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Association of serum Klotho with the severity and mortality among adults with cardiovascular-kidney-metabolic syndrome

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Abstract

Background Cardiovascular-kidney-metabolic (CKM) syndrome is characterized as a systemic disease resulting from the pathophysiological interplay among metabolic risk factors, chronic kidney disease (CKD), and cardiovascular disease (CVD). The Klotho protein may serve as a novel biomarker. However, the utility of serum Klotho levels as an indicator of severity and mortality risk in CKM syndrome remains uncertain.

Methods This study involved 9,871 participants from the National Health and Nutrition Examination Survey (NHANES) conducted between 2007 and 2016. Serum Klotho levels were measured using an enzyme-linked immunosorbent assay kit. The optimal cutoff value was established through the maximum Youden's index. Multivariable weighted regression models were employed to calculate the odds ratio and hazard ratio, along with the 95% confidence interval, to evaluate the association between serum Klotho levels and the severity of CKM syndrome, as well as all-cause and cardiovascular mortality. Additionally, the receiver operating characteristic curve and restricted cubic spline curves were utilized to assess predictive efficacy and to explore nonlinear relationships.

Results After adjusting for potential confounding factors, a non-linear relationship was seen between the Klotho protein, and CKM syndrome. In the multivariable, piecewise logistic regression, when the Serum klotho was less than 801, the risk of CKM syndrome decreased with the increase in Serum klotho (OR = 0.82, 95%CI 0.70, 0.96; $p < 0.001$). Furthermore, we observed the association when the Serum klotho was greater than 801 (OR = 0.94, 95%CI 0.89, 0.99; $p = 0.035$). The relationship between serum Klotho levels and all-cause mortality was U-shaped, while the relationship with cardiovascular mortality was L-shaped. Specifically, low serum Klotho levels were associated with an increase in all-cause mortality by 21% and cardiovascular mortality by 76% among patients with CKM syndrome. Furthermore, serum Klotho levels demonstrated excellent predictive efficacy for both the severity and mortality associated with CKM syndrome.

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Conclusions This study indicates that low serum Klotho levels serve as reliable indicators of both the severity of CKM syndrome and the associated risk of mortality.

Highlights

- This study is the first to reveal the relationship between serum Klotho levels and the severity and mortality of CKM syndrome.
- Serum Klotho is significantly associated with the severity of CKM syndrome.
- Serum Klotho was U-shaped related to all-cause mortality in patients with CKM syndrome.
- Serum Klotho was L-shaped associated with cardiovascular mortality in patients with CKM syndrome stage 0–3.
- Low serum Klotho level is an independent risk factor for the progression of CKM syndrome as well as all-cause and cardiovascular mortality.

Keywords Cardiovascular-kidney-metabolic syndrome, Metabolic syndrome, Klothos, NHANES

Introduction

The intricate relationship among metabolic syndrome (MS), cardiovascular disease (CVD), and chronic kidney disease (CKD) has been highlighted by numerous observational studies and epidemiological data [1–4]. This triad frequently co-occurs in CVD patients, who often present with CKD and type 2 diabetes mellitus (T2DM). Furthermore, individuals with CKD and/or T2DM demonstrate worsened cardiovascular prognoses [4]. As understanding of the relevant pathophysiological mechanisms deepens, the connections among these conditions become increasingly clear. The American Heart Association emphasized the significant interactions by releasing a scientific statement in October 2023 [5], which is defined as a systemic condition that is primarily characterized by complex interactions among various metabolic risk factors, CVD and CKD. These interrelated components contribute significantly to the overall health status of individuals affected by the syndrome. The syndrome is stratified into four progressive stages, which ranges from stage 0 to 4, serving to illustrate the potential for worsening multiorgan dysfunction over time. As the stages progress, there is an associated increase in the risk of experiencing adverse cardiovascular events. This classification system is crucial for understanding the severity of the syndrome and for guiding appropriate clinical interventions [5, 6].

Klotho is a critical factor in mammalian aging and encodes a protein that exists in multiple isoforms, each possessing distinct functions [7]. Currently, three distinct isoforms of Klotho have been identified: the full-length transmembrane form, the truncated soluble variant, and the secreted version [8]. The full-length version serves as a co-receptor for fibroblast growth factor 23 and is vital for maintaining phosphate equilibrium [8]. The circulating soluble and secreted forms of Klotho, collectively referred to as α-Klotho, are thought to exert endocrine or paracrine effects on various organ systems, including the kidneys, bones, brain, cardiovascular system, lungs, and vascular endothelium [9–13]. A decline in Klotho levels is generally associated with the aging process [7]. Recent retrospective analyses indicate that Klotho has the potential to serve as a novel biomarker for a variety of age-related conditions. These conditions encompass a spectrum of health issues, including heart failure, hypertension, diabetes, and cardiovascular mortality [14]. The identification of Klotho as a biomarker could facilitate early detection and intervention for these ailments, ultimately contributing to better management and improved outcomes for affected individuals.

Given the rising incidence of CKM syndrome and its significant impact on CVD progression, assessing the predictive value of serum Klotho levels is of paramount importance. This assessment is vital for predicting the severity of the condition and the associated mortality risk in patients with CKM syndrome, thereby facilitating more effective risk stratification and timely interventions.

Methods

The study used data from the National Health and Nutrition Examination Survey (NHANES), spanning five cycles from 2007 to 2016. NHANES provides a comprehensive overview of the health and nutritional status among the non-institutionalized civilian population in the United States [15, 16]. Approval for the survey protocol was obtained from the National Center for Health Statistics Research Ethics Review Board, ensuring the ethical conduct of the study [17].

Study Population

The analysis excluded 37,197 participants who lacked sufficient information on Klotho, 2,641 participants under the age of 20, 547 individuals with missing values for CKM indicators, and 709 individuals with missing covariable and mortality data. Consequently, the final sample comprised 9,871 participants with complete data (Fig. S1).

Klotho concentrations assessment

In this investigation, we focused on quantifying Klotho protein levels in serum samples, which were meticulously stored at minus 80 degrees Celsius at the Centers for Disease Control and Prevention. Following proper sample preservation, the specimens were transported on dry ice to Laboratories at the University of Washington in Seattle, Washington, for a comprehensive analysis conducted between 2019 and 2020 [7]. The Klotho levels were ascertained utilizing a ELISA kit procured from IBL International in Japan. Data obtained from the ELISA assays were systematically entered into the Oracle management system, ensuring a high level of data integrity. Subsequent verification of the data was performed by a designated regional supervisor, further validating the precision and reliability of our findings [8, 18]. Each serum sample was subjected to duplicate ELISA measurements to ensure the robustness of our results. The mean values of these measurements were calculated in strict accordance with the manufacturer's instructions.

Any discrepancies noted between the two measurements that exceeded 10% warranted a reanalysis of the sample in question. The laboratory procedures, including quality assurance and monitoring for Klotho concentration analysis, adhered to the NHANES guidelines [19].

CKM syndrome assessment

CKM syndrome is defined by the simultaneous occurrence of subclinical or clinical CVD, CKD and MS, with specific definitions outlined in Table S1 [6, 20, 21]. Clinical CVD includes a history of myocardial infarction, chronic heart failure, stroke or coronary heart disease, while subclinical CVD is identified by a 10-year CVD risk of ≥20% or the presence of high-risk CKD [22]. To assess estimated Glomerular Filtration Rate(eGFR), we utilized 2021 Chronic Kidney Disease Epidemiology Collaboration creatinine equations, which do not account for race or ethnicity [23]. The 10-year CVD risk was estimated through a simplified CKM risk algorithm that incorporated factors such as age, sex, tobacco use, blood pressure, cholesterol levels, diabetes status, kidney function, and the use of antihypertensive medications and statins. Metabolic disorders include overweight/obesity, abdominal obesity, prediabetes, diabetes, hypertension, dyslipidemia, and metabolic syndrome [24, 25].

To account for the varying clinical severity of CKM syndrome, participants were classified into four distinct CKM stages [26]. Stage 0 indicated the absence of abnormalities, while stage 1 was characterized by obesity or prediabetes alone. Stage 2 included individuals with at least one additional metabolic disorder or CKD. Stage 3 was defined by the presence of subclinical CVD alongside metabolic disorders or CKD. Finally, stage 4 represented clinical CVD in conjunction with metabolic disorders or CKD. The specific criteria for each stage are detailed in Table S1 [27].

Assessments of other covariables

The study investigated various covariables including gender, race, age, BMI, education level, PIR, smoking and alcohol consumption to evaluate their potential impact on obscuring the association between serum Klotho and symptoms of CKM syndrome. Educational attainment was classified into the following categories: less than a 9th-grade education, 9-11th grade and individuals with post-secondary education. Alcohol intake was divided into two categories: those who do not consume alcohol and those who do. Meanwhile, smoking habits were classified as individuals who have never smoked and those who are current smokers. Within the scope of this study, hypertension was defined by SBP measurements averaging 140 mmHg or greater, and DBP readings averaging 90 mmHg or greater. This definition was applied alongside either a confirmed medical diagnosis or documented use of antihypertensive medications. For the diagnosis of diabetes mellitus, this study considered fasting glucose levels of 7.0 mmol/L or above, levels of glycohemoglobin >6.5%, the presence of antidiabetic medications or insulin use, or a diagnosis recorded by a healthcare provider. These parameters align with standard clinical practices for identifying these conditions [28].

Statistical analysis

Conducting this study in alignment with the analytical protocols of the 2007–2016 NHANES, we utilized strata, primary sampling units, and sample weights to adeptly navigate the intricacies of the survey's design. Continuous variables describing the population were reported as means (±SE) or as percentages. Measurement data not conforming to normal distribution were expressed as median (upper quartile, lower quartile) [M (Q1, Q3)]. Subjects were stratified into two categories according to their Klotho concentrations, using the group with superior Klotho levels as the comparative reference. This categorization was established through a rigorous ROC analysis to ensure its clinical significance.

This study utilized multiple linear regression analyses, adjusted for all covariables, to investigate the connection between Klotho and CKM syndrome stages ranging from 0 to 4. Additionally, adjusted for various covariables, cox proportional hazards models were employed to ascertain the association between Klotho levels and the risks of all-cause and CVD mortality in participants with CKM syndrome at stages 0–3 and stage 4. The association between Klotho levels and all-cause or cardiovascular mortality was explored using restricted cubic splines to model the dose-response relationship across CKM syndrome stages

0–4 and in the comparison between stages 0–3 and stage 4.

To enhance the robustness of the findings, this study conducted supplementary analyses, including subgroup analyses, to address demographic differences between individuals with varying Klotho levels and those with CKM syndrome. This study evaluated the predictive power of Klotho levels for CKM syndrome stages 0 to 4 with all-cause and CVD mortality, utilizing ROC curve analysis. The values of AUC were determined to signify the predictive accuracy of these models. Analyses were performed using R (version 4.2.3).

Results

Characteristics of participation

The basic features of study participants, categorized according to the CKM syndrome group, are detailed in Table 1. This study comprised 18,373 participants with the median age of 57.00 (48.00–66.00) years old. Within this group, 50.58% (n=4,993) were women, and 16.64% (n=1,643) had diabetes. Importantly, notable differences were found among the CKM groups regarding sex, age, BMI, race, alcohol consumption, smoking status, educational attainment, diabetes prevalence, hypertension, physical activity, and the distribution of the PIR.

Association of Klotho and CKM syndrome

After adjusting for potential confounding factors, a nonlinear relationship was seen between the Klotho protein, and CKM syndrome via restricted cubic spline (RCS) curves(Fig S2). In the multivariable, piecewise logistic regression, when the Serum klotho was less than 801, the risk of CKM syndrome decreased with the increase in Serum klotho (OR=0.82, 95%CI 0.70, 0.96; p<0.001). Furthermore, we observed the association when the Serum klotho was greater than 801 (OR=0.94, 95%CI 0.89, 0.99; p=0.035). Moreover, the optimal cutoff of Klotho was determined through a rigorous ROC analysis aimed at maximizing the Youden index, establishing a threshold at 722. When using the higher Klotho group as a reference, the lower Klotho group demonstrated a significantly elevated odds ratio for CKM syndrome, reporting the OR of 1.20 (95% CI, 1.11–1.30, P<0.001) (Table 2). This reveals that those with Klotho levels falling below the threshold of 722 are more likely to develop cardiorenal metabolic syndrome, underscoring the possibility of using Klotho as a biomarker for evaluating risk.

Associations between Klotho levels and all-cause and CVD mortality

Table 3 delineates the study's findings on the association between Klotho and rates of mortality attributable to all causes and cardiovascular diseases. This table demonstrates the association between Klotho concentrations and likelihood of all-cause and CVD mortality across stages 0–3 and stage 4 of CKM syndrome. Designating the group with elevated Klotho levels as the reference, individuals with decreased Klotho levels displayed a significantly heightened risk for all-cause mortality (HR=1.21; 95% CI, 1.08–1.36, P<0.001) and mortality due to cardiovascular causes (HR=1.76; 95% CI, 1.41–2.19, P<0.001). These findings highlight the protective function of Klotho against mortality risks, particularly concerning cardiovascular health, and imply that lower Klotho levels may act as predictive markers for adverse outcomes in CKM syndrome.

Nonlinear relationships of Klotho with all-cause and CVD mortality

Figure 1 illustrates the results of the RCS analysis, which investigates the nonlinear connection between the levels of Klotho and the risks associated with all-cause and cardiovascular mortality among individuals with CKM syndrome stages 0–3 and stage 4. Following adjustments for all relevant covariables in the comprehensive analytical model 3, the findings revealed that Klotho levels demonstrated a nonlinear relationship with both all-cause mortality and CVD mortality across the CKM syndrome stages (P nonlinear<0.05). Notably, a U-shaped relationship between Klotho levels and the risk of all-cause mortality has been identified, suggesting that both extremely high and low Klotho levels are associated with an increased likelihood of all-cause mortality. Conversely, patients at CKM syndrome stages 0–3 exhibited an L-shaped association with cardiovascular mortality association. Within this framework, elevated Klotho levels were associated with a lower likelihood of cardiovascular mortality.

The predictive performance of the Klotho for CKM syndrome, all-cause mortality and CVD mortality

Figure 2 and Table S2 present the ROC curves from the analysis. The fully adjusted model for CKM syndrome yielded an AUC of 0.748, with a 95% CI spanning 0.733 to 0.763. This AUC reflects a sensitivity of 0.663, suggesting that the model correctly identifies approximately 66.3% of those with CKM syndrome, while a specificity of 0.710 indicates that about 71.0% of individuals without the syndrome are also accurately recognized by the model. In the analysis of all-cause mortality, the AUC was determined to be 0.769, accompanied by a 95% CI of 0.755 to 0.783. This AUC indicates a model sensitivity of 0.679 and a specificity of 0.733. For cardiovascular mortality, the AUC was found to be 0.741, with a 95% CI from 0.715 to 0.767, corresponding to a sensitivity of 0.722 and a specificity of 0.667.

Table 1 Baseline characteristics of individuals classified by stages of CKM

Characteristic	Cardiovascular- Kidney- Metabolic Syndrome					*P*-value
	0, *N* = 846	**1, *N* = 1,545**	**2, *N* = 4,500**	**3, *N* = 1,954**	**4, *N* = 1,026**	
Median (IQR)						
Age	51.00 (45.00, 60.00)	52.00 (45.00, 61.00)	60.00 (50.00, 67.00)	54.00 (46.00, 63.00)	66.00 (58.00, 72.00)	< 0.001
BMI	22.40 (20.80, 23.60)	28.20 (26.10, 31.31)	28.91 (25.76, 33.00)	30.40 (26.60, 34.61)	30.10 (26.47, 34.50)	< 0.001
PIR	3.03 (1.26, 5.00)	2.78 (1.31, 4.92)	2.40 (1.22, 4.63)	1.98 (1.05, 3.91)	1.65 (0.98, 3.31)	< 0.001
TC (mg/dl)	195.50 (172.00, 220.00)	197.00 (173.00, 221.00)	195.00 (168.00, 224.00)	212.00 (184.00, 243.00)	173.00 (147.00, 211.00)	< 0.001
TG (mg/dl)	79.00 (58.00, 101.00)	90.00 (70.00, 109.00)	158.00 (107.00, 218.00)	182.00 (124.00, 275.00)	146.00 (95.00, 216.00)	< 0.001
Scr (mg/dl)	0.82 (0.72, 0.95)	0.82 (0.70, 0.96)	0.91 (0.76, 1.06)	0.79 (0.67, 0.92)	0.98 (0.82, 1.19)	< 0.001
HBA1C (%)	5.40 (5.20, 5.50)	5.50 (5.30, 5.80)	5.70 (5.40, 6.10)	5.80 (5.40, 6.30)	5.90 (5.60, 6.60)	< 0.001
HDL-C (mg/dl)	65.00 (55.00, 76.00)	57.00 (48.00, 68.00)	49.00 (41.00, 60.00)	46.00 (38.00, 55.00)	46.00 (39.00, 56.00)	< 0.001
eGFR (ml/(min×1.73m2)	96.87 (85.85, 106.55)	95.40 (83.52, 105.87)	89.83 (74.19, 100.80)	94.54 (77.87, 105.91)	77.38 (62.43, 93.35)	< 0.001
Klotho (pg/ml)	852.55 (702.53, 1,047.70)	830.50 (686.10, 1,029.50)	807.00 (660.78, 996.20)	818.60 (664.43, 1,000.40)	771.05 (622.65, 946.73)	< 0.001
Number (%)						
Gender (%)						< 0.001
Male	426 (50.35%)	676 (43.75%)	2,750 (61.11%)	421 (21.55%)	605 (58.97%)	
Female	420 (49.65%)	869 (56.25%)	1,750 (38.89%)	1,533 (78.45%)	421 (41.03%)	
Race (%)						< 0.001
Non-Hispanic White	76 (8.98%)	242 (15.66%)	708 (15.73%)	353 (18.07%)	112 (10.92%)	
Non-Hispanic Black	67 (7.92%)	174 (11.26%)	484 (10.76%)	265 (13.56%)	77 (7.50%)	
Mexican	431 (50.95%)	707 (45.76%)	1,962 (43.60%)	880 (45.04%)	536 (52.24%)	
Other Hispanic	139 (16.43%)	324 (20.97%)	958 (21.29%)	292 (14.94%)	232 (22.61%)	
Others	133 (15.72%)	98 (6.34%)	388 (8.62%)	164 (8.39%)	69 (6.73%)	
Education (%)						< 0.001
Less Than 9th Grade	188 (22.22%)	383 (24.79%)	1,276 (28.36%)	617 (31.58%)	370 (36.06%)	
9-11th Grade	353 (41.73%)	720 (46.60%)	2,199 (48.87%)	999 (51.13%)	511 (49.81%)	
High school grades and more	305 (36.05%)	442 (28.61%)	1,025 (22.78%)	338 (17.30%)	145 (14.13%)	
Marital (%)						0.117
Married/Living with a partner	574 (67.85%)	994 (64.34%)	2,935 (65.22%)	1,250 (63.97%)	639 (62.28%)	
Widowed/Divorced/Never married	272 (32.15%)	551 (35.66%)	1,565 (34.78%)	704 (36.03%)	387 (37.72%)	
Diabetes (%)						< 0.001
No	846 (100.00%)	1,545 (100.00%)	3,665 (81.44%)	1,501 (76.82%)	671 (65.40%)	
Yes	0 (0.00%)	0 (0.00%)	835 (18.56%)	453 (23.18%)	355 (34.60%)	
Hypertension (%)						< 0.001
No	846 (100.00%)	1,545 (100.00%)	1,888 (41.96%)	911 (46.62%)	236 (23.00%)	
Yes	0 (0.00%)	0 (0.00%)	2,612 (58.04%)	1,043 (53.38%)	790 (77.00%)	
Current smoking (%)						< 0.001
No	458 (54.14%)	922 (59.68%)	2,215 (49.22%)	1,056 (54.04%)	364 (35.48%)	
Yes	388 (45.86%)	623 (40.32%)	2,285 (50.78%)	898 (45.96%)	662 (64.52%)	
Alcohol (%)						< 0.001
Yes	674 (79.67%)	1,168 (75.60%)	3,398 (75.51%)	1,281 (65.56%)	743 (72.42%)	
No	172 (20.33%)	377 (24.40%)	1,102 (24.49%)	673 (34.44%)	283 (27.58%)	
CHF (%)						< 0.001
Yes	0 (0.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	382 (37.23%)	
No	846 (100.00%)	1,545 (100.00%)	4,500 (100.00%)	1,954 (100.00%)	644 (62.77%)	
CHD (%)						< 0.001
Yes	0 (0.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	509 (49.61%)	

Table 1 (continued)

Characteristic	Cardiovascular- Kidney- Metabolic Syndrome					*P*-value
	0, *N* = 846	**1, *N* = 1,545**	**2, *N* = 4,500**	**3, *N* = 1,954**	**4, *N* = 1,026**	
No	846 (100.00%)	1,545 (100.00%)	4,500 (100.00%)	1,954 (100.00%)	517 (50.39%)	
Stroke (%)						< 0.001
Yes	0 (0.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	417 (40.64%)	
No	846 (100.00%)	1,545 (100.00%)	4,500 (100.00%)	1,954 (100.00%)	609 (59.36%)	
CVD (%)						< 0.001
Yes	846 (100.00%)	1,545 (100.00%)	4,500 (100.00%)	1,954 (100.00%)	0 (0.00%)	
No	0 (0.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	1,026 (100.00%)	
CKD (%)						< 0.001
Yes	843 (99.65%)	1,537 (99.48%)	4,059 (90.20%)	1,815 (92.89%)	801 (78.07%)	
No	3 (0.35%)	8 (0.52%)	441 (9.80%)	139 (7.11%)	225 (21.93%)	

Abbreviations: IQR- interquartile range; BMI - Body Mass Index; PIR Poverty income ratio; CHF - Congestive Heart Failure; CHD- Coronary Heart Disease; CVD - Cardiovascular Disease; CKD - Chronic Kidney Disease; TC - Total Cholesterol; TG – Triglycerides; Scr - Serum Creatinine; HDL-C - High-Density Lipoprotein Cholesterol; eGFR - Estimated Glomerular Filtration Rate

Table 2 Levels of Klotho in Relation to CKM Stage 0 to 4

	Model1		**Model2**		**Model3**	
	OR (95%CI)	***P***	**OR (95%CI)**	***P***	**OR (95%CI)**	***P***
Two-piecewise logistic regression model (Infection point = 801)						
Serum klotho<801	0.74(0.64, 0.85)	< 0.001	0.78(0.68, 0.90)	< 0.001	0.82(0.70, 0.96)	< 0.001
Serum klotho ≥ 801	0.91(0.87, 0.94)	< 0.001	0.90(0.86, 0.93)	< 0.001	0.94(0.89, 0.99)	0.035
Binary Variable (Cut-off point = 722)						
Serum klotho ≥ 722	Ref		Ref		Ref	
Serum klotho<722	1.23(1.14, 1.32)	< 0.001	1.19(1.11, 1.28)	< 0.001	1.20(1.11, 1.30)	< 0.001

Abbreviations: OR, odds ratio; CI, confidence interval. Adjusted Mode: Model 1 (unadjusted), Model 2 (adjusted for sex, age, and race), and Model 3 (further adjusted for sex, age, race, education, PIR, BMI, smoking, alcohol consumption, hypertension, and diabetes). Bold indicates P value < 0.05

Table 3 Regression analysis of Klotho and all-cause mortality, cardiovascular mortality among CKM stage 0–3 and stage 4 populations

Characteristic	**Model 1**	***P***	**Model 2**	***P***	**Model 3**	***P***
	HR (95% CI)		**HR (95% CI)**		**HR (95% CI)**	
All-cause mortality						
Serum klotho ≥ 722	Ref		Ref		Ref	
Serum klotho<722	1.29 (1.15, 1.44)	< 0.001	1.24 (1.10, 1.39)	< 0.001	1.21(1.08, 1.36)	< 0.001
Cardiovascular mortality						
Serum klotho ≥ 722	Ref		Ref		Ref	
Serum klotho<722	1.84 (1.48, 2.28)	< 0.001	1.79 (1.44, 2.22)	< 0.001	1.76 (1.41, 2.19)	< 0.001

Abbreviations: HR, hazard ratio; CI, confidence interval. Adjusted Mode: Model 1 (unadjusted), Model 2 (adjusted for sex, age, and race), and Model 3 (further adjusted for gender, age, race, education, PIR, BMI, smoking, alcohol consumption, hypertension, and diabetes). Bold indicates P value < 0.05

Stratification connection of Klotho for all-cause and CVD mortality

Table S3-4 provides a comprehensive stratified analysis of the correlation between Klotho levels and the hazards associated with both all-cause and CVD mortality, categorized by gender, smoking habits, alcohol consumption, hypertension and diabetes. This analysis aimed to determine whether the association between Klotho and mortality risk differed among these subgroups. The findings indicated that there were no significant interactions detected between Klotho levels and any of the subgroups analyzed (*P* for interaction > 0.05).

Discussion

This research pioneers an investigation into the association between Klotho and the severity and mortality associated with CKM syndrome. Involving 9,871 adults, the research demonstrated the significant association between serum Klotho and the severity of CKM syndrome. The study reveals that decreased Klotho levels are independently associated with an increased risk of CKM syndrome. Furthermore, the results suggest that lower Klotho levels may elevate all-cause mortality by 21% and CVD mortality by 76% in individuals diagnosed with CKM syndrome.

Klotho, originally discovered by Kuro-o and colleagues in 1997, plays a crucial role in modulating various age-related phenotypes in mice [29]. Mice with impaired

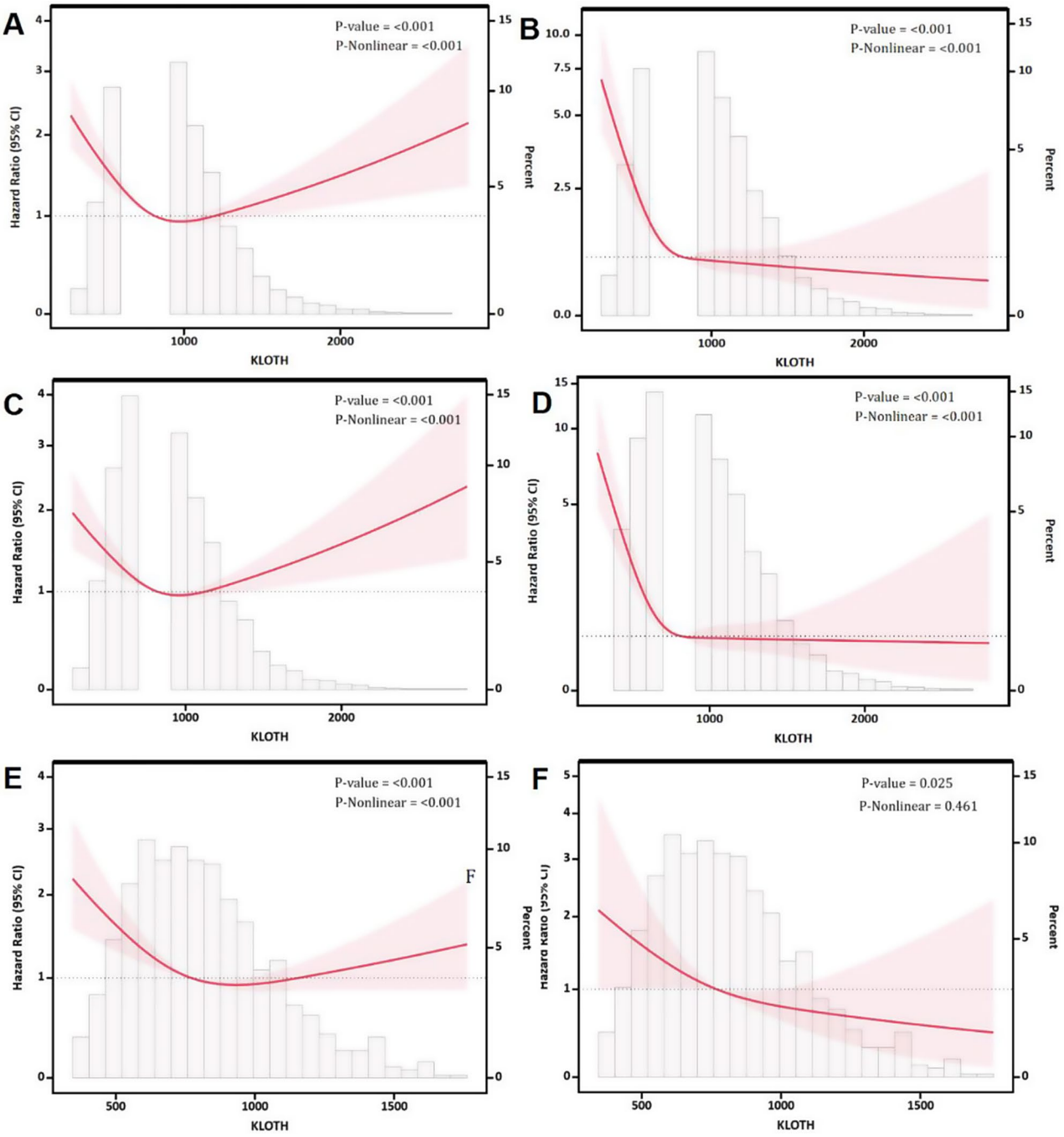

Fig. 1 Nonlinear associations Klotho and all-cause mortality, cardiovascular mortality among CKM stage 0–3 and stage 4 populations **A**: The relationship between Klotho and all-cause mortality among CKM stage 0–4 populations (fully adjusted RCS Model). **B**: The relationship between Klotho and cardiovascular mortality among CKM stage 0–4 populations (fully adjusted RCS Model). **C**: The relationship between Klotho and all-cause mortality among CKM stage 0–3 populations (fully adjusted RCS Model). **D**: The relationship between Klotho and cardiovascular mortality among CKM stage 0–3 populations (fully adjusted RCS Model). **E**: The relationship between Klotho and all-cause mortality among CKM stage 4 populations (fully adjusted RCS Model). **F**: The relationship between Klotho and cardiovascular mortality among CKM stage 4 populations (fully adjusted RCS Model). Adjustments in the model accounted for the following variables: age, gender, educational level, ethnicity, marital status, family PIR, smoking status, drinking status, BMI, diabetes, and hypertension

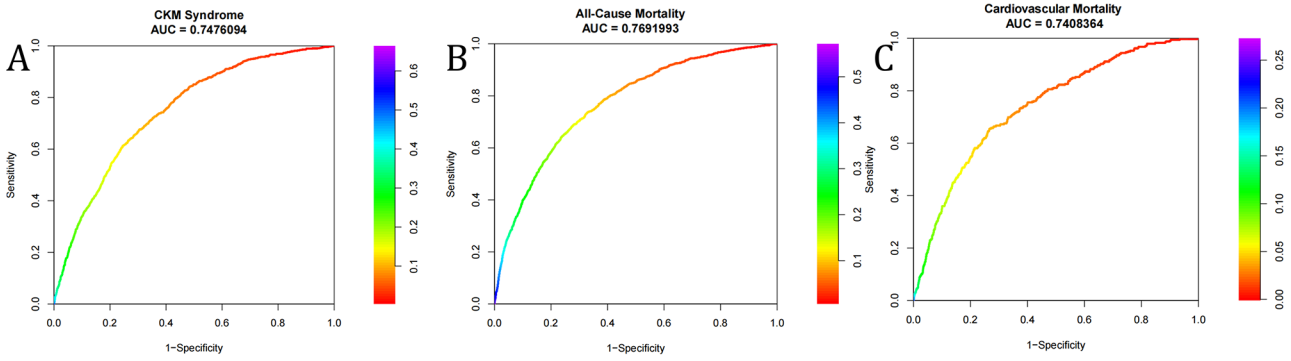

Fig. 2 ROC curve analysis of Klotho with age, gender, race, and BMI in predicting CKM Stage 0 to 4, all-cause or cardiovascular mortality. The AUC for CKM syndrome, all-cause and cardiovascular mortality were 0.748, 0.769, and 0.741, respectively

expression of the Klotho gene exhibit a syndrome resembling accelerated aging, characterized by a shortened lifespan, sterility, atherosclerosis, osteoporosis, skin atrophy, and emphysema [29]. Conversely, studies have indicated that when Klotho is overexpressed in mice, there is a corresponding extension of their lifespan, highlighting the gene's potential impact on longevity [30]. The Klotho gene gives rise to a transmembrane protein, predominantly found in the kidney. This protein comprises a sizable extracellular domain, a transmembrane region, and a brief intracellular segment, playing pivotal roles in renal function and mineral metabolism [8, 31]. The transmembrane Klotho interacts with the fibroblast growth factor receptor (FGFR) to form a complex that enhances its binding affinity for FGF23, thereby regulating phosphate excretion and the production of active vitamin D in the kidney [8, 19]. Additionally, the transmembrane Klotho is cleaved by membrane-bound proteases, such as α- and β-secretase, resulting in the release of the entire extracellular domain, which circulates as soluble Klotho. This soluble form exerts a wide range of effects on tissue and cellular functions, even in the absence of Klotho expression [8, 31].

CKM syndrome is a systemic disease characterized by complex pathophysiological processes, including metabolic risk factors, CKD, and CVD [5]. Previous studies have demonstrated that Klotho influences numerous metabolic pathways that are crucial for both the pathogenesis and prevention of CVD [32]. These pathways include the inhibition of lipid peroxidation and inflammation [33], the prevention of cardiac fibrosis development [34], the protection against endothelial damage and vascular calcification, and the reduction of vascular stiffness [13]. Importantly, expression levels of Klotho mRNA have been found to be markedly reduced in the kidneys of individuals with CKD [35]. In the early stages of CKD (stage≤2), there is an initial decline in serum and urinary Klotho levels, which is subsequently accompanied by an increase in serum FGF23 levels [36]. The extent of the reduction in urinary Klotho is positively correlated with the severity of eGFR decline in CKD patients [36, 37]. Further sensitivity analysis was conducted to address the potential confounding effect of eGFR on Klotho levels within this study population but failed to demonstrate a substantial relationship between these two variables (Fig.S3). Elevated circulating FGF23 has been independently associated with adverse renal and cardiovascular outcomes [36, 38, 39]. The Klotho/FGF23 axis is therefore pivotal in the context of CKD and CVD [36, 40]. Accordingly, it becomes imperative to explore the intricate interplay between Klotho levels and the associated risk of progression and mortality within CKM syndrome.

Furthermore, this study employed RCS curve analysis to reveal a U-shaped link between Klotho levels and all-cause mortality. This finding is consistent with a prior prospective study that identified a similar U-shaped association in patients with rheumatoid arthritis (RA) [41]. Recently, some studies have explored the clinical application of serum Klotho as a marker and therapeutic target. This study underscoring the necessity of monitoring serum Klotho level and maintaining it within an optimal range to mitigate early mortality potentially. Reduced serum Klotho may increase the risk of all-cause mortality in patients with CKM syndrome by affecting renal function, increasing the risk of cardiovascular events, exacerbating the progression of the metabolic syndrome, as well as triggering inflammation and oxidative stress, and disturbances in the regulation of calcium and phosphorus metabolism, among many other factors. Additionally, the RCS curves indicated an L-shaped association between Klotho and cardiovascular mortality in patients experiencing CKM syndrome stages 0–3. This implies that reduced Klotho levels significantly elevate the risk of CVD mortality, which is attributed to a variety of mechanisms such as serum Klotho exerting vascular protection, anti-inflammation, improvement of ventricular remodeling, and blood pressure.

Therefore, serum Klotho could act as a vital biomarker for screening high-risk groups and forecasting disease progression and mortality in individuals with CKM

syndrome. Meanwhile, it was revealed that increasing klotho levels could significantly reduce the risk of death within a certain range, and serum klotho could be an important target for CKM syndrome treatment in the future.

Addressed

This study is the first to investigate the relationship between serum Klotho levels and the severity and mortality of CKM syndrome. It underscores the potential of serum Klotho as a biomarker for predicting both the severity and mortality associated with CKM syndrome. Notably, among individuals with CKM syndrome stage 4, serum Klotho levels demonstrate a linear association with cardiovascular mortality, suggesting its viability as a therapeutic target.

This study has several limitations. Firstly, retrospective studies inherently cannot eliminate the possibility of bias, and cross-sectional designs do not allow for causal inferences. Secondly, the study's focus on European populations limits the generalizability of the findings to other demographic groups. Consequently, future large-scale prospective studies are essential to explore the longitudinal association between Klotho levels and the severity and mortality risk of CKM syndrome.

Conclusions

This study underscores the significant association between serum Klotho levels and the severity of CKM syndrome. Low serum Klotho levels serve as a reliable indicator of both CKM syndrome severity and associated mortality risk. Furthermore, serum Klotho levels may be utilized as a novel biomarker for staging CKM syndrome and guiding various treatment strategies. Elevating serum Klotho levels could represent a promising therapeutic approach for individuals with stage 4 CKM syndrome.

Supplementary Information

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Supplementary Material 1

Supplementary Material 2

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Author contributions

Jiao Tang and Zhehao Xu planned and designed the study, analyzed and interpreted the data, and wrote the manuscript. Li Ren, Jiahua Xu, Xin Chen, Yian Jin, and Ruiyun Liang analyzed the data and created the tables presented in the manuscript. Huanji Zhang, as the corresponding author, provided important advice in the study design, supervised and coordinated the study conduct process, and revised the manuscript and tables. All authors have contributed to the manuscript and approved the submitted version.

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Data availability

No datasets were generated or analysed during the current study.

Declarations

Disclosure Statement

The authors have nothing to disclose.

Competing interests

The authors declare no competing interests.

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