kidney disease. *Am J Clin Pathol* 2012;137:479–85.
21 Inci A, Sari F, Coban M, *et al*. Soluble Klotho and fibroblast growth factor 23 levels in diabetic nephropathy with different stages of albuminuria. *J Investig Med* 2016;64:1128–33.
22 Tanaka S, Fujita S, Kizawa S, *et al*. Association between FGF23, α-Klotho, and Cardiac Abnormalities among Patients with Various Chronic Kidney Disease Stages. *PLoS One* 2016;11:e0156860.
23 Kim HR, Nam BY, Kim DW, *et al*. Circulating α-klotho levels in CKD and relationship to progression. *Am J Kidney Dis* 2013;61:899–909.
24 National Kidney Foundation. K/DOQI clinical practice guidelines for chronic kidney disease: evaluation, classification, and stratification. *Am J Kidney Dis* 2002;39:S1–266.
25 Levey AS, Stevens LA, Schmid CH, *et al*. CKD-EPI (Chronic Kidney Disease Epidemiology Collaboration). A new equation to estimate glomerular filtration rate. *Ann Intern Med* 2009;150:604–12.
26 Yamazaki Y, Imura A, Urakawa I, *et al*. Establishment of sandwich ELISA for soluble alpha-Klotho measurement: age-dependent change of soluble alpha-Klotho levels in healthy subjects. *Biochem Biophys Res Commun* 2010;398:513–8.
27 Zhou L, Li Y, Zhou D, *et al*. Loss of Klotho contributes to kidney injury by derepression of Wnt/β-catenin signaling. *J Am Soc Nephrol* 2013;24:771–85.
28 Doi S, Zou Y, Togao O, *et al*. Klotho inhibits transforming growth factor-beta1 (TGF-beta1) signaling and suppresses renal fibrosis and cancer metastasis in mice. *J Biol Chem* 2011;286:8655–65.
29 Kuro-o M. Klotho as a regulator of oxidative stress and senescence. *Biol Chem* 2008;389:233–41.
30 Buendía P, Carracedo J, Soriano S, *et al*. Klotho prevents NFβB translocation and protects endothelial cell from senescence induced by uremia. *J Gerontol A Biol Sci Med Sci* 2015;70:1198–209.
31 Chen J, Zhang X, Zhang H, *et al*. Indoxyl sulfate enhance the hypermethylation of Klotho and promote the process of vascular calcification in chronic kidney disease. *Int J Biol Sci* 2016;12:1236–46.
32 Rotondi S, Pasquali M, Tartaglione L, *et al*. Soluble α -Klotho serum levels in chronic kidney disease. *Int J Endocrinol* 2015;2015:1–8.
33 Drew DA, Katz R, Kritchevsky S, *et al*. Association between soluble Klotho and change in kidney function: the health aging and body composition study. *J Am Soc Nephrol* 2017;28:1859–66.
34 Zhang Q, Liu L, Lin W, *et al*. Rhein reverses Klotho repression via promoter demethylation and protects against kidney and bone injuries in mice with chronic kidney disease. *Kidney Int* 2017;91:144–56.
35 Heijboer AC, Blankenstein MA, Hoenderop J, *et al*. Laboratory aspects of circulating α-Klotho. *Nephrol Dial Transplant* 2013;28:2283–7.
36 Gattineni J, Bates C, Twombley K, *et al*. FGF23 decreases renal NaPi-2a and NaPi-2c expression and induces hypophosphatemia in vivo predominantly via FGF receptor 1. *Am J Physiol Renal Physiol* 2009;297:F282–91.
37 Isakova T, Xie H, Yang W, *et al*.Fibroblast growth factor 23 and risks of mortality and end-stage renal disease in patients with chronic kidney disease. *JAMA* 2011;305:2432–9.
38 Chathoth S, Al-Mueilo S, Cyrus C, *et al*. Elevated fibroblast growth factor 23 concentration: prediction of mortality among chronic kidney disease patients. *Cardiorenal Med* 2015;6:73–82.
39 Kalaitzidis RG, Duni A, Siamopoulos KC. Klotho, the Holy Grail of the kidney: from salt sensitivity to chronic kidney disease. *Int Urol Nephrol* 2016;48:1657–66.