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Meta-analysis

Potential application of Klotho as a prognostic biomarker for patients with diabetic kidney disease: a meta-analysis of clinical studies

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Abstract

Background: Diabetic kidney disease (DKD) is a serious diabetic complication and the performance of serum Klotho in DKD's prognostic evaluation is controversial.

Objective: To assess the association of serum Klotho with adverse kidney and non-kidney clinical outcomes in patients with DKD.

Design: Clinical studies regarding the relationship of serum Klotho with DKD were included. Study quality was assessed using the Newcastle–Ottawa scale. Subgroup and sensitive analyses were performed to search for the source of heterogeneity.

Data sources and methods: We comprehensively searched PubMed, Embase, Web of Science, and Cochrane library databases up to 27 September 2022. The associations of Klotho with albuminuria, such as the urinary albumin creatinine ratio (UACR), kidney outcomes such as persistent albuminuria, estimated glomerular filtration rate decline, and non-kidney outcomes such as diabetic retinopathy, cardiovascular morbidity, and mortality, were evaluated. The indicators, such as the correlation coefficient (*r*), odds ratio (OR), relative risk, and hazard ratio, were retrieved or calculated from the eligible studies.

Results: In all, 17 studies involving 5682 participants fulfilled the inclusion criteria and were included in this meta-analysis. There was no significant association of serum Klotho with UACR in DKD patients [summary *r*, −0.28 (−0.55, 0.04)] with high heterogeneity. By contrast, a strong association was observed regarding serum Klotho with kidney outcomes [pooled OR, 1.60 (1.15, 2.23)], non-kidney outcomes [pooled OR, 2.78 (2.11, 3.66)], or combined kidney and non-kidney outcomes [pooled OR, 1.96 (1.45, 2.65)] with moderate heterogeneity. Subgroup analysis indicated that age, study design, and the estimated glomerular filtration rate may be the sources of heterogeneity.

Conclusion: A decreased serum Klotho level is possibly associated with an increased risk of developing kidney and non-kidney clinical outcomes in DKD patients; thus, Klotho may be a possible biomarker to predict DKD clinical outcomes. Additional studies are needed to clarify and validate Klotho's prognostic value.

Keywords: diabetic kidney disease, Klotho, outcomes, prognosis, urinary albumin creatinine ratio

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Introduction

Diabetic kidney disease (DKD) is a specific form of chronic kidney disease (CKD), which is caused by diabetic mellitus (DM)-induced microangiopathy. Approximately 30–40% of patients with DM may develop DKD; thus, DKD is a serious diabetic complication that has received considerable global attention.[1] There are often no

obvious clinical manifestations in early DKD, but it still irreversibly progresses to end-stage kidney disease (ESKD); furthermore, the rapid development of DKD confers substantial cardiovascular (CV) events and mortality.[2] Early identification and timely treatment of patients with progressive DKD are critical.[3] Although the understanding of DKD pathophysiology has evolved in recent years, effective management modalities and ideal diagnostic or prognostic tools, including better biomarkers for early diagnosis and prognostic risk stratification, remain lacking.[4,5]

DKD is characterized by the occurrence of persistent albuminuria such as the urinary albumin creatinine ratio (UACR) and/or a progressive decline of the estimated glomerular filtration rate (eGFR).[6] Accordingly, the UACR and eGFR are commonly used biomarkers for early and advanced DKD stages, respectively, in the clinical setting. However, these biomarkers in routine use are not specific to the DKD population, lacking sufficient specificity and sensitivity, particularly for early DKD diagnosis.[7] Moreover, regarding DKD prognostic biomarkers, a recent comprehensive review assessed the potential application of conventional and novel biomarkers for DKD progression monitoring.[8] Unfortunately, except for albuminuria and the eGFR, no other biomarkers showed the potential utility to predict DKD clinical outcomes. Similar findings were demonstrated by a recent clinical predictive model in a DKD population.[9] Therefore, it is necessary to screen and identify candidate biomarkers for monitoring DKD onset and progression.[10]

Klotho was identified as a kidney protective factor that exhibits pleiotropic biological functions.[11] It has been confirmed that the kidney, primarily the distal convoluted tubules, is the main organ that produces Klotho,[12,13] although lower Klotho levels were also detected in other organs.[13] Therefore, Klotho expression was associated with the state of kidney function, particularly tubular interstitial lesions.[14] Indeed, Klotho was downregulated universally under the condition of CKD, and this decrease preceded the changes in the traditional biomarkers, serum creatinine, and the eGFR, indicating that Klotho is an early diagnostic biomarker for CKD.[15] Furthermore, a lowered Klotho level was correlated with more adverse kidney outcomes such as an eGFR decline or serum creatinine doubling, indicating that Klotho is a prognostic biomarker for CKD.[16,17] DKD features an aggressive loss of kidney function and tubular interstitial injury[18] such that the Klotho level appears to be decreased in DKD, conferring on it a potential role for DKD diagnosis. Numerous studies have been conducted to investigate the clinical significance of Klotho in DKD patients but have contributed inconsistent results.[19–21] Interestingly, a recently published meta-analysis addressed this inconsistency and found that Klotho was indeed lower in this population, for the first time providing evidence of Klotho as an early biomarker in the DKD population.[22] However, whether a decline in the Klotho level is associated with increased DKD clinical outcomes is still unclear and the available clinical data remain controversial.[23–25] Therefore, we perform this meta-analysis and systematic review of clinical studies to address this issue and evaluate the prognostic performance of Klotho in the DKD population.

Methods

Search strategy

A comprehensive search of the PubMed, Embase, Web of Science, and Cochrane databases was conducted for eligible studies and relevant publications up to 27 September 2022. Our current study was in accordance with the International Prospective Register of Systematic Reviews (PROSPERO) statements and Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA).[26] The protocol of this meta-analysis is described in the Section 'Methods'; thus, it is not registered and registration information is not supplied. A search strategy based on PICOM was performed as follows:

Patients: DKD patients
Intervention: Klotho level
Comparison: Association of Klotho level with kidney and non-kidney outcomes
Outcomes: Kidney and non-kidney outcomes
Methods: Observational or cross-sectional or cohort or longitudinal study

DKD was defined as a UACR >30 mg/g, urinary albumin excretion rate >30 mg/24 h (20 mg/min), or eGFR <60 mL/min/1.73 m^2. Early DKD was defined as a UACR >30 mg/g and eGFR >60 mL/min.[6] Search terms in databases were (diabetic kidney disease or DKD or diabetic nephropathy

(DN) or DN or diabetic or diabetes) and (Klotho or sKlotho or KL or sKL) and (albuminuria or proteinuria or glomerular filtration rate or kidney function or creatinine doubling or end stage renal disease or end stage kidney disease or renal replacement therapy or dialysis or diabetic retinopathy or progression or decline or deterioration or morbidity or mortality or cardiovascular or cerebrovascular or death or outcome or survival or prognosis or prognostic or prediction or predictive). Other references included within these publications were also screened and identified if necessary.

Study selection

The searched publications were selected and checked, by three independent investigators (Li Xia Yu, Min Yue Sha, and Yue Chen) based on the inclusion and exclusion criteria. After screening the titles and abstracts of the publications, those potentially eligible were chosen for full-text reading. After excluding irrelevant studies, the eligible studies were included for a quality check and data extraction. Disagreements were resolved and consensus was reached by discussion with a third author (Qi-Feng Liu). Inclusion criteria were as follows: (1) adult participants with DKD (age $\geqslant$ 18 years); (2) studies that investigated the relationship between Klotho and DKD (albuminuria or kidney function or clinical outcomes); and (3) English studies. Exclusion criteria were as follows: (1) participants with renal dialysis or kidney transplantation; (2) studies with inadequate data; and (3) studies with irrelevance (i.e. studies on animals, the association between Klotho in renal tissue or in urine and DKD, case reports, letters, reviews, and duplicated studies).

Data extraction and quality assessments

The extracted data included first author, publication year, country, study design, age, sample size, sKlotho level, clinical outcomes including the UACR, urinary protein creatinine ratio, albumin excretion rate, eGFR, renal replacement therapy, CV events, morbidity, mortality, correlation coefficient *r* (Pearson or Spearman), odds ratio (OR), relative risk (RR), hazard ratio (HR), and 95% confidence interval (CI). Effectors such as the urinary protein creatinine ratio, albumin creatinine ratio, or urinary protein creatinine ratio all reflect the intensity of microalbuminuria in DKD; thus, the effectors were equivalent to the UACR. The data were collected by two authors (Fang Tan and Xi Liu) using a standardized form. If effectors were not obtained directly or insufficiently, the first author or correspondent author was contacted for the potential data by e-mail. The eligible studies were subjected to quality checks using the Newcastle–Ottawa scale.[27] This scale was designed and developed to assess the quality of non-randomized studies and incorporates three perspectives regarding the selection, comparability, and outcome of study groups by a 'star system' judgment. Studies awarded >7 stars were judged as high-quality studies. Any discrepancy in data extraction or quality assessments was addressed *via* discussion with a third reviewer (Shasha Li).

Statistical analysis

Pearson correlation coefficient (*r*) was first transformed into the Spearman correlation coefficient (*r*) because the Spearman correlation coefficient (*r*) was unaffected by logarithmic transformation in previous reports.[28,29] Due to the non-normal distribution of Spearman correlation coefficients (*r*), each Spearman correlation coefficient (*r*) was subjected to Fisher's transformation to obtain a normally distributed Z value and standard error of Z.[28,29] The Z value underwent inverse Fisher's conversion to generate the summary coefficient (*r*) and corresponding CI. Effectors of the OR, RR, or HR were combined to generate the pooled effect using an inverse variance method. The software of Manager 5.3 (Cochrane Collaboration, Copenhagen, Denmark) and Stata12.0 (StataCorp LP, College Station, TX, USA) were adopted for the meta-analysis. Heterogeneity among studies was checked according to the I^2 value. The fixed-effects model or random-effects model was applied dependent on the I^2 value. The random-effects model was chosen if the I^2 value was >50%, otherwise, the fixed-effects model was chosen. The publication bias was examined by Begg's and Egger's tests. Sensitivity and subgroup analyses were performed to identify the potential source of heterogeneity. The statistical significance level was defined as $p < 0.05$.

Results

Study selection and eligible studies

Our initial search strategy yielded 2313 potential citations, and the study selection process is presented in Figure 1. After removing duplications

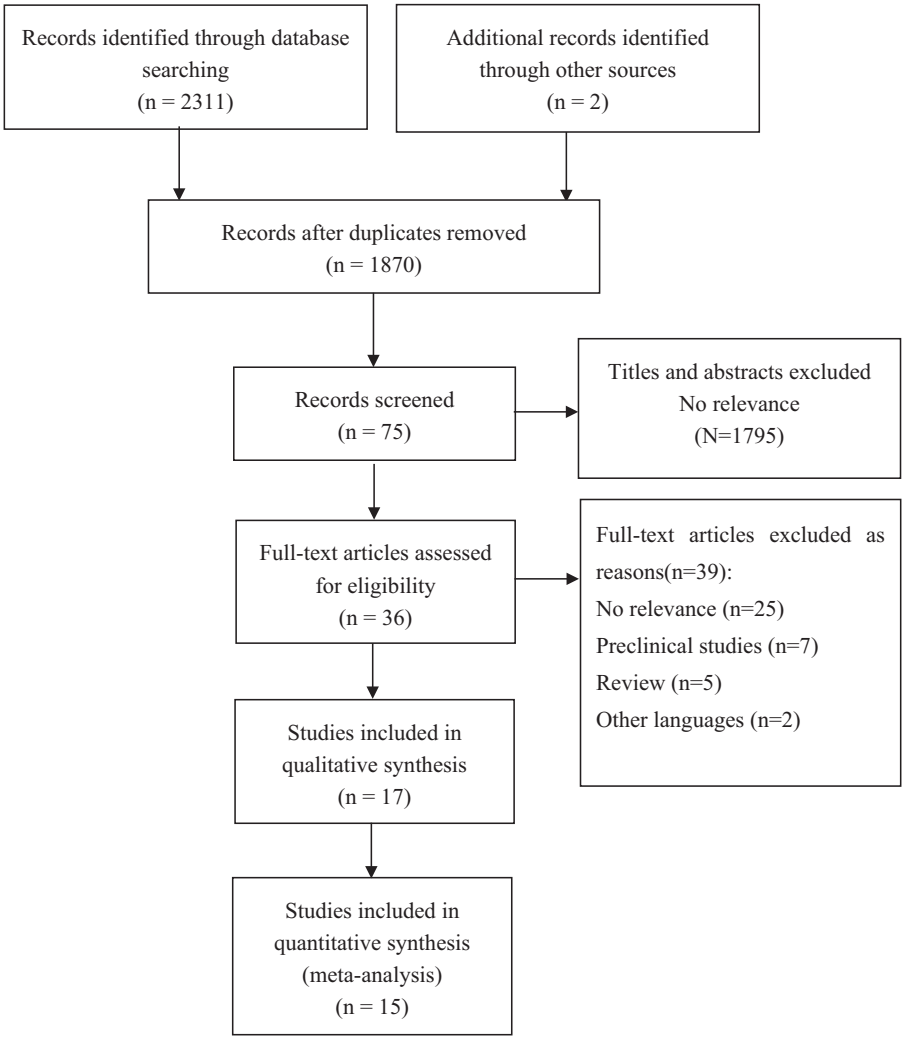

Figure 1. Flow chart of the included studies in the meta-analysis.

and screening titles and abstracts, 2238 irrelevant studies were excluded. After reading the full texts of the remaining 75 studies, 36 of them were extracted as initially eligible studies. On examining the remaining 36 full texts, 17 of them met the inclusion criteria and were identified as eligible studies and included in this study.[19,20,23,24,30–42] These studies were published from 2014 to 2022 and enrolled 5682 participants with DM with or without albuminuria. Ten studies were cross-sectional[19,20,30,34,36,37,40–42] and seven studies were a retrospective or prospective cohort.[23,24,31–33,35,39] The study characteristic is listed in detail in Table 1. The mean score of the cross-sectional studies was 7 stars and that of the cohort studies was 7.4 stars (Tables 2 and 3).

Association of Klotho with the UACR in early DKD

Nine studies reported an association of Klotho with the UACR.[19,20,24,30,35–37,41,42] The effect indicator was displayed as the Pearson or Spearman correlation (r) in eight studies.[19,20,24,35–37,41,42] One study reported that Klotho was not associated with the UACR, lacking sufficient data.[40] Another study reported an insignificant association, but this was presented as an unstandardized regression coefficient (β).[30] Therefore, these two studies were not pooled for meta-analysis due to inadequate data. Following data conversion, the pooled Fisher's Z value and 95% CI was −0.29 (−0.62, 0.05) (Figure 2) and the calculated summary r and 95% CI was −0.28 (−0.55, 0.04). The result was generated using the random-effects model because of the

Table 1. Characteristics of the included studies.

Author	Year	Country	Design	*N*	Age	eGFR	Klotho assays	Outcomes	Effectors	Conclusion
Lee *et al.*[42]	2014	Korea	CS	172 (25 cons)	55.8 ± 10.4	90.62 (87.42, 93.94) 85.49 (81.70 8,9.45)	IBL, Japan	ACR eGFR	$r = -0.214$, $p = 0.009$ $r = -0.018$, $p = 0.827$	Klotho was high in DKD and inversely correlated with ACR, but not with eGFR
Wu *et al.*[41]	2014	China	CS	622 (160 cons)	52.58 ± 5.96	eGFR > 90	EIAab, China	UACR Scr	$r = -0.732$, $p < 0.01$★ $r = -0.503$, $p < 0.01$★	Klotho was low in DKD and inversely correlated with UACR
Kim *et al.*[39]	2016	Korea	PH	109	56.4 ± 10.8	93.0 ± 23.2	IBL, Japan	GFR ACR	$r = -0.324$, $p = 0.004$ HR, 4.0 (1.3–12.7)	Reduced Klotho predicted annual GFR decline or albuminuria persistence or progression
Dogan *et al.*[40]	2016	Turkey	CS	147 (76 cons)	34.1 ± 9.2	eGFR > 90	Cusabio-Biotech, China	Early DN	Not obtained	Klotho was not related to early DN onset
Inci *et al.*[20]	2016	Turkey	CS	142 (32 cons)	61.0 ± 9.77	51.71 ± 23.11	YH Biosearch, China	eGFR UPCR eGFR	$r = -0.16$, $p = 0.097$ $r = -0.06$, $p = 0.541$ β = 0.074; $p = 0.27$	Klotho was high in DKD but was not related to eGFR or UPCR
Silva *et al.*[36]	2017	Portugal	CS	107	59.0 ± 8.57	53.2 ± 10.15	IBL, Japan	eGFRs ACR ACR ACR	β = −0.09; $p = 0.886$ $r = -0.336$; $p < 0.001$ β = −0.64; $p = 0.036$ OR, 1.324 (1.061,1.721)	Klotho was not related to kidney function, but was associated with ACR progression

(Continued)

Table 1. (Continued)

Author	Year	Country	Design	*N*	Age	eGFR	Klotho assays	Outcomes	Effectors	Conclusion
Maltese *et al.*[38]	2017	UK	CS	78 (45 cons) 45 cons	54.4 ± 11.6 43.3 ± 9.6	90.2 ± 21.7	IBL, Germany	MA	OR, 0.13 (0.02, 0.79) OR, 7.69 (1.26, 50.0)	Klotho was not related to eGFRs, but was inversely with MA
Nie *et al.*[37]	2017	China	CS	367 (106 cons)	45–84	36.0 ± 16.7	Friendbio-Science China	eGFR UACR	$r = 0.437$; $p < 0.001$ $r = -0.661$; $p < 0.001$	Klotho was positively related to eGFR and negatively related to UACR
Pan *et al.*[23]	2018	Taiwan	PH (7 years)	252	57.2 ± 10.3	88.9 ± 31.7	Cusabio, China	CAD	OR, 3.85 (1.406, 10.526)	A high Klotho level was associated with a reduced risk of developing microangiopathies.
Farías-Basulto *et al.*[19]	2018	Mexico	CS	136	64 (58–69) 59 (51–65)	95 (83–106)	R&D Systems, USA	ACR ACR	OR, 1.75 (0.78, 3.85) $r = 0.114$, $p = 0.111$	A high Klotho level was not associated with reduced risk of developing ACR
Fountoulakis *et al.*[35]	2018	UK	PH (9 years)	101	60 (40–82)	90.7 ± 20.0	IBL, Germany	eGFR AEB eGFR decline Death	None $r = -0.245$, $p = 0.01$★ OR, 5.05 (1.33–19.20)▲ OR, 1.81 (0.58–5.88)▲	Klotho was not related to eGFR but was inversely correlated with AEB and predicted eGFR decline except for death
Donate-Correa *et al.*[34]	2019	Spain	CS	57	69.8 ± 9.7	77.5 ± 24.7	IBL, Japan	DFS eGFR	OR, 1.01 (1.01–1.02)▲ $r = 0.329$, $p < 0.01$★	Klotho was positively related to eGFRs and inversely correlated with DFS

(Continued)

Table 1. (Continued)

Author	Year	Country	Design	*N*	Age	eGFR	Klotho assays	Outcomes	Effectors	Conclusion
Silva *et al.*[33]	2019	Portugal	PH (2.8 years)	107	57.2 ± 7.1	52.89 ± 20.15	IBL, Japan	CLVH	OR, 1.35 (1.03–1.66)	Reduced Klotho was related to more CLVH and predicted more CV deaths or hospitalizations
								CV death hospitalization	HR, 2.38 (1.49, 11.59)	
									OR, 1.32 (1.06–1.46)	
Bob *et al.*[24]	2019	Romania	RH (1 year)	63	58.13 ± 12	65.15 ± 32.45	Abbexa, UK	UACR	$r = 0.52$, $p < 0.01$★	Klotho is not related to eGFRs and it was high in patients with eGFRs < 60 ml/min
								eGFR	Not obtained	
								△eGFR	$r = 0.714$, $p < 0.001$★	High Klotho was related to rapidly declined eGFR.
Ribeiro *et al.*[31]	2020	Portugal	PH (no)	152 (26 cons)	58.1 ± 6.8	CKD2-3	IBL, Japan	Fracture RRT	OR, 2.95 (2.00, 3.5) HR, 1.16 (1.01, 2.52)	Klotho was closely related to eGFRs, fracture, and risk of RRT
Corcillo *et al.*[32]	2020	UK	PH (3.7 years)	81	61 (48–78)	69.0 ± 27.3	IBL, Germany	DR	OR, 14.9 (1.44, 166.7)	Klotho was related to DR onset or progression
Ciardullo and Perseghin[30]	2022	Italy	CS	2989	60.0 ± 0.2	83.6 ± 0.5	IBL, Japan	eGFR	β = 2.211 (1.405, 3.017)	Klotho was related to eGFRs but was not related to CV disease or UACR
								CV disease	β = −13.086 (46.579,20.407)	
								UACR	β = −0.017 (−0.033, 0.000)	

ACR, urine albumin creatinine ratio; Scr, serum creatinine; AEB, albumin excretion rate; ▲, calculated effectors; CAD, coronary artery disease; CI, confidence interval; CKD, chronic kidney disease; UPCR, urine protein-to-creatinine ratio; CLVH, concentric left ventricular hypertrophy; RRT, renal replacement therpy; CS, cross-sectional; CV, cardiovascular; DFS, diabetic foot syndrome; DKD, diabetic kidney disease; DR, diabetic retinopathy; eGFR, estimated glomerular filtration rate; HR, hazard ration; IBL, immuno-biological laboratories; MA, microalbuminuria; MHD, maintenance hemodialysis; OR, odds ration; PH, prospective cohort; RH, retrospective cohort; ★, Spearman relation; UACR, urinary albumin to creatinine ratio.

Table 2. NOS scores of the cross-sectional studies included.

Case–control study	Is the case definition adequate?	Representativeness of the cases	Selection of controls	Definition of controls	Control for important factor[a]	Control for additional factor[a]	Ascertainment of exposure[a]	Same method of ascertainment for cases and controls	Non-response rate	Total quality scores
Lee 2014	★	★	★	★	★	–	★	★	–	7
Wu 2014	★	★	★	★	★	★	★	★	–	8
Dogan 2016	★	★	★	★	–	–	★	★	–	6
Inci 2016	★	★	★	★	★	★	★	★	–	8
Silva 2017	★	★	–	–	★	★	★	★	–	8
Maltese 2017	★	★	★	★	★	★	★	★	–	8
Nie 2017	★	★	★	★	★	★	★	★	–	8
Basulto 2018	★	★	–	–	★	–	★	★	–	5
Correa 2019	★	★	–	–	★	★	★	★	–	6
Ciardullo 2022	★	★	–	–	★	★	★	★	–	6

[a]Two stars could be awarded for this item. Studies that controlled for age or eGFR or ACR were awarded one star, respectively.
eGFR, estimated glomerular filtration rate; NOS: Newcastle–Ottawa Scale.

Table 3. NOS scores of the cohort studies included.

Cohort study	Representativeness of the exposed cohort	Selection of the unexposed cohort	Ascertainment of exposure	Outcome of interest not present at the start of the study	Comparability control for important factor or additional factor[a]	Outcome assessment	Was follow-up long enough for outcomes to occur	Adequacy of follow-up of cohorts	Total quality scores
Kim 2016	–	★	★	★	★★	★	★	★	8
Pan 2018	–	★	★	★	★★	★	★	★	8
Fountoulakis 2018	–	★	★	★	★★	★	★	★	8
Silva 2019	–	★	★	★	★★	★	★	★	8
Bob 2019	–	★	★	★	★★	★	–	–	6
Ribeiro 2020	–	★	★	★	★★	★	★	–	7
Corcillo 2020	–	★	★	★	★	★	★	★	7

[a]Two stars could be awarded for this item. Studies that controlled for age or eGFR or were awarded one star, respectively.
ACR, albumin creatinine ratio; eGFR, estimated glomerular filtration rate; NOS, Newcastle–Ottawa Scale.

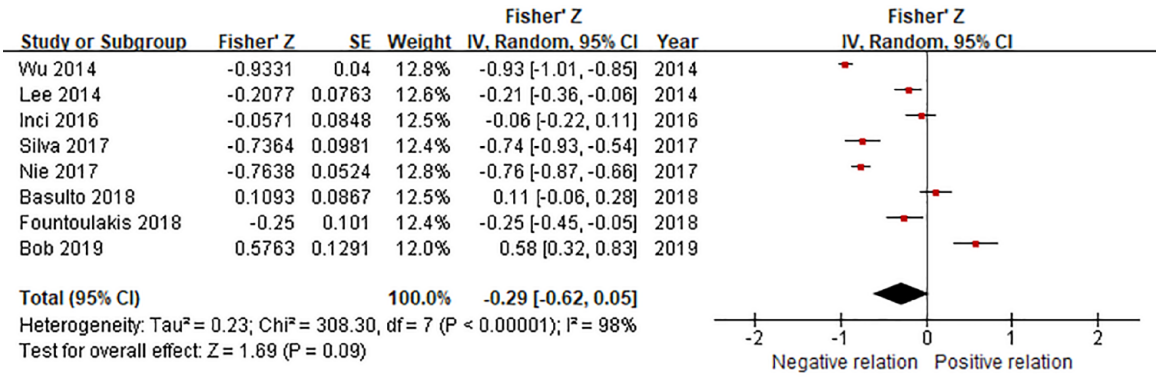

Figure 2. Forest plots of the pooled Fisher's *Z* for the association between Klotho level and UACR.
UACR, urinary albumin creatinine ratio.

presence of high heterogeneity ($I^2 = 98\%$, $p < 0.001$, Figure 2). Although Klotho was prone to be inversely correlated with the UACR, this association was lost in the final analysis. Publication bias was present based on the Begg's ($p = 0.063$) or Egger's tests ($p = 0.006$) (Supplemental Figure 1). Sensitivity analysis demonstrated the overall effect was significantly changed [pooled *Z* value, −0.41 (−0.72, −0.09)] by removing the Bob *et al.*'s study (Supplemental Figure 2).[24]

Association of Klotho with kidney or non-kidney clinical outcomes

Six studies reported an association of different Klotho levels (low *versus* high) with kidney outcomes, including the risks for albuminuria onset or progression, deteriorated kidney function, and renal replacement therapy.[19,31,35,36,38,39] Five studies reported an association of the Klotho level (low *versus* high) with non-kidney outcomes, including the risks for CV morbidity, mortality, diabetic retinopathy, diabetic foot syndrome, and fracture. Two studies reported the results of both kidney and non-kidney outcomes.[31,35] The indicators regarding the association of Klotho with clinical outcomes were presented as the adjusted OR, HR, calculated OR, or calculated HR and the corresponding 95% CIs. The pooled OR in terms of Klotho with kidney outcomes was 1.60 (1.15, 2.23) and there was moderate heterogeneity [$I^2 = 65\%$, $p = 0.01$, Figure 3(a)]. In terms of Klotho with non-kidney outcomes, the pooled OR was 2.78 (2.11, 3.66) and there was no obvious heterogeneity [$I^2 = 0\%$, $p = 0.49$, Figure 3(b)]. In terms of Klotho with combined clinical outcomes, the pooled OR was 1.96 (1.45, 2.65), and there was significant heterogeneity [$I^2 = 75\%$, $p < 0.001$, Figure 3(c)]. Sensitivity analysis showed that the pooled effect regarding the combined outcomes was not changed by excluding any one study included in the meta-analysis. Publication bias was also found according to Begg's ($p = 0.118$) or Egger's tests ($p = 0.021$) (Supplemental Figure 3).

Subgroup analysis of Klotho with adverse clinical outcomes

Considering the high heterogeneity regarding the Klotho level with composite outcomes, subgroup analysis was conducted to identify potential sources of heterogeneity. The included studies were divided into five subgroups based on age (⩾60 or <60), sample (⩾100 or <100), study design (cross-sectional or cohort), eGFR (⩾90 mL/min or <90 mL/min), and study quality (>7 stars or ⩽7 stars) independently (Table 4). Clear heterogeneity was not observed in the three subgroups categorized by age ⩾60 years ($p = 0.23$; $I^2 = 32\%$), cross-sectional study ($p = 0.14$; $I^2 = 49\%$), or eGFR ⩾ 90 mL/min ($p = 0.36$; $I^2 = 6\%$). Therefore, age, study design, and the eGFR may be the sources of heterogeneity (Table 4). Furthermore, the significant association of Klotho with clinical outcomes still persisted in all the subgroups except for the subgroups categorized by age ⩾60 years and case–control studies (Table 4).

Discussion

The results in this study demonstrated that reduced serum Klotho was significantly associated with increased clinical outcomes such as

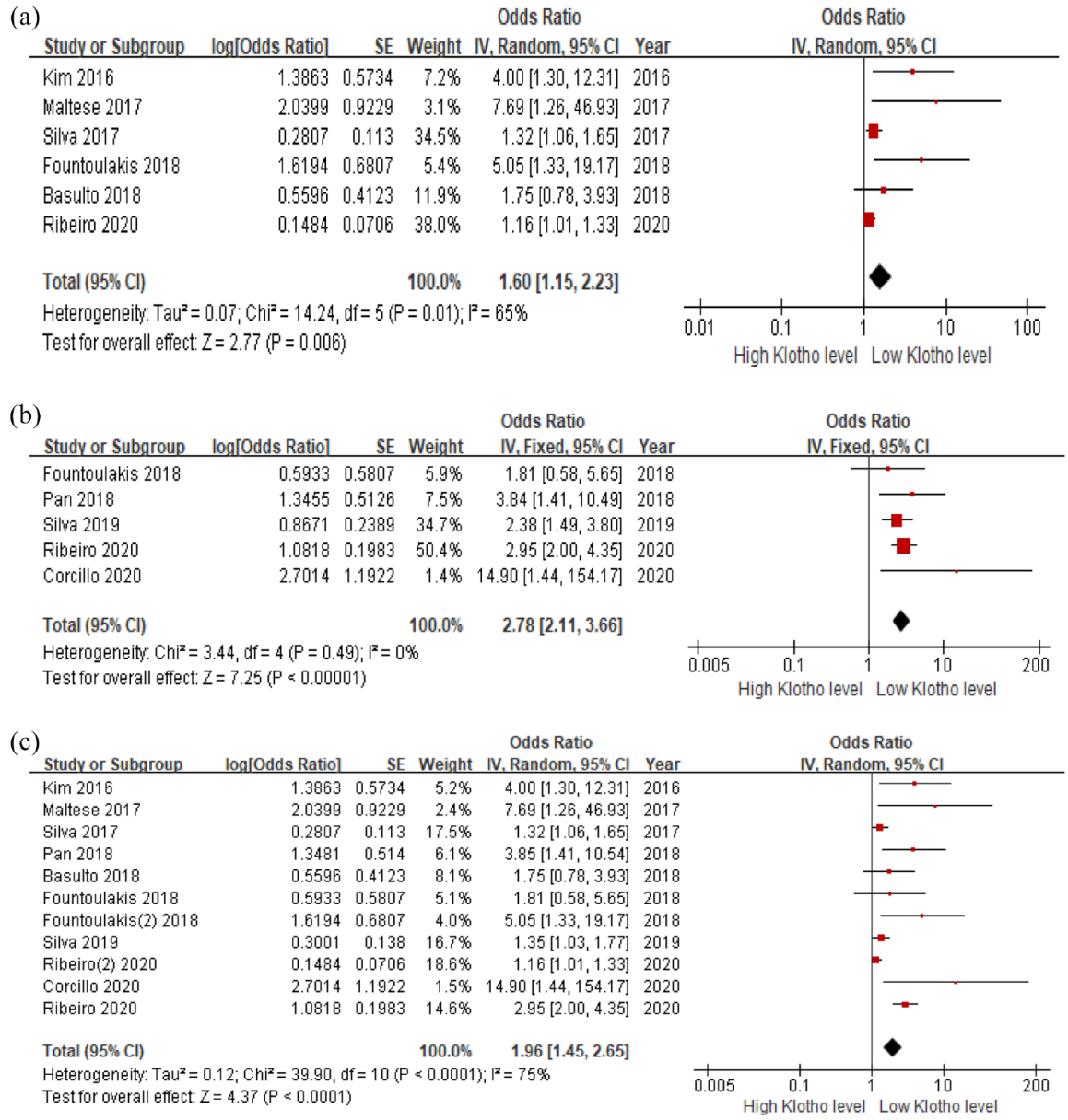

Figure 3. Forest plots of the association of Klotho level with DKD outcomes. (a) Forest plots of the pooled OR for the association between Klotho level and kidney outcomes. (b) Forest plot of the pooled OR the association between Klotho level and non-kidney outcomes. (c) Forest plots of the pooled OR for the association between Klotho level and combined clinical outcomes.
DKD, diabetic kidney disease; OR, odds ratio.

albuminuria progression, kidney function decline, morbidity, and mortality. This meta-analysis first indicated that Klotho was associated with the progression of DKD and that Klotho may be a useful prognostic biomarker for DKD.

Generally, DKD patients normally suffer severe adverse consequences, including an increased risk for ESKD, CV events, and mortality, even in the early stage of DKD.[43,44] Screening and identifying candidate biomarkers for early diagnosis of DKD or prognostic evaluation is an important step to improve DKD management.[45] Although albuminuria and the eGFR are pragmatic biomarkers for DKD diagnosis, these biomarkers in general lack sufficient specificity and sensitivity in

Table 4. Results of subgroup analysis by age, sample size, study design, eGFRs, and study quality.

Subgroup	Studies	Effect estimate (random-effects model) Pooled *r* [95% CI]	Heterogeneity between subgroup
Age			
Age ≧ 60 years	3	2.28 [0.97, 5.36]	$p = 0.23$; $I^2 = 32\%$
Age < 60 years	6	2.16 [1.42, 3.30]	$p < 0.001$; $I^2 = 77\%$
Sample size			
Sample size ≧ 100	6	1.40 [1.14, 1.72]	$p = 0.07$; $I^2 = 48\%$
Sample size < 100	3	9.85 [2.36, 41.19]	$p = 0.66$; $I^2 = 0\%$
Study design			
Cohort	6	2.59 [1.50, 4.46]	$p = 0.003$; $I^2 = 72\%$
Cross-sectional	3	1.71 [0.93, 3.13]	$p = 0.14$; $I^2 = 49\%$
eGFRs			
eGFRs ≧ 90	4	2.47 [1.40, 4.36]	$p = 0.36$; $I^2 = 6\%$
eGFRs < 90	5	2.02 [1.29, 3.15]	$p < 0.001$; $I^2 = 80\%$
Study quality			
>7 stars	6	1.76 [1.24, 2.51]	$p = 0.05$; $I^2 = 55\%$
≤7 stars	3	2.75 [1.48, 5.08]	$p = 0.19$; $I^2 = 40\%$

CI, confidence interval; eGFR, estimated glomerular filtration rate.

these aspects. Herein, there are still no ideal or consensus biomarkers available to help clinicians promptly identify DM patients who are at a high risk of developing DKD and other adverse clinical outcomes. Klotho is reduced during the early stage of CKD and predicts CKD progression in clinical studies, making it a potential early diagnostic and prognostic biomarker for CKD.[15,17,46] Similarly, reduced Klotho may also be involved in the pathogenesis of DM and its complications.[47]

In murine models with DM, *klotho* knockdown increased β-cell apoptosis and exacerbated glucose intolerance and hyperglycemia.[48,49] On the contrary, *klotho* transfer or systemic Klotho therapy increased β-cell replication and ameliorated diabetes.[48–50] This means Klotho has a protective effect on islets and plays a significant role in the occurrence of DM and its complications, such as DKD. Consistently, in clinics, a recent study reported that low Klotho is associated with a high risk for developing DM,[51] and another study reported that a high Klotho level is associated with remission of prediabetic patients, and most importantly, a high Klotho level is concomitant with a reduced UACR in prediabetic patients with normal kidney function.[52] The above findings mean that a Klotho decline precedes the occurrence of DM and albuminuria strongly indicating Klotho may be an early marker of kidney injury in DKD.

Subsequently, there are increasing studies conducted to investigate the relationship between serum Klotho and DKD, yet yielding inconsistent or even contradictory results. For example, previous studies reported that the Klotho level continued to decline together with increasing degrees of albuminuria and Klotho was inversely associated with the UACR in early DKD patients.[36,42,53] These studies revealed that the decrease in the Klotho level is earlier than the occurrence of the

albuminuria or the decline of the eGFR, demonstrating the role of Klotho in the timely or early diagnosis of DKD. However, other studies suggested no clear association of the Klotho level with the UACR or eGFR.[19,23,40] In this context, Xin *et al.*[22] conducted a meta-analysis to address this inconsistency. This study included 14 articles and investigated the association of Klotho with early DKD. They found that the Klotho level declined in DM patients and it continued to do so in early DKD, suggesting that Klotho has the potential ability to be a novel marker for early DKD detection. It is well demonstrated that Klotho expression is modulated negatively by numerous risk factors such as inflammation, oxidative stress, and renin–angiotensin system (RAS),[54,55] which were commonly observed and universally increased in DM.[56] Theoretically, Klotho level should be decreased due to the presence of such negative regulators, especially in early DKD with normal kidney function, meaning that Klotho can be adopted as a candidate marker of DKD onset in DM. However, the result in Xin's study had not been validated in our study. The inconsistent finding presented in the current study does not certainly rule out the performance of Klotho in DKD diagnosis. Instead, there are several probable reasons to explain this inconsistency. First, it is of note that a high heterogeneity was observed, and the result was reversed in sensitivity analysis, indicating a meta-analysis may not be appropriate in this aspect and thus, the conclusion drawn may not be reliable in the current study. Second, the differences in study methodologies and measurement effectors may also contribute to this inconsistency between the two studies. In the study of Xin *et al.*, standardized mean differences were used as the effect indicator, which is different from our indicator, the correlation coefficient (*r*). Therefore, the above evidence indicates that the diagnostic performance of serum Klotho for early DKD is still not determined to some extent and further clinical investigation is warranted to clarify their association.

The inexorable albuminuria development and progressive eGFR reduction in DKD can lead to devastating consequences, including ESKD and increased CV morbidity and mortality.[57] Early detection of patients who are at high risk for DKD progression is critical for prompt treatment and improved clinical outcomes. Unfortunately, no other new biomarkers have been found that have the potential ability to precisely recognize this patient population beyond albuminuria and eGFR.[4,8] Therefore, developing and validating new biomarkers for DKD prognostic evaluation is still ongoing. Previously, a number of studies demonstrated an association between a decreased serum Klotho level with more severe clinical outcomes, and Klotho is proposed as a candidate for monitoring CKD progression.[16,17] Similarly, a reduced serum Klotho level is also implicated in DKD development,[53] and possibly Klotho has the potential to predict DKD outcomes.

Indeed, a considerable number of studies have been performed to examine this association of Klotho with kidney and non-kidney outcomes and evaluate serum Klotho's prognostic performance in DKD patients, but these studies provided inconsistent results. A recent large sample study conducted by Zhang and Liu showed that the Klotho level was decreased in DM patients, and continued to decrease in DKD patients, indicating that Klotho was implicated in the development of DM and DKD.[58] Kim *et al.* conducted a prospective study that enrolled 147 DM patients and followed them up for 36 months according to Klotho tertiles and found that a reduced Klotho level predicted the progression of albuminuria or an annual decline of the eGFR in DM patients.[39] Moreover, a reduced Klotho level was also associated with an increased risk of non-kidney outcomes, including diabetic retinopathy, CV morbidity, and mortality.[32,33] Therefore, Klotho is proposed as a possible marker associated with adverse clinical outcomes in DKD. However, other authors presented discrepant results. In Zubkiewicz-Kucharska *et al.*'s study, sKlotho was inversely with hemoglobin Alc but was not linked with the duration of DM, which is a major risk factor for chronic complications of DM.[59] Among other studies, no significant association was found in terms of Klotho with eGFR decline or CV morbidity.[30,41] And even, Bob *et al.* reported contrary results. They found that a high Klotho level, but not a low Klotho level, was positively associated with a progressive annual eGFR decline.[24] The available evidence indicated that the prognostic role of serum Klotho in DKD remains under debate.

To address this issue, this current meta-analysis was conducted to evaluate serum Klotho's predictive role for DKD. We included 11 eligible studies with 1016 DM patients and assessed the

role of serum Klotho in predicting DKD kidney or non-kidney outcomes. Our results showed that a decline in the Klotho level was concomitant with an increase in undesirable clinical outcomes, including kidney and non-kidney outcomes and combined outcomes. Therefore, we demonstrated a lowered serum Klotho level was associated with more adverse DKD outcomes, and first provided evidence for the application of Klotho in predicting DKD consequences.

Mechanically, DKD develops progressively from diabetic vascular disease,[60] which is initiated by multiple pathological processes such as inflammation, oxidative stress, RAS, and hyperlipidemia in addition to traditional hyperglycemia and blood pressure.[61,62] If these risk factors are not controlled or properly treated, they can cause vascular lesions and subsequently the onset of albuminuria and/or eGFR decline, ultimately contributing to ESKD or other outcomes.[62] Klotho is expressed in vascular tissue and is implicated in vascular homeostasis.[13,63] Numerous studies demonstrated that Klotho is vascular and extravascular protective. First, it protects vascular endothelial cells (VEC) from damage induced by high glucose or reactive oxygen species or inflammation.[64–67] Second, Klotho is emerging as a novel negative regulator of VC,[28] and suppressed VC by inhibiting osteogenic transformation of vascular smooth muscle cells or by targeting fibroblast growth factor receptors in osteoblastic cells.[68,69] Third, Klotho maintained VEC integrity, mediated angiogenesis, and prevented arteriolar hyalinosis by other various mechanisms.[70,71] Moreover, Klotho has been previously reported to exhibit other pleiotropic extravascular beneficial actions, including protection against podocyte injury and inhibition of RAS and renal fibrosis.[14,72] Through these actions, Klotho attenuated microangiopathies, protect kidney function, and retarded DKD progression. Klotho deficiency largely abolished Klotho-induced vascular and extravascular protective effects and accelerated the development of DKD or DKD adverse outcomes.[23,72–75] This may be the underlying mechanism by which reduced Klotho was associated with an increased risk for disease progression and other adverse complications. This meant that decreased Klotho concentration could be used as an indicator of developing dismal DKD consequences along with DM progression, although the result from the current meta-analysis did not support Klotho as a diagnostic biomarker for early DKD onset.

Limitations

The reliability of our meta-analysis may have been attenuated due to a number of limitations. First, the included studies were cross-sectional in nature, with seven cohort studies. Consequently, it is impossible to demonstrate a causal relationship, particularly with respect to the association of Klotho and albuminuria. Second, the meta-analysis exhibited moderate heterogeneity regarding the relationship of Klotho with DKD clinical outcomes. The source of heterogeneity was possibly ascribed to variations in study characteristics such as age, study design, and the eGFR. The heterogeneities among studies undoubtedly limited the power of the overall findings. Third, the systematic review enrolled a small number of eligible studies, and with a relatively small sample and short follow-up periods, particularly in terms of the relationship of Klotho with clinical outcomes, which probably influenced the interpretation of the results. Fourth, it must be noted that DKD is a heterogeneous disease that involves multiple pathogenetic mechanisms,[61] thus a single biomarker does not fully reflect the complexity of the disease pathogenesis and progression. Many potential biomarkers tightly interact with each other and biomarker panels or clusters possibly have a better performance in this aspect. Finally, there was considerable publication bias, and the overall effect would not be stable in the sensitivity analysis. This strongly indicated that the results obtained in this study must be interpreted with caution and further study is urgently required to clarify this association in the future.

Conclusion

In conclusion, this study first investigated the association of the serum Klotho level with DKD clinical outcomes. Despite the nonsignificant association of Klotho with the UACR, a low Klotho level was associated with poorer kidney or non-kidney clinical outcomes, meaning that Klotho influenced DKD clinical outcomes and can be adopted as a potential biomarker in predicting DKD outcomes. Owing to the limitations above, cautious interpretation should be applied and further well-designed clinical studies should be conducted to clarify and identify this association.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Author contributions
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Competing interests
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Availability of data and materials
All data used during the study appear in the submitted article.

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