The prognostic value of serum α-klotho in age-related diseases among the US population: A prospective population-based cohort study

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ABSTRACT

Objective: α-Klotho is a potential biological marker of aging with satisfactory clinical applicability. However, its prognostic significance in age-related diseases has largely been undermined. Therefore, we aimed to report the prognostic value of serum α-klotho levels in age-related diseases.
Methods: Participants with available serum α-klotho data from the National Health and Nutrition Examination Survey (2007–2016) were included. Their survival status was collected at 7.62 ± 2.99 years after serum α-klotho data was collected, and the endpoint was all-cause and cardiovascular mortality. A Cox regression model was established to examine the association between serum α-klotho levels and all-cause and cardiovascular mortality.
Results: The present study included 13,746 U.S. adults with a survey-weighted mean age of 56.19 ± 10.42 years old. Of these, 52.2 % were female and 72.9 % were non-Hispanic whites. The optimal cutoff value of serum α-klotho for predicting all-cause mortality risk in the general population was 603.5 pg/ml. Individuals with low serum α-klotho (<603.5 pg/ml) had a significantly higher risk of all-cause (adjusted HR: 1.34(1.18–1.52), P < 0.001) and cardiovascular mortality (adjusted HR: 1.63(1.27–2.10), P < 0.001). Subgroup analysis showed that low serum α-klotho level was an independent risk factor for all-cause and cardiovascular mortality in people with hypertension, congestive heart failure, diabetes mellitus, and emphysema, while it was an independent risk factor for all-cause mortality in patients with renal insufficiency.
Conclusion: A low serum α-klotho concentration (<603.5 pg/ml) could serve as a marker of all-cause and cardiovascular mortality in the general population and in people with age-related diseases, including hypertension, congestive heart failure, diabetes mellitus, and emphysema.

1. Introduction

Since physical aging is a significant risk factor for age-related diseases, the outcome prediction models of these diseases commonly include age as an essential risk factor (Jylhävä et al., 2017). However, the observation that individuals do not age at the same pace led to only chronological aging, which refers to the passage of time, might be suboptimal for estimating aging (Colloca et al., 2020; Wagner et al., 2016). More attention should be paid to the development of biological markers of aging (Hamczyk et al., 2020). Several hallmarks have been identified representing common features of aging at the molecular and cellular levels (López-Otín et al., 2023), among which telomere attrition and epigenetic alterations, or rather, telomere length shortening and DNA methylation (Hamczyk et al., 2020), have been frequently used to

determine the biological age of humans. Moreover, age-dependent telomere shortening (Blackburn et al., 2015; De Meyer et al., 2018) and age-related DNA methylation (Fransquet et al., 2019; Marioni et al., 2015) have been proven to be satisfactory predictors of all-cause and cardiovascular mortality in population-based studies, inspiring us to investigate the potential of biological aging markers to facilitate the evaluation of patients in a clinical setting. Nevertheless, difficulties with nuclear DNA extraction, an inability to standardize the tissue source for analysis, and a lack of standardized procedures, reagents, and testing protocols impede their clinical application (Fasching, 2018; Martin-Ruiz et al., 2015; Wagner, 2022). Therefore, it is necessary to explore biological aging markers with satisfactory clinical applicability.

Accumulating evidence suggests that α-klotho is a potential biological marker of aging. Defects in α-klotho gene expression in mice lead to multiple age-like phenotypes, including shortened lifespan, arteriosclerosis, pulmonary emphysema, and multiple organ degeneration or failure, whereas overexpression extends lifespan (Kuro-o et al., 1997; Kurosu et al., 2005). Previous population-based studies have reported that low serum α-klotho is associated with higher long-term all-cause mortality in patients with chronic kidney disease (Charoenngam et al., 2020; Kim et al., 2013; Valenzuela et al., 2019), older people (Semba et al., 2011), and even in the general population (Kresovich and Bulka, 2022). In addition, as cleaved from the extracellular domain of transmembrane α-klotho by protease (Drew et al., 2021), tthe soluble α-klotho protein can be measured in the peripheral circulation using a commercial enzyme-linked immunosorbent assay (ELISA) system (Yamazaki et al., 2010). Based on the facts mentioned above, low serum α-klotho level could serve as a clinical biomarker for mortality risk stratification in the general population, older people, and individuals with chronic kidney disease. However, as a biological aging marker, the prognostic significance of serum α-klotho in age-related diseases remains largely undermined.

In the current study, based on a nationally representative survey, the National Health and Nutrition Examination Survey (NHANES), we reported the prognostic value of serum α-klotho in the prediction of all-cause and cardiovascular mortality in several age-related diseases, such as hypertension, congestive heart failure, diabetes mellitus, emphysema, malignant tumors, and renal insufficiency. Furthermore, we proposed an optimal cutoff value for serum α-klotho to assist its clinical application.

2. Methods

2.1. Study population

This was a population-based, prospective cohort study. All populations enrolled in this study were obtained from NHANES (2007–2016), an ongoing study to evaluate the health and nutritional status of US citizens with a complex, stratified, multistage, probability-cluster design to represent non-institutionalized individuals in the US, which is conducted by the National Center for Health Statistics of the Centers for Disease Control and Prevention (CDC, 2023). The survey protocol was approved by the Institutional Review Board of the Centers for Disease Control and Prevention. All the participants provided written informed consent for the use of their data. In total, 50,588 individuals were included in this study. After excluding participants aged < 18 years (N = 19,864), missing follow-up information (N = 72), questionnaire data (N = 1,527), and α-klotho (N = 15,379), 13,746 participants were analyzed (Fig. S1).

2.2. Serum α-klotho measurement

Pristine serum samples were collected from 40 to 79 years old participants in the NHANES 2007–2016 and transferred to the laboratory on dry ice. After receiving, the samples were scanned, and data compared with those on the received electronic manifest were entered into the laboratory information system and stored at −80 °C. Serum α-klotho levels were analyzed at the period 2019–2020 by the Northwest Lipid Metabolism and Diabetes Research Laboratories, Division of Metabolism, Endocrinology, and Nutrition, University of Washington, using a commercially available ELISA kit (IBL International, Gunma, Japan (Yamazaki et al., 2010). Each sample was analyzed in duplicate, and the average value of the two concentrations was used as the final value. The detailed laboratory method is available at NHANES website (CDC, 2021).

2.3. Baseline assessment

The covariates included in this study were sociodemographic information (age, sex, ethnicity, education level, and annual family income), lifestyle (current smoking status, alcohol consumption, physical activity, and eating habits), medical history (hypertension, diabetes, stroke, emphysema, malignant tumors, congestive heart failure, and coronary heart disease), medication history (use of antihypertensive drugs and hypoglycemic agents), body measurement (systolic blood pressure (SBP), diastolic blood pressure (DBP), and body mass index (BMI)), and laboratory data (fasting blood glucose (FBG), glycohemoglobin (HbA1C), serum creatinine, and urinary albumin-to-creatinine ratio (UACR)). Demographic, lifestyle, medical, and medication information was obtained through questionnaires. Physical activity was assessed by whether the participant met the national physical activity guidelines of ≥150 min of moderate activity per week, ≥75 min of vigorous activity per week, or an equivalent combination. Hypertension was defined as a history of hypertension, use of antihypertensive medications, SBP ≥ 140 mmHg, or DPB ≥ 90 mmHg (Williams et al., 2018). Diabetes mellitus was defined as having a history of diabetes mellitus, taking hypoglycemic medications currently, HbA1c level ≥6.5 %, or FBG level ≥7.0 mmol/L (American Diabetes Association Professional Practice Committee, 2022). The estimated glomerular filtration rate (eGFR) was calculated using the Chronic Kidney Disease Epidemiology Collaboration equation (Levey et al., 2009). Renal insufficiency was defined as an eGFR < 60 ml/min/1.73 m^2. Coronary heart disease was defined as a self-reported history of coronary heart disease, angina pectoris, or heart attack in medical history questionnaires. Congestive heart failure, stroke, emphysema, and malignant tumors were defined using self-reported disease history in the medical history questionnaires.

2.4. Outcomes

The endpoints were all-cause and cardiovascular mortality. The mortality data of participants were obtained from the NHANES public-used linked mortality files (LMF), which recorded the survival status and cause of death of survey participants from the NHANES interview until death or December 31, 2019. Cardiovascular death was determined based on the International Classification of Diseases, 10th Edition, Clinical Modification System codes (I00–I09, I11, I13, and I20–I51).

2.5. Statistical analysis

Baseline characteristics for all subjects were presented as survey-weighted mean ± standard deviation for normally distributed continuous variables, survey-weighted median (interquartile range) for skewly distributed continuous variables and number (survey-weighted percentage) for categorical variables. The *t*-test was used to compare α-klotho levels between the groups. We used X-tile (Camp et al., 2004) to estimate the optimal cut-off for α-klotho, which was then used to divide the cohort into two groups according to all-cause mortality risk (High: serum α-klotho ≥ 603.5 pg/ml vs. Low: serum α-klotho < 603.5 pg/ml). The X-tile was used to assess all possible cutoffs of serum α-klotho by plotting Kaplan–Meier curves and log-rank test, and the most significant cutoff was chosen as the best cutoff. Compared to the method of using the receiver operating characteristic (ROC) curve to determine the

cutoff, the outcome in this method included both survival time and status. In contrast, the outcome in ROC was only the survival status at a particular time. Survival analysis was performed using standardized Kaplan-Meier curves and the log-rank test. Both univariate and multivariate Cox proportional hazards regression models were used to estimate hazard ratios (HRs) and 95 % confidence intervals (CIs) for all-cause and cardiovascular mortality. The dose–response relationships between serum α-klotho and mortality were assessed using a restricted cubic spline based on the Cox proportional hazards model. All analyses used survey sampling weights and accounted for clustered sampling design and oversampling. All statistical analyses were performed using SPSS v25.0 (IBM, Armonk, NY, USA), X-tile (Yale School of Medicine, New Haven, CT, USA), and R (V.4.2.1). Two-sided P < 0.05 was considered statistically significant. The statistical power analysis was performed with R package of "pwr," "powerSurvEpi," and the statistical power for all significant findings was >0.80 (Table S1). All the assumptions associated with statistical tests in current study were checked and verified.

3. Results

3.1. Baseline characteristics of the study population

The baseline characteristics of the enrolled subjects were listed in Table 1. The analysis included 13,746 U.S. adults with a survey-weighted mean age of 56.19 ± 10.42 years old, while 52.2 % were female. The mean serum α-klotho level of all participants was 845.75 ± 293.12 pg/ml. In the follow-up, 8.5 % of participants (n = 1569) died during the survey, with a mean follow-up time of 7.62 ± 2.99 years, and 2.0 % of participants (n = 353) died from cardiovascular causes.

Table 1
Baseline clinical characteristics of included U.S. adults from NHANES 2007–2016.*

Variables	Total (n = 13,746)
Age, years	56.19 ± 10.42
Female gender, n (%)	7091 (52.2)
Ethnicity, n (%)	
Mexican American	2184 (6.7)
Non-Hispanic White	5719 (72.9)
Non-Hispanic Black	2726 (9.2)
Others	2919(11.2)
Education level, n (%)	
Less than 9th grade	1874(6.3)
9th grade to high school	5059(32.6)
College or above	6805(61.2)
BMI, kg/m^2	29.53 ± 6.59
Current smoke, n (%)	2694 (18.5)
Alcohol consumption, n (%)	9009(77.4)
Physical activity meets guidelines, n (%)	4157(33.7)
Hypertension, n (%)	7425(48.5)
Congestive heart failure, n (%)	558(3.0)
Coronary heart disease, n (%)	1252(7.5)
Diabetes mellitus, n (%)	3543(19.6)
Stroke, n (%)	623(3.4)
Emphysema, n (%)	388(2.6)
Malignant tumors, n (%)	1602(13.5)
eGFR (ml/min/1.73 m^2)	87.86 ± 19.83
Renal insufficiency, n (%)	1344(8.1)
UACR, mg/gCr	6.95(7.92)
Serum α-klotho, pg/ml	845.75 ± 293.12
Low α-klotho (<603.5 pg/ml), n (%)	2464(17.3)
Follow-up time, years	7.62 ± 2.99
Long-term mortality, n (%)	
All-cause	1569(8.5)
Cardiovascular	353(2.0)

Abbreviations: BMI, Body Mass Index; eGFR, estimated glomerular filtration rate; UACR, urinary albumin-to-creatinine ratio.
* Means ± standard deviation and percentages were adjusted for NHANES survey weights.

3.2. Serum α-klotho level in different population

Serum α-klotho concentrations were significantly lower in people with age ≥ 65 years old, male gender, reporting consuming alcohol, reporting current smoking, hypertension, congestive heart failure, coronary artery disease, stroke, emphysema, renal insufficiency, and malignant tumors (all P value < 0.05), and but not in people with diabetes mellitus (Table 2).

3.3. Serum α-klotho for predicting survival in the general population

Univariate Cox regression analysis of α-klotho (after log-transformation, Fig. 1) for long-term mortality in the general population showed that α-klotho was a protective factor for all-cause (HR 0.62 (0.54–0.72), P < 0.001) and cardiovascular mortality (HR 0.47 (0.34–0.64), P < 0.001). Lower serum α-klotho (in 1st tertile or quartile) was associated with higher all-cause and cardiovascular mortality (shown in Fig. S2). The ROC curve for 1-year, 5-year, and 10-year mortality rates revealed similar results (Table S2). A U-shaped correlation was observed between serum α-klotho levels and all-cause mortality, as plotted by the restricted cubic spline (P for non-linearity < 0.001).

3.4. Serum α-klotho for predicting survival in age-related diseases

The relationship between serum α-klotho and all-cause mortality in different age-related diseases was plotted using a restricted cubic spline (Fig. 2), and a U-shaped correlation was observed for hypertension (P for non-linearity < 0.001), congestive heart failure (P for non-linearity 0.002), stroke (P for non-linearity 0.015), renal insufficiency (P for non-linearity < 0.001), malignant tumors (P for non-linearity 0.015), diabetes mellitus (P for non-linearity < 0.001), and emphysema (P for non-linearity 0.018). Lower serum α-klotho levels (lower than the median or in the 1st tertile or quartile) were associated with higher all-cause mortality in different age-related diseases (Fig. S3).

Table 2
Serum α-klotho concentration of included U.S. adults from NHANES 2007–2016 stratified by age, gender, lifestyles and medical history.*

Variables	Yes	No	P	Statistical power
Age ≥ 65 years old	808.08 ± 269.71	857.59 ± 299.13	<0.001	1.00
Female	863.53 ± 309.75	826.29 ± 272.46	<0.001	1.00
Alcohol drinking	831.99 ± 284.61	879.83 ± 312.48	<0.001	1.00
Current smoking	819.21 ± 297.43	851.78 ± 291.80	<0.001	1.00
Hypertension	834.33 ± 292.91	856.48 ± 292.93	<0.001	1.00
Congestive heart failure	775.24 ± 283.94	847.87 ± 293.09	<0.001	1.00
Coronary artery disease	813.52 ± 262.69	848.37 ± 295.31	<0.001	1.00
Diabetes mellitus	845.37 ± 309.59	845.84 ± 288.97	0.949	1.00
Stroke	796.60 ± 263.03	847.53 ± 294.06	<0.001	1.00
Emphysema	821.14 ± 302.92	855.73 ± 309.97	0.030	1.00
Renal insufficiency	745.47 ± 247.03	854.62 ± 295.22	<0.001	1.00
Malignant tumor	813.54 ± 272.87	850.74 ± 295.88	<0.001	1.00

* Means ± standard deviation and percentages were adjusted for NHANES survey weights.

(A) All-cause mortality

Univariate Cox regression HR 0.62(0.54−0.72) P<0.001

P for non−linearity < 0.001

Ln klotho = 6.83

(B) Cardiovascular mortality

Univariate Cox regression HR 0.47(0.34−0.64) P<0.001
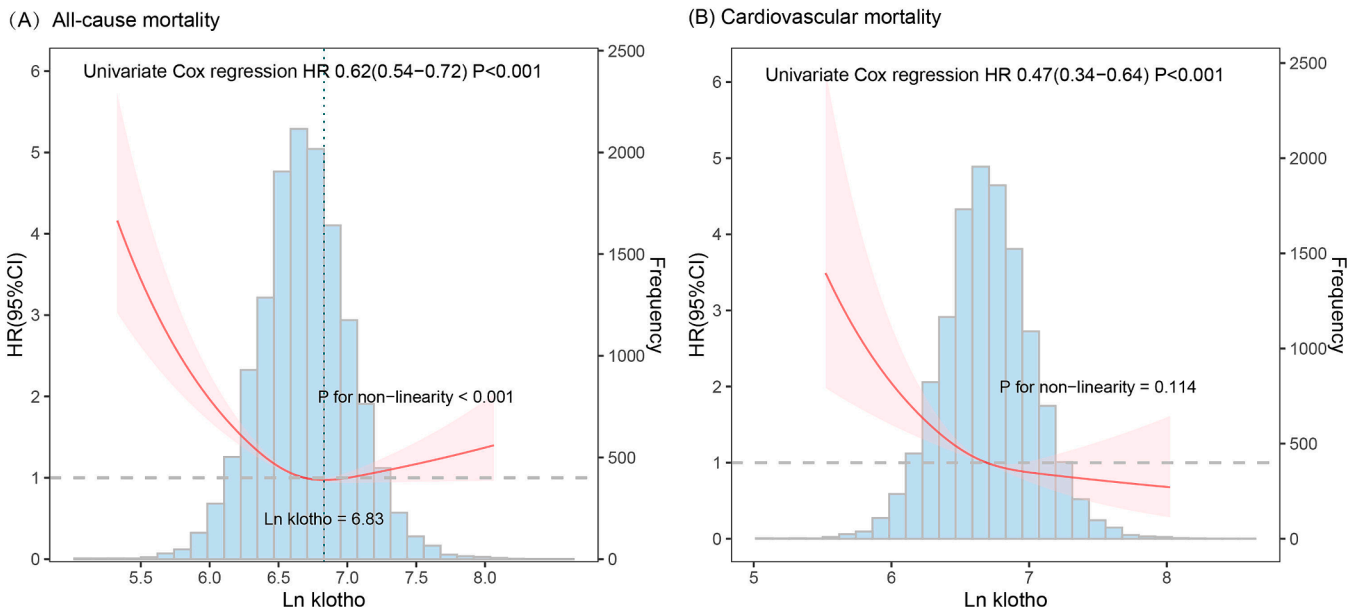
P for non−linearity = 0.114

Fig. 1. Effect of serum α-klotho concentration on mortality in included U.S. adults from NHANES 2007–2016. (A and B) Unadjusted effect of serum α-klotho concentration on all-cause mortality (A) and cardiovascular mortality (B) hazard function. The red solid line shows the estimated relationship between the hazard ratio and ln(klotho), and the pink area shows the 95% confidence limits, as estimated by P-splines. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Hypertension

P for non-linearity <0.001

Congestive heart failure

P for non-linearity 0.002

Coronary artery disease

P for non-linearity 0.055

Stroke

P for non-linearity 0.015

Renal insufficiency

P for non-linearity <0.001

Malignant tumor

P for non-linearity 0.015

Diabetes mellitus

P for non-linearity <0.001

Emphysema

P for non-linearity 0.018

Fig. 2. Effect of serum α-klotho concentration on all-cause mortality among different age-related diseases in included U.S. adults from NHANES 2007–2016. The red solid line shows the estimated relationship between the hazard ratio and ln(klotho), and the pink area shows the 95% confidence limits, as estimated by P-splines. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)
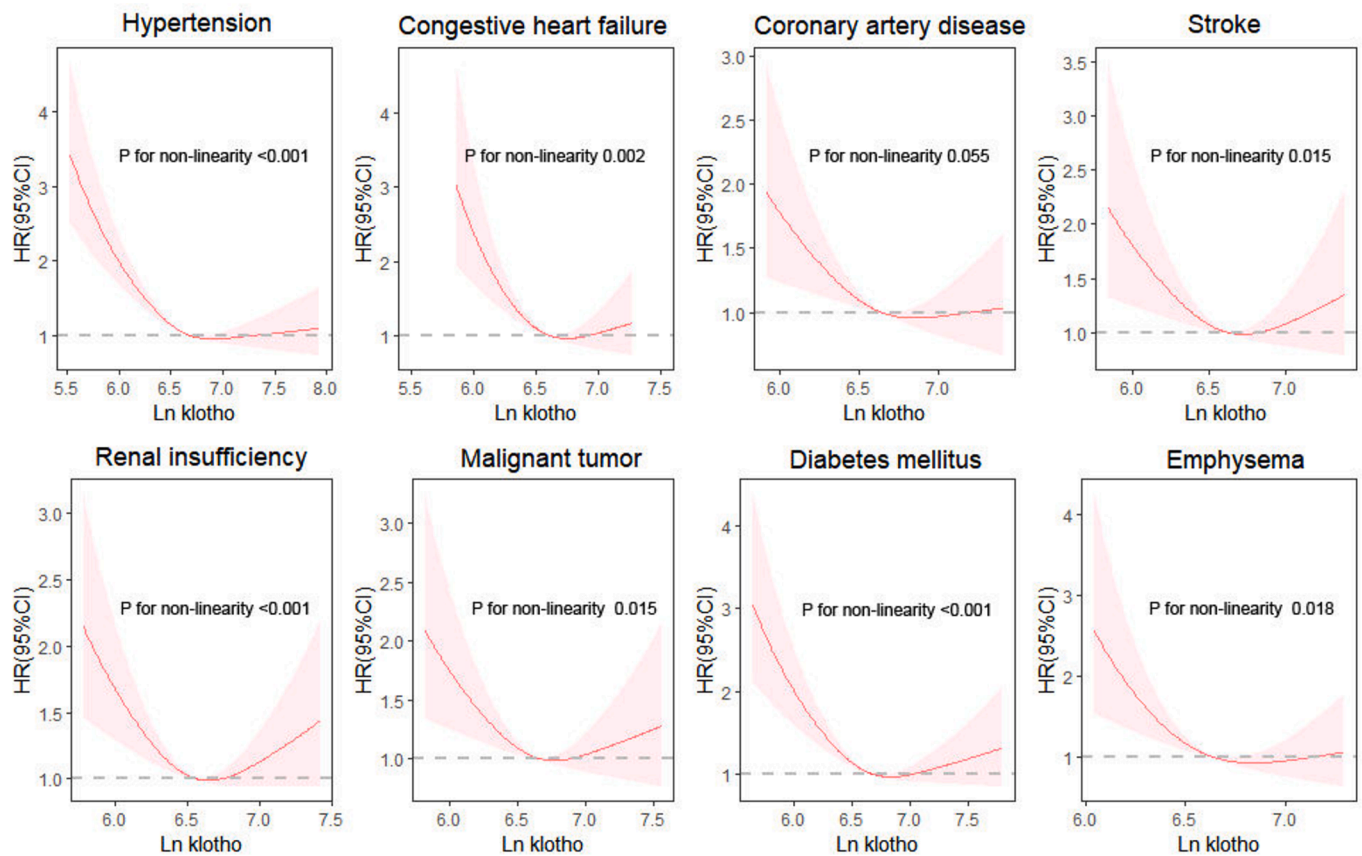
3.5. Optimal cut-off of serum α-klotho level and its prognostic value in the general population

X-tile plots were used to determine the optimal cut-off value of serum α-klotho concentration (603.5 pg/ml), which divided the subjects into two groups: high-risk (low α-klotho group) and low-risk (normal α-klotho group) all-cause mortality. As shown by Kaplan–Meier survival curves (Fig. 3) and univariate and multivariate Cox proportional hazard regression (Table 3), compared to those with normal serum α-klotho, those with low serum α-klotho (<603.5 pg/ml) had a significantly higher risk of all-cause mortality (adjusted HR: 1.34(1.18–1.52), P < 0.001) and cardiovascular mortality (adjusted HR: 1.63(1.27–2.10), P < 0.001), even after adjusting for covariates including sociodemographic factors, lifestyle, medical histories, BMI, eGFR, and UACR.

3.6. Subgroup analysis

We further explored the prognostic value of the proposed optimal serum α-klotho cut-off in the elderly and in age-related diseases. Multivariate Cox regression (shown in Table 3 and Fig. S4) indicated that low serum α-klotho (<603.5 pg/ml) was an independent risk factor for both all-cause and cardiovascular mortality in people aged > 65 years, hypertension, congestive heart failure, diabetes mellitus, and emphysema, whereas it could only independently predict all-cause mortality in patients with coronary artery disease and renal insufficiency.

4. Discussion

In this study, we explored the optimal cut-off of serum soluble α-klotho and evaluated its prognostic value in the general population and in age-related diseases. Our results revealed that the optimal cut-off of serum α-klotho for predicting long-term mortality in the general population was 603.5 pg/ml, and low serum α-klotho (<603.5 pg/ml) was a significant independent risk factor for all-cause and cardiovascular mortality in the general population and individuals with age-related diseases, including hypertension, congestive heart failure, diabetes mellitus, and emphysema. Our study demonstrated that serum α-klotho concentration could be clinically utilized for mortality risk stratification in age-related diseases and provided a cut-off of low serum α-klotho to facilitate its clinical application.

We found that serum α-klotho was a clinically applicable biological aging marker for mortality risk prediction in age-related diseases. Our findings are partially supported by previous evidence that serum α-klotho concentration is inversely associated with all-cause mortality in the general population (Kresovich and Bulka, 2022) and individuals with chronic kidney disease (Charoenngam et al., 2020). The prognostic significance of serum α-klotho concentration in different age-related diseases might be due to the anti-aging function of α-klotho through the regulation of phosphate homeostasis and suppression of oxidative stress (Ohnishi and Razzaque, 2010; Stubbs et al., 2007; Xu and Sun, 2015). In addition, α-klotho deficiency had been reported to participate in the development and progress of age-related diseases, such as hypertension (Gao et al., 2016; Kanbay et al., 2021; Kawarazaki et al., 2020; Lim et al., 2012; Zhou et al., 2016), congestive heart failure (Chen et al., 2021), coronary artery disease (Bäck et al., 2019; Liu et al., 2011), diabetes mellitus (Lin and Sun, 2012, 2015), and emphysema (Gao et al., 2015).

To facilitate the clinical application of serum α-klotho concentration in different age-related diseases, an optimal cutoff for low serum α-klotho concentration based on all-cause mortality risk in the general population was generated. Compared to the definition of low serum α-klotho in previous studies, which was mainly based on its distribution (Charoenngam et al., 2020; Kresovich and Bulka, 2022; Semba et al., 2011), the definition of low serum α-klotho (<603.5 pg/ml) in the present study was more applicable in the clinical setting, and its prognostic significance in the general population and several age-related diseases, including hypertension, diabetes mellitus, and emphysema, did not attenuate while adjusting for covariates. Additionally, the current cut-off was not an extreme value because there were quite a few individuals with serum α-klotho < 603.5 pg/ml. Thus, low serum α-klotho, defined as a serum α-klotho concentration < 603.5 pg/ml, could serve as a biological marker to identify high-risk patients with age-related diseases.

It should be highlighted that all-cause mortality rose marginally but insignificantly when the serum α-klotho concentration exceeded a certain range. Because α-klotho deficiency is involved in premature aging and early death (Typiak and Piwkowska, 2021), previous studies have primarily focused on the prognostic significance of low serum α-klotho levels. In contrast, observations of the effect of high or extremely high serum α-klotho on mortality risk are limited. Similar to the current investigation, a recent study reported a U-shaped relationship between serum α-klotho concentration and all-cause mortality in individuals with diabetes mellitus (Chen et al., 2022). Theoretically, the overexpression of soluble α-klotho might be due to upregulated fibroblast growth factors-23(FGF-23) levels, which leads to hypovitaminosis D, a significant risk factor for all-cause mortality in the general population (Amrein et al., 2020). This phenomenon was observed in shift
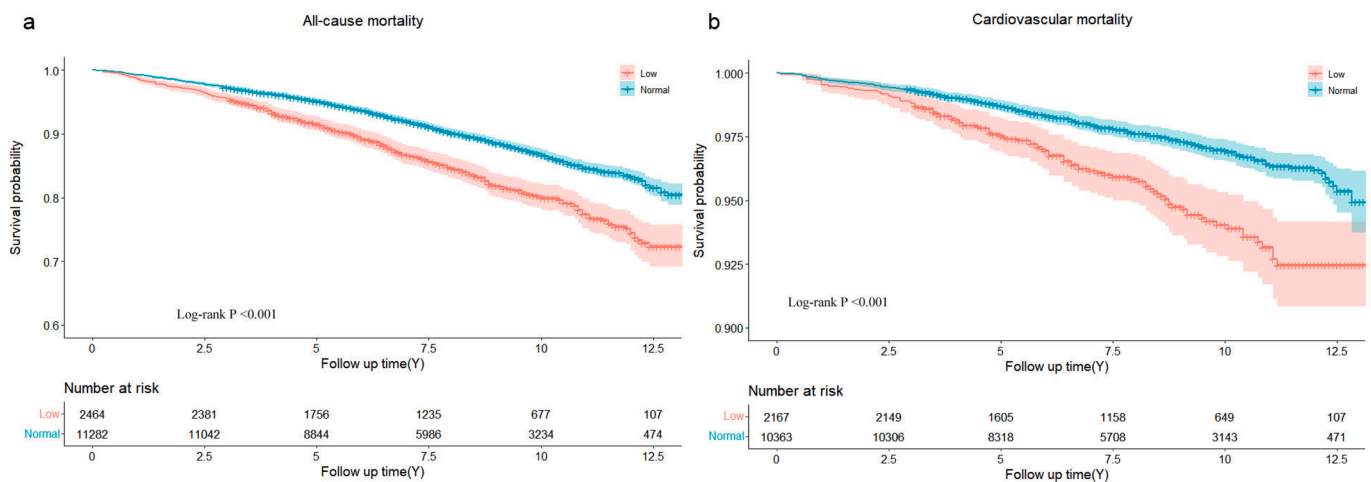

Fig. 3. Kaplan–Meier curves of long-term all-cause and cardiovascular mortality for low (<603.5 pg/ml) and normal (≥603.5 pg/ml) serum α-klotho concentration in included U.S. adults from NHANES 2007–2016.

Table 3

Crude and adjusted HR(95 %CI) of low serum α-klotho (<603.5 pg/ml) for all-cause and cardiovascular mortality of included U.S. adults from NHANES 2007–2016, stratified by age, gender, lifestyles and medical history.

Variables	Crude		Adjusted*	
	HR (95 %CI)	P	HR (95 %CI)	P
All-cause mortality				
All population	1.61 (1.44–1.81)	<0.001	1.34 (1.18–1.52)	<0.001
Age				
<65 years old	1.50 (1.24–1.82)	<0.001	1.22 (0.99–1.52)	0.062
≥65 years old	1.43 (1.24–1.65)	<0.001	1.38 (1.18–1.62)	<0.001
Sex				
Male	1.40 (1.20–1.63)	<0.001	1.15 (0.97–1.36)	0.103
Female	1.90 (1.60–2.26)	<0.001	1.65 (1.36–2.01)	<0.001
Comorbidities				
Hypertension	1.58 (1.38–1.80)	<0.001	1.37 (1.18–1.59)	<0.001
Congestive heart failure	1.68 (1.27–2.22)	<0.001	1.82 (1.29–2.57)	0.001
Coronary artery disease	1.41 (1.12–1.80)	0.004	1.51 (1.15–1.99)	0.003
Diabetes mellitus	1.60 (1.34–1.91)	<0.001	1.26 (1.03–1.54)	0.022
Stroke	1.49 (1.09–2.04)	0.013	1.31 (0.88–1.94)	0.183
Emphysema	1.90 (1.36–2.65)	<0.001	1.95 (1.32–2.89)	0.001
Renal insufficiency	1.42 (1.23–1.63)	<0.001	1.33 (1.05–1.69)	0.016
Malignant tumors	1.50 (1.17–1.91)	0.001	1.22 (0.92–1.61)	0.164
Cardiovascular mortality				
All population	1.91 (1.51–2.40)	<0.001	1.63 (1.27–2.10)	<0.001
Age				
<65 years old	2.05 (1.38–3.02)	<0.001	1.70 (1.10–2.63)	0.017
≥65 years old	1.57 (1.17–2.09)	0.002	1.57 (1.14–2.16)	0.005
Sex				
Male	1.75 (1.30–2.35)	<0.001	1.43 (1.03–1.98)	0.033
Female	2.10 (1.44–3.04)	<0.001	1.97 (1.31–2.96)	0.001
Comorbidities				
Hypertension	1.90 (1.47–2.45)	<0.001	1.70 (1.28–2.26)	<0.001
Congestive heart failure	1.77 (1.12–2.79)	0.015	1.87 (1.06–3.31)	0.031
Coronary artery disease	1.20 (0.76–1.88)	0.432	/	/
Diabetes mellitus	1.73 (1.24–2.42)	0.001	1.50 (1.03–2.17)	0.034
Stroke	1.26 (0.64–2.47)	0.504	/	/
Emphysema	2.29 (1.12–4.71)	0.024	3.40 (1.29–8.93)	0.013
Renal insufficiency	1.38 (0.93–2.05)	0.105	/	/
Malignant tumors	2.03 (1.16–3.56)	0.013	1.26 (0.62–2.60)	0.525

* Adjusted for age, sex, race, education, family income, current smoking, alcohol drinking, physical activity, BMI (body mass index), hypertension, diabetes mellitus, stroke, emphysema, malignant tumors, congestive heart failure, coronary artery disease, eGFR (estimated glomerular filtration rate), UACR (urinary albumin-to-creatinine ratio).

workers with higher serum FGF-23 and α-klotho levels and lower vitamin D3 levels than in day workers (Min et al., 2020). In addition, the slightly increased all-cause mortality might also be due to confounding factors; for instance, excessively high serum α-klotho concentrations were observed in patients with acromegaly (Sze et al., 2012), which was associated with reduced life expectancy. Therefore, further studies should focus on whether extremely high serum α-klotho concentration is significantly associated with higher all-cause mortality.

This study has several limitations that should be noted. First, as mentioned previously, the relationship between serum α-klotho concentration and the risk of all-cause death by restricted cubic spline appeared to be U-shaped. However, the association between the high concentrations and increased mortality risk was not significant. Therefore, we propose only a unilateral cutoff. Second, although low serum α-klotho could independently predict mortality risk, the area under the ROC curve suggested that serum α-klotho concentration alone was not an ideal predictor of long-term mortality. Therefore, further efforts should be made to explore new biological aging markers or to develop an ideal aging indicator by incorporating age with biological aging markers, including serum α-klotho. Third, our results might not apply to younger populations because serum α-klotho was analyzed only in individuals aged 40–79 in the NHANES. Fourth, owing to the retrospective study design, there might be recall bias in information collection, especially medical history and lifestyle. Finally, the measurement of serum α-klotho in the present study was based on a sandwich ELISA test (IBL), which is frequently used in population-based studies (Drew et al., 2021; Hughes-Austin et al., 2020) because of its high reproducibility of measurements compared to other ELISA assays (Heijboer et al., 2013). Therefore, our results might not apply to populations with serum α-klotho levels measured using other test methods.

5. Conclusion

In summary, a low serum α-klotho concentration (<603.5 pg/ml) was independently associated with higher all-cause and cardiovascular mortality in the general population and in people with age-related diseases, including hypertension, congestive heart failure, diabetes mellitus, and emphysema. This implies that low serum α-klotho (<603.5 pg/ml) could serve as a biological aging marker to identify individuals at high mortality risk in these populations.

CRediT authorship contribution statement

Zhiwen Yang: Writing – original draft, Software, Methodology, Data curation, Conceptualization. **Yusheng Ma:** Writing – original draft, Methodology, Formal analysis, Data curation. **Yanbing Wang:** Writing – original draft. **Ming Jin:** Formal analysis, Data curation. **Jianping Bin:** Writing – review & editing, Funding acquisition. **Zhiyong Chen:** Writing – review & editing, Writing – original draft. **Zhonghua Teng:** Writing – review & editing, Conceptualization.

Declaration of competing interest

Data availability

Data is from Centers for Disease Control and Prevention, National Center for Health Statistics. National health and nutrition examination

survey data. Available at: https://www.cdc.gov/nchs/nhanes/.

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None.

Author contributions

ZWY, ZHT contributed to the conception or design of the study. ZWY and MJ contributed to the acquisition, analyses, and interpretation of data. ZWY, YSM, YBW and ZYC drafted the manuscript. ZYC, JPB and ZHT revised the manuscript critically. ZYC, JPB and ZHT had all access to the data and is responsible for the overall content as guarantor. All authors contributed to refinement of the study protocol and approved the final manuscript.

Prior presentations

None.

Ethics approval and consent to participate

All NHANES study protocols survey protocol was approved by the Ethics Review Committee of NCHS of the Centers for Disease Control and Prevention. All participants had provided written, informed consent for the use of their data. All procedures in this study were conducted in accordance with all the relevant guidelines.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.pmedr.2024.102730.

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