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Association between serum α-klotho levels and the incidence of diabetic kidney disease and mortality in type 2 diabetes: evidence from a Chinese cohort and the NHANES database

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Abstract

Background The α-klotho is crucial in diabetes and its related complications. This study seeks to explore the link between α-klotho levels and the risk of diabetic kidney disease (DKD) as well as all-cause and cardiovascular mortality among individuals with type 2 diabetes mellitus (T2DM).

Methods The investigation involved 126 Chinese T2DM patients and 4,451 individuals from the National Health and Nutrition Examination Survey (NHANES) database. To evaluate the relationship between α-klotho levels and DKD risk, multivariate logistic regression was utilized. Additionally, restricted cubic spline (RCS) regression analysis was conducted to examine the nonlinear relationship between α-klotho levels and DKD incidence. RCS analysis was employed to explore the correlation between α-klotho and both all-cause and cardiovascular mortality.

Results In the Chinese cohort, α-klotho levels were notably elevated in T2DM group compared to DKD group. The NHANES data revealed a significant inverse relationship between α-klotho levels and DKD risk. Nonlinear analysis further illustrated a substantial nonlinear connection between α-klotho levels and DKD risk. Serum α-klotho levels below 880.78 pg/mL were linked to increased DKD risk in T2DM patients. When compared to the T2DM group, the DKD group had markedly higher all-cause and cardiovascular mortality rates, with the α-klotho low group (e.g., Q1) exhibiting lower survival compared to other groups. Cox regression findings indicated that elevated α-klotho levels could mitigate all-cause mortality in T2DM patients. The relationship between α-klotho levels and all-cause mortality

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was also nonlinear, with the minimal risk found at α-klotho levels between 776.95 pg/mL and 812.69 pg/mL, varying by gender.

Conclusion There exists a notable association between α-klotho levels and DKD risk, along with mortality in T2DM patients, with varying effects based on gender. These results highlight the potential importance of α-klotho as both a biomarker and a therapeutic target.

Keywords α-klotho, Type 2 diabetes, Diabetic kidney disease, All-cause mortality, Cardiovascular mortality

Introduction

Diabetes, a growing metabolic disorder, is increasingly prevalent each year. The International Diabetes Federation estimates that in 2021, approximately 537 million individuals aged 20–79 globally were affected by diabetes, and this trend is projected to rise in the coming years [1]. The incidence of cardiovascular events in diabetic patients is significantly greater than in non-diabetic individuals, particularly among those with chronic kidney disease (CKD) [2–4]. Diabetic kidney disease (DKD) remains one of the most prevalent complications of diabetes; notably, its prevalence has not declined over the past three decades, unlike many other complications [5]. During the disease process, the interplay between diabetes, cardiovascular disease, and kidney disease creates a vicious cycle that significantly increases the risk of all-cause and cardiovascular mortality [6, 7]. The concept of cardiovascular-kidney-metabolism syndrome represents a deeper understanding within the medical community of the intricate relationships between cardiovascular, kidney, and metabolic diseases [8].

α-klotho is a transmembrane protein predominantly expressed in the kidney and heart; its extracellular domain is released from the cell surface into the bloodstream, participating in oxidative stress response, inflammation, and vascular protection, and is closely related to aging phenotypes and lifespan [9, 10]. It is considered as an emerging biomarker and therapeutic target for cardiovascular, kidney and metabolic diseases [11–13]. Soluble α-klotho levels are reduced in DKD patients, and α-klotho levels are negatively correlated with the annual decline in estimated glomerular filtration rate (eGFR) and the occurrence of albuminuria [14, 15], making it a potential biomarker for the early diagnosis of DKD [16]. Emerging treatments for DKD, such as sodium-glucose co-transporter-2 inhibitors (SGLT2i), have demonstrated the ability to prevent the downregulation of α-klotho expression in renal tubular epithelial cells induced by elevated glucose levels, thereby enhancing α-klotho availability and promoting renal and cardiac protection in diabetic patients [17]. Preclinical studies in animal models have shown that augmenting α-klotho expression or mimicking its effects may open new pathways for preventing or mitigating CKD progression [18].

Numerous investigations indicate a significant association between serum α-klotho levels and the prevalence and mortality of metabolic syndrome, cardiovascular diseases, and CKD [13, 19–21]. Recent meta-analyses, which included 14 cohort studies, have confirmed that lower serum α-klotho levels are a robust predictor of heightened risk for all-cause mortality, cardiovascular mortality, and progression to end-stage renal disease (ESRD) in CKD patients [22]. However, in the context of diabetes, kidney and cardiovascular damage tends to be more pronounced, and the relationship between serum α-klotho levels and the risks of DKD and mortality in patients with type 2 diabetes mellitus (T2DM) remains to be elucidated. Accordingly, the objective of this study is to examine the associations between serum α-klotho levels and the incidence of DKD, as well as all-cause and cardiovascular mortality in individuals with T2DM.

Method

Study design and population

This study analyzed two distinct population datasets: one from a cohort of Chinese patients and the other from the NHANES database in the United States.

Chinese cohort

The Chinese cohort comprised hospitalized patients with T2DM and DKD at Dongzhimen Hospital of Beijing University of Chinese Medicine from May 2021 to March 2023. Approval was granted by the Ethics Committee of Dongzhimen Hospital (Approval No: 2022DZMEC-062-03), and informed consent was obtained from all participants. The diagnostic criteria for T2DM followed the "Chinese Guidelines for the Prevention and Treatment of Type 2 Diabetes" (2020 Edition) [23]; the DKD diagnostic criteria were based on the 2007 National Kidney Foundation (NKF-KD/OQI) guidelines [24], the 2020 Clinical Practice Guidelines for the Evaluation and Management of Chronic Kidney Disease published by the Kidney Disease: Improving Global Outcomes (KDIGO) [25] and the 2021 "Clinical Guidelines for the Diagnosis and Treatment of Diabetic Kidney Disease in China"[26]. DKD was defined in T2DM patients as having an eGFR < 60 ml/min/1.73 m^2 or the presence of albuminuria (urine albumin to creatinine ratio (UACR) ≥ 30 mg/g). Exclusion

criteria included severe liver disease, cancer, and other significant comorbidities.

NHANES database

NHANES is a national survey conducted by the Centers for Disease Control and Prevention (CDC) to evaluate the health and nutritional status of the non-institutionalized U.S. population. Utilizing a stratified multi-stage probability sampling method [27]. NHANES databse has received approval from relevant ethics committees. This analysis included data from five consecutive NHANES cycles (2007–2016), encompassing 50,588 participants. Inclusion criteria required adults aged ≥ 20 years diagnosed with T2DM, confirmed by a physician or laboratory results indicating fasting blood glucose (FPG) levels ≥ 7.0 mmol/L or glycosylated hemoglobin (HbA1c) levels ≥ 6.5%. Exclusion criteria consisted of cancer patients, pregnant women, and individuals without α-klotho or creatinine data. DKD was defined in diabetic patients as an eGFR < 60 ml/min/1.73 m^2 or the presence of albuminuria, indicated by a UACR ≥ 30 mg/g. The creatinine equation from the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) was employed to estimate eGFR [28] (Fig. 1).

Study variables

α-klotho measurement

In the Chinese cohort, all participants had fasting venous blood collected on the morning of the second day after admission. The blood was placed in anticoagulant tubes and centrifuged at 3000 *r*/min for 10 min at 4 ℃. The upper serum layer was collected and stored in EP tubes, and α-klotho was measured using enzyme-linked immunosorbent assay (ELISA). In the NHANES laboratory, commercially available ELISA kits produced by IBL International were used for measurement.

Covariates

In the Chinese cohort, biochemical measurements were taken from baseline venous blood samples at the time of admission. The diagnosis of cardiovascular disease (CVD) is based on the medical record system, including heart failure, angina pectoris, myocardial infarction, or coronary artery disease. Covariates considered in the NHANES database analysis included demographics (age, gender, race, socioeconomic factors (education level [high school or less, some college, and college graduate or above] [29], marital status, family poverty income ratio [PIR]), body mass index (BMI), lifestyle factors (drinking state [never, low to moderate, and heavy], smoking status [never, current, and former]), low-density lipoprotein (LDL), Triglycerides (TG), high-density lipoprotein (HDL), total cholesterol (TC) and uric acid (UA). Medical history variables included hypertension and CVD, with hypertension defined as self-reported hypertension, physician-diagnosed hypertension, or the need for medication. CVD was determined based on self-reported diagnoses of congestive heart failure, angina, myocardial infarction, or coronary heart disease.

Missing data

In the Chinese cohort, there is no missing data for categorical variables. For continuous variables, missing values are imputed using the mean. For the NHANES database, the approach to handling missing data varied by the type of variable: for missing categorical variables, we created a separate category to ensure all samples were included in the analysis, thus preventing a reduction in sample size due to missing values. For missing continuous variables, we used mean imputation.

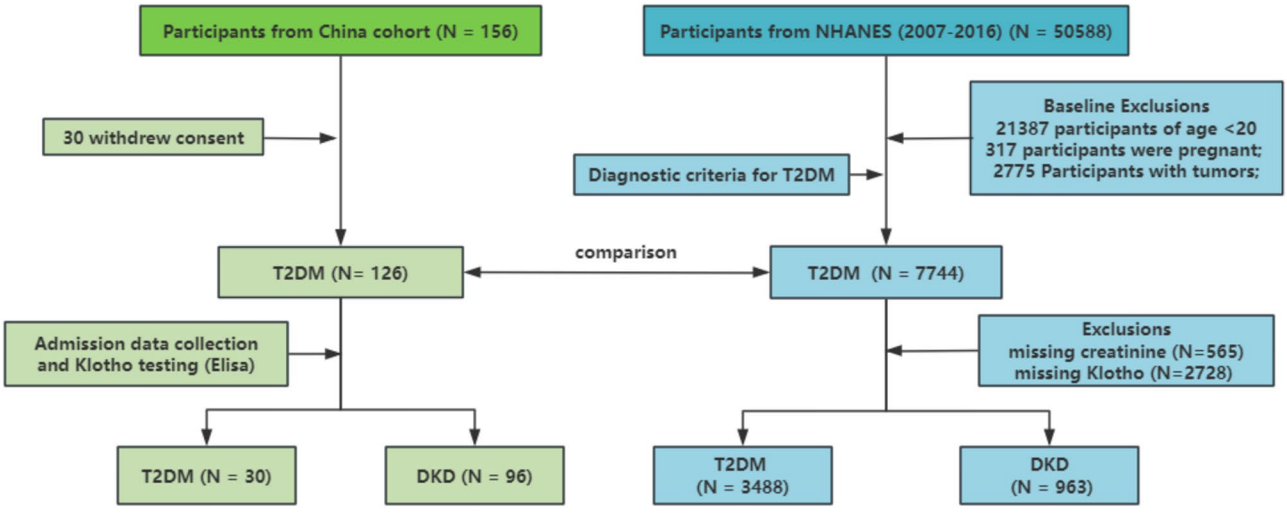

Fig. 1 Study flowchart

Statistical analysis

Statistical analyses were conducted using R Studio 4.3.0, Stata 17, and EmpowerStats software. To accommodate the complex and multi-stage survey design of NHANES, we applied the recommended weighting methods. Weighted chi-square tests were utilized for categorical data, while weighted Student's t-tests were used for continuous data. The association between serum α-klotho levels and the presence of DKD was assessed through weighted logistic regression. Model 1 was unadjusted, while Model 2 was adjusted for gender, age, and race. Model 3 included the adjustments from Model 2 and further controlled for education, marital status, PIR, BMI, drinking status, smoking status, hypertension, CVD, HDL, LDL, TC, TG and UA. To explore the dose-response relationship between serum α-klotho levels and DKD risk in patients with T2DM, as well as all-cause and cardiovascular mortality associated with T2DM, restricted cubic spline (RCS) regression was employed. Additionally, weighted Cox proportional hazards regression models were used to evaluate the association between α-klotho levels and mortality outcomes in this population. Two-sided $P < 0.05$ were considered statistically significant.

Result

Results in Chinese population

Baseline characteristics of the chines population

In the Chinese cohort, 126 T2DM patients were included, consisting of 80 males and 46 females, with a mean age of 59.50 ± 11.29 years. Participants were categorized into two groups: T2DM ($n = 30$) and DKD ($n = 96$) (Table 1). The DKD group exhibited significantly lower levels of FPG and eGFR, while CREA levels were markedly elevated compared to the T2DM group. The cardiovascular morbidity rate in the T2DM group is lower than that in the DKD group. Serum α-klotho levels were significantly higher in T2DM patients($P < 0.001$), and quartile distribution of α-klotho levels also differed significantly between the groups ($P < 0.001$), indicating a potential link between α-klotho and the progression of diabetes and its complications. To further evaluate α-klotho as a biomarker for DKD in T2DM, receiver operating characteristic (ROC) curve analysis was conducted, revealing an area under the curve (AUC) of 0.731 (Fig. 2), which suggests great sensitivity and specificity for identifying DKD patients.

Table 1 Baseline characteristics of the study participants in Chinese Corhort

Variables	Overall	T2DM	DKD	*P* value
	(*n* = 126)	(*n* = 30)	(*n* = 96)	
Age (years)	59.500 ± 11.290	58.800 ± 11.616	59.719 ± 11.240	0.857
Gender (%)				0.374
Female	46 (36.508%)	13 (43.333%)	33 (34.375%)	
Male	80 (63.492%)	17 (56.667%)	63 (65.625%)	
BMI (kg/m^2)	25.808 ± 3.680	25.591 ± 3.424	25.876 ± 3.771	0.525
SBP (mmHg)	138.889 ± 19.318	134.800 ± 18.357	140.167 ± 19.526	0.159
DBP (mmHg)	78.365 ± 12.462	81.033 ± 14.371	77.531 ± 11.762	0.166
FPG (mmol/L)	10.334 ± 5.392	11.904 ± 4.401	9.843 ± 5.596	0.007
eGFR (ml/min/1.73m^2)	61.295 ± 41.165	94.228 ± 16.943	51.003 ± 41.140	< 0.001
CREA (μmol/L)	211.671 ± 221.242	67.640 ± 17.948	256.680 ± 236.027	< 0.001
UA (μmol/L)	381.098 ± 97.354	353.593 ± 91.642	389.693 ± 97.947	0.082
TP (g/L)	66.378 ± 8.246	71.040 ± 6.765	64.906 ± 8.152	< 0.001
ALB (g/L)	38.344 ± 6.366	42.607 ± 3.871	37.011 ± 6.420	< 0.001
TG (mmol/L)	2.282 ± 1.870	2.261 ± 1.941	2.289 ± 1.857	0.921
LDL (mmol/L)	2.847 ± 1.040	2.904 ± 1.101	2.829 ± 1.025	0.798
HDL (mmol/L)	1.208 ± 0.334	1.180 ± 0.272	1.217 ± 0.353	0.870
Cardiovascular disease(%)				0.017
NO	87 (69.048%)	26 (86.667%)	61 (63.542%)	
Yes	39 (30.952%)	4 (13.333%)	35 (36.458%)	
Serum α-klotho levels(pg/mL)	412.021 ± 191.994	511.957 ± 142.174	380.791 ± 195.429	< 0.001
Serum α-klotho quartile				< 0.001
Q1	32 (25.397%)	0 (0.000%)	32 (33.333%)	
Q2	31 (24.603%)	5 (16.667%)	26 (27.083%)	
Q3	31 (24.603%)	13 (43.333%)	18 (18.750%)	
Q4	32 (25.397%)	12 (40.000%)	20 (20.833%)	

BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; FPG, fasting plasma glucose; eGFR, estimated glomerular filtration rate; CREA, creatinine; TG, triglycerides; HDL-C, high-density lipoprotein cholesterol; TP, total protein

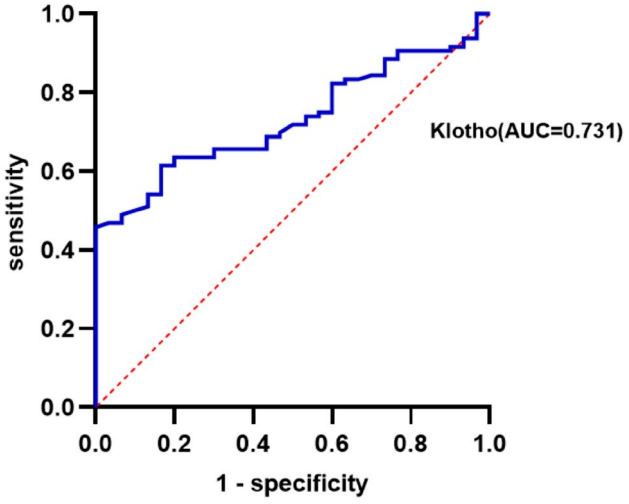

Fig. 2 ROC curve of α-klotho in patients with T2DM and DKD

Table 2 Logistic regression analysis of α-klotho and DKD risk

	OR	95%CI	*P* value
Model 1	0.996	0.994–0.999	0.002
Model 2	0.997	0.994–0.999	0.006
Model 3	0.997	0.995-1.000	0.049

CI, confidence interval. Model 1: Adjusted for age and gender; Model 2 further adjusted for BMI, CVD, SBP, and DBP; Model 3 further adjusted for UA, TP, ALB, TG, LDL and HDL based on Model 2

Logistic regression analysis of α-klotho and DKD risk

We further analyzed the relationship between α-klotho levels and the risk of DKD occurrence through logistic regression analysis, adjusting for confounding factors. The β values were negative in all models, indicating a negative correlation between α-klotho levels and DKD risk. In Model 3, after adjusting for BMI, CVD, SBP, DBP, and laboratory indices, the results showed a significant negative correlation between α-klotho levels and DKD occurrence (β = -0.003, $P = 0.049$). The OR value was 0.997 (95% CI: 0.995-1.000), indicating that for every unit decrease in α-klotho, the risk of DKD increases by approximately 0.3%. These results suggest that α-klotho levels may play a protective role in the occurrence of DKD, and a decrease in α-klotho levels may be associated with an increased risk of DKD (See Table 2).

Results from NHANES databases

Study population description

This study included a total of 4,451 patients with T2DM, of whom 963 were diagnosed with DKD. Age significantly impacted DKD prevalence, the DKD group being notably older than T2DM group; a higher prevalence was observed among patients aged 60 and older. Other factors influencing DKD incidence included educational level, marital status, PIR. The obesity rate was significantly elevated in the DKD group compared to the T2DM group, and the prevalence of hypertension and CVD was also markedly higher ($P < 0.001$). Patients with DKD exhibit lower levels of LDL and TC, higher levels of TG and UA, as well as slightly lower HDL levels. Serum α-klotho levels were significantly lower in DKD group, corroborating findings from the Chinese cohort. The prevalence of DKD in the lowest quartile group of α-Klotho (Q1) was 31.774%, which showed an increasing trend compared with other quartile groups. This suggests that we may need to pay attention to the potential association between α-klotho levels and DKD (Table 3).

Associations between α-klotho and the risk of DKD

Notably, 21.6% of T2DM patients had DKD. Serum α-klotho levels were categorized into quartiles, and weighted logistic regression analyzed DKD risk. In Model 1 (unadjusted), the OR for α-klotho levels in Q2, Q3, and Q4 compared to Q1 were 0.783 (95% CI: 0.600-1.022, $P = 0.072$), 0.447 (95% CI: 0.338–0.592, $P < 0.001$), and 0.635 (95% CI: 0.484–0.833, $P = 0.001$), respectively. Model 2 further adjusted for gender, age, and race based on Model 1. Compared to Q1, the OR for quartiles Q2, Q3, and Q4 were 0.924 (95% CI: 0.705–1.211, $P = 0.566$), 0.520 (95% CI: 0.390–0.694, $P < 0.001$), and 0.751 (95% CI: 0.567–0.996, $P = 0.047$), respectively. In Model 3, additional adjustments were made for various factors including education, marital status, PIR, BMI, drinking status, smoking status, hypertension, CVD, LDL, TG, HDL, UAmg and TG. In this model, the OR for Q2, Q3, and Q4 compared to Q1 were 1.073(95% CI: 0.652–1.768, $P = 0.781$), 0.531(95% CI: 0.315–0.897, $P = 0.018$), and 0.899(95% CI: 0.528–1.531, $P = 0.696$), respectively. After adjustment for these variables, the risk of developing DKD in patients from Q3 groups remained significantly lower (Table 4).

Multivariable logistic regression identified several risk factors for DKD, including advanced age (≥ 60 years), being non-Hispanic White, having hypertension, CVD, and heavy alcohol consumption (Fig. 3).

The nonlinear relationship between serum α-klotho levels and DKD risk

This study examined the nonlinear relationship between α-klotho levels and the risk of DKD using the RCS model. For the overall population (Fig. 4A), there was a significant negative correlation between α-klotho levels and DKD risk ($P_{\text{non\_linear}} = 0.0001$), indicating that increased α-klotho levels were associated with a reduced risk of DKD, reaching a minimum risk at approximately 800.78 pg/mL. Beyond this point, while the risk slightly increased, it remained within a lower risk range. Gender-based analysis showed similar trends: in females (Fig. 4B), higher α-klotho levels significantly reduced DKD risk ($P_{\text{non\_linear}} = 0.0023$), with a critical value of 816.02 pg/mL. In males (Fig. 4C), elevated α-klotho levels were also

Table 3 Demographic characteristics of patients in NHANES Databse

Characteristic	Overall	T2DM	DKD	*P* value
	(*n* = 4,451)	(*n* = 3,488)	(*n* = 963)	
Age (years)	56.468 ± 10.286	54.954 ± 9.707	63.291 ± 10.059	< 0.001
Age group (%)				< 0.001
<60 years	61.491	67.68	33.587	
≥ 60 years	38.509	32.32	66.413	
Gender (%)				0.057
Female	49.502	48.829	52.538	
Male	50.498	51.171	47.462	
Race (%)				0.147
Mexican American	8.928	8.759	9.688	
Other Hispanic	5.564	5.596	5.420	
Non-Hispanic White	66.220	66.795	63.625	
Non-Hispanic Black	11.753	11.218	14.163	
Other/Multi-Racial	7.536	7.632	7.105	
Education (%)				< 0.001
High school or less	45.667	43.741	54.35	
Some College	29.446	30.173	26.168	
Graduate or above	24.853	26.073	19.353	
Others	0.034	0.013	0.129	
Marital status (%)				0.003
Married/Living with partner	68.345	69.402	63.577	
Widowed/Divorced/Separated	23.365	22.249	28.398	
Never Married	8.182	8.232	7.956	
Others	0.108	0.117	0.069	
PIR (%)				< 0.001
0-1.3	21.775	19.392	32.298	
1.3–3.5	33.625	33.154	35.705	
> 3.5	44.600	47.454	31.997	
BMI (%)				< 0.001
Underweight (< 18.5 kg/m^2)	0.886	0.933	0.668	
Normal (18.5 to < 25 kg/m^2)	18.898	19.956	13.988	
Overweight (25 to < 30 kg/m^2)	31.329	32.714	24.901	
Obese (≥ 30 kg/m2)	48.888	46.397	60.443	
Smoking Status(%)				< 0.001
Never smoker	49.191	49.131	49.459	
Former smoker	31.975	31.083	35.994	
Current smoker	18.835	19.786	14.547	
Drinking state (%)				< 0.001
Non-drinker	53.172	60.024	22.276	
Low to moderate drinker	21.941	17.062	43.937	
Heavy drinker	19.181	16.930	29.330	
Not clear	5.706	5.983	4.457	
Hypertension (%)				< 0.001
No	48.395	53.054	27.387	
Yes	51.605	46.946	72.613	
Cardiovascular disease(%)				< 0.001
No	86.595	89.707	72.561	
Yes	13.405	10.293	27.439	
LDL (mg/dL)	112.865 ± 35.912	114.859 ± 34.924	102.946 ± 38.979	< 0.001
TG(mg/dL)	188.667 ± 165.101	182.247 ± 162.488	217.616 ± 173.476	< 0.001
HDL(mg/dL)	50.059 ± 16.558	50.354 ± 15.689	48.725 ± 19.958	0.011
UA(mg/dL)	5.647 ± 1.470	5.496 ± 1.356	6.325 ± 1.745	< 0.001
TC(mg/dL)	195.148 ± 45.390	196.906 ± 43.762	187.221 ± 51.361	< 0.001

Table 3 (continued)

Characteristic	Overall	T2DM	DKD	*P* value
	(*n* = 4,451)	(*n* = 3,488)	(*n* = 963)	
Glycohemoglobin(%)	6.471 ± 1.579	6.281 ± 1.422	7.329 ± 1.927	< 0.001
eGFR (ml/min/1.73m^2)	86.000 ± 20.698	91.197 ± 15.052	62.568 ± 25.841	< 0.001
Serum α-klotho levels(pg/mL)	858.164 ± 300.639	858.164 ± 300.639	814.968 ± 319.005	< 0.001
Serum α-klotho quartile				< 0.001
Q1 (156.6–649.5)	24.027	22.310	31.774	
Q2 (649.7–802.4)	26.132	25.598	28.54	
Q3 (802.8–1006.9)	25.974	27.807	17.712	
Q4 (1007–5038.3)	23.866	24.286	21.974	

Note: data are presented as weighted percentages (%) or mean ± SE. PIR: poverty income ratio; BMI: body mass index; LDL: low-density lipoprotein; TG: Triglycerides; HDL: high-density lipoprotein; UA: uric acid: TC: total cholesterol; eGFR: estimated glomerular filtration rate

Table 4 Associations between α-klotho and the risk of DKD

Characteristic	Model 1			Model 2			Model 3		
	OR	95% CI	*p*-value	OR	95% CI	*p*-value	OR	95% CI	*p*-value
α-klotho tertiles									
Q1	Ref			Ref			Ref		
Q2	0.783	0.600-1.022	0.072	0.924	0.705–1.211	0.566	1.073	0.652–1.768	0.781
Q3	0.447	0.338–0.592	< 0.001	0.520	0.390–0.694	< 0.001	0.531	0.315–0.897	0.018
Q4	0.635	0.484–0.833	0.001	0.751	0.567–0.996	0.047	0.899	0.528–1.531	0.696

CI, confidence interval. Model 1 is unadjusted; Model 2 is adjusted for gender, age, and race; Model 3 is further adjusted for education, marital status, PIR, BMI, drinking status, smoking status, hypertension, CVD, LDL, HDL, TG, TC and UA

linked to a decreased risk ($P_{\text{non\_linear}}$ = 0.0084), with a critical point of 789.35 pg/mL, slightly lower than that for females. Overall, α-klotho levels demonstrated a negative nonlinear relationship with DKD risk in T2DM, with variations between genders.

Kaplan–Meier survival curves of α-klotho and all-cause and cardiovascular mortality

During a median follow-up of 120 months, 720 participants (16.18%) died, with 239 (5.37%) deaths attributed to CVD. The survival rate was significantly lower in the DKD group compared to T2DM group (Fig. 5A). Patients in higher α-klotho quartiles (Q3, Q4) had better survival rates than those in lower quartiles (Q1, Q2) (Fig. 5C). The DKD group exhibited a markedly higher cardiovascular mortality rate compared to the T2DM group, with a hazard ratio (HR) of 4.42 (95% CI: 3.41, 5.73). Additionally, higher α-klotho levels were associated with a significantly reduced cardiovascular mortality risk, with an HR of 0.82 (95% CI: 0.73, 0.92) in the Q4 group versus Q1. Overall, DKD patients showed poor prognoses for both all-cause and cardiovascular mortality, while higher α-klotho levels correlated with improved survival outcomes.

Association between α-klotho and all-cause mortality and cardiovascular mortality

A weighted Cox regression model was employed to evaluate the relationship between α-klotho levels and both all-cause and cardiovascular mortality (Table 5). In the analysis of all-cause mortality, Model 3 showed that α-klotho levels in the Q2 and Q3 groups were associated with a lower risk of death. The HRs for the Q2 and Q3 groups were 0.608 (95% CI: 0.402–0.921, $P = 0.019$) and 0.646 (95% CI: 0.442–0.944, $P = 0.024$), respectively, indicating that higher α-klotho levels were significantly associated with a reduced risk of all-cause mortality. However, the highest α-klotho level (Q4 group) did not show a significant reduction in risk. For cardiovascular mortality, in the overall population, the risk ratios for the Q2, Q3, and Q4 groups were significantly lower than for the Q1 group, but none reached statistical significance. Regarding the gender effect, the results were more significant in females than in males. For example, in the analysis of all-cause mortality, females in the Q2 group (HR = 0.299, 95% CI: 0.160–0.559, $P < 0.001$) and Q3 group (HR = 0.466, 95% CI: 0.274–0.791, $P = 0.005$) both showed a lower risk of death, whereas males did not show similar statistical significance. In the analysis of cardiovascular mortality, females in the Q2 group and Q3 group also showed lower risk (Q2 group HR = 0.299, 95% CI: 0.160–0.559, $P < 0.001$; Q3 group HR = 0.466, 95% CI: 0.274–0.791, $P = 0.005$), suggesting that α-klotho may have a stronger protective effect in females. Overall, α-klotho had a more significant impact on all-cause mortality, with a smaller effect on cardiovascular mortality and a clear gender difference.

Variable	Reference	Group	OR.95.CI.	P.value
α-klotho	Q1	Q2	1.073(0.652-1.768)	0.781
		Q3	0.531(0.315-0.897)	0.018
		Q4	0.899(0.528-1.531)	0.696
Age	<60 years	≥60 years	3.801(2.530-5.710)	<0.001
Gender	Female	Male	0.806(0.527-1.233)	0.32
Race	Mexican American	Other Hispanic	0.732(0.391-1.372)	0.33
		Non-Hispanic White	0.841(0.512-1.384)	0.497
		Non-Hispanic Black	0.762(0.429-1.353)	0.353
		Other/Multi-Racial	0.739(0.364-1.502)	0.403
Education	High school or less	Some College	0.552(0.364-0.836)	0.005
		College Graduate or above	0.812(0.442-1.491)	0.501
Marital_status	Married/Living with partner	Widowed/Divorced/Separated	0.873(0.59-1.291)	0.496
		Never Married	0.739(0.38-1.437)	0.373
PIR	0-1.3	1.3-3.5	1.004(0.675-1.491)	0.986
		>3.5	0.624(0.363-1.073)	0.088
BMI	Underweight (<18.5)	Normal (18.5 to <25)	0.698(0.222-2.195)	0.539
		Overweight (25 to <30)	0.47(0.145-1.525)	0.209
		Obese (≥30)	0.462(0.141-1.514)	0.202
Smoking Status	Never smoker	Former smoker?	0.914(0.58-1.442)	0.699
		Current smoker	0.968(0.58-1.616)	0.901
Drinking state	Non-drinker	low to moderate?drinker	5.247(3.314-8.306)	<0.001
		heavy drinker	4.105(2.465-6.834)	<0.001
		other	1.882(0.919-3.855)	0.084
Hypertension (%)	NO	Yes	1.331(0.891-1.990)	0.163
CVD(%)	NO	Yes	1.331(1.266-2.983)	0.002
LDL(mg/dL)			1.003(0.98-1.027)	0.779
TG(mg/dL)			1.003(0.998-1.008)	0.214
HDL(mg/dL)			1.019(0.993-1.045)	0.152
UA(mg/dL)			1.414(1.211-1.651)	<0.001
TC(mg/dL)			0.995(0.973-1.018)	0.691

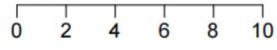

Fig. 3 Forest plot of multivariable logistic regression analysis

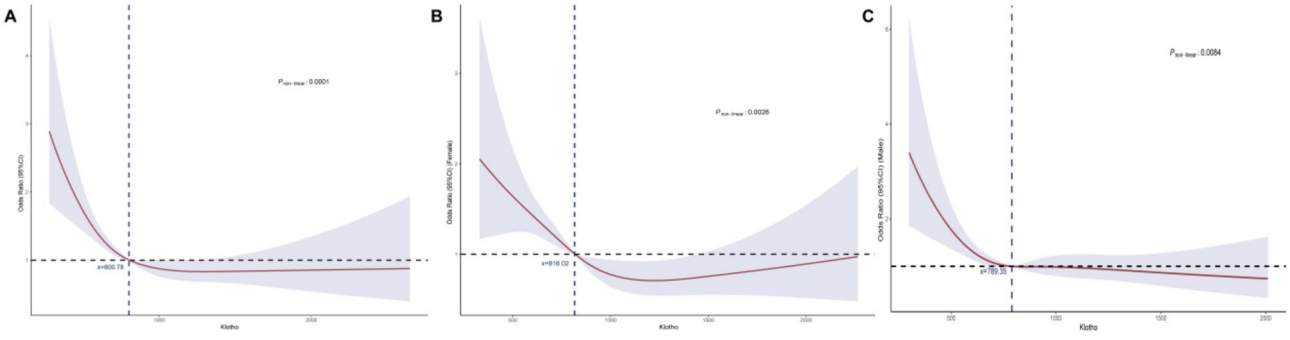

Fig. 4 The nonlinear relationship between α-klotho levels and DKD risk. (**A**) Overall population; (**B**) Female group; (**C**) Male group. The solid line represents the regression curve, and the shaded area indicates the 95% confidence interval

The nonlinear relationship between serum α-klotho levels and mortality

In RCS analysis, a significant nonlinear relationship was observed between α-klotho levels and all-cause mortality. The analyses for the overall population (Fig. 6A), females (Fig. 6B), and males (Fig. 6C) demonstrated that lower α-klotho levels markedly elevate the risk of all-cause mortality, with the risk reaching its nadir at specific thresholds (776.95 pg/mL to 812.69 pg/mL for the overall group, 738.26 pg/mL to 825.74 pg/mL for females, and

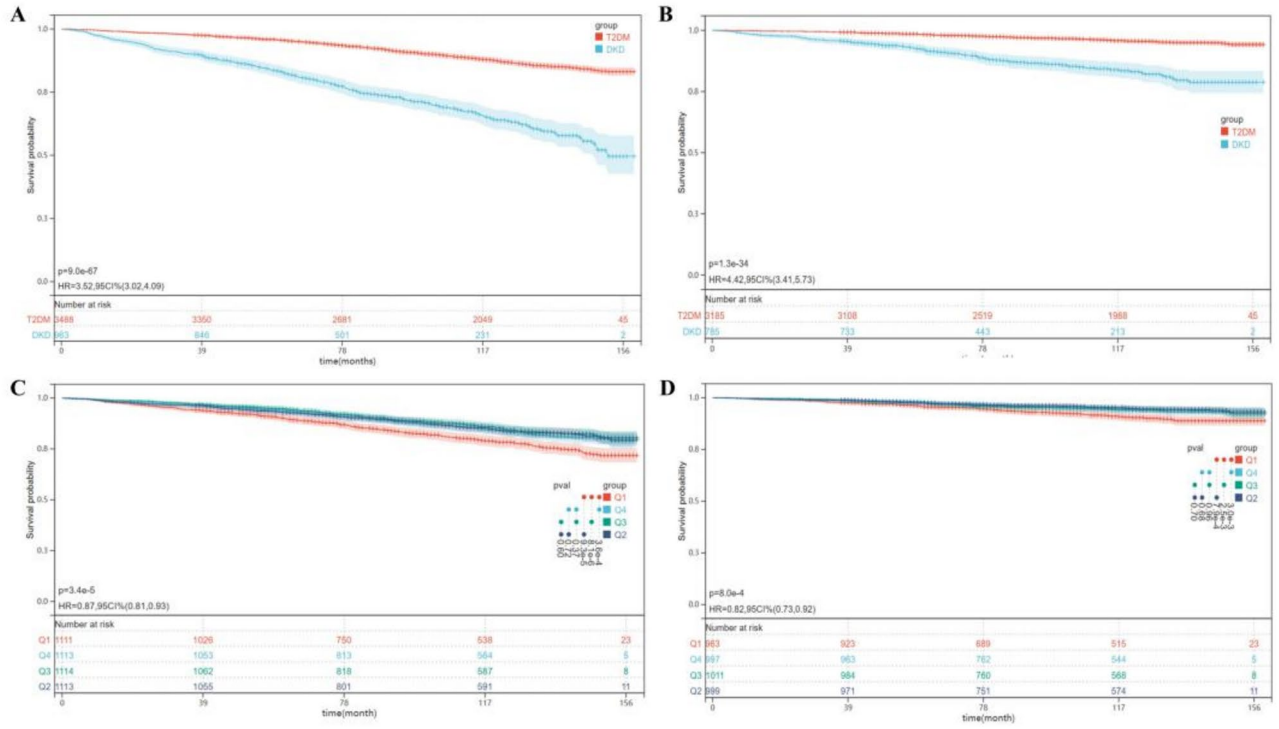

Fig. 5 Survival analysis curves for all-cause mortality and cardiovascular mortality in different populations. **A-B**: survival curves for T2DM and DKD patients (A: all-cause mortality; B: cardiovascular mortality). **C-D**:Curves for patients based on different quartiles of α-klotho levels (Q1-Q4) (C: all-cause mortality; D: cardiovascular mortality)

789.35 pg/mL to 1021.53 pg/mL for males). Beyond these thresholds, as α-klotho levels increased, mortality risk gradually rose. For cardiovascular mortality, the overall and female populations showed no significant nonlinear relationship ($P_{non-linear} > 0.05$, Figs. 6D-E). However, in the male population, the relationship was consistent with a nonlinear pattern (Fig. 6F, $P_{non-linear} = 0.0103$), with the lowest cardiovascular mortality occurring at α-klotho levels between 763.55 pg/mL and 797.95 pg/mL.

Discussion

DKD often lacks sensitive and specific predictive biomarkers in early stages, leading to diagnosis only after significant progression [30]. DKD patients may progress to ESRD, but many may die from cardiovascular-related events before receiving renal replacement therapy [31–33]. Therefore, identifying effective biomarkers for DKD and CVD risk is clinically crucial. α-klotho is emerging as a potential biomarker for inhibiting DKD progression and reducing cardiovascular event risk [15, 34], underscoring its predictive value in DKD patients (Fig. 7). To our knowledge, this study is the first large cohort study to compare and analyze serum α-klotho levels with mortality in patients with T2DM.

This study comprehensively analyzed the relationship between α-klotho levels and the risk of DKD, as well as all-cause and cardiovascular mortality in the T2DM population using multiple methods. In the Chinese cohort, α-klotho levels were significantly higher in T2DM patients compared to DKD patients, with an area under the ROC curve of 0.731, indicating good discrimination ability of α-klotho between T2DM and DKD. Next, we analyzed data from 4,451 T2DM participants in the NHANES database, and the results were similar to those of the Chinese cohort, showing that serum α-klotho levels in DKD patients were significantly lower than T2DM patients. In the lowest α-klotho quartile (Q1), the proportion of DKD patients was highest (31.774%), which showed an increasing trend compared with other quartile groups. This suggests that we may need to pay attention to the potential association between α-klotho levels and DKD. This is consistent with previous meta-analyses and clinical observations [35, 36]. Further nonlinear analysis indicated a significant nonlinear association between α-klotho levels and the risk of DKD, with serum α-klotho levels < 880.78 pg/mL increasing DKD risk in the overall population, and differing thresholds in females (816.02 pg/mL) and males (789.35 pg/mL). Subsequently, as α-klotho levels continued to rise, the risk slightly increased, but the overall trend remained within a lower risk range. In summary, these findings not only validate the important role of α-klotho in T2DM patients but also provide empirical support for its potential as a biomarker for DKD.

Table 5 Association between α-klotho and all-cause mortality and cardiovascular mortality

	Model 1			Model 2			Model 3		
All-cause mortality	**HR**	**95% CI**	**p-value**	**HR**	**95% CI**	**p-value**	**HR**	**95% CI**	**p-value**
Q1	reference			reference			reference		
Q2	0.597	0.450–0.791	< 0.001	0.684	0.520–0.901	0.007	0.608	0.402–0.921	0.019
Q3	0.594	0.448–0.786	< 0.001	0.728	0.552–0.96	0.025	0.646	0.442–0.944	0.024
Q4	0.676	0.511–0.892	0.006	0.815	0.623–1.066	0.135	0.766	0.518–1.133	0.182
Female									
Q1	reference			reference			reference		
Q2	0.537	0.335–0.860	0.010	0.606	0.383–0.958	0.032	0.299	0.160–0.559	< 0.001
Q3	0.662	0.437–1.003	0.052	0.730	0.488–1.090	0.124	0.466	0.274–0.791	0.005
Q4	0.766	0.509–1.153	0.202	0.865	0.584–1.282	0.470	0.695	0.384–1.259	0.230
Male									
Q1	reference			reference			reference		
Q2	0.643	0.451–0.918	0.015	0.739	0.522–1.048	0.090	0.874	0.5126–1.491	0.622
Q3	0.548	0.374–0.803	0.002	0.708	0.483–1.037	0.076	0.844	0.515–1.382	0.500
Q4	0.616	0.421-0.900	0.012	0.762	0.526–1.103	0.150	0.913	0.525–1.586	0.747
Cardiovascular mortality									
Q1	reference			reference			reference		
Q2	0.492	0.306–0.79	0.003	0.574	0.362–0.91	0.018	0.643	0.326–1.267	0.202
Q3	0.514	0.331–0.798	0.003	0.653	0.424–1.004	0.052	0.715	0.369–1.387	0.321
Q4	0.553	0.35–0.873	0.011	0.671	0.428–1.052	0.082	0.923	0.440–1.933	0.831
Female									
Q1	reference			reference			reference		
Q2	0.660	0.309–1.409	0.283	0.761	0.365–1.589	0.467	0.299	0.160–0.559	< 0.001
Q3	0.57	0.297–1.093	0.091	0.649	0.343–1.228	0.184	0.466	0.274–0.791	0.005
Q4	0.588	0.298–1.16	0.126	0.657	0.337–1.281	0.218	0.695	0.384–1.259	0.230
Male									
Q1	reference			reference			reference		
Q2	0.386	0.217–0.685	0.001	0.454	0.006–1.589	0.006	0.752	0.292–1.932	0.553
Q3	0.484	0.265–0.882	0.018	0.658	0.156–1.228	0.156	1.247	0.530–2.937	0.613
Q4	0.543	0.293–1.007	0.053	0.69	0.225–1.281	0.225	1.713	0.633–4.635	0.289

CI, confidence interval. Model 1 is unadjusted; Model 2 is adjusted for gender, age, and race; Model 3 is further adjusted for education, marital status, PIR, BMI, drinking status, smoking status, hypertension, CVD, LDL, HDL, TG, TC and UA

Research has shown that serum α-klotho levels are negatively correlated with the prevalence of metabolic syndrome, with higher α-klotho levels strongly indicating a lower risk of all-cause mortality in patients with metabolic syndrome [13]. Compared to the T2DM population, the DKD population has significantly higher all-cause and cardiovascular mortality rates, and the survival rate in the lower α-klotho group (e.g., Q1) is significantly lower than in other groups. Cox regression analysis shows that higher α-klotho levels can reduce all-cause mortality in T2DM patients, with this trend being more pronounced in females. Further RCS analysis indicated a nonlinear relationship between α-klotho and all-cause mortality, with the lowest risk observed at α-klotho levels between 776.95 pg/mL and 812.69 pg/mL. Serum α-klotho levels are nonlinearly associated with cardiovascular mortality in males, with the lowest cardiovascular mortality occurring at α-klotho levels between 763.55 and 797.95 pg/mL. Related studies support our inference; for instance, Wang Z et al. [37] found gender differences in α-klotho levels, with triglycerides being independently associated with serum α-klotho levels in females, while high-density lipoprotein cholesterol showed an independent correlation with α-klotho in males. Therefore, further exploration of the mechanisms by which gender influences α-klotho's regulation of cardiovascular and renal function could provide a significant theoretical basis for personalized medicine.

The various biological functions of α-klotho can explain how changes in its levels contribute to the progression of DKD and the increased all-cause and cardiovascular mortality in T2DM patients (1). The most classic function of α-klotho is its role as a co-receptor for FGF23, regulating calcium, phosphorus, vitamin D, and parathyroid hormone levels [38]. α-klotho deficiency is characterized by hyperphosphatemia and vascular calcification, both of which increase the risk of declining kidney function [39] and CVD [40]. In individuals with T2DM and early DKD, lower levels of α-klotho are associated with increased aortic pulse wave velocity [33] and there is a reverse and

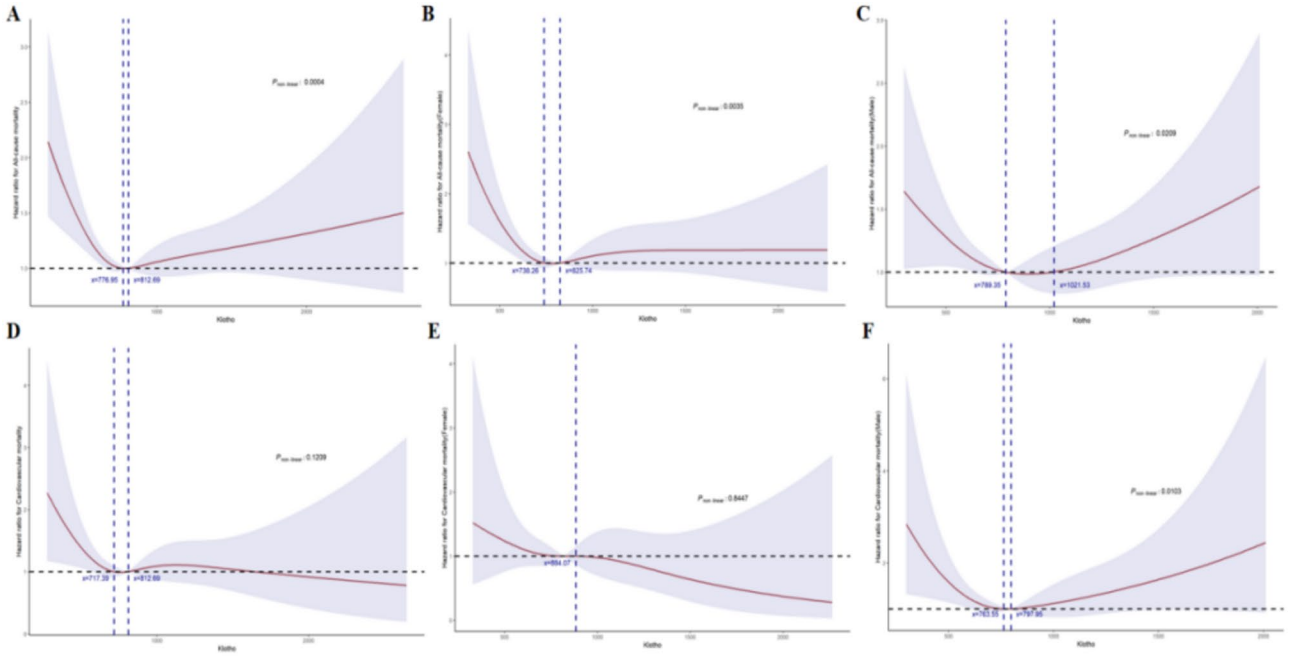

Fig. 6 The nonlinear relationship between α-klotho and all-cause as well as cardiovascular mortality. (**A-C**) Nonlinear relationship analysis of α-klotho with all-cause mortality. (A)overall population; (B) females (C) males. (**D-F**) Nonlinear relationship analysis of α-klotho with cardiovascular mortality. (D) overall population; (E) females; (F) males. The red line represents the hazard ratio (HR) of α-klotho levels, the blue dashed line indicates the α-klotho values at different thresholds, and the shaded area represents the 95% confidence interval

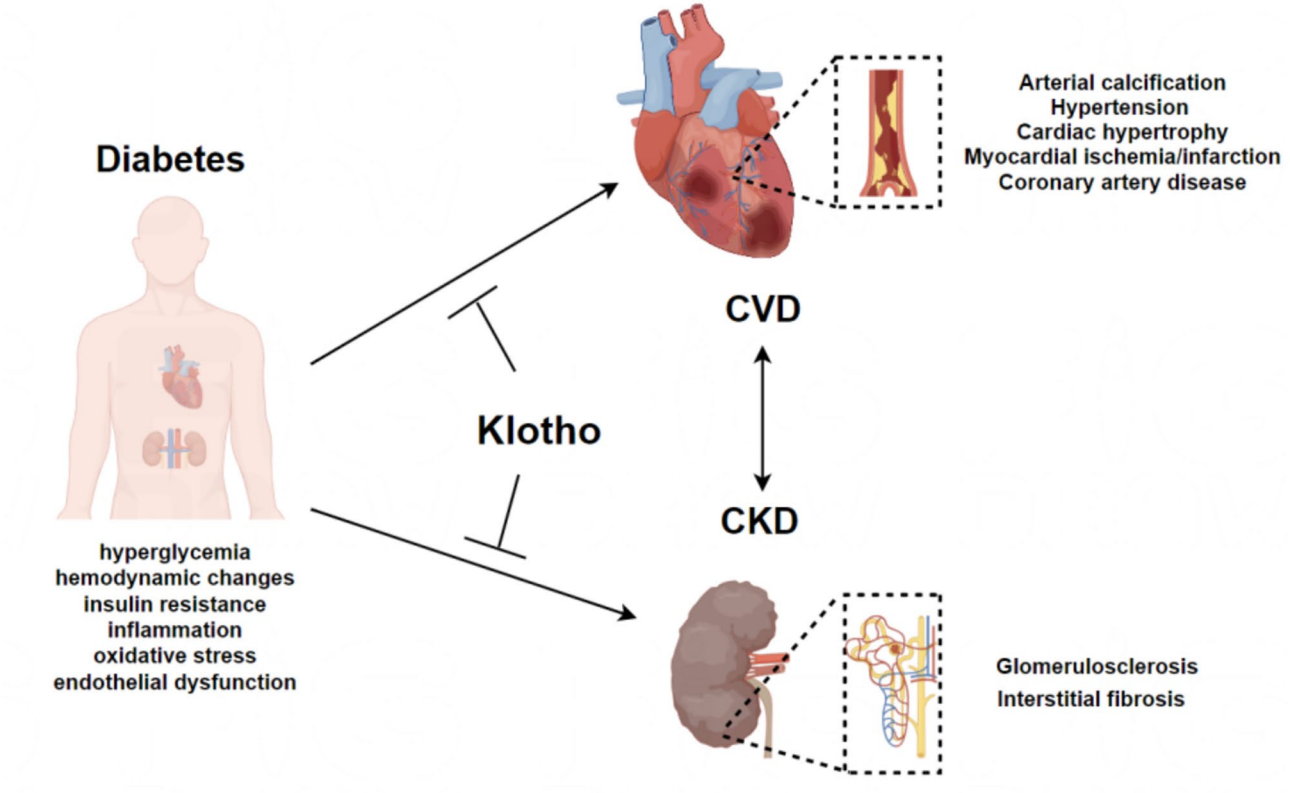

Fig. 7 The relationship between α-klotho and the heart and kidney. CVD: Cardiovascular disease; CKD: Chronic kidney disease

independent association between serum α-klotho levels and pulse pressure [41], suggesting that low α-klotho is a significant factor in the occurrence of atherosclerosis and cardiovascular mortality (2). α-klotho provides renal protection through its anti-fibrotic, anti-inflammatory, and antioxidant effects, as well as vascular protection, metabolic regulation, and anti-apoptotic and autophagy-modulating properties [42] (3). α-klotho alleviates hyperglycemia-induced cardiac injury and diabetic cardiomyopathy by inhibiting inflammation, mitochondrial dysfunction, reactive oxygen species production, and apoptosis in diabetic mice [43] (4). Additionally, α-klotho is involved in the homeostatic regulation of lipid metabolism, glucose metabolism, and energy balance. Impaired α-klotho function is associated with dyslipidemia and obesity, both of which are significant risk factors for the occurrence of DKD and cardiovascular disease [44].

Previous studies on the association between α-klotho levels and mortality risk have yielded inconsistent results. In a nationally representative sample of American adults, a negative correlation between circulating α-klotho levels and mortality was found [45]. Pan et al. [46] found that low baseline circulating α-klotho levels were associated with an increased risk of long-term outcomes of major vascular complications in T2DM, while high circulating α-klotho levels were strongly negatively correlated with the incidence of coronary artery disease, stroke, and left ventricular hypertrophy. In older adults and cardiovascular disease patients in the U.S., the relationship between serum α-klotho levels and all-cause mortality exhibited a U-shaped association [19, 47]. Our study confirms that the association between α-klotho and all-cause and cardiovascular mortality in diabetic patients is not strictly negative; when serum α-klotho concentrations exceed a certain range, mortality slightly increases, though not significantly. The heterogeneity among different studies, cross-reactivity between α-klotho subtypes, and detection biases may contribute to the inconsistent results. However, the association between low α-klotho levels and mortality risk is consistent across studies. Our previous research found that under diabetic conditions, kidney aging accelerates due to hyperglycemia, inflammation, oxidative stress, and hypertension, which induce renal cellular senescence and downregulation of α-klotho [48]. These discrepancies also suggest that in studies exploring therapeutic methods to enhance α-klotho expression or mimic its effects, careful attention should be paid to appropriate concentration ranges.

This study is based on clinical exploration within the Chinese population, combined with the nationally representative NHANES cohort in the U.S., thus offering valuable insights. This comprehensive dataset allows us not only to investigate the impact of serum α-klotho levels on DKD but also to analyze the effects of α-klotho on the overall survival of T2DM patients. Additionally, we employed RCS analysis to explore the nonlinear relationship between serum α-klotho levels and DKD risk, providing a more nuanced understanding of this complex interaction. Furthermore, our study uniquely compares these relationships across different genders, revealing significant gender-specific differences in α-klotho's protective role against DKD.

Limitations

However, this study has several limitations. First, the relatively small sample size, particularly in the Chinese cohort, may impact the robustness of the statistical results. Future studies should aim to increase the sample size to enhance the generalizability of the findings.Second, although we controlled for potential confounding factors using multivariable regression analysis, we cannot rule out the influence of other unmeasured factors on the results. Furthermore, this study is a cross-sectional design, which precludes the establishment of causal relationships. Additionally, the measurement of serum α-klotho was conducted at a single time point, which may be subject to biological variability. These findings require further validation through prospective cohort studies and mechanistic investigations.

Conclusion

In summary, this study indicates a significant association between α-klotho levels and the risk of DKD and mortality in T2DM patients, suggesting its potential as a biomarker. Future research should further explore the mechanisms of α-klotho action and optimize clinical management to improve the quality of life and prognosis for diabetic patients. By integrating multidisciplinary research findings, α-klotho may emerge as an important focus for research on diabetes-related complications.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13098-025-01711-x.

Supplementary Material 1

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Author contributions

Yi Kang: Contributed to the study design, data collection, statistical analysis, and interpretation of results. Drafted the manuscript and coordinated revisions. Qian Jin: Assisted in data collection and analysis, and contributed to the manuscript preparation and editing. Mengqi Zhou: Participated in the literature review and contributed to data interpretation and manuscript revision. Huijuan Zheng: Conducted biochemical measurements and contributed to the analysis of the Chinese cohort data. Danwen Li: Provided critical feedback on the study design and analysis, and contributed to manuscript revisions. Jingwei Zhou: Supported the statistical analysis and assisted in the interpretation of results. Jie Lv: Oversaw the overall research project, provided expertise in study design, and contributed to manuscript

writing and revisions. Yaoxian Wang: Contributed to the study's conception, design, and manuscript editing, ensuring the integrity of the research process. All authors read and approved the final manuscript.

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Data availability
All data included in this study are available upon request by contact with the corresponding author.

Declarations

Ethics approval and consent to participate
The Chinese corhort study was sanctioned by the Ethics Committee of Dongzhimen Hospital, Beijing University of Chinese Medicine (2022DZMEC-062-03), and all participants provided written informed consent. The NHANES study protocol was approved by the National Center for Health Statistics (NCHS) Research Ethics Review Board (ERB) with participants providing written informed consent at the time of enrollment.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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