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Review Article

Pathophysiologic Implications and Therapeutic Approach of Klotho in Chronic Kidney Disease: A Systematic Review

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ABSTRACT

The Klotho protein, known as an antiaging protein, is expressed mainly in the kidney, and kidney disorders may contribute to the disrupted expression of renal Klotho. The purpose of this systematic review was to determine if there are biological and nutraceutical therapies that increase the expression of Klotho and can help prevent complications associated with chronic kidney disease. A systematic literature review was carried out through the consultation of PubMed, Scopus, and Web of Science. Records between the years 2012 and 2022 in Spanish and English were selected. Cross-sectional or prevalence and analytical studies were included that evaluated the effects of Klotho therapy. A total of 22 studies were identified after the critical reading of these selected studies: 3 investigated the association between Klotho and growth factors, 2 evaluated the relationship between the concentration of Klotho and the type of fibrosis, 3 focused on the relationship between vascular calcifications and vitamin D, 2 assessed the relationship between Klotho and bicarbonate, 2 investigated the relationship between proteinuria and Klotho, 1 demonstrated the applicability of synthetic antibodies as a support for Klotho deficiency, 1 investigated Klotho hypermethylation as a renal biomarker, 2 investigated the relationship between proteinuria and Klotho, 4 linked Klotho as an early marker of chronic kidney disease, and 1 investigated Klotho levels in patients with autosomal dominant polycystic kidney disease. In conclusion, no study has addressed the comparison of these therapies in the context of their use with nutraceutical agents that raise the expression of Klotho.

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Introduction

Chronic kidney disease (CKD) is a serious public health problem, having a prevalence of approximately 11% in the adult population in Spain.[1] It is associated with high morbidity, especially due to cardiovascular diseases (CVDs).[2,3] CKD is characterized by the inability of the kidney to remove waste products and excess fluid from the body.

The term CKD replaced chronic renal failure and was defined in 2022 by the National Kidney Foundation Kidney Disease Quality Outcome Initiative[4] as a decrease in renal function, expressed by the glomerular filtration rate (GFR) or creatinine clearance >60/mL/min/1.73 m^2 or as the presence of persistent kidney damage for at least 3 months. Likewise, the National Kidney Foundation has divided CKD into 5 stages based on its GFR.

Kidney damage is usually diagnosed by urine markers, establishing the diagnosis of CKD by a decreased GFR or by markers of kidney damage. The main marker of kidney damage is elevated urinary albumin or protein excretion. This new vocabulary provides a platform for health care professionals caring for patients

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with CKD around the world to speak a common language. It also provides a simple definition of CKD and a classification system that distinguishes between groups of patients.[5]

The main causes of CKD are diabetes mellitus, renal vascular disease glomerulonephritis, and hypertension.[6] The risk of CKD increases with age, and lifestyle also plays a very important role in its development.[7] Obesity is known to lead to CKD through diabetes mellitus and hypertension. Still, evidence indicates that obesity can also contribute directly to kidney damage because of glomerular hyperfiltration, that is, by affecting the GFR and renal plasma flow.[8,9] In addition, there is evidence that smoking may be a risk factor for CKD.[10]

Furthermore, the prevalence of CKD is 1.5 times higher in men than that in women, suggesting a sex difference in susceptibility.[11]

The estimated global prevalence of CKD is 13.4% (11.7%-15.1%), and the number of patients with end-stage renal disease who need renal replacement therapy is estimated to be between 4.902 and 7.083 million.[12] Although data are scarce for developing countries, it is estimated that by 2030, 70% of patients with end-stage renal disease (stage 5 CKD) will be in developing countries, which increases the burden on the budgetary capacities of health care systems.[13]

Alterations in mineral metabolism that accompany CKD are an important cause of morbidity, especially cardiovascular mortality.[14] Approximately 50% of patients in dialysis die from cardiovascular events, not only in the terminal stage but even in the earliest stages.[15]

Cardiovascular pathology is mainly due to vascular calcification (VC) scores produced by parathyroid hyperfunction and high CaxP.[16] Elevated P serum levels associated with high-remodeling bone disease are also associated with VC in patients with CKD. It has been reported that for each mg/dL that serum phosphorus increases, the risk of death increases by 23%.[17] Some recent reports on vascular tissue cultures have studied how the chronic Ca/P metabolism disorder of CKD affects VC, finding that for the same CaxP product, the calcification induced by Ca is more powerful than that induced by P.[18]

In the VC of patients with CKD, in addition to the classic cardiovascular risk factors (diabetes, hypertension, obesity, dyslipidemia, smoking, age, and inflammation), other factors have been described, including Ca/P metabolism disorders, time on dialysis, treatment with phosphorous, and treatment with corticosteroids or warfarina.[19] Klotho is a protein that was discovered in 1997 in rapidly aging mice.[20] The defect in those mice was a lack of Klotho. Since then, it has been found that Klotho is present in the cell membrane and the bloodstream, where it functions as a hormone associated with the aging process; it participates in phosphorous metabolism and regulates the activity of fibroblast growth factor 23 (FGF23).[21] The main source of Klotho is the kidney. Kidney cells secrete Klotho into the environment that surrounds them, so that through circulation, it reaches the various organs of our body where it exerts its antiaging function.

In CKD, there is evidence of an early reduction in renal Klotho messenger RNA expression, affecting both transmembrane Klotho and soluble Klotho. This is responsible for the development of renal tubular cell resistance to FGF23. FGF23 regulates both renal phosphate handling and renal calcitriol synthesis. Soluble Klotho may have anti-inflammatory and antiapoptonic effects.[22] FGF23 and Klotho play an essential role in the regulation of mineral metabolism, and both are altered as a consequence of renal failure. FGF23 increases to increase phosphaturia, which prevents phosphate accumulation in the early stages of CKD. This effect of FGF23 requires the presence of Klotho in the renal tubules. However, its expression is reduced as soon as renal function begins to fail to generate a state of resistance to FGF23. Changes in these proteins directly affect other parameters of mineral metabolism. They can affect kidney function and cause damage to other organs, such as bone, the heart, and the vascular system (Fig. 1). Some of the mechanisms responsible for changes in FGF23 and Klotho levels are related to 2 changes in Wnt signaling. There seems to be a link between FGF23/Klotho and Wnt/β-catenin in different organs, such as the kidney, heart, and bone. Activation of canonical Wnt signaling produces changes in FGF23 and Klotho and vice versa; therefore, this pathway is of interest in complementing a project where new biological therapies, and nutraceuticals are created that increase the expression of Klotho. This can help prevent complications and CVD because it is still not possible to make up for a lack of Klotho. The aim of this systematic review was to determine if there are biological and nutraceutical therapies that increase the expression of Klotho and can help prevent complications associated with CKD.

Materials and Methods

A systematic review of the records of different bibliographic databases was carried out, following an established search and review protocol to minimize the risk of bias in both the choices and the publications themselves, thus ensuring optimal organization and content. The review followed the norms established in the Preferred Reporting Items for Systematic Reviews and Meta-Analyses[23] statement.

Information Sources and Search Equation

For the search, PubMed, Scopus, and the Web of Science databases were consulted, with the results restricted to documents published in the last 10 years. The search was conducted in March 2022, and the search equation was "(Klotho OR α-Klotho) AND (chronic kidney disease OR CKD) AND (therapy or treatment OR supplementation)." Search equation descriptors were obtained from the thesaurus Medical Subproject headings.

Inclusion and Exclusion Criteria

Studies published in Spanish or English in the last 10 years were selected in order to review the most current evidence. In this regard, cross-sectional or prevalence and analytical studies that evaluated the effects of Klotho therapy and its increase were included. Studies that were not related to the subject or that did not provide relevant statical information were excluded.

Selection and Evaluation of Methodological Quality

The selection was made in 4 phases: first, after performing the search, the data were entered into the Mendeley bibliographic manager to detect duplicate results. After reading the titles and abstracts, those that did not fit the subject of the research, or the objective of the study were excluded. Next, after reading the complete texts, those that provided data related to the study were chosen. Here, another article was discarded because it did not have a research study format (being informative). Finally, a complete reading of the articles that remained was carried out; they were analyzed for the extraction of data and the realization of this review.

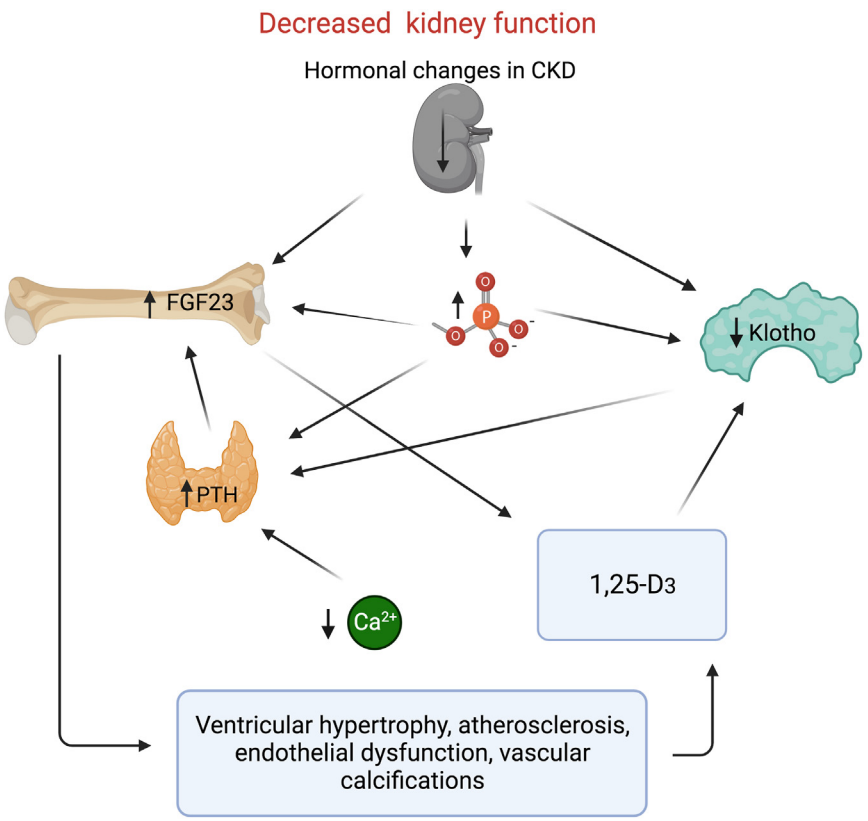

Figure 1.
The decrease in renal mass causes a decrease in Klotho, calcitriol, and an increase in phosphorus (P). The increase in P leads to a greater decrease in calcitriol, an increase in fibroblast growth factor 23 (FGF23), and parathyroid hormone. Parathyroid hormone also increases in an attempt to palliate the calcitriol deficit and increase FGF23. This network increases cardiovascular risk: ventricular hypertrophy, atherosclerosis, endothelial dysfunction, and vascular calcifications. CKD, chronic kidney disease; PTH, parathyroid hormone.

To assess the quality of the studies included in the review, the levels of evidence and grades of recommendation of the Oxford Center for Evidence-based Medicine [24] were followed. These guidelines made it possible to evaluate the evidence according to the thematic area of the clinical scenario in question, ensuring that the most relevant knowledge of each scenario was obtained, because of each scenario's high degree of specialization.

Data Analysis

During the analysis of the data, the study designs, years of publication, countries of publication of the studies, and the relationships of these studies with the objective proposed in this study were verified. Priority was given to those studies that referred to treatment in the different phases of CKD.

Results

Selection of Studies

After applying the different search equations indicated above, a total of 3365 articles were identified, of which 391 were obtained from the PubMed database, 716 from Scopus, and 2258 from Web of Science. In the first screening, duplicate articles were eliminated, leaving a total of 1569 articles, of which 80 were not related to CKD. In a second screening, articles that did not correspond to the inclusion criteria were eliminated, so 1716 studies were selected. These studies were read and analyzed to determine whether they could be included in this review. In total, 1345 articles were excluded after this evaluation, so the final number of articles used for this systematic review was 22 studies. These were then analyzed to assess their methodological quality. Figure 2 shows the flowchart of the selection and exclusion process of the research studies, which was carried out according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses system.

Among the articles included, 3 investigated the association between Klotho and growth factors,[25-27] 1 evaluated the relationship between oxidative stress and Klotho concentration,[28] 2 evaluated the relationship between Klotho concentration and fibrosis type,[29,30] 3 focused on the relationship between VCs and vitamin D,[31-33] 2 assessed the relationship between Klotho and bicarbonate,[34,35] 2 investigated the relationship between cardiovascular alterations and Klotho,[36,37] 1 demonstrated the applicability of synthetic antibodies as a support for Klotho deficiency,[38] 1 investigated Klotho hypermethylation as a renal biomarker,[39]. 2 investigated the relationship between proteinuria and Klotho,[40,41] 4 examined Klotho as an early marker of CKD,[42-45] and 1 investigated Klotho levels in patients with autosomal dominant polycystic kidney disease.[46]

The Table summarizes the most important results of these studies and the aspects assessed: the study in question; the type of design, objective, intervention, or treatment; the evaluation of Klotho and the concentration; and the results.

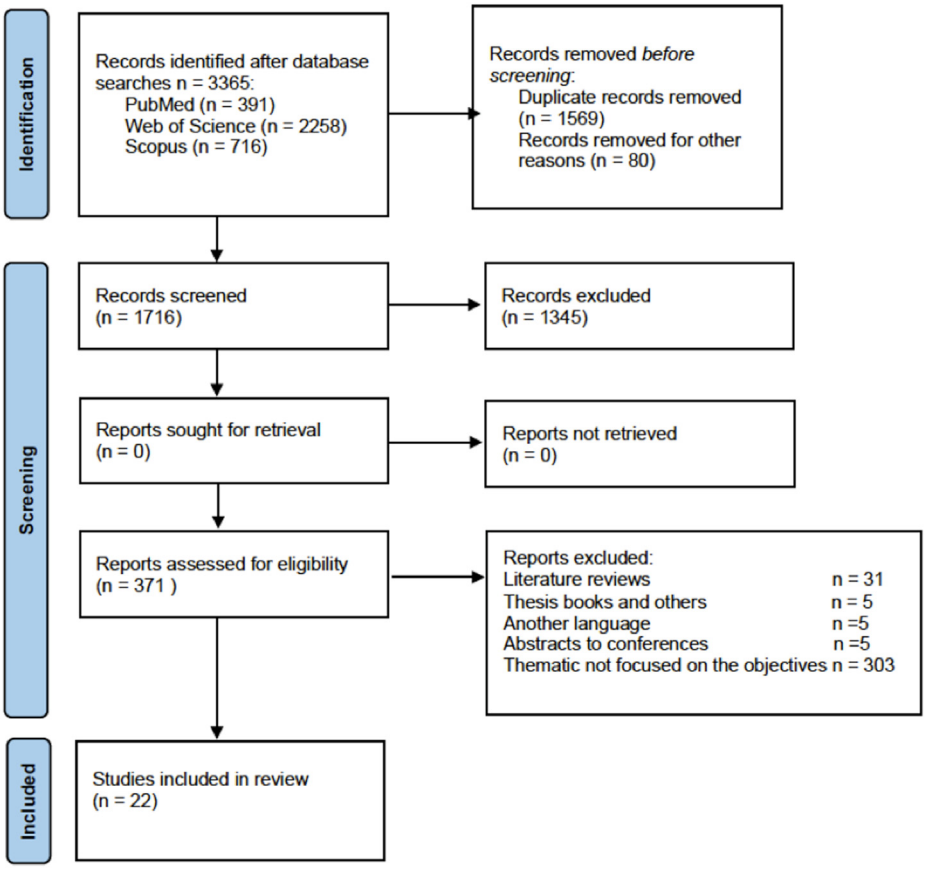

Figure 2.
Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA flowchart of study selection).

Quality Evaluation

The 22 included studies had been designed with adequate follow-ups, so they all had a level of evidence 2b and a grade of recommendation B (Table).

Discussion

The purpose of this systematic review was to classify and document all the possibilities of treatment with Klotho to complement a project where new biological therapies and nutraceuticals are being created that increase the expression of Klotho. This can help prevent complications associated with CKD because it is still not possible to make up for a lack of Klotho.

However, the studies analyzed were found to have focused on other lines of research.

Klotho Growth and Oxidative Stress

Some studies investigated the fluency of growth hormone administration with subcutaneous injections of GH (Genotropin, 20mcg/kg/d) for 7 consecutive days in patients with CKD to increase Klotho[25] levels, and this research only showed a modest increase in Klotho in patients with stage 3 CKD. Other research focused on FGF23 and reduced levels of Klotho.[26,27] The biological action of FGF23 depends on the Klotho gene, which acts as its coreceptor. In this study, renal replacement therapy reduced serum phosphorous and consequently FGF23 levels, but it did not increase the Klotho levels, which continued to remain low. In contrast, other studies in patients with polycystic kidney disease found elevated levels of FGF-23 and Klotho.[46]

Several studies have described the involvement of oxidative stress in the development of various kidney diseases,[47,48] such as diabetic kidney disease and acute kidney injury. However, some studies found no evidence that affected antioxidant therapy will increase α-Klotho concentrations in patients with CKD.[28]

Klotho Fibrosis and Vascular Calcifications

Klotho is a protein that is predominantly expressed in the kidney, where it acts as a coreceptor for FGF23. In its execrated form, Klotho exerts antifibrotic effects on various tissues. In clinical trials, differences in Klotho cause fibrosis and, conversely, overexpression or supplementation of Klotho protects against fibrosis in various models of fibrotic kidney and heart disease.[49,50] In this regard, some studies have shown the efficacy of dihydroartemisinin as a renoprotective agent, reversing Klotho repression and improving renal fibrosis.[29] Other studies have also indicated that overexpression of the Klotho protein protects the peritoneal membrane through alteration of the Wnt/b-catenin signaling pathway.[30] Klotho is an important regulator of mineral metabolism homeostasis as it increases renal phosphate reabsorption by acting as a coreceptor for the binding of FGF23 to FGFR1. It directly promotes the internalization and degradation of

Table
Characteristics and summary of the studies included in the review

Type of study (follow-up)	Aim	Intervention	Evaluation (Klotho concentration)	Results	Reference (first author)	Level of evidence (OCEBM)
Prospective, case-control (15 mo)	Effect of subcutaneous GH therapy on α-Klotho concentrations in subjects with or without mild CKD	Exogenous growth hormone administration	Nonfasting blood samples and first-morning spot urine were drawn at baseline, after 7 d of treatment and 1 wk after the treatment stopped (554 in CKD pg/m)	The difference in change of α-Klotho concentration was not statistically significant between the 2 sugroups	Adema et al,[25] 2018	2b/B
Post hoc and prospective randomized trial (12 mo)	A 12-mo antioxidant therapy compared to placebo induces an increase of α-Klotho	An antioxidant regime comprising of pravastatin (40 mg/d) starting at baseline followed by the addition of vitamin E (α-tocopherol acetate, 300 mg/d) at month 6, or placebo for 12 mo	α-Klotho and vitamin D (25(OH) D) and phosphate concentrations were measured in serum samples and parathyroid hormone (PTH) and c-terminal fibroblast growth factor-23 (cFGF23) concentrations In plasma samples, all at baseline and after 12 mo of treatment (placebo: 489.6 pg/m; intervention 492.7 pg/m)	Changes in α-Klotho concentrations were not different between both groups	Adema et al,[28] 2016	2b/B
Prospective randomized trial (12 mo)	Antifibrotic mechanisms of dihydroartemisinin (DHA), particularly its specific target in renal cells, reverse Klotho repression	DHA administration in mouse	Renal cells. Renal fibrosis was induced in mice by unilateral ureteral obstruction (UUO) or oral administration of adenine (80 mg/kg^{-1}), the mice received DHA (30 $mg/kg^{-1}/d^{-1}$, i.g.) for 14 or 21 d (high levels)	Downregulation of DNMT1 expression effectively reversed Klotho promoter hypermethylation, consequently restoring Klotho protein and ameliorating renal fibrosis	Zhou et al,[29] 2022	2b/B
Prospective randomized trial (15 mo)	How aldosterone and spironolactone influence Klotho gene expression	1º group of mice was subcutaneously injected with vehicle (soybean oil), DOCA (50mg/kg BW), spironolactone (75mg/kg BW) or DOCA and spironolactone together 2º group of mice: was treated with either spironolactone (80mg/L)[30] or with vehicle drinking water ad libitum	Kidney tissues, blood and plasma concentration (high levels)	Besides blocking the effects of aldosterone spironolactone upregulates Klotho gene expression by upregulation of 25-hydroxyvitamin D3 1-alpha-hydroxylase with subsequent activation of the vitamin D3 receptor by 1,25(OH)2 D3, an effect possibly independent from the mineralocorticoid receptor	Alesutan et al,[31] 2013	2b/B
Observational cohort study (12 mo)	α-Klotho deficiency has been shown to be an early biomarker as well as a pathogenic factor	Synthetic antibody library to identify a high-affinity human antigen-binding fragment that recognizes human, rat and mouse α-Klotho primarily in its native, rather than denatured, form	Measured both serum and urinary levels of full-length soluble α-Klotho (high levels)	Using synthetic antibody libraries, they furnish data in support of α-Klotho deficiency in human CKD, and we set the foundation for the development of diagnostic and therapeutic applications of anti−α-Klotho antibodies.	Barker et al,[38] 2015	2b/B

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Table (*continued*)

Type of study (follow-up)	Aim	Intervention	Evaluation (Klotho concentration)	Results	Reference (first author)	Level of evidence (OCEBM)
Observational cohort study (12 mo)	IMD1−53 attenuates vascular calcification by upregulating α-Klotho	Application IMD1−53 inhibits vascular calcification by upregulating α-Klotho	Aortas of rats with CKD and vascular calcification (high levels)	IMD1−53 treatment reduced vascular calcification and increased α-Klotho protein level in calcified vascular smooth muscle cells	Chang et al,[32] 2016	2b/B
Prospective randomized trial (7 mo)	Determine renal and peripheral blood mononuclear cells (PBMC) levels of Klotho (KL) promoter methylation and analyze their relationship with clinical and histologic severity in patients with CKD	Using bisulfite pyrosequencing, renal and PBMC levels of KL promoter methylation	Renal biopsy samples DNA and methylation analyses by quantitative bisulfite pyrosequencing, blood and plasma concentration (high levels)	PBMC level of KL promoter methylation correlated positively with renal level of KL promoter methylation	Chen et al,[39] 2013	2b/B
Prospective randomized trial (15 mo)	Investigate the clinical value of serum Klotho and FGF23 in cardiac valve calcification in patients with CKD	Patients were divided into 3 groups: CKD2~3 group, CKD4 group, and CKD5 group. In each group, ultrasound was used to evaluate the cardiac valve calcification, and the independent risk factors for cardiac valve calcification	Levels of hemoglobin and blood calcium. (high levels)	FGF23 and Klotho are independent risk factors for heart valve calcification in patients with CKD	Chen et al,[33] 2021	2b/B
Prospective randomized trial (12 mo)	Study the mechanism of decreased Klotho expression by albuminuria using an animal model of high-grade albuminuria generated through podocyte apoptosis, and cultured kidney epithelial cells (HEK-293 and HK-2)	Mice at the C57BL6 background were injected intraperitoneally with 2 doses of Tunicamycin (3 μg/g/d) to induce ER stress-mediated acute kidney injury and the kidneys were harvested 48 hours later	Kidney tissue, cell culture and transfection, total RNA extraction and real-time PCR analysis (high levels)	Pharmacologic inhibition of ER stress significantly rescues Klotho expression at the post-transcriptional level in proteinuric kidney disease	Delitsikou et al,[40] 2020	2b/B
Observational cohort study (120 mo)	Evaluated the association of soluble serum klotho with decline in kidney function as well as the development of incident CKD in 3075 patients	Just research	Klotho and kidney function at baseline, and at least one repeat measure of kidney function (low levels)	Demonstrated an association between low soluble Klotho and decline in kidney function.	Drew et al,[42] 2017	2b/B
Observational cohort study (7 mo)	Measure the relationship of serum α-Klotho with renal function, acid-base status, bone biomarkers, and proteinuria in CKD patients	Just research	Blood and plasma concentration (478 pg/m)	Serum α-Klotho is related to serum bicarbonate and proteinuria and not to renal function	Hage et al,[34] 2014	2b/B
Prospective, interventional, nonrandomized, open-label trial study (1 mo)	Tested the hypothesis that correcting acidosis may improve urinary Klotho excretion and serum α-Klotho	Patients were then prescribed 1 g of oral sodium bicarbonate 3 times per day for 4 wk	Blood and plasma concentration (615 pg/m)	Correcting acidosis by oral administration of sodium bicarbonate rapidly increases the urine excretion of soluble α-Klotho in CKD patients	Hage et al,[35] 2019	2b/B

Prospective randomized trial (2 mo)	Investigated the activation of Wnt/β-catenin signaling in peritoneal fibrosis in β-catenin-activated transgene (BAT) promoter mice and the effect of Klotho on peritoneal fibrosis in Klotho-overexpression mice.	For 5 wk, 5 mice were given 1.5 mL saline daily (saline) and another 5 mice were given 1.5 mL standard PD fluid buffered with lactate and containing 4.25% glucose; another set of mice received the same volume of PD combined with 5 mg/kg/d of ICG-001 (an inhibitor of β-catenin/T-cell factor [TCF] and cyclic adenosine monophosphate response element binding protein interaction)	Peritoneal membrane sections, blood and plasma concentration (high levels)	Klotho mRNA expression and protein expression increased in KLTG mice more than in WT mice	Kadoya et al,[30] 2020	2b/B
Prospective single-arm study (6 mo)	Effects of hemodialysis initiation on the levels of Klotho an FG-23 hormones and other parameters of mineral metabolism	Hemodialysis	Blood and plasma concentration (297 pg/m)	Soluble Klotho levels were decreased in patients with chronic kidney failure, but these levels did not change after the initiation of hemodialysis	Kawabata et al,[26] 2020	2b/B
Prospective, case-control (15 mo)	Study serum soluble α-Klotho in different stages of CKD and its correlation with FGF-23 to focus more light on its clinical importance	Just research	Blood and plasma concentration (Stage I 10 ng/ml, Stage II 8.7, Stage III 4.5, Stage IV 1.5, Estage V 1.5)	Soluble α-Klotho was early sensitive parameter for the early diagnosis of stages of CKD and extra renal bone complication	Khodeir et al,[27] 2020	2b/B
Observational cohort study (60 mo)	The association between serum Klotho and cardiac parameters from a large-scale patients CKD cohort	Just research	Blood and plasma concentration, left ventricular mass index (LVMI) (667 pg/m)	Serum Klotho was an independent biomarker of LVMI, but not arterial stiffness	Kim et al,[36] 2018	2b/B
Cross-sectional study (30 mo)	Investigate whether the soluble Klotho (s-Klotho) level in patients with CKD is related to kidney function and whether a low s-Klotho level can predict adverse renal outcomes or CKD progression in patients with advanced CKD	Just research	Blood and plasma concentration (mean in control 9.81; mean in CKD 2.44)	s-Klotho level was closely correlated with kidney function, further, low s-Klotho level could predict adverse kidney disease outcomes in patients with progressive CKD	Liu et al,[43] 2018	2b/B
Prospective, randomized, controlled comparative study (14 mo)	To compare the possibilities of LPD + KA and isolated LPD in their impact on morphogenetic proteins FGF-23 and Klotho, as well as the safety of these options in relation to both the risk of CVC and LVH in patients with CKD stage 3b to 4	The first group (42 patients) received LPD + KA. The second group (37 patients) continued the LPD alone	Blood and plasma concentration (group 1: 429.9 pg/m group 2: 433.2 pg/m)	Aggravation of mineral-bone disorders (increase in serum FGF-23, PTH and phosphate levels and decrease in serum Klotho)	Milovanova et al,[41] 2018	2b/B
Observational cohort study (6 mo)	Investigate mechanisms that regulate Klotho release by the human kidney	Measured plasma Klotho across the kidney, splanchnic organs and lung in 22 patients (71 ± 2 years, estimated glomerular filtration rate 60 ± 5.4 mL/min 1.73 m^2) during elective diagnostic cardiac	Blood and plasma concentration (280 pg/mL)	Kidney s-Klotho release is part of a homeostatic system that links kidney energy metabolism to the release of survival factor(s) to extrarenal tissues	Picciotto et al,[44] 2019	2b/B

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Table (*continued*)

Type of study (follow-up)	Aim	Intervention	Evaluation (Klotho concentration)	Results	Reference (first author)	Level of evidence (OCEBM)
Observational cohort study (6 mo)	Confirm the reduction of circulating levels of *s*-Klotho and at verifying the links with FGF23 and with other markers of mineral metabolism derangements	Just research	Blood and plasma concentration (519 pg/mL)	Klotho represents an early marker of renal damage and of ensuing CKD-MBD, indicative of the cross-talk between bone and kidney	Rotondi et al,[45] 2015	2b/B
Observational cohort study (6 mo)	Describe the role of FGF-23 and soluble α-Klotho in bone and mineral abnormalities in patients with ADPKD	Just research	Blood and plasma concentration (high levels)	Elevated FGF-23 and soluble α-Klotho levels were present in patients with ADPKD	Sari et al,[46] 2017	2b/B
Prospective randomized trial (12 mo)	Explored the effect of α-Klotho on prevention and treatment on post-AKI-to-CKD progression and cardiovascular disease	Administration of recombinant α-Klotho	Renal and cardiac tissue (prevents CKD)	Thus, recombinant α-Klotho protein is safe and efficacious, and might be a promising prophylactic or therapeutic option for prevention or retardation of AKI-to-CKD progression and uremic cardiomyopathy	Hu et al,[37] 2017	2b/B

the NaPi2a cotransporter in the renal proximal tubules and suppresses vitamin D signaling.[51] In this regard, some of the studies indicated that both aldosterone and spironolactone increase the expression of Klotho messenger RNA,[31] whereas other studies indicated that endocrine factors, such as intermedin 1-53 (a peptide related to calcitonin/calcitonin), can attenuate VC by the positive relation of Klotho through the calcitonin receptor, by modifying the protein complex and signaling of calcitonin protein kinase A.[32] Other studies instead indicated that GF23 and Klotho are independent risk factors for heart valve calcification in patients with CKD.[33]

Klotho Acid—Base Balance and Cardiovascular Disorders

In CKD, proteinuria and metabolic acids are produced. The pathogenesis is based on the lack of synthesis of serum bicarbonate with the accumulation of organic and inorganic acids, causing tubulointerstitial damage through ammonia retention and complement deposition.[52] In this regard, some studies showed that in CKD, serum Klotho is related to serum bicarbonate and proteinuria and not to renal function.[34] Other studies showed that correction of acidosis by oral administration of sodium bicarbonate rapidly increases the urinary excretion of soluble Klotho. However, during 4 weeks of treatment, this did not increase serum Klotho.[35]

Much research has shown that CKD constitutes a state of severe Klotho deficiency accompanied by an extremely high incidence of CVD cardiac hypertrophy and uremic vaculopathy.[22,53]

In this review, we found that increasing serum Klotho exerts a cardioprotective effect during CKD, improving kidney function and preventing mortality,[36-38,40,41] confirming that Klotho is an early marker of CKD.[42,45]

As we have seen, CVD is one of the leading causes of morbidity and mortality in patients with CKD. Inflammation and alterations in bone mineral metabolism are pathologic conditions that lead to increased cardiovascular risk in CKD. FGF23 and Klotho play an essential role in the regulation of mineral metabolism, both of which are altered as a consequence of renal failure. FGF23 increases to increase phosphaturia, which prevents phosphate accumulation in the early stages of CKD. This effect of FGF23 requires the presence of Klotho in the renal tubules. However, the expression of Klotho occurs as soon as renal function begins to fail to generate a state of resistance to FGF23. Changes in these proteins directly affect other parameters of mineral metabolism; they can affect kidney function and can cause damage to other organs, such as bone, the heart, and the vascular system. Some of the mechanisms responsible for changes in FGF23 and Klotho levels are related to changes in Wnt signaling. There seems to be a link between FGF/Klotho and Wnt/β-catenina in different organs, such as the kidney, heart, and bone.

Activation of canonical Wnt signaling produces changes in FGF23 and Klotho and vice versa; this may be of interest to a project where new biological therapies and nutraceuticals are being created that increase the expression of Klotho. Lately, nutraceuticals have gained increasing recognition; they are considered crucial to improve life and provide molecules that contain antioxidants. One of these nutraceutical compounds is derived from blue lupine and olive; these are associated with an improvement in parameters associated with CVD, in patients with CKD. This improvement may be based on a combination of biological therapies and/or nutraceuticals that leads to a decrease in parameters associated with CVD (reduction of mediators, such as proinflammatory cytokines and typical proteins of VC), which may increase the expression of Klotho (Fig. 3).

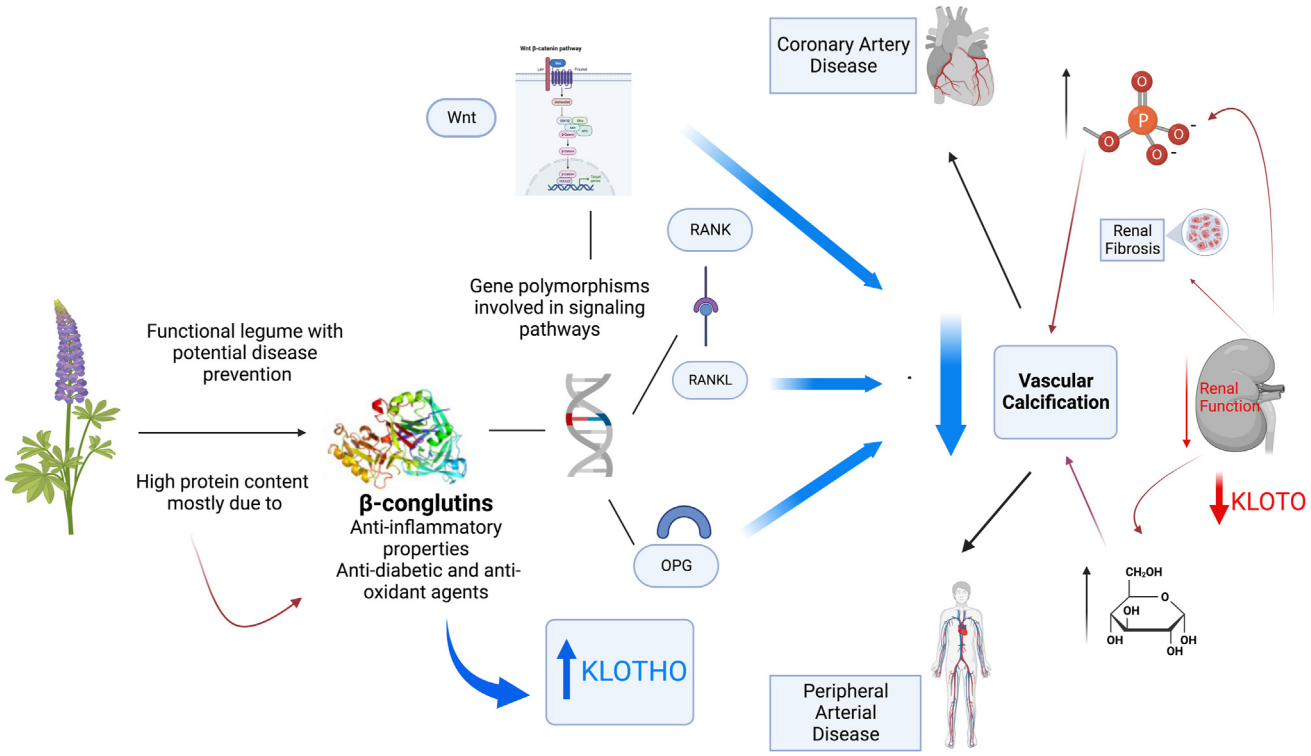

Figure 3.
Hyperphosphatemia and hyperglycemia present in patients with **c**hronic kidney disease can stimulate calcification. Vascular calcification is an important factor that connects cardiovascular events with bone loss, sharing pathophysiologic mechanisms and genetic causes. Conglutin proteins, from the leguminous plant blue lupin (Lupinus angustifolius L.), possess anti-inflammatory activity and could be useful in inflammatory processes such as **c**hronic kidney disease by increasing Klotho concentration. Polymorphisms of genes involved in the Wnt and RANK/RANKL/OPG signaling pathways will be associated with cardiovascular disease. The genotypes of some polymorphisms will be associated with a better response to biological therapy with nutraceutical compounds decreasing cardiovascular calcifications and consequently cardiovascular disease.

The search for natural compounds that offer beneficial properties in chronic diseases has increased in recent years. Thus, it has been proven that conglutin proteins, from the leguminous plant blue lupin (Lupinus angustifolius L.), possess anti-inflammatory activity and reduce insulin resistance, properties that could be useful in diabetes mellitus 2 and any other pathology with inflammatory processes, such as CVD and CKD. In addition, extra v/irgin olive oil is a potential protector against several pathologies also related to inflammatory processes and oxidative damage, mainly due to its content of bioactive compounds, and mainly to its phenolic fraction (oleuropein, oleocanthal, hydroxytyrosol, tyrosol, etc.) characterized by its antioxidant properties. Therefore, the enrichment of optimized virgin olive oil obtained by processes that enhance its biological value with other potentially bioactive components would create a functional oil with high biological potential. Thus, the ability to reduce the risk of CVD during the consumption of extra virgin olive oils and metabolic syndrome has been determined, improving these results when extra virgin olive oils are supplemented with different amounts of these compounds. The most interesting compounds from olive processing that have beneficial effects on health in different diseases have been extensively studied by members of this group. These molecules include betulinic acid, ursolic acid, oleanolic acid, and their derivatives. However, the potential effect of many derivatives of these molecules in the treatment of CKD has not been addressed, nor has their possible potentiating effect on therapies for CVD.

In this review, we explored the many facets of Klotho biology. The best-characterized aspects relate to renal physiology, especially phosphate and calcium homeostasis. The importance of the FGF23/Klotho/FGFR interaction was evident in Klotho-deficient mice. The major outcomes were hyperphosphatemia and hypervitaminosis D and cardiovascular disorders. Despite all these summarized lines of research and the impressive, documented advance in the knowledge of the role of the RANK/OPG/RANKL and Wnt signaling pathway and its inhibitors in the regulation of bone and cardiovascular homeostasis, no study included in this review compared the framework of their use with nutraceutical agents that elevate Klotho expression in human.

This review is not without limitations. First, although the search strategy was broad, there may be relevant studies that were missed and therefore not included in the review. Because this review consisted of a critical reading of studies, it is possible that limitations secondary to the personal criteria applied were ignored.

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Author Contributions

R.F.C. wrote and prepared the original draft, conducted analysis, visualization, resources, software, methodology, and

conceptualization; The author read and approved the published version of the manuscript.

Data Availability

The data sets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

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Declaration of Competing Interest

None reported.

References

1. Otero A, Gayoso P, Garcia F, de Francisco AL, EPIRCE study group. Epidemiology of chronic renal disease in the Galician population: results of the pilot Spanish Epirce study. *Kidney Int Suppl*. 2005;99:16–19.
2. US Renal Data System: USRDS 2009 Annual Data Report. Bethesda, MD. National Institutes of Health, National Institute of Diabetes and Digestive and Kidney Diseases.
3. Rucker D, Tonelli M. Cardiovascular risk and management in chronic kidney disease. *Nat Rev Nephrol*. 2009;5(5):287–296.
4. National Kidney Foundation. K/DOQI clinical practice guidelines for chronic kidney disease: evaluation, classification, and stratification. *Am J Kidney Dis*. 2002;39(2 Suppl 1):S1–S266.
5. Levin A. The advantage of a uniform terminology and staging system for chronic kidney disease (CKD). *Nephrol Dial Transplant*. 2003;18(8): 1446–1451.
6. Anavekar NS, McMurray JJ, Velazquez EJ, et al. Relation between renal dysfunction and cardiovascular outcomes after myocardial infarction. *N Engl J Med*. 2004;351(13):1285–1295.
7. Go AS, Chertow GM, Fan D, McCulloch CE, Hsu CY. Chronic kidney disease and the risks of death, cardiovascular events, and hospitalization. *N Engl J Med*. 2004;351(13):1296–1305.
8. Astor BC, Matsushita K, Gansevoort RT, et al. Lower estimated glomerular filtration rate and higher albuminuria are associated with mortality and end-stage renal disease. A collaborative meta-analysis of kidney disease population cohorts. *Kidney Int*. 2011;79(12):1331–1340.
9. Gansevoort RT, Matsushita K, van der Velde M, et al. Lower estimated GFR and higher albuminuria are associated with adverse kidney outcomes. A collaborative meta-analysis of general and high-risk population cohorts. *Kidney Int*. 2011;80(1):93–104.
10. Levey AS, de Jong PE, Coresh J, et al. The definition, classification, and prognosis of chronic kidney disease: a KDIGO Controversies Conference report. *Kidney Int*. 2011;80(1):17–28.
11. Stevens PE, Levin A. Evaluation and management of chronic kidney disease: synopsis of the kidney disease: improving global outcomes 2012 clinical practice guideline. *Ann Intern Med*. 2013;158(11):825–830.
12. Lv JC, Zhang LX. Prevalence and disease burden of chronic kidney disease. *Adv Exp Med Biol*. 2019;1165:3–15.
13. Pearson ER, McCrimmon RJ. Diabetes mellitus. In: Stuart R, Ian P, Mark S, Richard H, eds. *Davidson's Principles and Practice of Medicine*. 22nd ed. 21.
14. Di Lullo L, Barbera V, Bellasi A, et al. Vascular and valvular calcifications in chronic kidney disease: an update. *EMJ Nephrol*. 2016;4:84–91.
15. Yun HR, Joo YS, Kim HW, et al. KoreaN Cohort Study for Outcomes in Patients With Chronic Kidney Disease (KNOW-CKD) investigators. Coronary artery calcification score and the progression of chronic kidney disease. *J Am Soc Nephrol*. 2022;33(8):1590–1601.
16. Chen J, Budoff MJ, Reilly MP, et al. Coronary artery calcification and risk of cardiovascular disease and death among patients with chronic kidney disease. *JAMA Cardiol*. 2017;2(6):635–643.
17. Kestenbaum B, Sampson JN, Rudser KD, et al. Serum phosphate levels and mortality risk among people with chronic kidney disease. *J Am Soc Nephrol*. 2005;16(2):520–528.
18. Coen G, De Paolis P, Ballanti P, et al. Peripheral artery calcifications evaluated by histology correlate to those detected by CT: relationships with fetuin-A and FGF-23. *J Nephrol*. 2011;24(3):313–321.
19. Neto R, Pereira L, Magalhães J, et al. Sclerostin and DKK1 circulating levels associate with low bone turnover in patients with chronic kidney disease Stages 3 and 4. *Clin Kidney J*. 2021;14:2401–2408.
20. Kurosu H, Yamamoto M, Clark JD, et al. Suppression of aging in mice by the hormone Klotho. *Science*. 2005;309(5742):1829–1833.
21. Hu MC, Kuro-o M, Moe OW. The emerging role of Klotho in clinical nephrology. *Nephrol Dial Transplant*. 2012;27(7):2650–2657.
22. Kuro OM. The Klotho proteins in health and disease. *Nat Rev Nephrol*. 2019;15(1):27–44.
23. Page MJ, McKenzie JE, Bossuyt PM, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. *BMJ*. 2021; 372:n71.
24. OCEBM Levels of Evidence Working Group. *The Oxford Levels of Evidence 2*. Oxford, UK: Oxford Centre for Evidence- Based Medicine; 2011. Accessed May 10, 2022. https://www.cebm.ox.ac.uk/resources/levelsof-evidence/ocebm-levels-of-evidence
25. Adema AY, de Roij van Zuijdewijn CLM, Hoenderop JG, et al. Influence of exogenous growth hormone administration on circulating concentrations of α-klotho in healthy and chronic kidney disease subjects: a prospective, single-center open case-control pilot study. *BMC Nephrol*. 2018;19(1):327.
26. Kawabata C, Komaba H, Ishida H, et al. Changes in fibroblast growth factor 23 and soluble klotho levels after hemodialysis initiation. *Kidney Med*. 2020;2(1):59–67.
27. Khodeir SA, Okda HI, Abdalal HM. Clinical significance of fibroblast growth factor-23 and soluble alpha klotho in different stages of chronic kidney disease. *Saudi J Kidney Dis Transpl*. 2019;30(1):108–118.
28. Adema AY, van Ittersum FJ, Hoenderop JG, et al. Reduction of oxidative stress in chronic kidney disease does not increase circulating α-Klotho concentrations. *PLoS One*. 2016;11(1):e0144121.
29. Zhou W, Chen MM, Liu HL, et al. Dihydroartemisinin suppresses renal fibrosis in mice by inhibiting DNA-methyltransferase 1 and increasing Klotho. *Acta Pharmacol Sin*. 2022;43(10):2609–2623.
30. Kadoya H, Satoh M, Nishi Y, et al. Klotho is a novel therapeutic target in peritoneal fibrosis via Wnt signaling inhibition. *Nephrol Dial Transplant*. 2020;35(5):773–781.
31. Alesutan I, Feger M, Pakladok T, et al. 25-Hydroxyvitamin D3 1-α-hydroxylase-dependent stimulation of renal klotho expression by spironolactone. *Kidney Blood Press Res*. 2013;37(4-5):475–487.
32. Chang JR, Guo J, Wang Y, et al. Intermedin1-53 attenuates vascular calcification in rats with chronic kidney disease by upregulation of α-klotho. *Kidney Int*. 2016;89(3):586–600.
33. Chen Y, Chen YX, Huang C, Duan ZB, Xu CY. The clinical value of klotho and FGF23 in cardiac valve calcification among patients with chronic kidney disease. *Int J Gen Med*. 2021;14:857–866.
34. Hage V, Pelletier S, Dubourg L, et al. In chronic kidney disease, serum alpha-klotho is related to serum bicarbonate and proteinuria. *J Ren Nutr*. 2014;24(6):390–394.
35. Hage V, Villain C, Pelletier S, Laville M, Drai J, Fouque D. Bicarbonate supplement restores urinary klotho excretion in chronic kidney disease: a pilot study. *J Ren Nutr*. 2019;29(4):285–288.
36. Kim HJ, Kang E, Oh YK, et al. The association between soluble klotho and cardiovascular parameters in chronic kidney disease: results from the KNOW-CKD study. *BMC Nephrol*. 2018;19(1):51.
37. Hu MC, Shi M, Gillings N, et al. Recombinant α-klotho may be prophylactic and therapeutic for acute to chronic kidney disease progression and uremic cardiomyopathy. *Kidney Int*. 2017;91(5):1104–1114.
38. Barker SL, Pastor J, Carranza D, et al. The demonstration of αKlotho deficiency in human chronic kidney disease with a novel synthetic antibody. *Nephrol Dial Transplant*. 2015;30(2):223–233.
39. Chen J, Zhang X, Zhang H, et al. Elevated klotho promoter methylation is associated with severity of chronic kidney disease. *PLoS One*. 2013;8(11), e79856.
40. Delitsikou V, Jarad G, Rajaram RD, et al. Klotho regulation by albuminuria is dependent on ATF3 and endoplasmic reticulum stress. *FASEB J*. 2020;34(2): 2087–2104.
41. Milovanova L, Fomin V, Moiseev S, et al. Effect of essential amino acid κetoanalogues and protein restriction diet on morphogenetic proteins (FGF-23 and Klotho) in 3b-4 stages chronic κidney disease patients: a randomized pilot study. *Clin Exp Nephrol*. 2018;22(6):1351–1359.
42. Drew DA, Katz R, Kritchevsky S, et al. Association between soluble klotho and change in kidney function: the health aging and body composition study. *J Am Soc Nephrol*. 2017;28(6):1859–1866.
43. Liu QF, Ye JM, Yu LX, et al. Plasma s-klotho is related to kidney function and predicts adverse renal outcomes in patients with advanced chronic kidney disease. *J Investig Med*. 2018;66(3):669–675.
44. Picciotto D, Murugavel A, Ansaldo F, et al. The organ handling of soluble klotho in humans. *Kidney Blood Press Res*. 2019;44(4):715–726.
45. Rotondi S, Pasquali M, Tartaglione L, et al. Soluble α-klotho serum levels in chronic kidney disease. *Int J Endocrinol*. 2015;2015, 872193.
46. Sari F, Inci A, Dolu S, Ellidag HY, Cetinkaya R, Ersoy FF. High serum soluble α-klotho levels in patients with autosomal dominant polycystic kidney disease. *J Investig Med*. 2017;65(2):358–362.

47. Hou YC, Zheng CM, Chiu HW, Liu WC, Lu KC, Lu CL. Role of calcimimetics in treating bone and mineral disorders related to chronic kidney disease. *Pharmaceuticals (Basel)*. 2022;15(8):952.
48. Rapa SF, Di Iorio BR, Campiglia P, Heidland A, Marzocco S. Inflammation and oxidative stress in chronic kidney disease-potential therapeutic role of minerals, vitamins and plant-derived metabolites. *Int J Mol Sci*. 2019;21(1):263.
49. Wang K, Li Z, Li Y, et al. Cardioprotection of klotho against myocardial infarction-induced heart failure through inducing autophagy. *Mech Ageing Dev*. 2022;207, 111714.
50. Jena S, Sarangi P, Das UK, Lamare AA, Rattan R. Serum α-klotho protein can be an independent predictive marker of oxidative stress (os) and declining glomerular function rate in chronic kidney disease (ckd) patients. *Cureus*. 2022;14(6), e25759.
51. Gupta M, Orozco G, Rao M, Gedaly R, Malluche HH, Neyra JA. The role of alterations in alpha-klotho and fgf-23 in kidney transplantation and kidney donation. *Front Med (Lausanne)*. 2022;9, 803016.
52. Shah SN, Abramowitz M, Hostetter TH, Melamed ML. Serum bicarbonate levels and the progression of kidney disease: a cohort study. *Am J Kidney Dis*. 2009;54(2):270–277.
53. Yang K, Du C, Wang X, et al. Indoxyl sulfate induces platelet hyperactivity and contributes to chronic kidney disease-associated thrombosis in mice. *Blood*. 2017;129(19):2667–2679.