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Evaluation of the Predictive Value of Klotho in Patients with Type 2 Diabetes Mellitus and Cardiovascular Disease

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Abstract

Type 2 Diabetes Mellitus (T2DM) is a chronic metabolic disorder characterized by hyperglycemia due to insulin resistance and relative insulin deficiency, and is associated with serious cardiovascular complications. Klotho is an anti-ageing protein involved in metabolic regulation and cellular protection.

The study aimed to investigate klotho levels in patients with T2DM and in those with T2DM complicated by cardiovascular disease.

Subjects and methods This case-control study was conducted to evaluate serum klotho levels in patients with T2DM and those with T2DM complicated by cardiovascular disease (CVD). A total of 112 participants were enrolled and categorized into three groups: 41 patients with T2DM, 41 patients with T2DM and CVD, and 30 apparently healthy controls. Serum klotho, glucose, HbA1c, triglycerides, total cholesterol, HDL-c, LDL-c, creatinine (Cr), eGFR, and hs-CRP were measured. Correlation and receiver operating characteristic (ROC) analyses were performed to assess the diagnostic performance of Klotho.

Serum Klotho levels were significantly elevated in both diabetic groups compared with healthy controls ($p<0.001$). Significant correlations were observed between klotho levels and several metabolic and inflammatory parameters. Receiver operating characteristic (ROC) analysis demonstrated excellent diagnostic performance of serum klotho in discriminating patients from healthy controls. For both T2DM and T2DM with CVD groups, the area under the curve (AUC) reached 1.000, with 100% sensitivity and 100% specificity at the optimal cut-off values.

Serum Klotho levels were significantly altered in patients with T2DM and T2DM-associated CVD. These findings suggest that Klotho may represent a promising biomarker for disease progression and cardiovascular risk assessment; however, larger prospective studies are required to validate its predictive value.

Keywords: Klotho, Type 2 Diabetes Mellitus, Cardiovascular Diseases.

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Introduction

Type 2 diabetes mellitus (T2DM) is the most prevalent form of diabetes, accounting for over 90% of cases worldwide. It is characterised by chronic hyperglycemia resulting from insulin resistance and progressive impairment of pancreatic β-cell function. The growing global burden of T2DM and its strong association with cardiovascular morbidity and mortality have highlighted the need for identifying novel biomarkers involved in disease progression and complication risk (1).

Diabetes mellitus and cardiovascular disease (CVD) are closely related. Compared to healthy persons, those with (T2DM) have twice the risk of cardiovascular death, and CVD accounts for around half of DM-related deaths. Thus, one of the main goals of diabetes care is the early identification and treatment of CV hazards in diabetic patients (2,3).

Type 2 diabetes, dyslipidemia, hypertension, and obesity are known risk factors for (CVD). Although traditional cardiovascular risk factors such as dyslipidemia, hypertension, obesity, and smoking are widely used for cardiovascular risk assessment, accumulating evidence suggests that these markers may not adequately identify subclinical cardiovascular events in all patients with T2DM, emphasizing the need for novel biomarkers (4).

The KL gene encodes the multipurpose protein Klotho, which is well known as an anti-ageing biomolecule with important functions in preserving physiological homeostasis. The kidney is the main organ where it is expressed and secreted into the bloodstream. It can exist in both membrane-bound and soluble forms (5). Klotho regulates phosphate and vitamin D metabolism, which are critical for mineral balance and overall health, by acting as a co-receptor for fibroblast growth factor-23 (FGF23) (6). Reduced klotho expression is linked to a shorter lifespan and a higher risk of developing chronic illnesses like cardiovascular, renal, and neurological disorders, according to new research that emphasises its role in ageing-related processes (7).

Klotho plays an important role in regulating metabolic balance through its effect on insulin resistance (8), inflammation, and oxidative stress (9).

Previous clinical studies have produced inconsistent findings regarding circulating Klotho levels in T2DM. While many studies have reported reduced Klotho concentrations and cardiovascular disease, others observed elevated circulating Klotho levels that may represent a compensatory response to ongoing metabolic and vascular stress. For example, Pan et al. reported that increased circulating Klotho levels were associated with long-term macrovascular outcomes in patients with T2DM (10). Recent clinical evidence highlighted the potential role of soluble Klotho as a biomarker of vascular and metabolic health in patients with T2DM. In a prospective observational study, Corcillo et al. demonstrated that lower circulating Klotho concentrations were associated with the development and progression of diabetic microvascular complications, suggesting that Klotho deficiency may reflect ongoing endothelial dysfunction and oxidative stress. These findings support the concept that Klotho is involved in the pathophysiology of diabetic complications and may have prognostic value in risk stratification of diabetic patients (11).

Several biological mechanisms may explain the association between Klotho and cardiovascular disease. Klotho has been shown to enhance endothelial function, suppress oxidative stress,

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inhibit vascular calcification, and attenuate inflammatory signaling pathways. Dysregulation of these protective mechanisms may contribute to atherosclerosis progression and cardiovascular complications in patients with T2DM (12). Despite increasing evidence supporting the role of Klotho in metabolic and cardiovascular regulation, its relationship with cardiovascular complications among Iraqi patients with T2DM remains insufficiently characterized. Furthermore, the diagnostic and prognostic value of circulating Klotho levels in identifying cardiovascular risk within this population has not been fully established. Therefore, this study aimed to evaluate circulating Klotho levels and investigate their association with cardiovascular complications in patients with T2DM.

Materials & Methods

The participants in this case-control study were chosen from Al-Ramadi Teaching Hospital between December 2025 and February 2026. The participants were divided into three groups according to disease status: the first group included 30 apparently healthy controls; the second group included 41 patients with type 2 diabetes mellitus (T2DM); and the third group included 41 patients with T2DM and concomitant cardiovascular disease (T2DM+CVD). Cardiovascular disease (CVD) was defined based on documented clinical diagnosis of coronary artery disease, myocardial infarction, ischemic heart disease, heart failure, hypertension-related cardiovascular disease, or other physician- confirmed cardiovascular conditions. Diagnosis was established according to medical records, clinical examination findings, electrocardiographic data, and relevant imaging studies when available. Kidney function was evaluated in all studied groups by estimating the glomerular filtration rate (eGFR) using the following equation (13).

$$\mathbf{eGFR} = \mathbf{142} \times \mathbf{min}\left(\frac{\mathbf{Scr}}{\boldsymbol{\kappa}}, \mathbf{1}\right)^{\boldsymbol{\alpha}} \times \mathbf{max}\left(\frac{\mathbf{Scr}}{\boldsymbol{\kappa}}, \mathbf{1}\right)^{\mathbf{-1.200}} \times \mathbf{0.9938}^{\mathbf{Age}} \times \mathbf{1.012}\ \ (\mathbf{if\ female})$$

Scr: serum creatinine concentration(mg/dL)
K: Is 0.7 for females and 0.9 for males
min: minimum value between Scr/k or 1
max: maximum value between Scr/k or 1
Age: is expressed in years

Inclusion criteria

Adults above the age of thirty were eligible to participate in this study. individuals who have been diagnosed with T2DM using recognized clinical and biochemical criteria. Based on clinical assessment and pertinent diagnostic tests, participants were divided into groups of people with T2DM alone and those with concurrent cardiovascular disorders. Before being enrolled, each subject gave their informed consent.

Exclusion criteria

Individuals with type 1 diabetes (T1DM), gestational diabetes mellitus, acute or chronic inflammatory diseases, hepatic or renal failure, cancer, or any endocrine abnormalities other than diabetes that could affect klotho levels were excluded. Additionally, patients taking drugs that are known to alter oxidative stress indicators or mineral metabolism were not included. The study did not include people who were pregnant or nursing.

Study variable and anthropometric measurements

Age, sex, duration of T2DM, and smoking status were among the clinical and demographic variables considered in the study. Body weight and height were measured using conventional

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methods, and the body mass index (BMI) was computed by dividing the weight in kilograms by the square of the height in meters (kg/m²) (14). These parameters were recorded for all participants to assess their potential association with circulating klotho levels and the presence of cardiovascular diseases.

Blood sample collection

Venous blood samples were taken from each participant after they had fasted for at least eight hours. After being separated for 10 minutes at 1500 × g in a centrifuge, the serum was kept in Eppendorf tubes at -20 °C until the assays were carried out. HbA1c was measured using whole blood.

Biochemical evaluation

A biochemical analyser c4000 (Abbott company) was used to assess FBG levels and lipid profiles, including total cholesterol (TC), triglycerides (TG), and high-density lipoprotein (HDL-c). LDL-c levels were calculated using the Friedewald equation (15). HbA1c levels were measured using the Automated Fluorescent Immunoassay System (AFIAS; Boditech Med Inc., Korea), and serum klotho concentrations were assessed using an enzyme-linked immunosorbent test (ELISA), FineTest, China company.

Statistical analysis

GraphPad Prism version 8.02 and SPSS version 26 were used for the statistical analysis of the data from the current investigation. Results were expressed as mean ± standard deviation (SD). The study data were statistically analyzed using one-way ANOVA to compare differences among groups, and the area under the curve (AUC) for each variable was measured using receiver operating characteristic (ROC) curve analysis; cut-off values, sensitivity, and specificity were calculated. $p \leq 0.05$ was considered statistically significant. For each variable, the sensitivity (Sen%), specificity (Spec%), likelihood ratio (LR), and cut-off value were calculated.

Results:

The demographic and clinical characteristics of the studied groups are summarized in Table 1. Statistically significant differences among the groups were observed in age ($p = 0.003$), gender distribution ($p = 0.013$), and disease duration ($p < 0.001$). Conversely, no significant differences were found regarding BMI ($p = 0.636$) or smoking status ($p = 0.704$).

Table 1: Demographic, Anthropometric, and Clinical Characteristics of the Study Groups

Variable	Control (n=30)	DM (n=41)	HD (n=41)	P-value
Age (years)	49.93 ± 9.98	54.87 ± 9.23	58.00 ± 9.34	**0.003**
BMI (kg/m²)	31.62 ± 7.28	30.87 ± 5.58	30.27 ± 5.10	**0.636**
Gender (M/F)	10 / 20	20 / 21	28 / 13	**0.013**
Smoking (%)	6.7%	12.2%	12.2%	**0.704**
Duration of disease (months)	-------	65.5 ± 64.3	64.3 ± 50.0	**0.922**

Table 2 presents the biochemical characteristics of the study groups. Significant differences were observed in FBG, HbA1c, TG, HDL-c, hs-CRP, and Klotho levels, whereas TC, LDL-c, creatinine, and eGFR showed no statistically significant differences among the groups.

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Table 2. Comparison of biochemical and clinical parameters among healthy controls, patients with T2DM, and CVD

variable	Control (N=30)	T2DM Group N=41	T2DM+CVD Group N=41	p-value
FBG (mg/dL)	94.4± 7.8	188.9 ± 73.4	227.4 ±103.5	<0.001
HbA1c%	5.05±0.54%	7.65±1.66%	7.57±1.90%	<0.001
TG (mg/dL)	126.8 ±30.1	165.8 ±72.5	205.3 ±72.4	<0.001
TC (mg/dL)	173.8 ±21.8	185.3 ± 52.4	189.4 ±39.5	0.283
HDL-c (mg/dL)	53.6 ± 12.3	43.8 ± 11.9	39.3 ±8.6	<0.001
LDL-c (mg/dL)	94.8 ±17.8	110.8 ± 48.7	108.8 ±35.0	0.323
Cr (mg/dL)	0.73 ± 0.14	0.77 ± 0.17	0.81 ± 0.14	0.097
Egfr (mL/min/1.73 m²)	101.9 ±15.6	97.4 ±16.1	91.7 ±22.0	0.068
HS-CRP (mg/dL)	16.19 ± 2.87	27.05 ± 4.34	31.01 ± 5.86	<0.001
Klotho (pg/mL)	77.56 ± 22.66	185.34 ± 27.12	171.54 ± 17.62	<0.001

T2DM: Type 2 Diabetes Mellitus, CVD: Cardiovascular Disease. FBG, fasting blood glucose; HbA1c, glycated hemoglobin; TG, triglycerides; TC, total cholesterol; HDL-c, high-density lipoprotein cholesterol; LDL-c, low-density lipoprotein cholesterol; Cr, creatinine; eGFR, estimated glomerular filtration rate; hs-CRP, high-sensitivity C-reactive protein; Klotho, α-Klotho protein.

Correlation analysis revealed that serum klotho correlates significantly with glycemic markers (FBG, HbA1c), lipid profile (TG, HDL-c), and hs-CRP. As for the remaining variables, klotho protein did not show a clear or strong correlation. Table 3.

Receiver operating characteristic curve analysis was performed to evaluate the diagnostic performance of serum Klotho in distinguishing patients with T2DM, with and without cardiovascular complications, from healthy controls. Serum Klotho demonstrated an apparently excellent discriminative performance (AUC = 1.00). At optimal cut-off values (132.8 pg/mL and 148.5 pg/mL, respectively), both sensitivity and specificity were 100%. Although this result should be interpreted cautiously because perfect diagnostic accuracy is uncommon in clinical biomarker studies and may be influenced by the relatively small sample size and the single-centre study design

Table 3: Correlation of serum klotho level with the studied parameters.

		FBG	TC	TG	Cr	HDL-c	LDL-c	HbA1c	eGFR	hs-CRP	klotho	Sst2
klotho	Pearson Correlation	0.505**	0.146	0.305**	0.066	-0.381**	0.130	0.548**	0-.125	0.632**	1	0.747**
	Sig. (2-tailed)	0.000	0.123	0.001	0.487	0.000	0.171	0.000	0.190	0.000		0.000
**. Correlation is significant at the 0.01 level (p-value).												
*. Correlation is significant at the 0.05 level (p-value).												

FBG, fasting blood glucose; TC, total cholesterol; TG, triglycerides; Cr, creatinine; HDL-c, high-density lipoprotein cholesterol; LDL-c, low-density lipoprotein cholesterol; HbA1c, glycated hemoglobin (hemoglobin A1c); eGFR, estimated glomerular filtration rate; hs-CRP, high-sensitivity C-reactive protein; Klotho, α-Klotho protein; sST2, soluble suppression of tumorigenicity 2.

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Table4. Area under the curve for serum klotho in T2DM patients.

Bi-omarker	Cut-off Value	Sensitiv-ity	Specific-ity	AUC	95%CI for AUC	Interpretation
Klotho	132.8 pg/mL	100%	100%	1.000	1.000– 1.000	good diagnostic perfor-mance

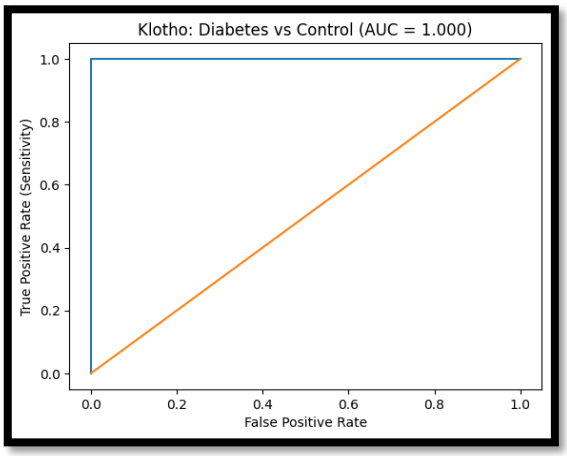

Figure 1: ROC curves of serum Klotho for diagnostic performance in T2D patients compared with controls

Table 5: Area under the curve for serum klotho in T2D and CVD patients

Bi-omarker	Cut-off Value	Sensitiv-ity	Specific-ity	AUC	95% CI for AUC	Interpretation
Klotho	148.5 pg/mL	100%	100%	1.000	1.000-1.000	Good diagnostic perfor-mance

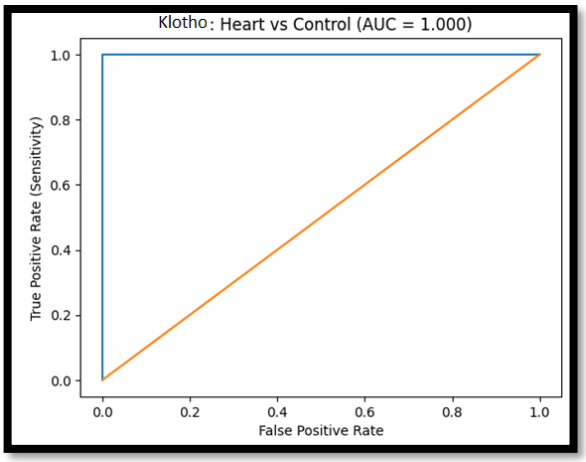

Figure 2: ROC Curves of Klotho for Patients with T2D and CVD and control

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Discussion:

Chronic hyperglycemia is a hallmark of diabetes mellitus (DM), a metabolic disorder. Reduced insulin secretion, resistance to insulin's peripheral effects, or a combination of the two could cause this. When combined with other metabolic abnormalities, chronic hyperglycemia can harm several organ systems in people with diabetes mellitus, leading to the development of severe and often fatal health issues, most notably microvascular (retinopathy, nephropathy, and neuropathy) and macrovascular issues, which elevate the risk of cardiovascular diseases by two to four times (3,15). Due to increased insulin resistance and vascular dysfunction, aging appears to be a major contributing factor to metabolic and cardiovascular illnesses, as seen by the marked increase in age among T2D and CVD patients (16).

Although BMI is still a well-established risk factor for diabetes and cardiovascular illnesses, the lack of substantial changes in BMI between groups may suggest a fairly comparable distribution of body weight among individuals (17). Hormonal and metabolic differences between males and females, which affect illness susceptibility and cardiovascular disorders, may be the cause of the notable gender disparity (18).

Despite smoking's established significance as a cardiovascular risk factor, the non-significant association may be explained by similar distribution across groups or a small sample size (19). Patients' disease duration was noticeably longer, indicating a higher risk of complications and accumulated metabolic burden (20). Experimental evidence indicates that Klotho exerts antioxidant, anti-inflammatory, and anti-fibrotic effects, protecting organs such as the kidneys, heart, vasculature, and retina. This protection is mediated through its regulation of key metabolic signaling pathways, including Nrf2, Wnt/β-catenin, PI3K/Akt, and TGFβ/Smad, providing a mechanistic basis for its role in mitigating diabetes-induced organ damage (21). Klotho is a protein with protective effects on the kidneys and vascular system. While numerous studies have demonstrated that its levels are low in type 2 diabetes, more recent research has revealed that it may be raised in response to stress and inflammation or in some compensatory circumstances (22). Klotho is strongly linked to cardiovascular diseases and vascular function, and it may have a significant role in evaluating cardiac risk in individuals with type 2 diabetes, according to another recent study (23). Recent studies have shown that elevated levels of Klotho may occur in the early stages of the disease (10,24). Klotho is involved in insulin signaling, oxidative stress regulation, and glucose metabolism (25,26). The results of the current study showed that increased Klotho levels were significantly associated with higher levels of both glucose and HbA1c (27,28).

Klotho and the TyG index, a measure of insulin resistance based on glucose and triglycerides, have a complicated relationship, according to recent National Health and Nutrition Examination Survey (NHANES)-based research (29). An increased risk of vascular disease is indicated by a drop in HDL. In this situation, the increase in Klotho might be a compensatory reaction meant to lessen vascular damage brought on by low HDL levels. Additionally, a recent study has established links between Klotho and lipid markers and cardiovascular risk factors (23) On the other hand, neither total cholesterol nor LDL-c levels showed any discernible variation. This implies that rather than total cholesterol or LDL,

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Klotho may be more strongly linked to glycemic disorders, insulin resistance, triglycerides, and vascular function in the current study.

Preserved renal function in the studied patients may explain the maintained or elevated circulating klotho levels observed in the current study (30). Several recent studies indicate that reduced Klotho protein levels are associated with a decrease in estimated glomerular filtration rate (eGFR) and the progression of chronic kidney disease (CKD), although this decline may not be evident in the early stages of the disease (22). Additionally, a sizable population-based study showed that Klotho is negatively correlated with chronic renal disease and favorably correlated with eGFR, especially in diabetic individuals (31). Therefore, elevated Klotho and unaltered creatinine levels may suggest that kidney damage has not yet reached the point of Klotho depletion and that the body still has a strong renal defensive capacity (32).

In terms of hs-CRP, patients with type 2 diabetes had higher levels than healthy individuals, and those with type 2 diabetes who also had cardiovascular conditions had even higher levels. Given that hs-CRP is a recognized indicator of atherosclerosis and systemic inflammation, this is biologically conceivable (33). Elevated Klotho levels may represent a compensatory anti-inflammatory response, as Klotho inhibits pro-inflammatory cytokines such as Tumor Necrosis Factor alpha and Interleukin 6 and regulates inflammatory pathways including NF-kappa B in diabetes-related chronic inflammation (34). Its preventive function is supported by recent research showing that lower Klotho levels are linked to an increased risk of cardiovascular events and mortality (35). Elevated hs-CRP and elevated Klotho levels together indicate that inflammation is occurring but has not yet completely overwhelmed Klotho's compensating defensive response.

Correlation analysis revealed significant associations between Klotho levels and fasting glucose, triglycerides, Hemoglobin A1c, HDL-C, and High-sensitivity C-reactive protein, indicating that elevated Klotho levels may represent a compensatory response to metabolic and inflammatory disturbances associated with diabetes and cardiovascular disease. In contrast, the absence of significant correlations with creatinine and eGFR, in the setting of no marked renal impairment, suggests that Klotho alterations in this population are more strongly linked to metabolic and inflammatory status than to renal function (36).

The current study's results are in line with a recent study that suggests Klotho is impacted by oxidative stress, glycemic control, and inflammatory status, with the direction of association possibly changing based on renal function, disease stage, and study population characteristics (37).

Roc curve analysis demonstrated good diagnostic performance of serum klotho in distinguishing patients with T2D and those with cardiovascular complications. These findings suggest that klotho may serve as a useful biomarker associated with metabolic and cardiovascular alteration in diabetic patients. However, the findings should be interpreted cautiously due to the relatively small sample size and require further validation in larger multicenter studies (38,39).

However, the findings should be interpreted cautiously due to the potential influence of sample size and the characteristics of the studied population, which necessitates further validation in larger multicenter studies (10).

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Conclusion

In conclusion, serum klotho may represent a potential biomarker associated with metabolic and cardiovascular alterations in patients with type 2 diabetes mellitus.

Ethical Approval: The Iraqi Ministry of Health and the Ethics Committee of the College of Education for Pure Science, Ibn-Al-Haitham, University of Baghdad, granted ethical approval for this study (Approval No. 20250147, dated DD Month 2025/11/27), which was carried out in compliance with the Declaration of Helsinki. Before being included in the study, each subject provided written informed consent. Every piece of information was handled with the utmost confidentiality and used only for study.

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Conflicts of interest: The authors declare no competing of interest.

Author contributions: All authors contributed to the collection of data, the preparation of the draft, and the review and approval of the final version of the manuscript.

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