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A cohort study on the correlation between serum Klotho levels and all-cause mortality in American diabetic populations

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Abstract

Background The global prevalence of diabetes is on an upward trajectory. The management of complications related to the condition has seen limited progress in recent years. Klotho, characterized as an anti-aging protein that mitigates oxidative stress and inflammation, has previously been correlated with all-cause mortality in the broader United States population. The objective of this research was to investigate the persistence of this relationship among diabetic patients.

Methods This study meticulously analyzed data (2007–2016) sourced from the National Health and Nutrition Examination Survey, encompassing a cohort of 3,560 individuals. To elucidate the links of Klotho with all-cause mortality in diabetic patients, a multivariate Cox proportional hazards regression model was employed. The relationship was further explored using the restricted cubic spline model, threshold analysis, and subgroup analysis. Additionally, a mediation analysis was conducted to unravel the influence of age on the observed correlations.

Results Throughout the observation period, which had a median duration of 84 months, the incidence of all-cause mortality reached 18.511%. The Cox model analysis revealed a statistically significant association between Klotho levels and all-cause mortality. Further, the application of restricted cubic splines revealed a nuanced, nonlinear relationship between exposure factors and outcome across the entire study population (nonlinear $P < 0.001$), pinpointing a critical threshold at 829.138 pg/mL. Subgroup analyses showed consistent correlation between Klotho levels and mortality across various groups. Intriguingly, mediation analysis indicated that age was a significant mediator, accounting for 76.1% of the observed correlation of Klotho levels with all-cause mortality among diabetic patients.

Conclusions Low levels of Klotho were found to be strongly associated with an increased risk of all-cause mortality in individuals with diabetes (Klotho levels < 829.138 pg/ml), and a nonlinear relationship was observed between these two variables. These associations were largely mediated by age.

Keywords Klotho, Diabetes, All-cause mortality

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Introduction

Diabetes, now recognized as a global health crisis, is distinguished by heightened blood glucose levels resulting from compromised insulin secretion and diminished cellular responsiveness to this essential hormone [1, 2]. Over the past few decades, the diabetes epidemic has surged dramatically, with the global count of individuals affected rising from 108 million in 1980 to 463 million in 2019. This escalation has placed an intense strain on healthcare infrastructures worldwide, underscoring the condition's escalating public health challenge [3]. The progression of diabetes is notably marked by the emergence of chronic complications that compromise the well-being of those diagnosed and severely deteriorate their quality of life. Among them, vascular complications are the main cause of mortality [4]. While diabetes management strategies have been effective in mitigating adverse outcomes associated with the disease and reducing all-cause mortality [5], advancements in treating diabetes-induced vascular complications have lagged since 2010 [6]. Hence, the prompt identification and subsequent intervention are imperative to diminish the mortality rates in diabetics identified as being at a high risk of death.

In 1997, a groundbreaking discovery was made with the identification of an anti-aging molecule, termed Klotho, in mice for the first time [1]. Primarily expressed in the kidneys and brain, Klotho is also found in the pituitary gland, parathyroid gland, heart, and reproductive organs [1, 7]. Of the α-, β-, and γ-Klotho isoforms, α-Klotho is the only circulating form mentioned in this article [8]. The soluble variant of Klotho protein (S-Klotho), produced through the proteolytic cleavage of membrane-bound Klotho proteins, is predominantly sourced from kidney cells but can also originate from the ependymal cells of the choroid plexus [9]. Compared to their membrane-bound counterparts, S-Klotho proteins are more prevalent within the human body, detectable in urine, blood, and cerebrospinal fluid [10]. Klotho exerts an anti-aging role through its multifaceted functions, including anti-inflammatory activities, antioxidant properties, and regulation of signal transduction pathways [11]. Clinical studies utilizing animal models have demonstrated that Klotho possesses antioxidant and anti-inflammatory effects, which offer protection against kidney and cardiovascular diseases [12, 13]. Evidence suggests that a deficiency in Klotho levels is correlated with predictors of cardiovascular outcomes and overall mortality in both patients with chronic kidney disease (CKD) and the general population [14, 15]. In studies conducted among older adults in Europe and the general population in the United States, an inverse relationship has been observed between Klotho concentrations and all-cause mortality, yielding similar outcomes [16, 17]. However, the relationship between Klotho and all-cause mortality in individuals with diabetes remains to be definitively established. Our cohort study aims to fill this research gap by systematically exploring the relationship between Klotho levels and prognosis in people with diabetes. Utilizing patient data from the NHANES database, our study aims to provide robust evidence of the prognostic value of Klotho levels in diabetic patients.

Diabetes and its associated complications continue to pose a significant clinical challenge, as current treatment modalities do not fully address the clinical needs of diabetic patients, necessitating the urgent need for early risk stratification [6, 18]. To explore the correlation of serum Klotho concentrations with overall mortality among diabetic patients, a retrospective cohort study was carried out in the United States. Our hypothesis posited an inverse relationship between serum Klotho concentrations and all-cause mortality in diabetic patients, suggesting that lower Klotho levels correlate with an increased risk of mortality.

Method

Study population

The National Health and Nutrition Examination Survey (NHANES), a stratified, multi-stage probabilistic survey targeting the general U.S. population, served as the source of data for this study [19]. This nationally representative survey, conducted in two-year cycles, assesses the nutrition and health status of the American civilian non-institutionalized population [20]. In addition to conducting home visits and physical examinations, the NHANES also performed clinical and laboratory analyses of serum, plasma, urine, and DNA samples during data collection. Informed consent was secured from all participants, and the survey protocol received approval from the National Center for Health Statistics Research Ethics Review Board [21]. All study processes complied with NHANES: plans and operations' requirements, ensuring adherence to relevant standards and regulations [22]. To investigate whether serum Klotho levels were correlated with all-cause mortality among American diabetics, we utilized data from the NHANES database (2007–2016). Furthermore, the data of 60 diabetic patients were collected from The Second Affiliated Hospital and Yuying Children's Hospital of Wenzhou Medical University (WMU) to enhance and validate the findings of this study, the follow-up period was 60 months. Patients with diabetes were identified based on the response to the question "Doctor told you have diabetes" (DIQ010) from the NHANES database. To strengthen diagnostic validity, additional verification was conducted using biochemical criteria (fasting glucose ≥ 126 mg/dL or HbA1c ≥ 6.5%) for participants without self-reported diagnosis. Individuals without diabetes, lacking serum Klotho concentration data, or missing relevant follow-up information were

excluded. The final study cohort consisted of 3560 participants. The recruitment process is depicted in Fig. 1.

Exposure factor

This study focused on serum Klotho concentrations, and samples were stored at the Atlanta Centers for Disease Control and Prevention. During 2019 and 2020, The specimens were transported to the Northwestern Lipid Metabolism and Diabetes Research Laboratory, located at the University of Washington. The concentration of Klotho in the samples was accurately measured utilizing an enzyme-linked immunosorbent assay (ELISA) kit (IBL International, Gunma, Japan) [23]. The assay exhibited a minimum detection limit of 6 pg/mL. All sample values met this threshold, eliminating the need for imputation.

Clinical outcomes

Certificate data from the National Death Index database, which is compiled by state vital statistics offices, through the National Center for Health Statistics. Follow-up commenced from the interview date and concluded on the earlier of the date of death or December 31, 2019, marking the end of the study period.

Covariate information

In this study, a methodical categorization of covariates was performed: (1) Demographic Characteristics, which include age, gender, race, the poverty income ratio (PIR), marital status, and smoking status. Smoking status is categorized as: never smoker (defined as smoking < 100 cigarettes), former smoker (defined as smoking ≥ 100

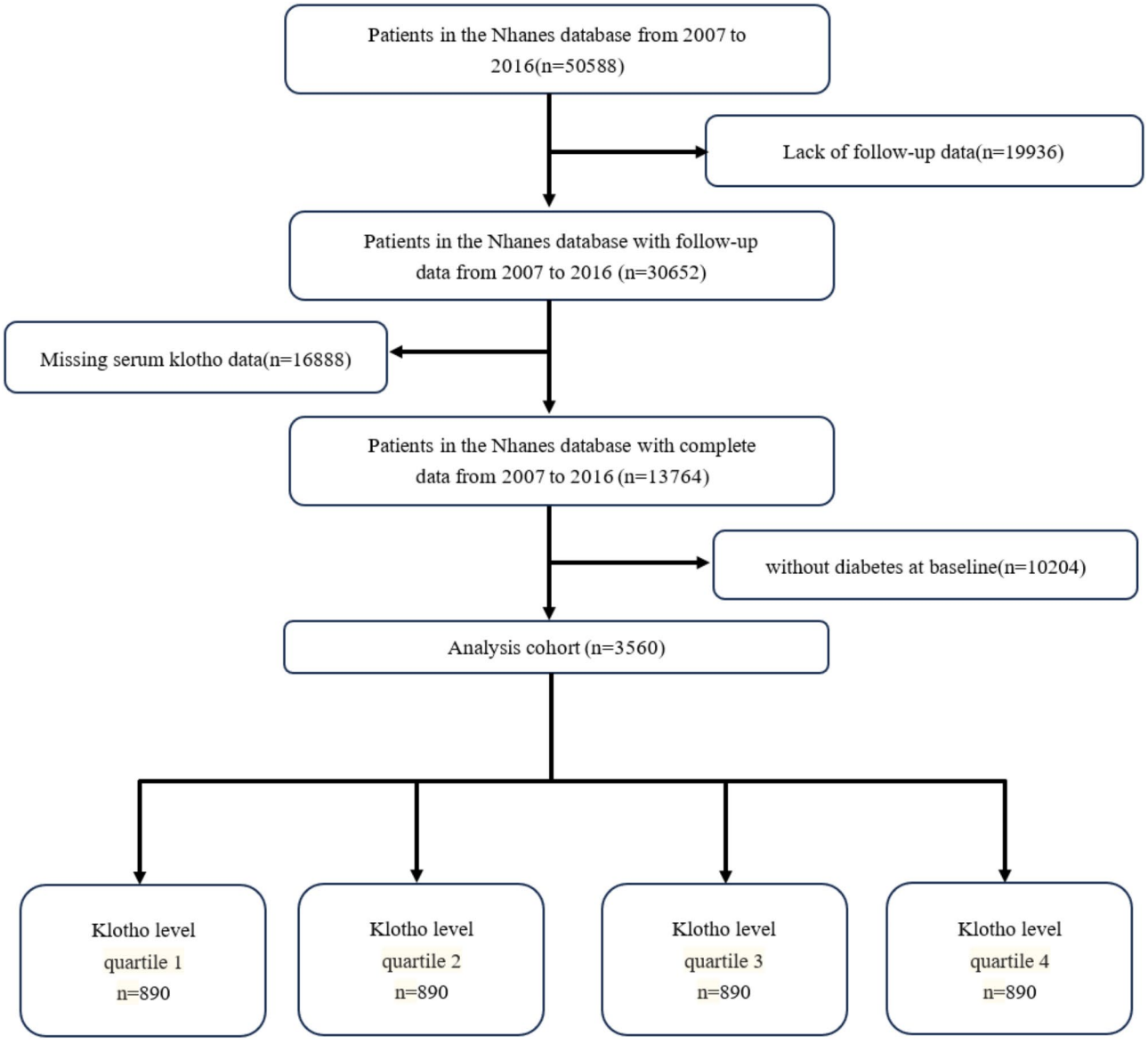

Fig. 1 Procedure for selecting trial participants

cigarettes historically but having ceased smoking now), and current smoker (defined as smoking ≥ 100 cigarettes and currently smoking some days or every day). Alcohol status is defined as: never drinking (never had 12 drinks), former drinking (had at least 12 drinks, but none in the past 12 months), and current drinking (had at least 12 drinks per year or had 12 drinks in their lifetime and had drinks in the past 12 months). (2) Vital Signs, which encompass body mass index (BMI), diastolic blood pressure (DBP), and systolic blood pressure (SBP); (3) Laboratory Parameters, including alanine aminotransferase (ALT), albumin (Alb), creatinine, total cholesterol (TC), high-density lipoprotein (HDL), HbA1c, estimated glomerular filtration rate (eGFR); (4) Complications, featuring hyperlipidemia, stroke, coronary heart disease, and hypertension. The eGFR was calculated using the standardized creatinine equation (CKD-EPI), developed by the Chronic Kidney Disease Epidemiology Collaboration in 2009 [24]. Variables exhibiting more than 10% missing data were excluded from the analysis, and the remaining variables underwent multiple imputation to address missing values.

Statistical analysis methods

The characteristics of the study population were analyzed through stratification into Klotho quartiles. Data were presented as percentages for categorical variables and as mean values with standard errors (mean ± SE) for continuous variables. To evaluate the differences among different groups, analysis of variance (ANOVA) or the Kruskal-Wallis test was utilized for continuous variables, and the Chi-square ($\chi 2$) test was employed for categorical variables. Cox proportional hazard regression models were utilized to estimate hazard ratios (HR) and 95% confidence intervals (CI) to explore the association between serum Klotho levels and all-cause mortality. We developed three models for analysis: Model 1, which includes only serum Klotho levels; Model 2, which adjusts for demographic characteristics (age, gender, and race); and Model 3, which builds on Model 2 by further adjusting for the poverty income ratio (PIR), marital status, smoking status, alcohol status, selected laboratory indicators (eGFR), and comorbidities (hypertension, Coronary heart disease). To investigate the potential nonlinear relevance between Klotho concentration and all-cause mortality, restricted cubic spline models were constructed. In cases where a nonlinear relationship was identified, the log-likelihood ratio test of threshold effect analysis was applied to ascertain the presence of a threshold effect. Subgroup analyses were carried out to investigate potential differences in correlations among subgroups, defined by various parameters such as age, gender, BMI, smoking status, alcohol status, hypertension, coronary heart disease, and hyperlipidemia. The mediating effect of age on the relationship between Klotho level and all-cause mortality among diabetic patients was assessed through mediating effect analysis. The mediation effect was analyzed primarily through the application of the following three equations: (1) $Y = cX + e_1$ (2) $M = aX + e_2$ (3) $Y = c'X + bM + e_3$.

Equation 1 delineated the quantitative relationship between the independent variable X and the dependent variable Y, elucidating the influence of X on Y. Equation 2 delineated the influence of the independent variable X on the mediator variable M. Equation 3 elucidated the effect of X on Y, adjusted for M, as well as the effect of M on Y, adjusted for X [25]. All statistical analyses were performed utilizing the R statistical software (version 4.3.2), and "rcssci" package for restricted cubic spline model. In this study, a *P*-value of less than 0.05 (two-sided) was deemed indicative of statistical significance.

Results

General characteristics of the participants

The baseline characteristics of the study participants were stratified by serum Klotho concentration quartiles (Q) (Q1: ≤ 645.7; Q2: 646.8–802.8; Q3: 803–1008.9; Q4: ≥ 1009). These data were presented in Table 1. The average Klotho concentrations for the four groups were respectively 525.9 (SE: 87.7), 723.5 (SE: 44.8), 897.4 (SE: 57.6), and 1290.7 (SE: 331.4). Compared to the lower Klotho group, participants in the higher Klotho group were generally younger, had fewer smokers, lower creatinine levels, and a reduced prevalence of coronary heart disease and hypertension, while DBP, ALT, eGFR, TC, and HbA1c levels were higher. In the WMU cohort, participants were stratified into two groups (group 1: ≤ 745.8; group 2: > 745.8) according to their serum Klotho concentrations. Diabetic patients with higher Klotho levels tended to be older (Supplementary Table S1).

Associations of Klotho with all-cause mortality in diabetic patients

According to the results obtained from the Cox proportional hazard analysis, serum Klotho, used as a classification predictor, was significantly associated with all-cause mortality in diabetic patients. In Model 1, without adjusting for any covariates, serum Klotho concentration across the four groups showed a significant correlation with all-cause mortality: for Q2, the hazard ratio (HR) was 0.66 (95% CI: 0.53, 0.81), $P < 0.0001$; for Q3, HR was 0.65 (95% CI: 0.53, 0.80), $P < 0.0001$; for Q4, HR was 0.65 (95% CI: 0.53, 0.80), $P < 0.0001$. The application of adjusted models yielded similar results: In Model 2 (adjusted for demographic characteristics), the HRs were: Q2: 0.68 (95% CI: 0.55, 0.84), $P = 0.0003$; Q3: 0.75 (95% CI: 0.60, 0.92), $P = 0.0068$; Q4: 0.80 (95% CI: 0.65, 0.99), $P = 0.0384$. However, in Model 3 (further adjusted for socioeconomic and

Table 1 Characteristics of participants in the NHANES (2007–2016) by Klotho levels

	Klotho levels				
Variables	**Q1**	**Q2**	**Q3**	**Q4**	***P*-value**
Participants, No.	890	890	890	890	
Klotho(pg/ml)	525.9(87.7)	723.5(44.8)	897.4(57.6)	1290.7(331.4)	< 0.001
Clinical parameters					
Age(years)	62.8(10.0)	61.8(10.1)	61.1(9.9)	59.8(10.0)	< 0.001
Gender, n (%)					0.272
Female	424 (47.6)	416 (46.7)	452 (50.8)	445 (50.0)	
Male	466 (52.4)	474 (53.3)	438 (49.2)	445 (50.0)	
Race, n (%)					< 0.001
Mexican American	184 (20.7)	170 (19.1)	195 (21.9)	178 (20.0)	
Non-Hispanic Black	217 (24.4)	187 (21.0)	173 (19.4)	253 (28.4)	
Non-Hispanic White	322 (36.2)	327 (36.7)	305 (34.3)	253 (28.4)	
Other Hispanic	92 (10.3)	119 (13.4)	119 (13.4)	134 (15.1)	
Other Race	75 (8.4)	87 (9.8)	98 (11.0)	72 (8.1)	
PIR	2.2 (1.5)	2.3 (1.5)	2.2 (1.5)	2.3 (1.6)	0.970
Marital status, n (%)					0.971
Divorced/ Separated	186 (20.9)	181 (20.3)	184 (20.7)	173 (19.4)	
Married/ Living with partner	547 (61.5)	555 (62.4)	547 (61.5)	549 (61.7)	
Never married/ Widowed	157 (17.6)	154 (17.3)	159 (17.9)	168 (18.9)	
Smoking, n (%)					0.003
Never smoker	405 (45.5)	393 (44.1)	467 (52.5)	457 (51.3)	
Former smoker	321 (36.1)	337 (37.9)	282 (31.7)	278 (31.2)	
Current smoker	164 (18.4)	160 (18.0)	141 (15.8)	155 (17.5)	
Alcohol status, n (%)					0.566
Never	148 (16.6)	163 (18.3)	179 (20.1)	163 (18.3)	
Former	278 (31.2)	260 (29.2)	247 (27.8)	260 (29.2)	
Current	464 (52.2)	467 (52.5)	464 (52.1)	467 (52.5)	
Vital signs					
BMI (kg/m2)	32.5 (7.0)	32.6 (7.2)	32.5 (7.4)	32.4(7.5)	0.984
DBP (mmHg)	67.6 (12.9)	68.7(13.1)	69.6(12.8)	70.8(12.9)	< 0.001
SBP (mmHg)	130.8(19.9)	130.4(18.8)	129.3(18.4)	131.0(19.3)	0.261
Laboratory parameters					
ALT(U/L)	24.5(14.0)	25.7(13.7)	28.2(33.4)	31.4 (25.7)	< 0.001
Alb(g/L)	41.3 (3.5)	41.8(3.2)	41.8(3.3)	41.4(3.5)	< 0.001
Creatinine(umol/L)	106.1(99.0)	86.1(40.6)	81.8(53.2)	80.0 (43.6)	< 0.001
eGFR (mL/min/1.73m2)	75.1(27.0)	83.0(24.7)	86.6(22.1)	89.5(23.3)	< 0.001
TC (mg/dl)	4.7 (1.2)	4.8 (1.2)	4.9(1.1)	5.0 (1.3)	< 0.001
HDL (mmol/L)	1.3 (0.4)	1.2 (0.4)	1.2 (0.4)	1.2 (0.4)	0.561
HbA1c (%)	6.9 (1.3)	7.1(1.6)	7.2(1.7)	7.7 (2.1)	< 0.001
Comorbidities					
Hyperlipidemia, n (%)					0.223
No	90 (10.1)	96 (10.8)	109 (12.2)	115 (12.9)	
Yes	800 (89.9)	794 (89.2)	781 (87.8)	775 (87.1)	
Stroke, n (%)					0.057
No	795 (89.3)	823 (92.5)	822 (92.4)	818 (91.9)	
Yes	95(10.7)	67 (7.5)	68 (7.6)	72 (8.1)	
Coronary heart disease, n (%)					0.007
No	780 (87.6)	804 (90.3)	812 (91.2)	821 (92.2)	
Yes	110 (12.4)	86 (9.7)	78 (8.8)	69 (7.8)	
Hypertension, n (%)					0.024
No	204 (22.9)	229 (25.7)	249 (28.0)	256 (28.8)	
Yes	686 (77.1)	661 (74.3)	641 (72.0)	634 (71.2)	

Abbreviations: PIR, poverty income ratio; BMI, body mass index; DBP, diastolic pressure; SBP, systolic pressure; ALT, alanine transaminase; Alb, albumin; eGFR, estimated glomerular filtration rate; TC, total cholesterol; HDL, high density lipoprotein; LDL, low density lipoprotein; HbA1c, glycohemoglobin

Table 2 HR (95% CI) for outcomes across groups of Klotho levels

	Model 1		Model 2		Model 3	
Klotho	**HR (95% CI)**	***P*-value**	**HR (95% CI)**	***P*-value**	**HR (95% CI)**	***P*-value**
Continuous	1.00 (1.00, 1.00)	0.0050	1.00 (1.00, 1.00)	0.3898	1.00 (1.00, 1.00)	0.2573
Q1	1.0		1.0		1.0	
Q2	0.66 (0.53, 0.81)	< 0.0001	0.68 (0.55, 0.84)	0.0003	0.71 (0.57, 0.88)	0.0017
Q3	0.65 (0.53, 0.80)	< 0.0001	0.75 (0.60, 0.92)	0.0068	0.94 (0.75, 1.16)	0.5434
Q4	0.65 (0.53, 0.80)	< 0.0001	0.80 (0.65, 0.99)	0.0384	0.97 (0.78, 1.20)	0.7533
P for trend		0.0003		0.0576		0.8030

Model 1: did not adjust for any confounding factors

Model 2: adjusted for age, gender, race

Model 3: adjusted for age, gender, race, PIR, Marital status, Smoking, Alcohol status, eGFR, Hypertension, Coronary heart disease

Footnote klotho levels quartile (Q): Q1:≤645.7; Q2:646.8–802.8; Q3:803- 1008.9; Q4:≥1009.5

Abbreviations: PIR, poverty income ratio; eGFR, estimated glomerular filtration rate; HR, hazard ratio; CI, confidence interval

health-related behaviors), the higher Klotho quartiles (Q3 and Q4), serum Klotho levels in diabetic patients were not significantly associated with all-cause mortality (Q3, $P = 0.5434$; Q4, $P = 0.7533$). Conversely, in the low Klotho quartile (Q2), serum Klotho levels were significantly associated with all-cause mortality, the HR was 0.71 (95% CI: 0.57, 0.88), $P = 0.0017$.

In the analysis of Klotho concentration quartiles using Cox proportional hazard models, a decreasing trend was observed between Klotho concentration and the incidence of overall mortality. However, this trend was not significant in Model 2 after adjusting for age, gender, and race (*P* for trend = 0.0576), and in Model 3 after including additional adjustments (*P* for trend = 0.8030).

In the WMU cohort, after adjusting for variables such as age, gender, smoking, alcohol status, eGFR, hypertensions and coronary heart disease., serum Klotho levels were found to be inversely associated with all-cause mortality in diabetic patients, the HR was 0.06 (95% CI: 0.01, 0.64), *P*-value = 0.0196 (Supplementary Table S2).

Exploration of nonlinear relationships

To further explore the nonlinear relevance between serum Klotho concentration and all-cause mortality in diabetic patients, a restricted cubic spline analysis approach was adopted. The analysis revealed non-linear associations (Fig. 2, Non-linear $P < 0.001$).

The model incorporated four knots, which corresponded to the positions of 0.05, 0.35, 0.65, and 0.95, respectively.

Threshold effect analysis

As demonstrated in Table 3, the log likelihood ratio test was employed to identify the threshold in the correlation between exposure and outcome variable by comparing non-segmented regression models (Model I) with segmented regression models (Model II), resulting in a *P*-value of 0.005. The threshold of Klotho concentration was determined to be 829.138 pg/ml, utilizing a two-piecewise linear regression model. Levels below this threshold were inversely associated with all-cause mortality (*P*-value < 0.0001), but this association did not persist for concentrations above the threshold (*P*-value = 0.1397). Of the participants, 1903 individuals exhibited Klotho levels below the specified threshold, whereas 1657 individuals demonstrated levels exceeding it.

Subgroup analysis

Subgroup analysis was conducted to further explore the relationship between Klotho levels and all-cause mortality. As depicted in Supplementary Table S3, no significant interactions were observed in the subgroup analysis across various factors such as age, gender, BMI, smoking status, alcohol status, HbA1c levels, eGFR, hypertension, coronary heart disease, and hyperlipidemia (*P* for interaction ranges from 0.2601 to 0.7939), indicating that the association between Klotho levels and all-cause mortality did not significantly vary across these subgroups.

Effect of mediators on the association between Klotho levels and all-cause mortality

Pearson's analysis revealed a negative correlation between age and serum Klotho (β= -0.1084, 95% CI:-0.1408, -0.0759, *P-value* < 0.001)(Table 4). Similarly, within the WMU cohort, Pearson's correlation analysis indicated a negative correlation between age and Klotho levels (β= -0.3294, 95% CI: -0.5383, -0.0823, *P-value* = 0.0102) (Supplementary Table S4). Furthermore, baseline characteristics were evaluated according to the presence or absence of all-cause mortality groups to identify potential adjustment variables (Supplementary Table S5). Cox regression analysis demonstrated that the age of diabetic patients was significantly associated with all-cause mortality both before and after adjusting for variables (*P-value* < 0.001) (Table 5).

A mediating analysis was undertaken to assess the extent to which age in diabetic patients mediates the relationship between serum Klotho concentration and

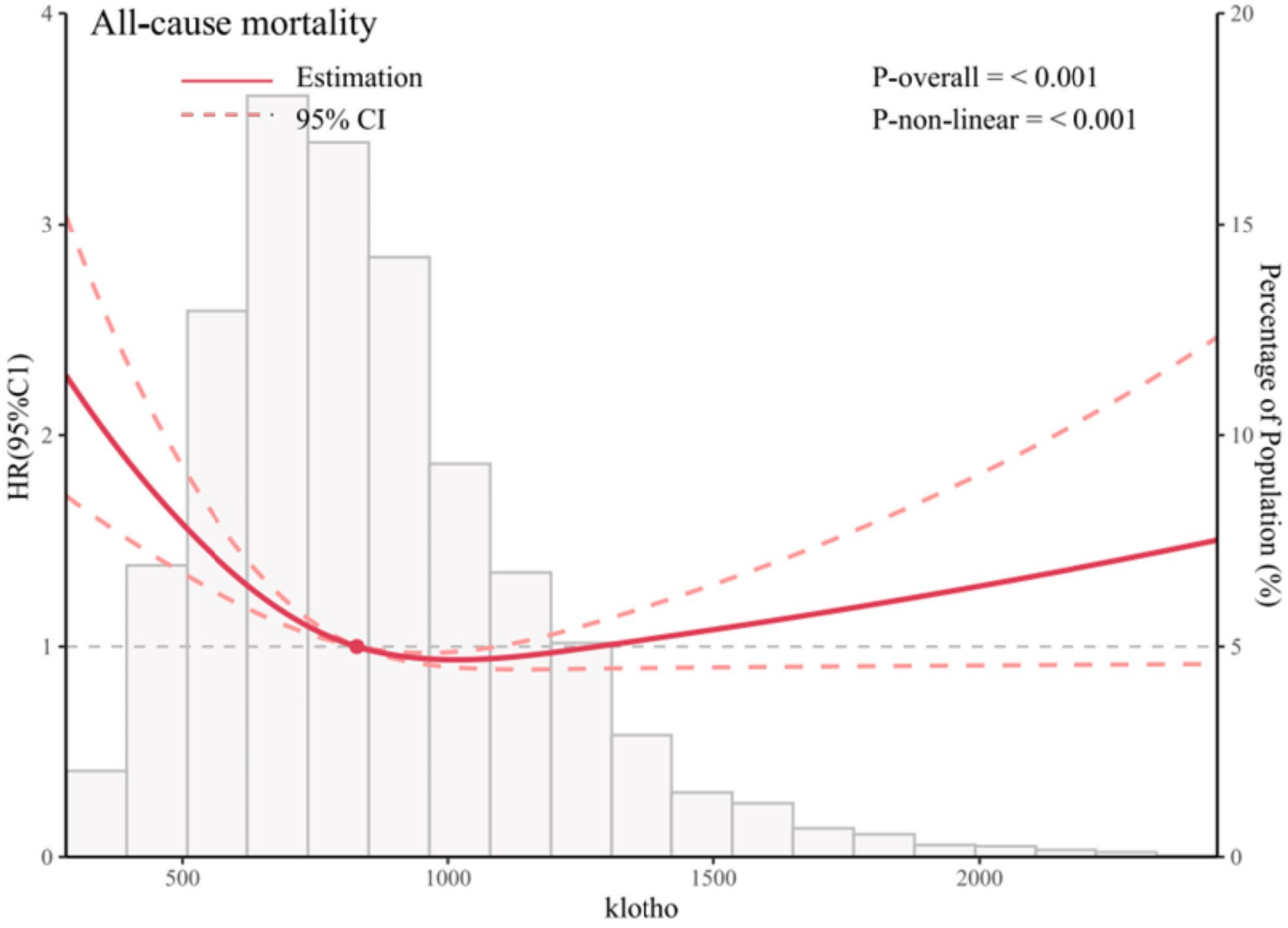

Fig. 2 Association between klotho levels and all-cause mortality.The hazard ratio was computed with a Klotho level of 829.138 as the reference. Abbreviations: HR, hazard ratio; CI, confidence interval

Table 3 Threshold effect analysis of the association between Klotho levels and all-cause mortality

		HR (95% CI)	*P*-value
All-cause mortality			
Klotho			
Model I		1.000 (0.999, 1.000)	0.0050
One line effect			
Model II			
Turning point(K)	829.138		
< K effect 1		0.998 (0.998, 0.999)	< 0.0001
> K effect 1		1.000 (1.000, 1.001)	0.1397
effect 2 – 1		1.002 (1.001, 1.003)	< 0.0001
Log likelihood ratio test			0.005

Abbreviations: HR, hazard ratio; CI, confidence interval

Table 4 Pearson correlation analysis of the age and Klotho

	β	95% CI lower	95% CI upper	*P*-value
Age— Klotho	-0.1084	-0.1408	-0.0759	< 0.001

Abbreviations CI, confidence interval

Table 5 Association between age and all-cause mortalityin diabetic patients

Characteristic	HR	95% CI lower	95% CI upper	*P*-value
Model 1	1.08	1.07	1.09	< 0.001
Model 2	1.07	1.06	1.08	< 0.001
Model 3	1.05	1.04	1.06	< 0.001

Abbreviations HR, hazard ratio; CI, confidence interval

Model 1: did not adjust for any confounding factors

Model 2: adjusted for gender, race

Model 3: adjusted for gender, race, PIR, Marital status, Smoking, Alcohol status, BMI, eGFR, TC, Stroke, Hypertension, Coronary heart disease

all-cause mortality. The analysis revealed that Klotho exhibited no significant direct effect on all-cause mortality (*P*-value = 0.620). However, the total effect was found to be significant (*P*-value = 0.018), with the portion of the effect mediated by age accounting for approximately 76.1% (0.761) of the total effect (Fig. 3).

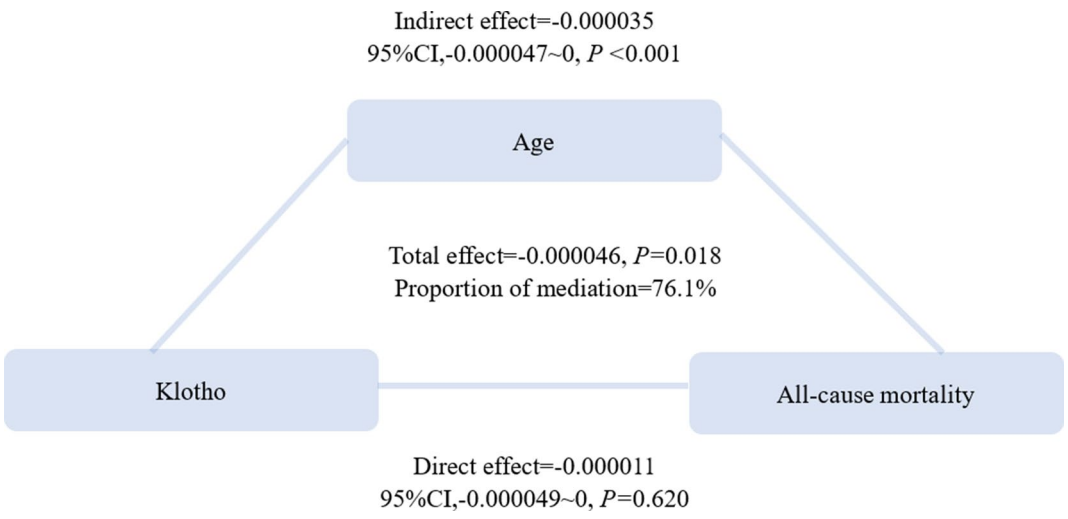

Fig. 3 Mediating analyse of the association between Klotho and all-cause mortality. Abbreviations: CI, confidence interval

Discussion

This study utilizes the NHANES database to examine the relationship between serum Klotho concentration and mortality risk of diabetic patients. Our findings indicate that increasing all-cause mortality rates are inversely correlated with lower serum Klotho concentrations (Klotho levels < 829.138 pg/ml), highlighting a non-linear relationship with mortality outcomes.

Previous research on the association between serum Klotho levels and the risk of all-cause mortality in the general United States population has shown that lower serum Klotho concentrations serve as a marker for increased all-cause mortality in a nationally representative sample of American adults [16]. Nevertheless, research exploring the correlation between serum Klotho levels and all-cause mortality rates remains scant. For instance, A study conducted on individuals with CKD identified a notable inverse correlation between decreased serum levels of Klotho and the risk of all-cause mortality [26]. Ke and colleagues found in their study that low serum Klotho levels in non-diabetic dialysis patients acted as an significant predictor of all-cause mortality [27], and research among elderly community residents demonstrated that serum Klotho concentration was independently associated with all-cause mortality [17]. Nonetheless, these studies are constrained by small sample sizes, duplication in study populations, or a lack of detailed statistical methodological interpretation regarding the correlation between Klotho concentration and mortality.

In our study, we observed a non-linear correlation between serum Klotho concentration and all-cause mortality, echoing the observations made by Jacob et al. and Yuqin et al. Jacob et al. identified a non-linear trend between Klotho levels and all-cause mortality after adjusting for covariates [16], yet they did not further explore this non-linear trend. In contrast, we employed threshold effect analysis to identify a specific threshold in the relationship between serum Klotho concentration and all-cause mortality. Our analysis indicated a negative association when Klotho levels were below 829.138pg/ml, with no significant association observed above this threshold. Similarly, Yuqin et al. reported a non-linear correlation between Klotho levels and all-cause mortality among patients with hypertension, successfully pinpointing the threshold of this non-linear relationship [28]. Building upon these studies, our research provides additional insight into the association between serum Klotho concentrations and all-cause mortality through mediation analysis. However, it is crucial to acknowledge that the link between Klotho and all-cause mortality remains complex and partially unresolved, influenced by factors such as differences in study populations, follow-up durations, and sample sizes.

Over the past three decades, the global prevalence of diabetes has seen a manifold increase, establishing it as a highly prevalent disease. Among the various forms of diabetes, type 2 diabetes is predominant [3, 29]. Diabetic complications emerge as the primary cause of mortality among diabetic patients. The mechanisms underlying diabetic complications due to hyperglycemia primarily involve the direct toxic effects of hyperglycemia and its derivatives on tissues, along with alterations in cell signaling pathways induced by glucose metabolites [30]. The inquiry into why Klotho levels exhibit a negative correlation with all-cause mortality in diabetic patients leads to several insightful mechanisms. The expression of the Klotho gene (mKL) in pancreatic cells is known to mitigate pancreatic β-cell apoptosis and ward off streptozotocin (STZ)-induced diabetes [31]. Klotho enhances the expression of antioxidant genes by inhibiting the insulin/insulin-like growth factor-1 (IGF-1) receptor signaling

pathway, thereby exerting anti-aging effects. Additionally, Klotho plays a pivotal role in reducing diabetes-related organ injury through its inhibitory effect on the inflammatory nuclear factor kappa-B (NF-κB) pathway, addressing chronic inflammation [32, 33]. In the context of type 2 diabetes mellitus (T2DM), Klotho is shown to prevent liver lipid accumulation, improve insulin sensitivity, and lower the incidence of diabetes complications [34]. The correlation between diabetes, cardiovascular disease and systemic inflammation is well-established, with diabetic patients facing a markedly higher risk of developing cardiovascular diseases. This increased risk is attributed to the inflammatory effects, which also constitute the primary cause of all-cause mortality among diabetes patients [35]. Klotho counteracts the impact of inflammation in atherosclerosis by inhibiting TNFα-induced expression of Intercellular Cell Adhesion Molecule-1 (ICAM-1) and Vascular Cell Adhesion Molecule-1 (VCAM-1), as well as suppressing TNFα-induced NF-κB activation and IkB phosphorylation. Furthermore, Klotho is recognized as an early predictor of atherosclerosis [36].

Evidence suggests that serum Klotho concentration can be elevated through both endogenous and exogenous pathways. Administering recombinant Klotho protein as an exogenous supplement may represent a safe and effective therapeutic strategy for addressing Klotho deficiency [37]. In addition, there are endogenous pathways. Animal experimental studies have demonstrated that histone deacetylase (HDAC) inhibitors can effectively improve and reduce the loss of targeted Klotho in mice, and Berberine has been shown to upregulate the expression of the Klotho gene and enhance autophagic processes, thereby exerting a protective effect in the context of acute kidney injury [38, 39].

An important and hitherto unexplored finding of our study is the delineation of the association between serum Klotho concentrations and all-cause mortality in diabetic patients, with a particular emphasis on age's mediating role. In light of the escalating prevalence of chronic diseases, including diabetes and cardiovascular diseases within an aging population, and given that type 2 diabetes mellitus—accounting for the bulk of diabetes incidences—is deemed an age-associated disease [40, 41], this facet is notably pertinent. Observations reveal that individuals with diabetes undergo a marked decrease in life expectancy, forfeiting approximately 7.5 to 20 years relative to their non-diabetic counterparts [42]. Prior research has illustrated a decline in serum Klotho levels concomitant with aging [43, 44], a consistency echoed in our study's baseline characteristics. Our inquiry into the mediating influence of age on the nexus between Klotho levels and all-cause mortality in diabetic patients unveils substantial evidence that a major fraction of Klotho's impact on all-cause mortality (circa 76.1%) is channeled through age. This accentuates the pivotal role of age as a mediating variable in the linkage between serum Klotho levels and all-cause mortality in diabetic individuals.

Our study has several strengths. The present study sample size from a large national database was large enough to determine the association between klotho levels and all-cause mortality in patients with diabetes and the findings represent a wide range. In addition, we provided further understanding of the association between serum klotho concentrations and all-cause mortality in patients with diabetes by mediating analysis with age as the mediating variable. However, our study still has many limitations. Firstly, as a retrospective cohort study, the effect of residual confounding factors could not be completely eliminated. Secondly, we restricted our study to persons with diabetes who were 40 years of age or older, it is unclear whether these findings apply to people of different ages. Lastly, the absence of causal models within our study framework precludes definitive conclusions regarding causality. Hence, prospective studies are warranted to delve deeper into the causal relationships.

Conclusion

Our investigation conclusively establishes that diminished serum Klotho levels are significantly correlated with an elevated risk of all-cause mortality among patients with diabetes (Klotho levels < 829.138 pg/ml). Importantly, we elucidated a nonlinear dynamic between serum Klotho concentrations and mortality risk, underscoring the complexity of this relationship. The predominance of these associations appears to be substantially mediated by age, indicating that the impact of Klotho on mortality is intricately intertwined with the aging process. This insight not only highlights the critical role of Klotho as a biomarker in diabetic populations but also underscores the significance of age as a determinant in the observed outcomes.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13098-025-01686-9.

Supplementary Material 1

Author contributions

KW wrote the manuscript. JC collected the data. KW and JC analyzed the data. YL and JW revised the manuscript. JW conducted the study design and quality control. All authors read and approved the final manuscript.

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Data availability

A public database was used in this study. The data of this study are available here: https://www.cdc.gov/nchs/nhanes/.

Declarations

Informal consent

Informal consent was obtained from all adult participants of the NHANES, and the NCHS Research Ethics Review Board approved the protocol.

Competing interests

The authors declare no competing interests.

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