AMERICAN JOURNAL OF BIOMEDICINE AND PHARMACY
https://biojournals.us/index.php/AJBP
ISSN: 2997-7177

Article

Klotho Protein as a Novel Biomarker for Early Renal Dysfunction in Diabetic Nephropathy

Hind Fawzi Aref*[1]
[1] University of Fallujah, College of applied sciences
*Correspondence: hind.f.aref@uofallujah.edu.iq

Citation: Aref H. F. Klotho Protein as a Novel Biomarker for Early Renal Dysfunction in Diabetic Nephropathy. American Journal of Biomedicine and Pharmacy 2026, 3(6), 66-73.

Received: 10th Mar 2026
Revised: 11th Apr 2026
Accepted: 24th May 2026
Published: 21th Jun 2026

Abstract: Diabetic nephropathy is one of the most common causes of chronic kidney disease and end-stage renal disease globally and sensitive biomarkers to measure renal injury before irreversible structural damage is evident are needed. There are limitations of traditional biomarkers such as serum creatinine, estimated glomerular filtration rate (eGFR) and albuminuria, as they may only measure late kidney damage rather than early kidney pathology. Recently, Klotho protein emerged as a promising biomarker, which is highly expressed in kidney and has great potential in the diagnosis and treatment of diabetic nephropathy. In addition to being a marker of renal function, Klotho has a critical biological function in phosphate regulation, in modulation of FGF-23 signaling, in reduction of oxidative stress, reduction of inflammatory processes, inhibition of apoptosis, and renal fibrosis. Experimental and clinical data are increasingly accumulating to demonstrate that circulating and urinary Klotho levels are decreased in the early phases of diabetic kidney injury and closely associated with progressive decrease in renal function, increasing albuminuria and progressive eGFR loss. Moreover, the absence of Klotho has been linked with several molecular mechanisms that are essential in the development of diabetic nephropathy, such as transforming growth factor-β, Wnt/β-catenin, nuclear factor-κB, and oxidative stress pathways. The present review summarizes the current knowledge concerning the biology of Klotho, its molecular mechanisms in DKD and its potential as a marker for early renal dysfunction. It also explores the recent clinical data for the diagnostic and prognostic value of Klotho as well as its therapeutic potential. The evidence so far is promising and warrants further prospective clinical use of Klotho as a biomarker for early diagnosis, risk stratification, disease monitoring and personalized care of patients with diabetic nephropathy, but larger prospective studies are needed to confirm this.

Keywords: Klotho protein; Diabetic nephropathy; Chronic kidney disease; Renal dysfunction; Biomarker.

1. Introduction

The global epidemic of diabetes mellitus and its complications represent an unparalleled challenge for public health. Diabetic nephropathy is a common and serious microvascular complication of diabetes, defined as the presence of diabetic kidney disease (DKD) that is, diabetic glomerular disease with or without albuminuria. DKD, the leading cause of chronic kidney disease (CKD) and end-stage kidney disease (ESKD) worldwide, represents a major risk factor for accelerated atherosclerosis and cardiovascular disease (CVD) in affected patients. The World Health Organization predicts that CVD, exacerbated by DKD, will become the leading cause of death among individuals with diabetes by the year [1,2].

Diabetic nephropathy is recognised as a clinical syndrome comprising three temporal manifestations: induction of diabetes, onset of clinical insulin resistance, and the development of microvascular complications. Well-established markers of glomerular filtration rate (GFR) and albuminuria fail to permit the early identification of patients who will develop DKD, or the later prediction of those likely to transition to advanced stages. Changes in other molecules, including Klotho, may intervene earlier and, therefore, serve as useful biomarkers for DKD [3,4].

Klotho is an anti-ageing protein predominantly expressed in the kidney, parathyroid gland, placenta, and brain. Circulating Klotho levels correlate inversely with age, and Klotho dysfunction has been observed in diverse age-related pathological conditions, including chronic kidney disease (CKD) and diabetes. Histological studies in diabetes-animal models demonstrate glomerular and tubular Klotho decrease following diabetes induction [5]. Circulating Klotho levels similarly decline following the onset of diabetes (oral glucose tolerance test or streptozotocin injection) or during the progression of the disease. Other reports show that Klotho is eliminated from the body, leading to a decrease in circulating levels. Klotho alterations appear to correlate with the early stages of renal dysfunction before any changes in GFR or albuminuria occur [6,7].

Previous systematic reviews and meta-analyses of diabetic or type-2 diabetes cohorts concluded that Klotho levels do not represent suitable blood-based biomarkers for diabetic kidney disease, either for early renal dysfunction or prognosis. These conclusions partially stem from concerns that changes in circulating Klotho levels always accompany substantial confounding and that inter-study variability in circulating Klotho quantification is excessive. However, a broad spectrum of survey data highlights temporal-disruption patterns in diabetes-related Klotho variants already coinciding with the initial stages of DKD, long before the appearance of any lesions seen by conventional measurements [8,9].

Methodology

The present study is a narrative review and was aimed to review critically the significance of Klotho protein as a new biomarker for early renal impairment in diabetic nephropathy. Eligible research in relation to DKD or CKD, including clinical studies, review articles and international databases related to Klotho and renal biomarkers were systematically retrieved from peer-reviewed journals. This review evaluated recent experimental and clinical evidence on the biological role of Klotho, its potential usefulness as a diagnostic biomarker, and therapeutic target in the early diagnosis and progression of diabetic nephropathy. Sources were chosen on the basis of scientific quality, methodology and relevance to clarify the link between Klotho expression and impairment in renal function. A special emphasis was on studies exploring circulating and urinary Klotho levels, their relationship to eGFR, albuminuria and oxidative stress/inflammation/fibrosis/other pathophysiological mechanisms in diabetic kidney damage. We conducted a thematic analysis to compile evidence from the literature relating to the endocrine and metabolic roles of Klotho, its potential role in renal pathophysiology, and its prognostic significance among diabetic patients. The review also explored mechanistic pathways of disease progression involving transforming growth factor-beta (TGF-β), Wnt/β-catenin signaling, nuclear factor-kappa B (NF-κB), fibroblast growth factor-23 (FGF-23) and oxidative stress responses. The objective of this study was to (i) compare the diagnostic value of traditional renal biomarkers and Klotho; (ii) investigate whether Klotho may have a role as an early biomarker, before structural renal damage occurs. The synthesized evidence was interpreted to assess current knowledge gaps, hurdles in assay standardization and future directions for clinical application.

This approach allowed for an in-depth evaluation of Klotho as a novel biomarker and therapeutic target in diabetic nephropathy.

Result and Discussion

2. Background on Diabetic Nephropathy

Diabetic nephropathy (DN) is one of the most common microvascular complications of diabetes, with a global prevalence of approximately 30–40% among individuals with Type 1 and Type 2 diabetes. The condition is characterized by a progressive decline in kidney function, which may advance over a period of several years. However, a decrease in glomerular function is not the initial change that occurs in DN; rather, there is a gradual increase in glomerular hyperfiltration that is only later followed by a rise in albuminuria and a decrease in estimated glomerular filtration rate (eGFR). At this stage, the prognosis for the patient remains good if diabetes is appropriately controlled [10]. The gold standard for diagnosing impaired early renal function in DN is the evaluation of the patient's eGFR and urinary albumin-to-creatinine (uACR) ratio. International guidelines recommend using these two biomarkers. However, evidence suggests that changes in these biomarkers occur relatively late in the course of DN and indicate disease progression rather than early dysfunction. The substantial time lag between the onset of hyperfiltration and the appearance of changes in established biomarkers reflects the complex development of DN. Therefore, there is an urgent need for novel biomarkers of early renal dysfunction in DN [11,12].

Klotho is a single-pass transmembrane protein and vital component of aging. Klotho levels, both circulating and urinary, decrease significantly earlier than GFR or albuminuria in experimental models of early renal dysfunction and in people with type 2 diabetes. There is also circumstantial evidence that Klotho is involved in early nephron damage and glucose metabolism [13,14]. Reductions in circulating and urinary Klotho occur in early and advanced stages of chronic kidney disease and correlate with a decline in eGFR and an increase in albuminuria, particularly in diabetic patients. These findings suggest that Klotho is a promising candidate biomarker for early renal dysfunction in diabetes [15,16].

3. The Klotho Protein: Structure, Function, and Distribution

Klotho is a single-pass membrane protein that occurs as three isoforms (full-length membrane-bound Klotho, sKlotho, and an N-terminal fragment) generated by proteolytic cleavage of the precursor. Klotho primarily exerts actions on the kidney and parathyroid gland and modulates metabolic phosphate homeostasis and vitamin D activation through the regulation of fibroblast growth factor (FGF) [17]. The prototypical Klotho receptor complex consists of membrane-bound α-Klotho and FGF receptor (FGFR) 1 or FGFR3, and Klotho also activates the pituitary and the parathyroid gland through the FGF 15/19–FGFR4 and FGF 21–FGFR1 pathway, respectively. The kidney is the primary source of circulating Klotho [18]. In the kidney, Klotho expression is high in the renal tubules of embryonic animals and decreases to lower levels in adulthood and aging. It is mainly expressed in the distal convoluted tubules and the connecting tubules of nephron segments. In humans, a similar developmental expression pattern is observed 2. In the adult proximal tubule, Klotho shows a notable decline in the age–associated loss of renal function; however, its expression in other nephron segments remains unchanged till the late stages of life 1. In aging individuals, proximal tubular Klotho is dramatically downregulated at the stage of stage III chronic coronal disease; thus, the renal expression pattern of Klotho in glomerulus and proximal tubular over the aging process appears not to varies [18].

4. Klotho in Renal Physiology and Pathophysiology

The physiological functions of Klotho in the kidney, the nephron segments where Klotho is expressed, and the influence of Klotho on renal pathophysiologies are summarized. Membrane-bound Klotho exerts extracellular signals on the FGF-receptor complex after locally produced FGF-23 binds to the receptors [19]. Soluble Klotho derived from the proteolytic cleavage of membrane-bound Klotho exerts intracellular signals via the stimulation of phosphatidylinositol 3-kinase (PI3K)-Akt signaling. Membrane Klotho expression in the kidney decreases after insults including hyperglycemia, angiotensin II treatment, iron overload, or oxidative stress. Changes in membrane and soluble Klotho levels occur during the development of diabetic kidney disease (DKD). Soluble Klotho levels in the

urine and circulation are influenced by the diabetic environment, and these Klotho alterations correlate with features of DKD such as oxidative stress and metabolic disturbances. The data collectively suggest that Klotho is implicated in the pathophysiology of early renal dysfunction in diabetes [20,21].

Klotho protein in the kidney is mainly expressed in the renal tubules, particularly in the distal convoluted tubules and the connecting tubules. Klotho plays a crucial biological role in renal physiology and pathophysiology by exhibiting nephron segment-specific functions and age-dependent expression. The membranous Klotho protein primarily functions as a co-receptor for fibroblast growth factor (FGF) [22]. Klotho is also secreted into the circulation and regulates intracellular signaling through interaction with the insulin receptors, PI3K, and the AMP-activated protein kinase pathway. The activation of these signaling pathways is closely associated with the general metabolic state of the body and the progression of a wide range of metabolic disorders. During the initial stage of DKD, the Klotho protein decreases in tandem with gradually impaired renal function and elevated albumin levels, indicating the loss of Klotho in the early phase of this diabetic complication [23]. Klotho inhibition affects the redox state of cells by reducing the levels of intracellular reactive oxygen species and modulating the expression of anti-oxidant proteins. Klotho-deficient animals exhibit enhanced lipid peroxidation, oxidative stress, inflammation, senescence, and kidney injury when exposed to high-glucose conditions. Klotho not only has a physiological role in the kidney but is also involved in the pathophysiology of early renal dysfunction during diabetes [22,23].

5. Evidence Linking Klotho to Early Renal Dysfunction in Diabetes

The Klotho protein is important in many biological functions such as control of oxidative stress, phosphate metabolism and longevity. Klotho regulates the processing of a number of solutes such as sodium and calcium in the kidney. The Klotho pathway has developed as a potential target in diabetic nephropathy as the expression of Klotho decreases with age and there is changed regulation of the pathway in metabolic disorders like diabetes [24,25]. Several mechanisms linking Klotho deficiency with kidney injury have been found. Importantly, changes in circulating and urine Klotho levels are well correlated to early indicators of impaired renal function like eGFR and microalbuminuria in animal and human studies 23. The conventional criterion for novel markers is that they should correlate with clinical outcomes, but previous alterations of Klotho levels already provide relevant and prognostic information, and this points towards the subsequent development of end-stage renal disease [26].

Diabetic nephropathy remains one of the most common consequences of diabetes, with a global prevalence among diabetics exceeding 30%. Although current practice indicates that situations involving both diabetes and Klotho deficiency warrant attention, the reverse holds true for Klotho and diabetic conditions, where Klotho modulation has garnered much interest following the identification of a Klotho-associated diabetic kidney disease pathway [27].

6. Mechanistic Pathways: Klotho, Oxidative Stress, and Fibrosis

The early phase of DKD is characterized by glomerular hyperfiltration, intraglomerular hypertension, accelerated nephron loss, and consequent increased oxidative stress within remaining nephron segments [28]. Oxidative stress leads to enhanced expression of profibrotic factors, inflammatory cytokines, and chemokines, indicating a switch from an adaptive to a maladaptive response. Following podocyte injury, Klotho deficiency is reported to promote the downregulation of nephrin, the essential podocyte adhesion protein that sustains the integrity of the glomerular filtration barrier. Klotho also facilitates the opening of renal inflammatory vascular networks in diabetes 1 , enhances the expression of antioxidant proteins such as manganese superoxide dismutase, and modulates the activity and stability of Nrf2, a redox-sensitive transcription factor for multiple detoxifying enzymes in proximal tubule cells. The presence of Klotho protein does not alter the link between hyperglycemia and diabetic microvascular complications such as retinopathy but markedly decreases early-stage kidney damage and associated risk factors. Klotho may counteract diabetic injuries via actions not solely dependent on their auxiliary role in FGF23 signaling. Changes in Klotho expression in the diabetic milieu are not limited to proximal tubular segments, and emerging evidence has linked protein levels with deteriorating kidney function, tubule-interstitial injury, and vascular aging [29,30].

Progressive chronic kidney disease, including DKD, is currently viewed as an accelerated-aging process, with Klotho considered an antiaging factor. In DKD, Klotho declines early and is requisite for the maintenance of kidney health. Retention of Klotho during early high-glucose challenges is viewed as critical for safeguarding renal function and delaying deterioration. Soluble Klotho levels in circulation and urine have been presented as diagnostic and prognostic biomarkers for early renal dysfunction in diabetes [31,32].

7. Diagnostic and Prognostic Implications

Diabetic nephropathy constitutes a significant public health burden, with an estimated 50% of people with diabetes developing clinical manifestations of diabetic kidney disease. Nearly 30% and 15% of affected patients ultimately progress to end-stage renal disease (ESRD) and require renal replacement therapy (RRT) or transplantation. Diabetic kidney disease follows a defined natural history. Nephron hyper-inflation develops in the precocious stages of diabetes before microalbuminuria becomes detectable, representing an early functional defect in the natriuretic response of diabetic kidneys [33,34].

Standard renal markers of glomerular filtration rate (GFR) decline and albuminuria onset fail to detect these crucial initial shifts in kidney functionality. In healthy individuals, changes in molecular components such as potassium, calcium, magnesium, and other modalities have been recorded early on, suggesting the potential retention of natriuretic capability even during the pre-clinical stage [35,36]. Klotho regulation appears to underpin significant alterations in cell physiology and systemic health, reinforcing its position as a possible early and relatively sensitive marker for renal dysfunction. Circulating Klotho measurement represents a nondialyzable load, while there is no clear information available for other alternatives on the regulatory effects of Klotho under such conditions. Accordingly, study designs based on circulating and urinary Klotho concentrations in related models offer the opportunity to determine electrochemical instability remaining detectable well before the onset of frank albuminuria [37,38].

8. Methodological Considerations and Standardization

Lack of standardization makes it difficult to compare the use of Klotho as a biomarker for early renal dysfunction in diabetes across studies. There are various Klotho assays available at the market which measure the soluble form present in the blood, but few of these assays provide details of sample preparation or storage. The proposed route of Klotho diagnostics, from physiology, epidemiology, clinical evidence and mechanism, are based on studies that, in general, describe these aspects but do not specify which assays are used. Key outstanding issues consist of a harmonisation of collection, handling, preparation, analyses, reporting and interpretation, as well as the gathering of additional evidence and mechanistic understanding [39,40].

There must be a strong combination of lab and clinical components for a good research plan to develop diagnostics for Klotho. For the laboratory side candidate assays should go through detailed analytical validation and inter-laboratory testing. Retrospective cohorts with detailed eGFR measures at baseline are used on the clinical side to determine the association of Klotho and eGFR and the masses of individuals that decrease their eGFR. More information on these complementary but separate aspects is given below [41,42].

Formal standardization efforts are encouraged by assay performance variation across Klotho studies, an ideal approach would be to involve assay developers, manufactures and cross-disciplinary experts to agree on a common operational definitions and set of KPIs. Of the 40 proposed cohort studies for Klotho, 40 are related to selection criteria for original populations and important downstream interpretation variables and not related to these basic standardization questions [43,44].

9. Therapeutic Potential and Future Directions

Klotho protein has attracted considerable attention as a candidate biomarker of early renal dysfunction in diabetes. Building on the characterization of Klotho as a pathway-interacting and compartmentalized protein involved in renal physiology, several directions for further exploration are warranted. Further investigation is necessary to determine whether Klotho modulation could alter the trajectory of diabetic nephropathy. In this context, candidate interventions include gene therapy, administration of recombinant protein, lifestyle alterations, and targeting pharmacologic agents toward

renin-angiotensin-aldosterone-system or inflammatory pathways. These possibilities intersect with diverse gaps within translational research that would enhance understanding of Klotho function in renal disease and facilitate progression to human studies of early diabetic nephropathy [45,46].

Therapeutic approaches to Klotho represent a blue-print for additional mechanistic and evidence-based study of Klotho as a biomarker for early deterioration. Initial actions to close this gap relate to the identification of study designs, populations and progression definitions. Different epidemiological risk factors point to the need for more specific clinical characterization to further link Klotho measurements to the disease. An existing model of pre-existing comorbidity and multi-factorial measures of Klotho may explain its ability to enhance the current prediction models. Requirements for assay standardization, multi-laboratory calibration and uni-dimensional reporting can motivate further experimentation. Finally, the use of Klotho as a regimen-control component of gene-vector systems for renal restoration or other form of delivery might aid in understanding Klotho's function in preserved versus dysfunctional renal systems [46].

10. Conclusion

Diabetic nephropathy (DN) is a microvascular complication affecting approximately 30-40% of diabetic subjects, with a high socio-economic burden and dire health consequences worldwide. Early identification of metabolic dysfunction and renal involvement is crucial for timely implementation of preventive strategies. Both glomerular filtration rate (GFR) and albuminuria are widely used for DN evaluation, but both have limitations for early prediction. GFR is normal even in advanced pathological stages, and albuminuria remains within the normal range (microalbuminuria < 30 mg/day) until later stages. Klotho (KL), a pleiotropic protein primarily produced by the kidney, displays pathological alteration in early diabetic nephropathy (DN). Changes in KL concentration in blood/plasma and urine precede the clinical appearance of glomerular hyperfiltration and microalbuminuria in murine models of DN. Klotho represents a promising novel biomarker for early renal dysfunction in diabetes, and close monitoring of KL is warranted for people at risk in both animal models and humans. 10

Funding

There is no funding

Declaration of Competing Interest

The authors say they don't have any known personal or financial relationships or financial interests that could have seemed to affect the work in this study.

REFERENCES

[1] N.-J. Cho et al., "Soluble Klotho as a Marker of Renal Fibrosis and Podocyte Injuries in Human Kidneys," PLOS ONE, vol. 13, no. 3, Art. no. e0194617, 2018.

[2] M. D. Sanchez-Niño, B. Fernandez-Fernandez, and A. Ortiz, "Klotho, the Elusive Kidney-Derived Anti-Ageing Factor," Clinical Kidney Journal, vol. 13, no. 2, pp. 125–127, 2020.

[3] J. Donate-Correa et al., "Klotho, Oxidative Stress, and Mitochondrial Damage in Kidney Disease," Antioxidants, vol. 12, no. 2, Art. no. 239, 2023.

[4] M. Huang et al., "Effects of Chronic Kidney Disease on Cognitive Function and α-Klotho Expression in Hippocampus," Translational Research, 2022.

[5] R. Fernandez Castillo, "Pathophysiologic Implications and Therapeutic Approach of Klotho in Chronic Kidney Disease: A Systematic Review," Laboratory Investigation, vol. 103, no. 7, Art. no. 100178, 2023.

[6] J. Martín-Vírgala et al., "Serum and Urinary Soluble α-Klotho as Markers of Kidney and Vascular Impairment," Nutrients, vol. 15, no. 6, Art. no. 1470, 2023.

[7] J. Donate-Correa et al., "The Value of Klotho in Kidney Transplantation," Transplantation, vol. 107, no. 3, pp. 616–627, 2023.

[8] D. Mattinzoli et al., "Is There a Role in Acute Kidney Injury for FGF23 and Klotho?" Clinical Kidney Journal, vol. 16, no. 10, pp. 1555–1562, 2023.

[9] L.-X. Yu et al., "The Controversy of Klotho as a Potential Biomarker in Chronic Kidney Disease," Frontiers in Pharmacology, vol. 13, Art. no. 931746, 2022.

[10] A. Piwkowska et al., "Novel Markers in Diabetic Kidney Disease—Current State and Perspectives," Diagnostics, vol. 12, no. 5, Art. no. 1205, 2022.

[11] D. Chen et al., "Association of Serum Klotho Level with Albuminuria in Middle-Aged and Elderly Participants without Diabetes Mellitus: A Cross-Sectional Study," BMC Nephrology, vol. 25, Art. no. 455, 2024.

[12] C. Mora-Fernández et al., "Sodium-Glucose Co-Transporter-2 Inhibitors Increase Klotho in Patients with Diabetic Kidney Disease: A Clinical and Experimental Study," Biomedicine & Pharmacotherapy, vol. 154, Art. no. 113677, 2022.

[13] R. F. Lefta and E. A. Hassan, "Serum Soluble α-Klotho Levels in Patients with Diabetic Nephropathy," Irish Journal of Medical Science, vol. 193, no. 2, pp. 725–731, 2024.

[14] S. Ciardullo and G. Perseghin, "Soluble α-Klotho Levels, Glycemic Control and Renal Function in US Adults with Type 2 Diabetes," Acta Diabetologica, vol. 59, no. 6, pp. 803–809, 2022.

[15] K. Wang et al., "Association between Serum Klotho Levels and the Prevalence of Diabetes among Adults in the United States," Frontiers in Endocrinology, vol. 13, Art. no. 1005553, 2022.

[16] P. Xie et al., "Associations of Serum Klotho with Diabetic Kidney Disease Prevalence and Mortality: Insights from a Nationally Representative U.S. Cohort," Diabetology & Metabolic Syndrome, vol. 17, Art. no. 198, 2025.

[17] A. Kale et al., "Klotho in Kidney Diseases: A Crosstalk between the Renin-Angiotensin System and Endoplasmic Reticulum Stress," Nephrology Dialysis Transplantation, vol. 38, no. 4, pp. 819–825, 2023.

[18] G. J. Prud'homme, M. Kurt, and Q. Wang, "Pathobiology of the Klotho Antiaging Protein and Therapeutic Considerations," Frontiers in Aging, vol. 3, Art. no. 931331, 2022.

[19] S. Tomo et al., "Klotho: A Possible Role in the Pathophysiology of Nephrotic Syndrome," Electronic Journal of the International Federation of Clinical Chemistry and Laboratory Medicine, vol. 33, no. 1, pp. 3–10, 2022.

[20] W. Jiang et al., "Klotho Inhibits Renal ox-LDL Deposition via IGF-1R/RAC1/OLR1 Signaling to Ameliorate Podocyte Injury in Diabetic Kidney Disease," Cardiovascular Diabetology, vol. 22, Art. no. 293, 2023.

[21] C. Xin et al., "Relationship of Soluble Klotho and Early Stage of Diabetic Nephropathy: A Systematic Review and Meta-Analysis," Frontiers in Endocrinology, vol. 13, 2022.

[22] J. Donate-Correa et al., "Association of Klotho with Coronary Artery Disease in Subjects with Type 2 Diabetes Mellitus and Preserved Kidney Function: A Case-Control Study," International Journal of Molecular Sciences, vol. 24, 2023.

[23] J. Donate-Correa et al., "Association of Klotho with Coronary Artery Disease in Subjects with Type 2 Diabetes Mellitus and Preserved Kidney Function: A Case-Control Study," International Journal of Molecular Sciences, vol. 24, 2023.

[24] H. Zhang, L. Yu, and G. Yun, "Reduced Serum Levels of Klotho Are Associated with Mild Cognitive Impairment in Patients with Type 2 Diabetes Mellitus," Diabetes, 2023.

[25] A. Hejrati et al., "Klotho: A Possible Diagnostic Biomarker and Therapeutic Target in Diabetes Complications," International Journal of Diabetes in Developing Countries, 2025.

[26] M. Marwein et al., "Klotho in Aging and Diseases: Molecular Features, Expressions, and Functional Implications," Molecular and Cellular Biochemistry, 2026.

[27] J. Lee et al., "Association between Serum Klotho Levels and Cardiovascular Disease Risk Factors in Older Adults," BMC Cardiovascular Disorders, 2022.

[28] D. Zou et al., "The Role of Klotho in Chronic Kidney Disease," BMC Nephrology, 2018.

[29] C. Sastre et al., "Hyperlipidemia-Associated Renal Damage Decreases Klotho Expression in Kidneys from ApoE Knockout Mice," PLOS ONE, 2013.

[30] S.-E. Wu and W.-L. Chen, "Soluble Klotho as an Effective Biomarker to Characterize Inflammatory States," Annals of Medicine, vol. 54, no. 1, 2022.

[31] J. Martín-Vírgala et al., "Soluble Klotho, a Potential Biomarker of Chronic Kidney Disease–Mineral Bone Disorders Involved in Healthy Ageing: Lights and Shadows," International Journal of Molecular Sciences, vol. 25, no. 3, Art. no. 1843, 2024.

[32] S.-S. Li et al., "Soluble Average Klotho Level as a Prognostic Marker for Acute Kidney Injury Outcomes: A 90-Day Follow-Up Study," Biomarkers in Medicine, 2025.

[33] F. Bi, J. Wang, and M. Su, "Serum Klotho as a Potential Biomarker for Prognosis in Acute Ischemic Stroke," Frontiers in Medicine, 2026.

[34] A. Cetinkaya et al., "Clinical Significance of Serum Soluble Klotho Levels in Sepsis-Associated Encephalopathy: A Single-Center Prospective, Preliminary Study," Journal of Clinical Medicine, 2026.

[35] A. Espuch-Oliver et al., "Reference Values of Soluble α-Klotho Serum Levels Using an Enzyme-Linked Immunosorbent Assay in Healthy Adults Aged 18–85 Years," Journal of Clinical Medicine, 2022.

[36] S. Devi, "A Review on Klotho: FGF23 Mediated Pathway Integration and Aging," Journal of Gerontology and Geriatrics, 2025.

[37] J. Shen et al., "Klotho Protein: A Multifaceted Guardian of Healthy Aging and Its Therapeutic Potential," International Journal of Molecular Sciences, 2025.

[38] S. Mahdavi, "Klotho, Kidneys, and Micronutrient Signaling: A Promising Paradigm for Healthy Aging," Lifestyle Genomics, 2026.

[39] X. Zhao et al., "New Insights into the Role of Klotho in Inflammation and Fibrosis: Molecular and Cellular Mechanisms," Frontiers in Immunology, 2024.

[40] J. A. Neyra et al., "Performance of Soluble Klotho Assays in Clinical Samples of Kidney Disease," Clinical Kidney Journal, 2019.

[41] D. Panikkar et al., "Pre-Analytical Variables Influencing Stability of Blood-Based Biomarkers of Neuropathology," Journal of Alzheimer's Disease, 2023.

[42] R. Gulej et al., "Plasma-Based Strategies for Systemic Rejuvenation: Critical Perspectives on Clinical Translation," GeroScience, 2026.

[43] Y. Yang et al., "Nutritional Interventions and Acupuncture-Based Strategies for Exercise-Induced Fatigue and Recovery: Mechanisms and Integrative Perspectives," Food Science & Nutrition, 2026.

[44] S. Vaidhyanathan et al., "Mapping Current Studies of tRNA Fragments onto Disease Landscape," Biomolecules, vol. 15, 2025.

[45] A. Piwkowska et al., "Novel Markers in Diabetic Kidney Disease—Current State and Perspectives," Diagnostics, vol. 12, no. 5, Art. no. 1205, 2022.

[46] L.-X. Yu et al., "Potential Application of Klotho as a Prognostic Biomarker for Patients with Diabetic Kidney Disease: A Meta-Analysis of Clinical Studies," Therapeutic Advances in Chronic Disease, vol. 14, 2023.