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Associations of serum Klotho with diabetic kidney disease prevalence and mortality: insights from a nationally representative U.S. cohort

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Abstract

Background Serum Klotho, a biomarker associated with anti-aging, has been implicated in kidney disease. However, there is a lack of robust evidence for the relationship between the serum Klotho and diabetic kidney disease (DKD). This study aimed to investigate the association of the serum Klotho levels with DKD and assess the relationship between serum Klotho and all-cause mortality in individuals with DKD.

Methods We utilized data from the 2007–2016 National Health and Nutrition Examination Survey (NHANES), incorporating both cross-sectional and cohort study designs. The association between the serum Klotho and DKD was examined using weighted logistic regression models. To estimate the hazard ratios (HRs) and 95% confidence intervals (95% CIs) for all-cause mortality, weighted Çox proportional hazards models were applied. Restricted cubic spline analysis was used to assess the linear or nonlinear relationships between the serum Klotho and DKD or all-cause mortality. Additionally, mediation analysis was conducted to determine whether the systemic immune-inflammatory index (SII) mediated the effect of serum Klotho on all-cause mortality.

Results Our findings revealed a significant reverse association between serum Klotho and DKD after adjusting for sociodemographic and lifestyle factors in Model 2 (odds ratio [OR] 0.65, 95% CI 0.47–0.90, $P=0.01$). However, this association was attenuated and lost statistical significance after further adjustment for comorbidities, SII, estimated glomerular filtration rate, and urine albumin/creatinine ratio in Model 3 (OR 0.65, 95% CI 0.32–1.31, $P=0.2$). During an average follow-up period of 76 months, a total of 795 individuals (34%) experienced mortality. Weighted multivariate Cox regression models indicated that each one-unit increase in the serum Klotho was associated with a reduced risk of all-cause mortality (HR 0.48, 95% CI 0.29–0.82, $P=0.008$) in DKD patients. Furthermore, restricted cubic spline

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analysis identified a nonlinear relationship between the serum Klotho and DKD (*P* for nonlinearity < 0.001), while a linear relationship was observed between serum Klotho and all-cause mortality (*P* for nonlinearity = 0.3480) among DKD populations. Stratified and interaction analysis confirmed the robustness of these core findings. Additionally, SII was found to partially mediate the association between serum Klotho and all-cause mortality, accounting for 5.7% of the effect.

Conclusions Serum Klotho is inversely associated with the prevalence of DKD and is also linked to reduced all-cause mortality in individuals with DKD.

Keywords Klotho, Diabetic kidney disease, Prevalence, Mortality, NHANES

Background

Klotho, a protein encoded by the Klotho gene and primarily expressed in the kidney, was initially identified for its role in aging regulation. It functions as a single-pass transmembrane co-receptor protein with a large extracellular domain that can be cleaved to form soluble Klotho, detectable in blood, urine, and cerebrospinal fluid, and acts as a hormone with systemic protective effects [1]. Studies using Klotho gene knock-out mice have demonstrated that Klotho's absence leads to premature aging phenotypes, whereas its overexpression extends lifespan and protects against pathologies, notably renal diseases [2]. These effects are attributed to Klotho's regulation of phosphate and calcium metabolism, reduction of oxidative stress, inhibition of apoptosis, and promotion of anti-inflammatory and antifibrotic responses [3, 4].

The kidney plays a crucial role in Klotho production and retrieval of soluble Klotho from circulation, possibly through tubular transcytosis [5]. Extensive research in genetic and experimental models of chronic kidney disease (CKD) has underscored Klotho's significant cardio-renal protective effects [6, 7]. As a transmembrane protein, Klotho is primarily expressed in renal tubular cells, but its expression declines early in CKD progression [8]. This has positioned Klotho not only as an early biomarker for CKD but also as a potential therapeutic target [9–11]. Low serum Klotho levels have been linked to faster declines in estimated glomerular filtration rate (eGFR) and increased mortality in CKD patients [12, 13], as well as heightened mortality risk in middle-aged and older adults [14]. Restoration of Klotho levels has been shown to mitigate chronic kidney injury [6], suggesting a direct link between Klotho depletion and the severity of renal damage.

Diabetic kidney disease (DKD), a serious microvascular complication affecting up to 40% of people with diabetes, is a leading cause of end-stage renal disease (ESRD) [15]. Patients with DKD face a significantly elevated risk of mortality, particularly due to cardiovascular complications that arise even before ESRD develops. This alarming mortality rate underscores the need for reliable biomarkers to identify high-risk individuals who could benefit from early intervention. Prior studies have associated decreased serum and urine Klotho levels with worse outcomes, including a rapid decline in eGFR, elevated urine albumin/creatinine ratio (UACR), and increased mortality [12, 13, 16–22]. However, despite the extensive research on Klotho, no large-scale studies have specifically examined its relationship with DKD.

Furthermore, recent reviews have reported conflicting results regarding the association between low serum Klotho levels and outcomes such as renal function decline, cardiovascular events, and mortality in CKD patients [23]. These discrepancies may be due to limitations in the studies, including small sample sizes, variability in Klotho stability during sample processing, and the lack of standardized assays for measuring circulating soluble Klotho. Our study aims to address these gaps by investigating the association between serum Klotho levels and all-cause mortality in patients with DKD, using updated data from the National Health and Nutrition Examination Survey (NHANES) from 2007 to 2016.

Materials and methods

Study design and participants

The NHANES is an ongoing national cross-sectional survey designed to evaluate the health and nutritional status in the U.S. This study was approved by National Center for Health Statistics Ethics Review Board and all participants provided their written informed consent. For this research, the inclusion criterion encompassed all individuals with diabetes, identified through NHANES data from 2007 to 2016. After the exclusion of participants with incomplete data on Klotho, mortality, and those without diabetes, our current analysis ultimately included 2474 participants (Fig. 1).

Definition of diabetes and DKD

Diabetes was defined by self-reported diagnosis, use of insulin or oral hypoglycemic medication, glycated hemoglobin level ≥ 6.5%, or fasting plasma glucose level ≥ 7.0 mmol/L [24, 25]. The UACR was determined to quantify albuminuria, while eGFR was ascertained employing the 2021 Chronic Kidney Disease

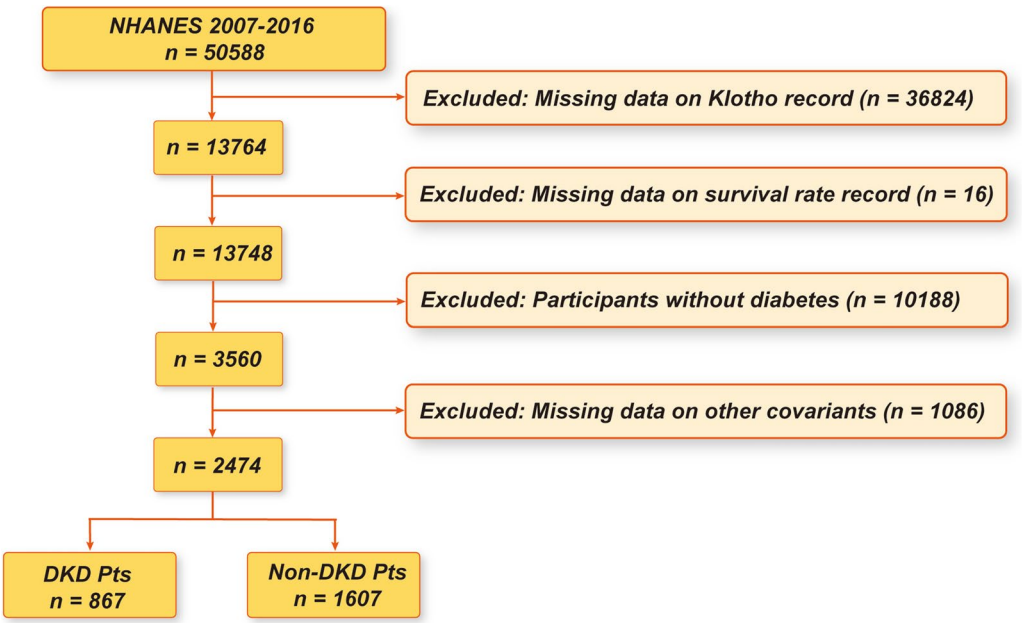

Fig. 1 Flowchart of participant selection. DKD, diabetic kidney disease; Pts, patients

Epidemiology Collaboration formula [26]. The presence of DKD in the diabetic cohort was diagnosed based on criteria indicating an UACR of 30 mg/g or higher and/or an eGFR below 60 mL/min/1.73 m^2 [28].

Mortality ascertainment

All participants from NHANES 2007–2016 were linked to death records in the National Death Index up to 31 December 2015. The follow-up duration was measured from the time of participation to the date of death for decedents or to the date of censoring for survivors. Mortality outcomes in the current study included all-cause mortality and deaths attributed to cardiovascular disease (CVD), as ascertained by ICD-10 codes recorded in NHANES.

Serum Klotho concentrations

The exposure variable of interest was the level of soluble α-Klotho, which was measured using a commercially available enzyme-linked immunosorbent assay kit manufactured by IBL International in Japan [29]. We categorized the participants into quartiles (Q1, Q2, Q3, Q4) based on soluble α-Klotho levels for all-cause mortality analysis, and the Q1 group was used as the reference group.

Covariate information

Covariates included age (years), sex, ethnicity (race), body mass index (BMI, kg/m^2 [2]), UACR, systemic immune-inflammation index (SII), eGFR (ml/min/1.73 m^2 [2]), physical activity (PA, metabolic equivalent minutes per week of activity), alcohol status, smoking status, history of CVD, hypertension, and hyperlipidemia. All covariates were considered potential confounders in the relationship between serum Klotho levels and the prevalence of morbidity. The racial groups included non-Hispanic White, non-Hispanic Black, Mexican American, Hispanic and other races. NHANES participants self-reported their PA information using the Global Physical Activity Questionnaire, a validated effective instrument for PA surveillance [30, 31]. Participants without any PA and performing < 600 metabolic equivalent (MET) min/week were classified as inactive [32]. Those performing ≥ 600 MET min/week were classified as active. The World Health Organization classified BMI into four categories: underweight (< 18.5 kg/m^2), normal (18.5–24.9 kg/m^2), overweight (25.0–29.9 kg/m^2), and obese (≥ 30.0 kg/m^2). The SII, an immune inflammation indicator, is calculated based on the peripheral blood counts of neutrophils, platelets, and lymphocytes using the formula SII = (platelet count × neutrophil count)/lymphocyte count [33, 34]. Alcohol consumption status was dichotomized into non-consumers and consumers. Smoking status was categorized as never (lifetime cigarette consumption less than 100), former (lifetime cigarette consumption more than 100 and not smoking at all now), and current (lifetime cigarette consumption less than 100 and currently smoking some days or every day). CVD was classified as having angina, heart attack, congestive

heart failure, coronary artery disease, or stroke. Hypertension was identified as meeting one of the following conditions: physician previously diagnosed hypertension, current prescription medication treatment, mean systolic blood pressure ≥ 140 mmHg or mean diastolic blood pressure ≥ 90 mmHg. Hyperlipidemia was defined as having total cholesterol levels of ≥ 200 mg/dL, triglyceride levels of ≥ 150 mg/dL, low-density lipoprotein levels of ≥ 130 mg/dL, or high-density lipoprotein levels of ≤ 50 mg/dL for women and ≤ 40 mg/dL for men [24]. Individuals who acknowledged using cholesterol-lowering medication were also classified as having hyperlipidemia [35].

Statistical analysis

All analyses were conducted adhering to the complex sampling design of the NHANES and the associated analytic guidelines. We employed χ^2 tests, nonparametric tests, and t-tests to assess the baseline characteristics of participants categorized by vital status. Continuous variables were depicted as mean ± standard deviation or median (interquartile range), whereas categorical variables were presented as n (%). Logistic regression analysis was utilized to assess the odds ratios (ORs) for the association between serum Klotho levels and DKD prevalence. Cox proportional hazard models were applied to estimate hazard ratios (HR) and 95% confidence intervals (CI) for the associations between serum Klotho with all-cause as well as cause-specific mortality, with the time scale set to months of follow-up. Model 1 included adjustments for age, sex, and race, while Model 2 incorporated additional adjustments for smoking status, physical activity levels, alcohol consumption status, and BMI. Model 3 was further adjusted for CVD, hypertension, hyperlipidemia, SII, UACR and eGFR. A trend test across increasing exposure groups was performed using integer values (1, 2, and 3). The dose–response relationships between serum Klotho and all-cause mortality in DKD patients were assessed and visualized by restricted cubic spline (RCS) regression with three knots placed at the 25th, 50th, and 75th percentiles. Subgroup analysis was stratified by age, gender, BMI, hypertension, hyperlipidemia, and CVD. Mediation analyses were conducted to investigate the potential of SII to accelerate the mortality elicited by lower serum Klotho. The mediated proportion referred to the average mediating effect of SII on mortality changes in relation to the overall effect. *P* value for mediated proportion was obtained from the quasi-Bayesian Monte Carlo simulation conducted 2000 times. All analyses were performed using R (Version 4.2.2, http://www.R-project.org, The R Foundation). $P < 0.05$ indicated a significant difference.

Results

Baseline characteristics of all participants

The baseline characteristics of the participants with and without DKD are shown in Table 1. A total of 2474 participants were included in our research; their mean age was 60.2 ± 10.0 years. The gender distribution was in a generally homogeneous manner, of which 52% were males and 48% were females. The overall prevalence of DKD in type 2 Diabetes Mellitus was 35%. The findings suggest that CKD patients among participants were more likely to be former smokers, former alcohol intake, taking insufficient PA. Specifically, a greater proportion of participants with DKD had a higher body mass index (BMI > 30), and a higher prevalence of hypertension and CVD. In addition, participants with DKD were more likely to have higher UACR, SII, a lower eGFR and serum Klotho.

Association between serum Klotho levels and DKD prevalence

The results of the multivariate logistic regression analysis are presented in Table 2. Serum Klotho, treated as a continuous variable, was significantly associated with a lower risk of DKD in Model 1 (OR = 0.65, 95% CI 0.48–0.89). This association remained robust and statistically significant after adjusting for sociodemographic and lifestyle factors in Model 2 (OR = 0.65, 95% CI 0.47–0.90). However, upon further adjustment for comorbidities, SII, eGFR, and UACR in Model 3, the association was attenuated and lost statistical significance (OR = 0.65, 95% CI 0.32–1.31). Moreover, we transformed serum Klotho from a continuous variable to a categorical variable and constructed several models to assess the independent effects of serum Klotho on the prevalence of DKD. Compared to individuals in the first quartile of serum Klotho (Q1), those in the third quartile (Q3) exhibited notably lower multivariate-adjusted ORs, as evidenced by Model 1 (OR = 0.52, 95% CI 0.39–0.71, *P* for trend < 0.001), Model 2 (OR = 0.52, 95% CI 0.38–0.70, *P* for trend = 0.003), and Model 3 (OR = 0.27, 95% CI 0.14–0.53, *P* for trend = 0.017). In addition, as illustrated in Fig. 2A, after adjustment for multiple potential confounders, we observed a statistically significant nonlinear and L-shaped association between the serum Klotho and DKD prevalence (*P* for nonlinear < 0.001).

Correlation between serum Klotho levels and all-cause mortality

Among the total participants, 795 individuals (34%) experienced mortality during a median follow-up

Table 1 The baseline characteristics of participants with or without diabetic kidney disease

Variables	Overall N = 2474 (100%)	Non-DKD N = 1607 (65%)	DKD N = 867 (35%)	P-value
Age (years)	60.2 (10.0)	58.9 (9.7)	63.2 (10.1)	< 0.001
Sex (%)				0.8
Female	1,218 (48%)	790 (48%)	428 (48%)	
Male	1,256 (52%)	817 (52%)	439 (52%)	
Race				0.034
White	815 (64%)	527 (65%)	288 (61%)	
Black	558 (12%)	329 (11%)	229 (15%)	
Mexican	523 (10%)	353 (10%)	170 (10%)	
Hispanic	326 (6.1%)	221 (6.0%)	106 (6.2%)	
Others	252 (7.8%)	177 (8.1%)	75 (7.2%)	
Smoking status (%)				0.094
Current smoker	408 (16%)	255 (15%)	153 (17%)	
Former smoker	838 (37%)	524 (35%)	314 (41%)	
Never smoker	1,228 (48%)	828 (50%)	400 (43%)	
Alcohol intake (%)				0.2
Former	665 (24%)	395 (23%)	270 (27%)	
Heavy	330 (13%)	211 (13%)	119 (13%)	
Mild	779 (38%)	544 (40%)	235 (34%)	
Moderate	241 (11%)	163 (11%)	78 (10%)	
Never	459 (15%)	294 (14%)	165 (16%)	
Obesity (%)				0.14
Normal (BMI < 25)	283 (9.5%)	171 (8.7%)	112 (11%)	
Obesity (BMI > 30)	1,468 (64%)	932 (63%)	636 (65%)	
Overweight (25 < = BMI < = 30)	723 (27%)	504 (28%)	219 (24%)	
Physical activity (%)				0.012
High	267 (12%)	194 (13%)	73 (8.2%)	
Insufficient	2,207 (89%)	1,413 (87%)	794 (92%)	
Hypertension (%)				< 0.001
No	660 (29%)	517 (33%)	143 (19%)	
Yes	1,814 (72%)	1,090 (67%)	724 (81%)	
Hyperlipidemia (%)				0.6
No	301 (11%)	207 (10%)	94 (11%)	
Yes	2,173 (89%)	1,400 (90%)	773 (89%)	
Cardiovascular disease (%)				< 0.001
No	2,138 (86%)	1,459 (90%)	679 (77%)	
Yes	336 (14%)	148 (10%)	188 (23%)	
UACR (mg/g)	95 (527)	10 (6)	286 (918)	< 0.001
SII	571 (363)	544 (341)	632 (400)	< 0.001
eGFR (ml/min•1.73m^2)	85 (21)	91 (15)	71 (27)	< 0.001
Klotho (pg/ml)	844 (314)	857 (314)	814 (315)	< 0.001
Psychogenic death				< 0.001
No	1,635 (64%)	1,113 (67%)	533 (58%)	
Yes	101 (3.6%)	30 (1.4%)	71 (8.5%)	
NA	738 (32%)	464 (32%)	274 (34%)	
Living status (%)				0.086
Living	1679 (66%)	1130 (68%)	549 (62%)	
Death	795 (34%)	477 (32%)	318 (39%)	

Data are shown as mean (SD), median (IQR), or n (%). Abbreviations: BMI, body mass index; UACR, urine albumin/creatinine ratio; DKD, diabetes kidney disease; SII, systemic immune-inflammation index; eGFR, estimated glomerular filtration rate; NA, not available

Table 2 ORs (95% CIs) for DKD according to the serum Klotho

	Model 1			Model 2			Model 3		
Characteristics	**OR**	**95% CI**	***P* value**	**OR**	**95% CI**	***P* value**	**OR**	**95% CI**	***P* value**
Continuous	0.65	0.48, 0.89	0.008	0.65	0.47, 0.90	0.01	0.65	0.32, 1.31	0.2
Category									
Q1	Ref			Ref			Ref		
Q2	0.86	0.60, 1.25	0.4	0.85	0.58, 1.24	0.4	0.53	0.27, 1.04	0.063
Q3	0.52	0.39, 0.71	< 0.001	0.52	0.38, 0.70	< 0.001	0.27	0.14, 0.53	< 0.001
Q4	0.75	0.58, 0.97	0.03	0.75	0.57, 1.00	0.046	0.59	0.31, 1.10	0.094
P for trend		< 0.001			0.003			0.017	

OR, odds ratio; CI, confidence interval. Model 1: Adjusted for sex, age and ethics. Model 2: Adjusted for sex, age, ethics, smoking status, activity status, alcohol consumption, and BMI. Model 3: Adjusted for sex, age, ethics, smoking status, activity status, alcohol consumption, BMI, hypertension, cardiovascular disease, hyperglycemia, systemic inflammation index, UACR and eGFR

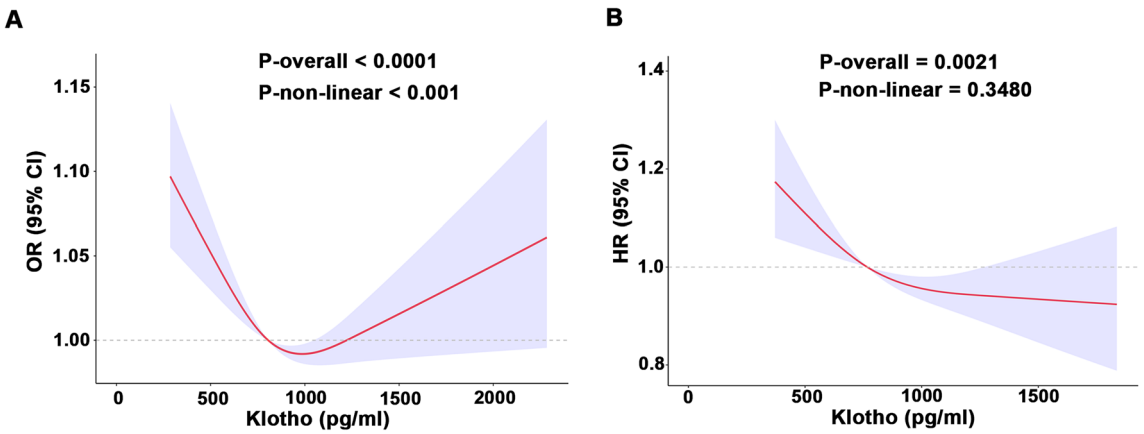

Fig. 2 **A** Restricted cubic spline curve for the association between the serum Klotho and the risk of DKD. **B** Restricted cubic spline curve for the association between the serum Klotho and the all-cause mortality of DKD. Red lines represent odds ratios, and the blue areas represent 95% confidence intervals

duration of 76 months. Multivariate Cox regression models indicated that each one-standard deviation increased in serum Klotho was associated with a 47% reduced risk of all-cause mortality for the DKD population in Model 1 (HR = 0.53, 95% CI 0.31–0.91). After adjusting for multiple variables, this correlation remained strong and statistically significant in both Model 2 (HR = 0.53, 95% CI 0.30–0.92) and Model 3 (HR = 0.48, 95% CI 0.29–0.82). In contrast to individuals in the first quartile of serum Klotho (Q1), those in the fourth quartile (Q4) showed significantly lower multivariate-adjusted HRs, as demonstrated by Model 1 (HR = 0.57, 95% CI 0.34–0.95, *P* for trend = 0.013), Model 2 (HR = 0.55, 95% CI 0.32–0.94, *P* for trend = 0.013), and Model 3 (HR = 0.48, 95% CI 0.28–0.83, *P* for trend = 0.003) (Table 3 and Table 4). The relationship between the serum Klotho and all-cause mortality was further assessed by RCS analysis (Fig. 2B). The RCS curves revealed an inverse association between serum Klotho levels and all-cause mortality in DKD patients (*P* for nonlinear = 0.3480). Subgroup analysis was also in accordance with these findings (Fig. 3). As depicted in Fig. 3, a subgroup analysis was performed considering age, sex, BMI, hypertension, hyperlipidemia, and CVD. Our subgroup analysis revealed significant interaction between serum Klotho and hypertension (*P* for interaction = 0.033). For those DKD patients with hypertension, the benefit of serum Klotho for lowering the risk of death was not significant. Furthermore, the inverse association between serum Klotho levels and all-cause mortality was consistent across other subgroups, without significant interaction differences observed (all *P* for interaction was less than 0.05).

Mediation analysis

Results of the causal mediation analysis investigating the role of SII in the association between serum Klotho and all-cause mortality of DKD patients are shown in Fig. 4 and Table S1 in Supplementary Material. We found that

Table 3 The baseline characteristics of participants with DKD according to the serum Klotho

	Overall	Serum klotho quartiles (pg/mL)				P value
Variables	**867**	**Q1 N = 202**	**Q2 N = 193**	**Q3 N = 235**	**Q4 N = 237**	
Age (years)	63.2 (10.1)	65.3 (9.0)	63.9 (10.3)	63.8 (9.8)	59.9 (10.3)	0.003
Sex (%)						0.4
Female	428 (48%)	98 (48%)	95 (55%)	120 (45%)	115 (45%)	
Male	439 (52%)	104 (52%)	98 (45%)	115 (55%)	122 (55%)	
Race						0.004
White	288 (61%)	74 (64%)	79 (70%)	73 (58%)	62 (54%)	
Black	229 (15%)	60 (16%)	38 (10%)	60 (15%)	71 (17%)	
Mexican	170 (10%)	31 (7.0%)	36 (9.5%)	46 (10%)	57 (15%)	
Hispanic	105 (6.2%)	15 (3.0%)	24 (5.9%)	33 (7.2%)	33 (8.9%)	
Others	75 (7.2%)	22 (10%)	16 (4.4%)	23 (9.5%)	14 (4.7%)	
Smoking status (%)						0.3
Current smoker	153 (17%)	36 (15%)	31 (13%)	36 (14%)	50 (24%)	
Former smoker	314 (41%)	73 (39%)	78 (44%)	91 (45%)	72 (34%)	
Never smoker	400 (43%)	93 (45%)	84 (43%)	108 (41%)	115 (42%)	
Alcohol intake (%)						0.4
Former	270 (27%)	63 (25%)	67 (29%)	64 (21%)	76 (33%)	
Heavy	119 (13%)	27 (11%)	27 (12%)	26 (12%)	39 (18%)	
Mild	235 (34%)	51 (32%)	48 (34%)	73 (41%)	63 (28%)	
Moderate	78 (10%)	22 (14%)	19 (11%)	18 (7.8%)	19 (7.9%)	
Never	165 (16%)	39 (18%)	32 (15%)	54 (18%)	40 (13%)	
Obesity (%)						0.9
Normal (BMI < 25)	112 (11%)	26 (11%)	23 (9.4%)	28 (11%)	35 (14%)	
Obesity (BMI > 30)	536 (65%)	121 (63%)	117 (64%)	153 (67%)	145 (66%)	
Overweight (25 < = BMI < = 30)	219 (24%)	55 (26%)	53 (27%)	54 (22%)	57 (21%)	
Physical activity (%)						0.7
High	73 (8.2%)	19 (9.0%)	14 (6.7%)	22 (10%)	18 (7.0%)	
Insufficient	794 (92%)	183 (91%)	179 (93%)	213 (90%)	219 (93%)	
Hypertension (%)						0.048
No	143 (19%)	24 (14%)	29 (20%)	36 (13%)	54 (27%)	
Yes	724 (81%)	178 (86%)	164 (80%)	199 (87%)	183 (73%)	
Hyperlipidemia (%)						0.4
No	94 (11%)	20 115%)	17 (8.3%)	30 (15%)	27 (11%)	
Yes	773 (89%)	182 (89%)	176 (92%)	205 (85%)	210 (89%)	
Cardiovascular disease (%)						0.3
No	679 (77%)	149 (72%)	155 (79%)	178 (74%)	197 (82%)	
Yes	188 (23%)	53 (28%)	38 (21%)	57 (26%)	40 (18%)	
UACR (mg/g)	286 (918)	346 (1,402)	258 (637)	260 (636)	279 (777)	0.091
SII	632 (400)	641 (419)	698 (410)	645 (451)	545 (288)	0.003
GFR (ml/min•1.73m^2)	69 (27)	60 (25)	66 (26)	70 (26)	82 (24)	< 0.001
Klotho (pg.ml)	814 (315)	498 (78)	674 (35)	833 (63)	1,247 (277)	< 0.001
Psychogenic death						0.065
No	522 (58%)	103 (48%)	113 (52%)	146 (64%)	160 (66%)	
Yes	71 (8.5%)	18 (9.8%)	16 (7.4%)	23 (9.5%)	14 (7.2%)	
Living status (%)						0.041
No	549 (61%)	113 (55%)	118 (54%)	151 (66%)	167 (70%)	
Yes	318 (38%)	89 (45%)	75 (46%)	84 (34%)	70 (30%)	

Data are shown as mean (SD), median (IQR), or n (%). Abbreviations: BMI, body mass index; UACR, urine albumin/creatinine ratio; DKD, diabetes kidney disease; SII, systemic immune-inflammation index; eGFR, estimated glomerular filtration rate; NA, not available

Table 4 HRs (95% CIs) for all-cause mortality according to the serum Klotho

	Model 1			Model 2			Model 3		
Characteristics	**HR**	**95% CI**	**P value**	**HR**	**95% CI**	**P-value**	**HR**	**95% CI**	**P value**
Continuous	0.53	0.31, 0.91	0.022	0.53	0.30, 0.92	0.025	0.48	0.29, 0.82	0.008
Category									
Q1	Ref			Ref			Ref		
Q2	1.04	0.56, 1.91	> 0.9	1.01	0.56, 1.85	> 0.9	1.01	0.56, 1.85	> 0.9
Q3	0.63	0.35, 1.16	0.14	0.65	0.36, 1.16	0.14	0.55	0.31, 1.97	0.04
Q4	0.57	0.34, 0.95	0.033	0.55	0.32, 0.94	0.028	0.48	0.28, 0.83	0.011
P for trend		0.013			0.013			0.003	

HR, hazard ratio; CI, confidence interval. Model 1: Adjusted for sex, age and ethics. Model 2: Adjusted for sex, age, ethics, smoking status, activity status, alcohol consumption, and BMI. Model 3: Adjusted for sex, age, ethics, smoking status, activity status, alcohol consumption, BMI, hypertension, cardiovascular disease, hyperglycemia, systemic inflammation index, UACR and eGFR

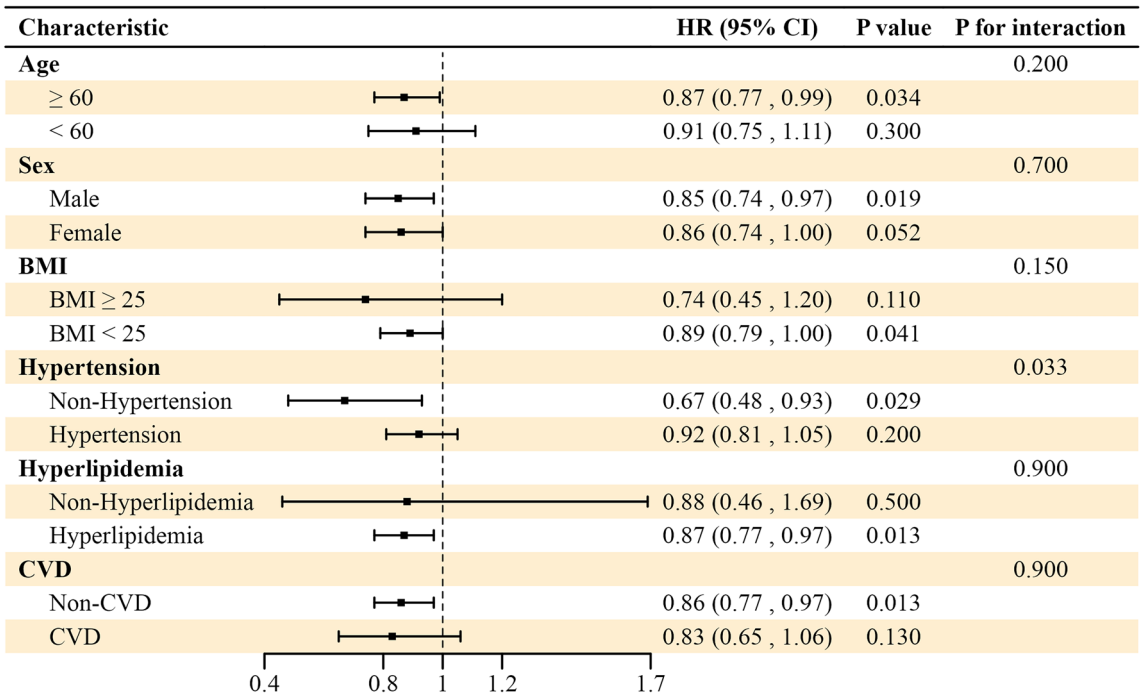

Fig. 3 Subgroup analysis of the association between serum Klotho and the all-cause mortality in DKD patients. HR, hazard ratio; CI, confidence interval

both direct and indirect effects played a significant role in the association of serum Klotho and all-cause mortality of DKD patients. Ultimately, Fig. 4 shows that 5.67% (95% CI 3.88%-7.00%) of the observational association of serum Klotho with risk of all-cause mortality was mediated through SII.

Discussion

This study explored the associations between serum Klotho levels and the prevalence of DKD, as well as all-cause mortality in DKD patients, using data from NHANES 2007–2016. We identified a nonlinear L-shaped relationship between serum Klotho levels and DKD prevalence in diabetic individuals, indicating that lower serum Klotho levels were linked to a higher risk of developing DKD. Additionally, we found a linear association between serum Klotho levels and all-cause mortality in DKD patients, with those having lower Klotho levels being at greater risk of mortality. These findings remained robust even after adjusting for potential confounders and were further supported by subgroup analyses. This suggests that serum Klotho

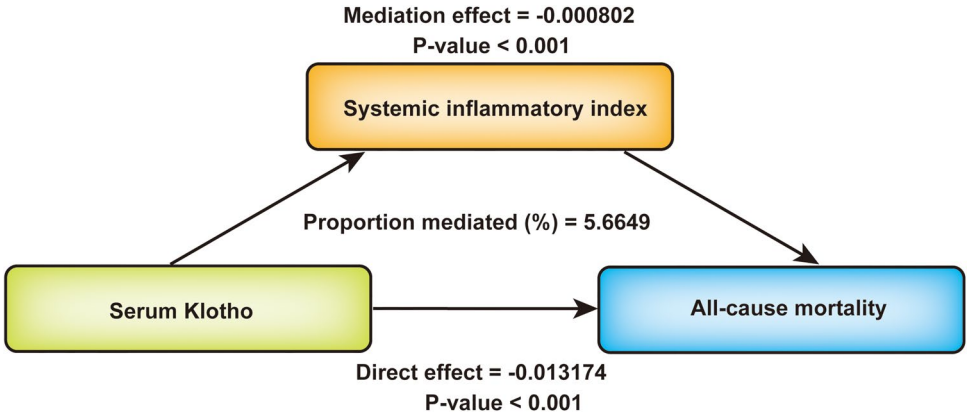

Fig. 4 The mediation effect of SII on the relationship between serum Klotho and all-cause mortality. SII, systemic immune-inflammation index

may serve as an emerging biomarker for DKD and its complications.

Klotho is well-recognized for its anti-aging properties, with soluble Klotho-produced by cleavage of the membrane-bound form-exerting physiological effects throughout the body. The Klotho gene was first cloned in mice by Kuro et al. [36], who found that Klotho knockout mice exhibited signs of premature aging, such as atherosclerosis and metabolic abnormalities. Subsequent studies showed that Klotho is primarily expressed in the kidney and brain and is closely related to conditions such as lipid metabolism disorders, kidney damage, and cardiovascular diseases [37–39]. Recent research has also suggested that Klotho helps regulate fibroblast growth factors, which are involved in tissue-specific energy metabolism [40–43]. Notably, numerous studies have demonstrated the relationship between Klotho and diabetes [44, 45], as well as cardiovascular disease and hypertension [46–48], but few have examined its specific role in DKD, despite smaller cohort studies suggesting that low Klotho levels may be a novel biomarker for DKD [12, 17, 49].

Our study is the first to demonstrate a strong association between low serum Klotho levels and increased DKD prevalence using a nationally representative sample. Previous evidence supports the role of Klotho in DKD development [12, 45, 50–53], with Klotho acting as a renoprotective factor by mitigating albuminuria [16–20], renal function decline [12, 13, 20, 21], and furthermore, kidney-related death [22]. Moreover, Janaka et al. found that lower serum Klotho levels were linked to a nearly fourfold increase in the cumulative incidence of renal function decline over a nine-year period, reinforcing the notion that Klotho is a reliable predictor of DKD prognosis [13]. Li and colleagues also showed that lower serum Klotho levels in DKD patients were associated with an elevated risk of cardiovascular morbidity and all-cause mortality [22]. Collectively, these findings suggest that a reduction in Klotho levels may signal the progression of DKD, highlighting its involvement in multiple disease mechanisms [12].

Furthermore, our study revealed a significant linear relationship between serum Klotho levels and all-cause mortality in DKD patients. This is consistent with findings from other studies that have linked low Klotho levels to increased mortality. For instance, Chuang et al. [14] found a nonlinear association between serum Klotho and mortality in middle-aged and elderly populations, while Han et al. [54] reported an L-shaped association between Klotho and mortality in CKD patients. Single-center studies have also demonstrated Klotho's predictive value for cardiovascular and all-cause mortality in CKD patients, although these studies were limited by small sample sizes and lack of adjustment for confounders [55–57]. While some inconsistencies exist in the literature [23], our findings align with the majority of studies linking low Klotho levels to higher mortality risk, offering important insights into the prognostic value of Klotho in DKD.

Moving forward, we aim to delve deeper into the molecular mechanisms underlying the impact of the serum Klotho on DKD and mortality. Potential mechanisms involve its roles in reducing inflammation and oxidative stress, preventing fibrosis, protecting endothelial function, inhibiting vascular calcification, regulating metabolism, and maintaining calcium and phosphate balance. Additionally, Klotho may influence cell fate by modulating autophagy, apoptosis, and pyroptosis pathways. Particularly, the downregulation of renal Klotho mRNA and protein expression has been shown to increase kidney inflammation in diabetic mice [58]. This aligns with evidence that reduced Klotho expression in

T-helper cells of elderly and rheumatoid arthritis patients coincides with decreased CD28 costimulatory molecules and elevated levels of proinflammatory tumor necrosis factor-α (TNF-α) [58]. Klotho also appears to enhance anti-inflammatory responses. In human monocytes stimulated by lipopolysaccharide to mimic immunosenescence, Klotho expression stimulates interleukin 10 secretion, a cytokine that suppresses various proinflammatory molecules including TNF-α [59]. Mechanistically, both transmembrane and soluble Klotho inhibit TNF-α-induced activation of nuclear factor kappa B by blocking RelA phosphorylation and its recruitment to the promoters of inflammatory genes like interleukin 6, interleukin 8, monocyte chemoattractant protein 1, and C–C motif chemokine ligand 5 [60]. This process helps prevent the production of these proinflammatory cytokines, thereby protecting kidney tissues from inflammation-related damage [60].

In our analysis, we employed mediation analysis to elucidate the underlying mechanisms linking serum Klotho levels to all-cause mortality in DKD patients. Specifically, the direct effect refers to the relationship between serum Klotho and all-cause mortality, while the indirect effect is mediated through systemic inflammation, assessed via the SII, a well-established marker of immune status and inflammatory burden. The proportion mediated quantifies the extent to which SII contributes to this relationship. Notably, recent analyses of NHANES data revealed a negative linear relationship between serum Klotho levels and the SII [61–64]. Additionally, previous studies from our group and others have consistently established a strong association between elevated SII and an increased risk of cardiovascular and all-cause mortality in DKD patients [34, 65, 66]. Our mediation analysis further reinforces these findings, as it demonstrated that including SII as a mediator in the model revealed a significant association between low serum Klotho levels and higher all-cause mortality in DKD patients. These results provide deeper insight into the intricate interplay between Klotho, inflammation, and mortality in this patient cohort, highlighting he potential role of systemic inflammation as a key mechanism underlying this relationship.

Despite the strengths of our study, including the use of a large, representative sample, there are some limitations. First, while we adjusted for many potential confounders, residual confounding cannot be ruled out. Second, a causal relationship between serum Klotho and DKD prevalence or mortality could not be established. Future randomized controlled trials or Mendelian randomization studies are needed to clarify causality. Third, the NHANES database does not allow for detailed exploration of DKD subtypes or the episodic versus persistent nature of the disease. Fourth, the study was limited to U.S. populations, and additional multicenter studies are necessary to evaluate external validity. Lastly, the NHANES questionnaire's reliance on self-reported data introduces the possibility of misclassification, and future studies should investigate the influence of Klotho on cause-specific mortality in DKD.

Conclusions

Our study found that serum Klotho levels were negatively correlated with the prevalence of DKD and the all-cause mortality in patients with DKD. Monitoring Klotho levels could be critical for reducing premature mortality in this patient population. As all-cause mortality represents a significant risk when serum Klotho levels decline, early intervention becomes essential. These findings contribute to the growing body of research on Klotho and underscore the need for future studies to confirm causal relationships, identify therapeutic targets to elevate Klotho levels, and evaluate the potential for clinical applications.

Abbreviations

BMI	Body mass index
CI	Confidence interval
CKD	Chronic kidney disease
CVD	Cardiovascular disease
DKD	Diabetic kidney disease
eGFR	Estimated glomerular filtration rate
ESRD	End-stage renal disease
HR	Hazard ratio
NHANES	National Health and Nutrition Examination Survey
OR	Odds ratio
Q	Quartiles
RCS	Restricted cubic spline
SII	Systemic immune-inflammation index
TNF-α	Tumor necrosis factor-α
UACR	Urine albumin/creatinine ratio

Supplementary Information

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Supplementary material 1.

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Author contributions

PX, DW, MZ and BL conceived and designed the study; PX, DW, MZ, LJ, YQ, YW, SY, MHZ, LT, SC, QL, HP, and SL analyzed the data; PX, DW, MZ, JL, QW, LJ, XW and BL drafted the original manuscript and created the figures; LT, SC, QL, HP, SL, and KWC helped data collection, and provided technical or material support; SCWT and WC reviewed and edited the writing. All authors approved the final version of the manuscript.

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Availability of data and materials
No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate
The National Health and Nutrition Examination Survey (NHANES) has been approved by the National Center for Health Statistics Ethics Review Board, and all participants provided informed written consent at enrollment.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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