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Association of serum klotho level with albuminuria in middle-aged and elderly participants without diabetes mellitus: a cross-sectional study

Dawei Chen[1†], Mengxing Chen[1†], Zhixiang Qi[2], Yumei Tang[2] and Xin Wan[1*]

Abstract

Background The relationship between serum klotho level and albuminuria is unknown in middle-aged and elderly participants without diabetes mellitus (DM). Therefore, we will investigate the association between serum klotho level and albuminuria in middle-aged and elderly participants without DM.

Methods Participants (aged 40–79) were from the five continuous cycles (2007–2016) of the National Health and Nutrition Examination Survey (NHANES). Multiple logistic regression was performed to investigate the association between serum klotho level and albuminuria.

Results 9217 participants were included in the present study. 47.6% of the participants were male. The average age of the overall participants was 56.3 years (40–79 years). Overall, 823 participants with albuminuria were identified. After adjusted confounders (age, gender, marital status, ethnicity, family income to poverty ratio, education, body mass index, smoke, charlson comorbidity index, hypertension, hyperlipidemia, angiotensin converting enzyme inhibitor/angiotonin receptor blocker, and estimated glomerular filtration rate), participants with a high serum klotho level had a decreased risk for albuminuria. Compared with the lowest serum klotho level (Tertile 1), participants in Tertile 2 (odds ratio [OR] 0.83, 95% CI 0.70–0.99, $P=0.044$) and Tertile 3 (OR 0.76, 95% CI 0.63–0.91, $P=0.003$) had a lower risk of albuminuria (P for trend = 0.002). The stratified analysis showed that serum klotho level was still negatively associated with albuminuria in the subgroups, and statistically significant interactions were not observed in the subgroups (all P values for interactions > 0.05, except for the hypertension subgroup).

Conclusions In middle-aged and elderly participants without DM, a high serum klotho level is associated with a decreased risk of albuminuria. In the future, the mechanism of the interaction between klotho and albuminuria needs to be elucidated to find new treatment targets for individuals without DM who suffer from albuminuria.

Keywords Klotho, Albuminuria, Participants without diabetes mellitus, NHANES

[†]Dawei Chen and Mengxing Chen contributed equally to this work.

*Correspondence:
Xin Wan
wanxin@njmu.edu.cn

[1]Department of Nephrology, Nanjing First Hospital, Nanjing Medical University, 68 Changle Road, Nanjing, Jiangsu, China
[2]Department of Nuclear Medicine, Nanjing First Hospital, Nanjing Medical University, Nanjing, Jiangsu, China

Introduction

Albuminuria is independently associated with an increased risk of progression to end-stage renal disease and cardiovascular and all-cause mortality [1, 2]. Moreover, several studies reported that even low-level albuminuria was an independent predictor of diabetic kidney disease [3], left ventricular hypertrophy [4], heart failure [5], and all-cause mortality [6]. Kang et al. reported that even albuminuria within the normal range could also predict all-cause and cardiovascular mortality [7]. Recently, researchers suggested the implementation of opportunistic or systematic albuminuria screening and therapy, which may have the potential to improve cardiorenal outcomes and mitigate the dismal 2040 projections for chronic kidney disease and related cardiovascular burden [8].

The klotho gene is originally identified as playing a role in aging suppression. There are three isoforms of the protein klotho: α-klotho, β-klotho, and γ-klotho, all of which are encoded by different genes [9]. Moreover, α-klotho can be further divided into membrane klotho and soluble klotho, which likely have distinct functions. Klotho is present in two general forms: a type 1 transmembrane protein and a secreted form that is derived from the same gene through alternative mRNA splicing [10]. The extracellular domain of membrane klotho is composed of two repeating klotho domains, KL-1 and KL-2, which can be cleaved by metalloproteinases and released into cerebrospinal fluid, urine, and blood. The secreted klotho, together with cleaved KL fragments, is collectively referred to as soluble klotho [11]. The membrane-bound Klotho is known to serve as a complex with fibroblast growth factor receptors and functions as a co-receptor for fibroblast growth factor 23, which plays a critical role in the maintenance of mineral ion and vitamin D homeostasis [11]. Soluble klotho plays important roles in a variety of processes, including preventing stress-induced cardiac remodeling, modulating ion transport, antisenescence, anti-oxidation, and anti-fibrotic actions [12]. Previous studies reported that serum klotho is not only associated with acute kidney injury [13, 14] but also cognitive performance [15], metabolic syndrome [16], and all-cause mortality [17] among individuals with CKD. Previously, most studies showed that klotho is related to albuminuria in diabetes mellitus (DM) patients [18–20]. However, the association between serum klotho level and albuminuria in participants without DM is still unknown. Therefore, in the present study, we will investigate the relationship between serum klotho level and albuminuria in middle-aged and elderly participants without DM.

Methods

Study participants

All data were obtained from five continuous cycles (2007–2016) of the National Health and Nutrition Examination Survey (NHANES). The present study design was carried out according to the Helsinki Declaration. The study protocol was approved by the National Center for Health Statistics Ethics Review Board, and each participant supplied signed informed permission [21]. Diabetes is described as having a doctor tell you that you have diabetes, having glycosylated hemoglobin greater than 6.5%, fasting glucose greater than or equal to 7.0 mmol/L, random blood glucose greater than or equal to 11.1 mmol/L, two-hour oral glucose tolerance test blood glucose greater than or equal to 11.1 mmol/L, and using diabetic medicine or insulin. 9217 eligible participants were included in the current study after participants with diabetes and those without complete study data were excluded. (Fig. 1)

Serum klotho concentrations

Serum samples from NHANES individuals (aged 40–79) collected between 2007 and 2016 were received and measured between 2019 and 2020, and klotho concentrations were measured using an enzyme-linked immunosorbent assay (ELISA; IBL international, Japan) [22]. All samples were tested twice, and the average of the two detection values was used to determine the final value. Additionally, each plate had two quality control samples with low and high klotho concentrations. Re-measurement was requested for samples when the difference between the duplicate results was more than 10%. The entire plate was repeated if the value of the quality control sample was not covered within two standard deviations of the known value. The detection limit for klotho was 6 pg/ml. All samples' final values exceed this limit.

Albuminuria

Urine samples were collected from NHANES participants at a standardized mobile examination center. A solid-phase fluorescence immunoassay and a modified Jaffe kinetic technique were used to detect urinary albumin and creatinine from a single spot urine sample. The urine albumin-to-creatinine ratio (UACR) was determined by dividing the concentration of urinary albumin in milligrams by the concentration of urinary creatinine in grams. Albuminuria was defined as a UACR of 30 mg/g or higher [23].

Covariates

The adjusted analyses included many covariates, such as age, gender, ethnicity (non-Hispanic white, non-Hispanic black, Mexican American, other Hispanic, and other), family income to poverty ratio, education levels

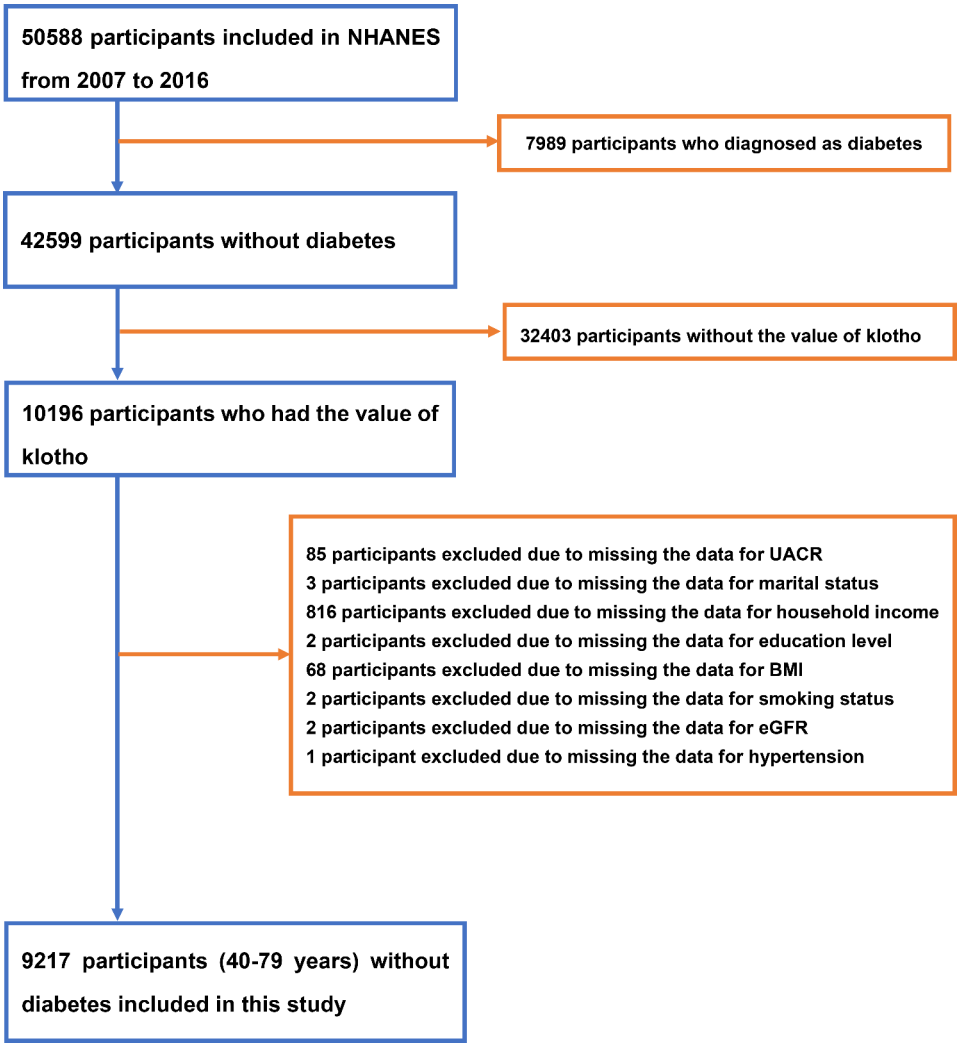

Fig. 1 Flow chart of patient selection. Abbreviations NHANES = National Health and Nutrition Examination Survey, BMI = body mass index, eGFR = estimated glomerular filtration rate, UACR = urine albumin-to-creatinine ratio

(college graduate or above, some college or AA degree, high school graduate/GED or equivalent, 9-11th grade, and less than 9th grade), smoking status (current smoker, never smoker, or former smoker), marital status (married, never married, living with a partner, widowed, separated, and divorced), body mass index (BMI), charlson comorbidity index (CCI), and estimated glomerular filtration rate (eGFR). The Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation was used to calculate eGFR [24]. CCI was used to assess a respondent's comprehensive health state, which was calculated by summing the scores of all the diseases [25, 26]. We defined diagnosed diseases based on affirmative answers to one or more queries, such as "Have you ever been told that you have the illness?". Hyperlipidemia is defined as the presence of total cholesterol levels of ≥200 mg/dL, triglycerides of ≥150 mg/dL, low-density lipoprotein levels of ≥130 mg/dL, or high-density lipoprotein levels of <50 mg/dL for females and <40 mg/dL for males, as well as receiving cholesterol-lowering drugs [27]. The definition for hypertension were as follows: (1) mean diastolic blood pressure of at least 90 mmHg; (2) mean systolic blood pressure of at least 140 mmHg; (3) currently receiving medications for hypertension; and (4) participants who self-reported having hypertension [28–30]. The information on receiving angiotensin converting enzyme inhibitor (ACEI) and angiotonin receptor blocker (ARB) was from the questionnaire.

Statistical analyses

Frequencies and percentages were used to present categorical data. Continuous variables were described as mean and standard deviation (SD) for data with a regularly distributed distribution. Continuous variables were shown as medians and interquartile ranges for non-normally distributed data. On the basis of the level of serum

klotho, the klotho variable was divided into three groups. The one-way ANOVA (normal distribution), Kruscal-Whallis H (skewed distribution) test, and chi-square test (categorical variables) were used to determine statistical differences between the means and proportions of the three groups. Student's t test (normal distribution), Mann-Whitney U test (skewed distribution), and chi-square (categorical variables) were used to examine differences between the albuminuria group and the non-albuminuria group. To assess the relationship between serum klotho level and albuminuria, univariate and multivariable logistic models were employed. We simultaneously displayed the results of unadjusted, minimally adjusted, and fully adjusted analyses in accordance with the STROBE statement's recommendation.

In addition, we also used a generalized additive model to identify the non-linear relationship between serum klotho level and UACR. If a non-linear association was detected, a two-piecewise linear regression model was utilized to determine the threshold influence of the serum klotho level on UACR, as shown in the smoothing plot. When the ratio between UACR and serum klotho level appeared obvious in the smoothed curve, the recursive method calculated the inflection point automatically, where the maximum model likelihood would be used [31]. To investigate the association between albuminuria and low serum klotho levels in different subgroups identified by all the variables, we performed stratification analyses. All the covariates, except for the stratification factor, were adjusted for in stratification and interaction analyses. Statistical significance was defined as P value less than 0.05. All statistical analyses were performed using R version 3.4.3 (R Foundation for Statistical Computing, Vienna, Austria; http://www.r-project.org), SPSS v22.0 (IBM Corporation, New York, USA), and Empower (X&Y solutions, Inc., Boston, MA; www.empowerstats.com).

Results

Participants characteristics

9217 middle-aged and elderly participants were enrolled in this study. 47.6% of the participants were male. The average age of the overall participants was 56.3 (SD: 10.8) years. In all, 823 subjects were identified as having albuminuria. The average serum klotho level was 852.9 (SD: 299.5) pg/ml. The proportions of proteinuria decreased across serum klotho level tertiles. Other information related to basic characteristics was presented in Table 1.

Albuminuria characteristics

The proportions of gender and hyperlipidemia were similar between the albuminuria and non-albuminuria groups. Participants with albuminuria had a greater likelihood of being older, as well as a higher BMI and CCI, compared to the non-albuminuria group. Nevertheless, the albuminuria group had lower eGFR and serum klotho level. In addition, there were also significant differences in ethnicity, marital status, education, family income to poverty ratio, hypertension, receiving ACEI/ARB, and smoking. (Table 2)

Association between serum klotho level and albuminuria

We employed a univariate logistic regression model to assess the correlation between serum klotho level and albuminuria. Meanwhile, the minimally adjusted and fully adjusted models were also shown in Table 3. In order to examine the relationship between serum klotho level and albuminuria, we categorized serum klotho level into tertiles. Our findings indicated a negative link between serum klotho level and albuminuria, with a consistent trend detected. In the fully adjusted model, compared with the lowest serum klotho level (Tertile 1), participants in Tertile 2 have a lower risk of albuminuria (odds ratio [OR] 0.83, 95% confidence interval [CI] 0.70–0.99, $P=0.044$), and Tertile 3 (OR 0.76, 95% CI 0.63–0.91, $P=0.003$) had the lowest risk of albuminuria (P for trend = 0.002).

Analyses of Non-linear relationship between serum klotho level and UACR

The analyses of non-linear relationship was necessary due to the fact that both serum klotho level and UACR were continuous variables. In the present study, we found that the relationship between serum klotho level and UACR was non-linear (after adjusting age, gender, marital status, ethnicity, family income to poverty ratio, education, BMI, smoke, CCI, hypertension, hyperlipidemia, ACEI/ARB, and eGFR). (Fig. 2) The inflection point was determined to be 589 μg/ml by a two-piecewise linear regression model. On the left of the inflection point, the effect size (β), 95% CI, and P value were −0.13, −0.23 to −0.02, and 0.024, respectively. Nevertheless, we did not observe a correlation between serum klotho level and UACR on the right of the inflection point (β = -0.01, 95% CI: −0.02 to 0.01, $P=0.450$). (Table 4)

Subgroup analyses

In order to further evaluate the relationship between serum klotho level and albuminuria in the subgroups, stratified analyses were carried out, as shown in Table 5. Subgroup analysis revealed that a high serum klotho level was still correlated with a decreased risk of albuminuria. All interaction tests were not statistically significant for age, gender, BMI, hyperlipidemia, ACEI/ARB, and eGFR subgroups (all P values for interactions > 0.05). However, the test for interaction was significant for hypertension (P for interaction = 0.015), which indicated that the effect

Table 1 Characteristics of participants without diabetes mellitus

Serum klotho level (pg/mL)	Tertile 1 (*N* = 3071)	Tertile 2 (*N* = 3072)	Tertile 3 (*N* = 3074)	*P*-value
Age (year)	57.5 ± 11.1	56.0 ± 10.7	55.3 ± 10.6	< 0.001
Gender (n,%)				< 0.001
Male	1576 (51.3)	1512 (49.2)	1297 (42.2)	
Female	1495 (48.7)	1560 (50.8)	1777 (57.8)	
BMI (kg/m^2)	28.9 ± 5.9	28.9 ± 6.3	28.6 ± 6.5	0.173
Ethnicity (n,%)				< 0.001
Mexican American	432 (14.1)	452 (14.7)	382 (12.4)	
Non-Hispanic White	1539 (50.1)	1507 (49.1)	1328 (43.2)	
Non-Hispanic Black	551 (17.9)	457 (14.9)	704 (22.9)	
Other Race	549 (17.9)	656 (21.3)	660 (21.5)	
Marital status (n,%)				0.357
Never married	247 (8.0)	240 (7.8)	274 (8.9)	
Widowed, Divorced, Separated	812 (26.5)	788 (25.7)	822 (26.7)	
Living with partner, Married	2012 (65.5)	2044 (66.5)	1978 (64.4)	
Education (n,%)				0.010
Less than 9th grade	340 (11.1)	335 (10.9)	308 (10.0)	
9-11th grade or High school	1150 (37.4)	1072 (34.9)	1045 (34.0)	
Some college or College or above	1581 (51.5)	1665 (54.2)	1721 (56.0)	
Family income to poverty ratio (n,%)				0.345
≤ 130%	896 (29.2)	839 (27.3)	885 (28.8)	
130–350%	1080 (35.2)	1069 (34.8)	1056 (34.3)	
> 350%	1095 (35.6)	1164 (37.9)	1133 (36.9)	
Smoking (n,%)				< 0.001
Never	1421 (46.3)	1595 (51.9)	1783 (58.0)	
Former	932 (30.3)	842 (27.4)	760 (24.7)	
Now	718 (23.4)	635 (20.7)	531 (17.3)	
Charlson comorbidity index (n,%)				< 0.001
0	1508 (49.1)	1638 (53.3)	1679 (54.6)	
1	724 (23.6)	664 (21.6)	641 (20.9)	
2	356 (11.6)	383 (12.5)	396 (12.9)	
≥ 3	483 (15.7)	387 (12.6)	358 (11.6)	
Hypertension (n,%)				< 0.001
No	1525 (49.7)	1693 (55.1)	1702 (55.4)	
Yes	1546 (50.3)	1379 (44.9)	1372 (44.6)	
Hyperlipidemia (n,%)				< 0.001
No	2425 (79.0)	2401 (78.2)	2277 (74.1)	
Yes	646 (21.0)	671 (21.8)	797 (25.9)	
ACEI/ARB (n,%)				0.095
No	2686 (87.5)	2741 (89.2)	2721 (88.5)	
Yes	385 (12.5)	331 (10.8)	353 (11.5)	
Albuminuria (n,%)				< 0.001
No	2737 (89.1)	2818 (91.7)	2839 (92.4)	
Yes	334 (10.9)	254 (8.3)	235 (7.6)	
eGFR (mL/min/1.73 m^2)	85.6 ± 20.0	88.7 ± 17.7	90.6 ± 17.2	< 0.001
Urine albumin-to-creatinine ratio (mg/g)	6.95 (4.62–12.61)	6.75 (4.55–12.02)	6.69 (4.68–11.46)	0.007

Abbreviation: BMI = body mass index, eGFR = estimated glomerular filtration rate, ACEI = angiotensin converting enzyme inhibitor, ARB = angiotonin receptor blocker

Table 2 Comparison between NHANES participants with and without albuminuria

Variable	Total (*N* = 9217)	Non-Albuminuria (*N* = 8394)	Albuminuria (*N* = 823)	*P* Value
Age (year)	56.3 ± 10.8	56.0 ± 10.7	59.6 ± 11.2	< 0.001
Gender (n,%)				0.973
Male	4385 (47.6)	3993 (47.6)	392 (47.6)	
Female	4832 (52.4)	4401 (52.4)	431 (52.4)	
BMI (kg/m^2)	28.8 ± 6.3	28.7 ± 6.2	29.6 ± 7.1	< 0.001
Ethnicity (n,%)				< 0.001
Mexican American	1266 (13.7)	1155 (13.8)	111 (13.5)	
Non-Hispanic White	4374 (47.5)	4037 (48.1)	337 (40.9)	
Non-Hispanic Black	1712 (18.6)	1493 (17.8)	219 (26.6)	
Other Race	1865 (20.2)	1709 (20.3)	156 (19.0)	
Marital status (n,%)				< 0.001
Never married	761 (8.2)	686 (8.2)	75 (9.1)	
Widowed, Divorced, Separated	2422 (26.3)	2151 (25.6)	271 (32.9)	
Living with partner, Married	6034 (65.5)	5557 (66.2)	477 (58.0)	
Education (n,%)				< 0.001
Less than 9th grade	983 (10.7)	868 (10.3)	115 (14.0)	
9-11th grade or High school	3267 (35.4)	2937 (35.0)	330 (40.1)	
Some college or College or above	4967 (53.9)	4589 (54.7)	378 (45.9)	
Family income to poverty ratio (n,%)				< 0.001
≤ 130%	2620 (28.4)	2326 (27.7)	294 (35.7)	
130–350%	3205 (34.8)	2885 (34.4)	320 (38.9)	
> 350%	3392 (36.8)	3183 (37.9)	209 (25.4)	
Smoking (n,%)				0.002
Never	4799 (52.1)	4414 (52.6)	385 (46.8)	
Former	2534 (27.5)	2298 (27.4)	236 (28.7)	
Now	1884 (20.4)	1682 (20.0)	202 (24.5)	
Charlson comorbidity index (n,%)				< 0.001
0	4825 (52.4)	4489 (53.5)	336 (40.8)	
1	2029 (22.0)	1836 (21.9)	193 (23.5)	
2	1135 (12.3)	1016 (12.1)	119 (14.5)	
≥ 3	1228 (13.3)	1053 (12.5)	175 (21.2)	
Hypertension (n,%)				< 0.001
No	4920 (53.4)	4662 (55.5)	258 (31.3)	
Yes	4297 (46.6)	3732 (44.5)	565 (68.7)	
Hyperlipidemia (n,%)				0.059
No	7103 (77.1)	6447 (76.8)	656 (79.7)	
Yes	2114 (22.9)	1947 (23.2)	167 (20.3)	
ACEI/ARB (n,%)				0.014
No	8148 (88.4)	7442 (88.7)	706 (85.8)	
Yes	1069 (11.6)	952 (11.3)	117 (14.2)	
Klotho (pg/ml)	803.1 (658.4-987.8)	806.9 (661.3-991.4)	758.8 (620.1-958.2)	0.001
eGFR (ml/min per1.73 m^2)	88.3 ± 18.5	88.9 ± 17.6	82.7 ± 24.7	< 0.001

Abbreviation: BMI = body mass index, eGFR = estimated glomerular filtration rate, ACEI = angiotensin converting enzyme inhibitor, ARB = angiotonin receptor blocker

of serum klotho level on albuminuria was significantly affected by hypertension status.

Discussion

Previous research had demonstrated the association between serum klotho level and albuminuria [32], particularly in individuals with type 1 diabetes mellitus (T1DM) and type 2 diabetes mellitus (T2DM). Maltese et al. found that T1DM patients with albuminuria had significantly lower serum klotho levels [33]. Several investigations revealed a negative correlation between serum klotho level and UACR [34] and were also significantly inversely associated with albuminuria stages in T2DM patients [35, 36]. Recently, a study reported that the association between serum klotho and eGFR was significantly different between the diabetes and non-diabetes groups

Table 3 Relationship between serum klotho level and albuminuria

Albuminuria	Non-adjusted model		Minimally adjusted model		Fully adjusted model	
Serum klotho level Tertiles	OR (95%CI)	P value	OR (95%CI)	P value	OR (95%CI)	P value
T1	Reference		Reference		Reference	
T2	0.74 (0.62–0.88)	< 0.001	0.77 (0.65–0.92)	0.003	0.83 (0.70–0.99)	0.044
T3	0.68 (0.57–0.81)	< 0.001	0.72 (0.60–0.86)	< 0.001	0.76 (0.63–0.91)	0.003
P for trend		< 0.001		< 0.001		0.002

Abbreviation: OR = odds ratio, CI = confidence interval, BMI = body mass index, ACEI = angiotensin converting enzyme inhibitor, ARB = angiotonin receptor blocker, eGFR = estimated glomerular filtration rate

Non-adjusted model: did not adjust variables

Minimally adjusted model: adjust for age and gender

Fully adjusted model: adjust for age, gender, BMI, ethnicity, marital status, education, family income to poverty ratio, smoking, charlson comorbidity index, hypertension, hyperlipidemia, ACEI/ARB, and eGFR

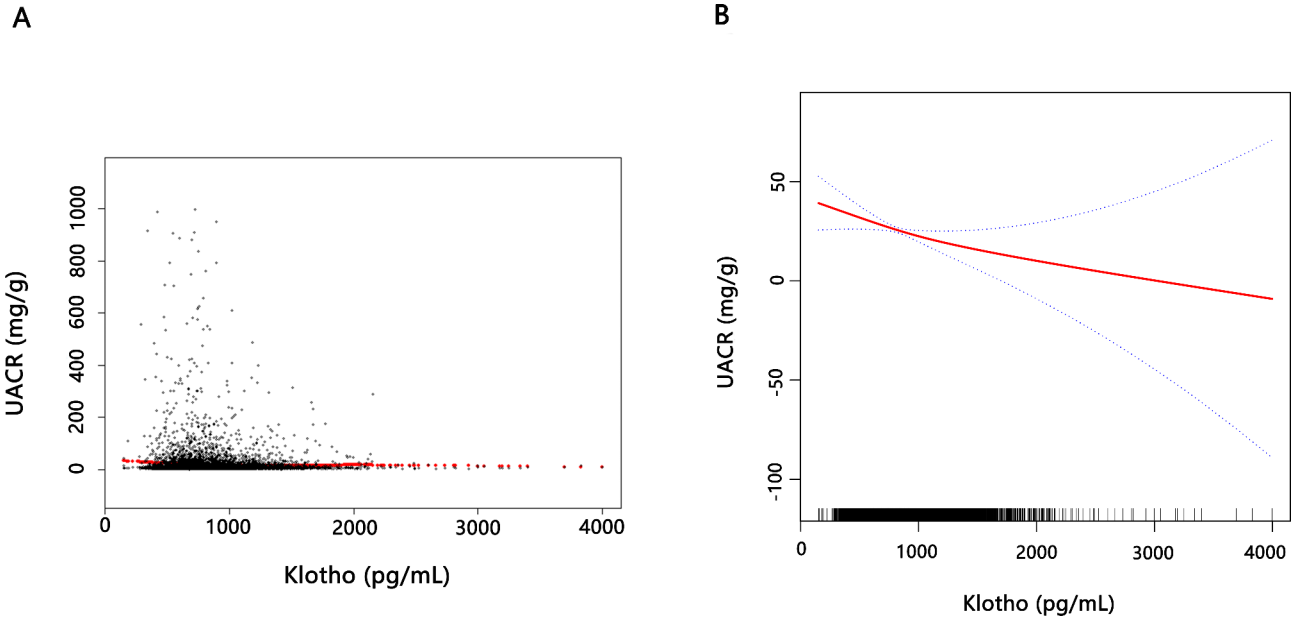

Fig. 2 The association between serum klotho level and UACR in the middle-aged and elderly participants without diabetes mellitus (**A**) Each black point represents a sample. (**B**) The solid red line represents the smooth curve fit between klotho level and UACR level, and blue dotted lines represent the 95% confidence interval from the fit. Abbreviations UACR = urine albumin-to-creatinine ratio

Table 4 The results of two-piecewise linear regression model

Inflection point of serum klotho level (pg/ml)	Effect size (β)	95%CI	*P* value
≤ 589	-0.13	-0.23 to -0.02	0.024
> 589	-0.01	-0.02 to 0.01	0.321

Abbreviation: CI = confidence interval, BMI = body mass index, eGFR = estimated glomerular filtration rate, ACEI = angiotensin converting enzyme inhibitor, ARB = angiotonin receptor blocker

Model adjust for age, gender, BMI, ethnicity, marital status, education, family income to poverty ratio, smoking, charlson comorbidity index, hypertension, hyperlipidemia, ACEI/ARB, and eGFR

by stratification and interaction analyses [37]. However, studies of serum klotho level and albuminuria risk in participants without DM were limited. To the best of our knowledge, this is the first investigation into the relationship between albuminuria in individuals without DM and serum klotho level. In this study, we found that participants with a high serum klotho level had a decreased risk for albuminuria. Compared with the lowest level of serum klotho level (Tertile 1), participants in Tertile 2 and Tertile 3 had a lower risk of albuminuria. The stratified analysis showed that serum klotho level was still negatively associated with albuminuria in the subgroups (age, gender, BMI, hypertension, hyperlipidemia, receiving ACEI/ARB, and eGFR), and statistically significant interactions were not observed in the subgroups (except for the hypertension subgroup).

However, the mechanism between serum klotho level and albuminuria has not been clearly elucidated. On the

Table 5 Association of serum klotho level with albuminuria in subgroups

Characteristic	Subgroups	Number	Klotho level	OR (95% CI)	*P* Value	*P* for interaction
Age (year)						0.222
	< 65	6928	TI	Reference		
			T2	0.80 (0.64-1.00)	0.053	
			T3	0.79 (0.63–0.99)	0.036	
	≥ 65	2289	TI	Reference		
			T2	0.93 (0.70–1.25)	0.644	
			T3	0.72 (0.52–0.99)	0.042	
Gender						0.144
	Male	4385	TI	Reference		
			T2	1.00 (0.79–1.30)	0.947	
			T3	0.77 (0.58–1.02)	0.066	
	Female	4832	TI	Reference		
			T2	0.70 (0.54–0.90)	0.005	
			T3	0.74 (0.58–0.94)	0.012	
BMI (kg/m^2)						0.504
	< 25	2568	TI	Reference		
			T2	1.00 (0.70–1.42)	0.990	
			T3	0.81 (0.57–1.16)	0.249	
	≥ 25	6649	TI	Reference		
			T2	0.78 (0.64–0.96)	0.020	
			T3	0.73 (0.59–0.90)	0.003	
Hypertension						0.015
	No	4920	TI	Reference		
			T2	0.81 (0.59–1.11)	0.190	
			T3	0.99 (0.73–1.35)	0.955	
	Yes	4297	TI	Reference		
			T2	0.85 (0.69–1.06)	0.144	
			T3	0.65 (0.52–0.82)	< 0.001	
Hyperlipidemia						0.372
	No	7103	TI	Reference		
			T2	0.82 (0.68–0.99)	0.048	
			T3	0.71 (0.58–0.88)	0.001	
	Yes	2114	TI	Reference		
			T2	0.88 (0.58–1.34)	0.554	
			T3	0.92 (0.62–1.36)	0.672	
ACEI/ARB						0.273
	No	8148	TI	Reference		
			T2	0.88 (0.73–1.07)	0.199	
			T3	0.76 (0.62–0.92)	0.006	
	Yes	1069	TI	Reference		
			T2	0.56 (0.34–0.92)	0.021	
			T3	0.72 (0.45–1.15)	0.165	
eGFR (ml/min per1.73 m^2)						0.087
	< 60	645	TI	Reference		
			T2	0.76 (0.47–1.22)	0.254	
			T3	0.51 (0.29–0.89)	0.017	
	≥ 60	8572	TI	Reference		
			T2	0.89 (0.73–1.07)	0.216	
			T3	0.83 (0.69–1.01)	0.068	

Abbreviation: OR=odds ratio, CI=confidence interval, BMI=body mass index, eGFR=estimated glomerular filtration rate, ACEI=angiotensin converting enzyme inhibitor, ARB=angiotonin receptor blocker

one hand, proteinuria was caused mostly by podocyte damage in primary glomerular diseases. Klotho is recognized as an antagonist of endogenous Wnt/β-catenin activity, while the Wnt/β-catenin signaling pathway plays an important role in podocyte injury and proteinuria formation. Loss of Klotho contributes to kidney injury by derepressing Wnt/β-catenin signaling [38]. Zhou et al. reported that blocking Wnt signaling with klotho protected podocytes from damage and albuminuria caused by advanced oxidation protein products [39]. Kim et al. demonstrated that klotho might ameliorate proteinuria by targeting transient receptor potential channel 6 in podocytes [40]. On the other hand, albuminuria may have contributed to the downregulation of klotho [41]. In this regard, albumin itself was shown to directly decrease klotho mRNA and cell protein in cultured tubular cells [42]. In fact, decreased kidney and/or urine klotho levels were observed in both animals and individuals with pathological albuminuria (but preserved eGFR) [42]. Currently, antialbuminuric therapy was the clinical practice standard for proteinuric nephropathies. Renin-angiotensin system antagonists, the most commonly prescribed medications for proteinuria, also increased kidney klotho gene expression in experimental animals and serum klotho in patients with proteinuria [43, 44]. Delitsikou et al. demonstrated that albuminuria alone might be sufficient to selectively downregulate klotho in vivo and in vitro at the mRNA and protein levels, which was partly associated with the activation of transcription factor 3 and partly associated with activating the endoplasmic reticulum stress pathway [45]. Given that the mechanism is not yet entirely clear, future studies should further explore the mechanism of the interaction between klotho and albuminuria to find new targets for the treatment of albuminuria.

In addition, the present study showed that the relationship between serum klotho level and albuminuria was more obvious in patients with a history of hypertension. The relationship between hypertension, serum klotho level, and albuminuria was complex. Classically, albuminuria has been attributed to hypertension [46]. On the other hand, a previous study reported that albuminuria was also a cause of hypertension [47]. Furthermore, Drew et al. found that higher klotho was linked to a decreased likelihood of incident hypertension [48]. Therefore, future studies can further explore the exact relationship between the three.

There are several limitations in our study. First, due to the cross-sectional nature of this investigation, a causal relationship between serum klotho and albuminuria could not be established. Second, the data is gathered from the official NHANES database, which exclusively represents the population of the United States. Third, the serum klotho is limited by only being measured among middle-aged and elderly participants. Fourth, this study lacked data on the cause of albuminuria. Fifth, in this study, we did not further explore the association between serum klotho level and persistent albuminuria, which is defined as UACR ≥ 30 mg/g in two consecutive measurements. However, two consecutive UACR measurements were only available in the NHANES 2009–2010 cycle. Therefore, due to the small sample size of participants with persistent albuminuria, we did not investigate the relationship between serum klotho level and persistent albuminuria.

Conclusions

In summary, a high serum klotho level is associated with a decreased risk of albuminuria. in middle-aged and elderly participants without diabetes. Future studies will be needed to determine the exact mechanism of the interaction between klotho and albuminuria in order to discover novel targets for the treatment of albuminuria in individuals without diabetes.

Abbreviations

DM	Diabetes mellitus
NHANES	National Health and Nutrition Examination Survey
OR	Odds ratio
UACR	Urine albumin-to-creatinine ratio
BMI	Body mass index
CCI	Charlson comorbidity index
eGFR	Estimated glomerular filtration rate
CKD-EPI	Chronic Kidney Disease Epidemiology Collaboration
ACEI	Angiotensin converting enzyme inhibitor
ARB	Angiotonin receptor blocker
SD	Standard deviation
T1DM	Type 1 diabetes mellitus
T2DM	Type 2 diabetes mellitus

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Author contributions

DC, MC, and XW contributed to the study conception and design. Material preparation, data collection and analysis were performed by ZQ and YT. The first draft of the manuscript was written by DC and XW and all authors commented on previous versions of the manuscript. All authors gave final approval of the version to be published.

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Data availability

No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate

Study protocols for NHANES were approved by the NCHS ethnics review board (Protocol #2011–17, https://www.cdc.gov/nchs/nhanes/irba98.htm). All participants provided written informed consent.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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