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RESEARCH ARTICLE

Inverse Correlation Between α-Klotho and Diabetic Biomarkers in Type 2 Diabetes: A Cross-Sectional Study

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Abstract: Evidence suggests a link between diabetes and Klotho protein, demonstrated by reduced Klotho gene expression in individuals with diabetes, α- klotho the potential modulator of kidney health in DM. Klotho is primarily expressed in the kidneys and has an anti-inflammatory and antioxidant role, which may protect against the pathological processes that damage renal function in diabetes. Research conducted in Japan on postmenopausal women revealed a strong inverse relationship between Klotho levels and the insulin resistance marker HOMA-IR. Nevertheless, there have been no studies examining the connection between Klotho and diabetes related biochemical factors like uric acid, urea, and creatinine. This study aims to investigate the correlation between α-Klotho and biochemical markers in patients with diabetes mellitus. Analysis of biochemical parameters showed a significant increase in insulin, HbA1c, FBG, VLDL, cholesterol, and triglyceride levels in patients (both male and female) compared to the control group. Conversely, HDL levels decreased significantly, while LDL remained unchanged. Gene expression analysis indicated an increase in klotho protein expression in patient groups compared to the controls. Fold change analysis further confirmed significantly higher klotho expression in patients. The analysis of the fold change test showed a substantial increase in fold change level for both male and female patients compared to the control group, with a significant level. The statistical evaluation indicated a highly negative correlation between α-klotho expression and blood urea, as well as α-klotho expression and creatinine, at a significance level of P≤ 0.01. Nevertheless, there was a significant inverse relationship between α-klotho expression and HbA1c, as well as α-klotho expression and FBS at P≤ 0.05, while there was a significant positive relationship with LDL but no noticeable correlation between α-klotho expression and the other parameters studied. This study demonstrated an inverse correlation between α-klotho and diabetes- related biochemical markers. High HbA1c is associated with decreased klotho expression, suggesting glucose levels may suppress α-klotho gene transcription. Klotho levels were negatively correlated with urea and creatinine.

Keywords: Ecosystem, Fish Pond Storage, Inert, Gas, Preservation Diabetes Mellitus, Alpha Klotho, HbA1c, Lipid Profile

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Introduction

Klotho, a glycoprotein, has been discovered to have connections to the process of aging and diseases associated with growing older. It is produced and formed in the kidney and choroid plexus of the brain. Research in the past has demonstrated that Klotho provides a protective benefit against aging and diseases related to age. Transgenic mice overexpressing Klotho

have an extended lifespan and display various phenotypic variations when compared to wild-type mice. One interesting characteristic of these mice is that regular mice have high blood sugar and high insulin levels, whereas mice with Klotho have normal glucose levels [1-4].

Diabetes mellitus is a widespread condition that has been rapidly increasing over recent decades. Since the latter half of the 20th century, it has been a significant health issue due to lifestyle changes, environmental factors, and the rising number of individuals with diabetes. Ultimately, it impacts other organs in the body that are metabolically active, which can result in potentially fatal complications [5, 6].

The connection between Klotho and diabetes has been established through the discovery that diabetes patients show reduced expression of the Klotho gene. Research conducted in Japan with postmenopausal women has shown that there is an inverse relationship between Klotho and the insulin resistance marker HOMA-IR. Nonetheless, studies examining the correlation between Klotho and diabetes-related biochemical markers like uric acid, urea, and creatinine are lacking [7, 8].

It seems that Klotho influences insulin levels and controls Phosphatidylinositol 3-kinase (PI3K) activity, leading to changes in glucose metabolism. A different research indicated that Klotho influences glucose absorption and insulin release by impacting the activity of Na+-glucose cotransporter 2 (SGLT2) and glucagon-like Peptide-1 (GLP-1), a hormone that regulates glucose metabolism. This causes higher absorption of glucose in the kidneys through SGLT, leading to a rise in gluconeogenesis, resulting in elevated levels of blood sugar and insulin [9-16].

Endothelial dysfunction is a commonly observed consequence of Klotho deficiency. Mice lacking Klotho show reduced ability of blood vessel relaxation due to the endothelium, which is believed to play a role in the onset of atherosclerosis [2, 17].

It has not been confirmed how α-klotho directly affects biochemical parameters. α-klotho may impact biochemical parameters by affecting insulin levels. Research using rodents found that the klotho gene boosts insulin sensitivity and reduces the development of insulin resistance over time. Since insulin controls blood sugar levels and high blood sugar can cause protein glycation, the impact of α-klotho on insulin might help prevent protein glycation, where a sugar molecule connects to a protein or lipid molecule without enzymatic control. One possible effect of α-klotho on biochemical parameters could be through the prevention of protein glycation. In rats, the overexpression of the klotho gene was found to prevent the rise in protein glycation and decrease other diabetes-related alterations in the kidney. A study on mice showed comparable results; klotho prevented kidney damage caused by diabetes and decreased proteinuria. This research also observed decreased oxidative stress levels in the kidneys of mice overexpressing α-klotho. Preventing protein glycation might also be connected to the reduction of oxidative stress and kidney injury, but the specific mechanisms in this scenario remain unclear [18-21].

This research investigation between α-klotho and diabetes is presented to find the relationship between α-klotho and some biochemical parameters in diabetes mellitus patients.

Patients and Methods

Study Population

Inclusion Criteria

Patients with type 2 diabetes mellitus of both sexes aged from 23-70 years were included in the study.

Exclusion Criteria

Patients with Thyroid gland disorder or cardiovascular diseases were excluded from this study.

Study Groups

Patients with Type 2 Diabetes Mellitus (T2DM) were classified based on glycemic condition by Fasting Blood Glucose (FBG) and HbA1c levels. Poor control was defined when FBG > 150 mg/dL and Hba1c > 7%, while good control was FBG 90- 120 mg/dL and HbA1c 6.5- 7%.

This study involved 96 participants divided into three groups. A healthy control group of 32 individuals (16 males and 16 females), 32 females who are diabetic patients and 32 males who are diabetic patients.

Information was gathered via both fundamental and applied research methods. Epidemiological situation, gender, age group, length of diabetes mellitus, presence of macrovascular complications, and results of lab tests. Generally, nutrition intake is the basis of informatics. Data on nutritional consumption was gathered through the use of a Food Frequency Questionnaire (FFQ) and a 24-hour food recall. Questionnaires were used to inquire about the frequency of diseases and the use of drugs. Fresh blood samples were examined for biochemical parameters.

This research received approval from Tikrit University - College of Pharmacy- Scientific Research Ethical Committee (SREC) on 04. April.2024 with number SREC 15., in accordance with the Declaration of Helsinki. All participants were informed of the study procedures and potential dangers before providing written consent to take part. Data was gathered for this research project from November 2024 to February 2025.

Blood Collection

Five mL of blood was collected from each participant, then was split into two portions. 1 mL was then transferred into EDTA tubes and preserved for RNA extraction. The 4 mL left was in a centrifuged at 4,000 × g for 10 minutes to obtain serum. The obtained serum samples were stored at −80°C until analysis of biochemical parameters, and the serum was kept for analyzing biochemical parameters.

Biochemical Parameters

Fasting Blood Sugar (FBS), total cholesterol, HDL, LDL, Triglycerides (TG), and kidney function were assessed through blood tests. Enzymatic colorimetric method was utilized to measure FBS, cholesterol, and TGs. The measurement of HDL was calculated through clearance techniques utilizing heparin manganese. LDL was determined using the formula (LDL = cholesterol - [HDL + TGs/5]) when TGs were below 400 mg/dL and directly measured using homogeneous enzymatic assay. Serum creatinine and blood urea tests were used to assess kidney function, and all measurements were done with a biochemical analyzer.

RNA Extraction and Gene Expression Analysis

Whole blood sample of approximately 1 mL was used, promptly submerged in 10 mL of RNAlater solution from Ambion (Europe) Limited and kept at 4°C for future RNA extraction. RNA was extracted from tissues by homogenizing them in TRI Reagent_ (Sigma-Aldrich, St. Louis, MO, USA) using TissueRuptor (Qiagen) and purified with RNeasy Mini kit (Qiagen), following the manufacturer's instructions.

RNA quality was assessed with an Experion TM Automated Electrophoresis System and quantified using a Thermo Scientific Nanodrop 2000 spectrophotometer. cDNA was synthesized using a High-Capacity RNA-to-cDNA kit from Applied Biosystems for qRT-PCR. Quantification of transcripts for Klotho and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was conducted through cybergreen qRT-PCR Universal PCR Master Mix (Applied Biosystems).

Running qRT-PCR

The mRNA levels of heat shock proteins and antioxidant enzymes were analyzed using qRT-PCR on a Chinese Bioevapeak Real-Time PCR SystemTM and the SYBR green technique on a GoScriptTM Reverse Transcription System. CT model in relation to something or someone was Statistical analysis is employed to determine relative gene expression using relative qPCR, which is measured by the number of cycles needed for the fluorescent signal to surpass the threshold. The RT-qPCR reaction involved exposing all materials to a total reaction mixture volume of 25µl, consisting of 12.5µl FastStart Universal SYBR Green Master (Rox), 1µl forward primer (20 pmol/l), 1µl reverse primer (20 pmol/l), 2µl cDNA (templates), and 8.5µl sterile water. The RT-qPCR cycling parameters were: a start denaturation for three minutes at 95 oC, then 35 rounds of annealing 95 o C for one minute, and 60 o C for one minute, and elongation at 72 o C for one minute. The Bioevapeak Real-Time qPCR Software (PCR-Q96-5) V. 2022 was utilized for comparing relative quantitative PCR, with the results of each specimen normalized to β actin expression as the housekeeping gene. qPCR has been utilized to evaluate the listed factors. Transcript-specific Gene Expression Assays for Klotho and GAPDH ([Klotho (f: CCTCCTTTACCTGAAAATCAGCC, r: CAGGTCGGTAAACTGAGACAGAG], and [GAPDH] (f: GTCTCCTCTGACTTCAACAGCG, r: ACCACCCTGTTGCTGTAGCCAA)) were analyzed using a Real-Time PCR System from Applied BioSystems. The amount of target mRNA was determined through relative quantification using the 2^-ΔΔCt method by adjusting for GAPDH expression.

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Statistical Analysis

SPSS In. utilized IBM SPSS Statistics 22 for data analysis. Chicago, Illinois, United States of America. Gene expression data was presented as the mean plus Standard Error (S.E). ANOVA is utilized to analyze the data in a single direction. The differences between the groups were analyzed with a significance level below 0.05, which is statistically significant.

Results

This study included 96 participants who were divided into 3 groups. A control group of 32 individuals, half of them males and half females, were in good health. 32 females who are diabetic patients and 32 males who are diabetic patients, all the groups are volunteers of the same age.

The results gene expression test of the α-klotho gene shows an increase in the concentration of α-klotho in Patients groups in comparison with the control group as shown in (Fig. 1).

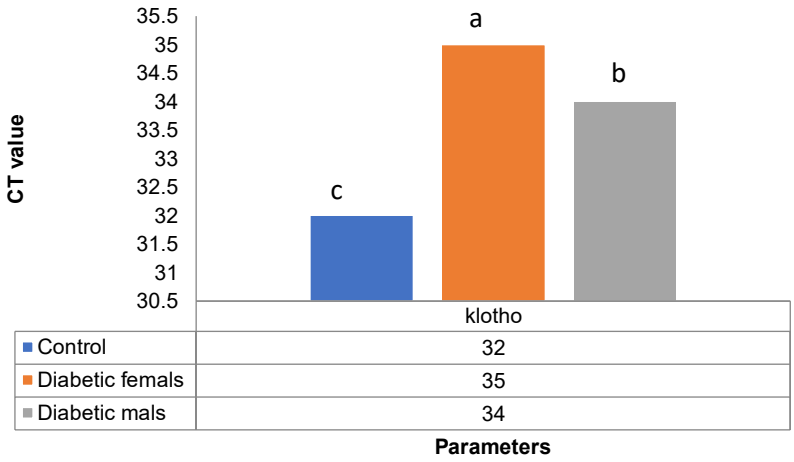

Fig. 1: α-klotho expression level of the study participants

As well as the fold change test results (Fig. 2) revealed a significant increase (P≤ 0.05) in the level of fold change for the patient's group (males and females) in comparison to the control group.

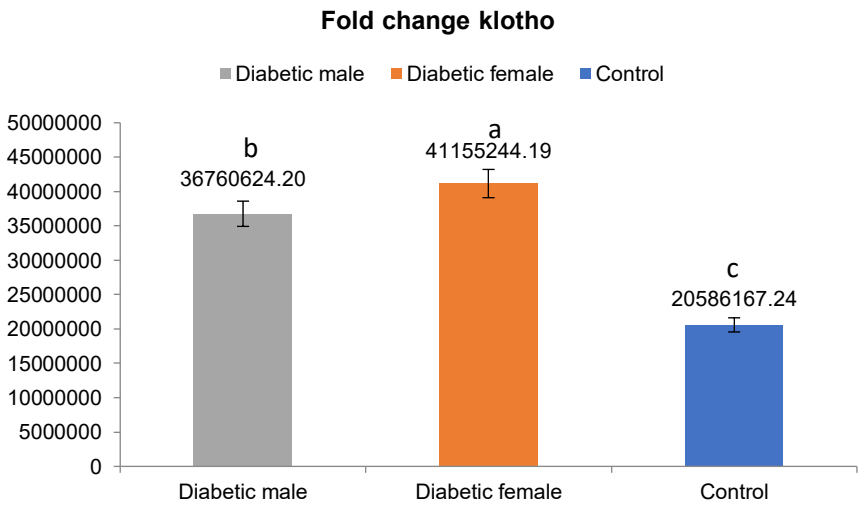

Fig. 2: α-klotho fold change of the study participants

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The results of the biochemical parameters Shows that when compared the Patients groups (male and females) to the control there was a significant increase in the levels of insulin, HbA1c, VLDL shown in (Fig. 3), as well as showed increase in the FBS and TG, however, there was a significant decrease in HDL and no significant change were observed in the cholesterol and LDL levels shown in (Fig. 4).

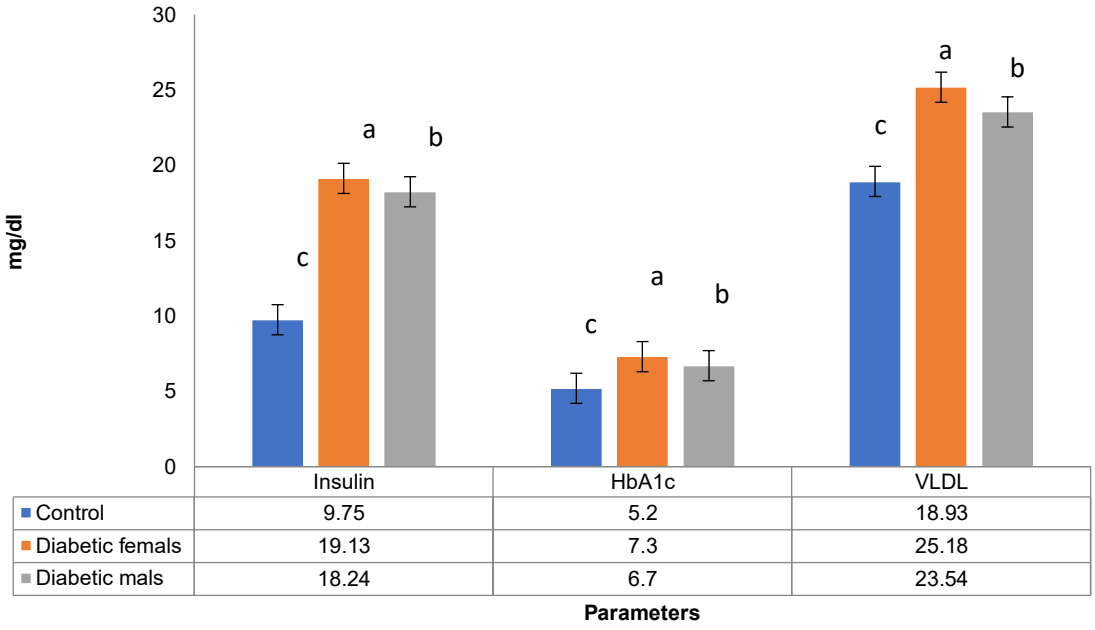

Fig. 3: Insulin, HbA1c and VLDL levels in the serum of study participants

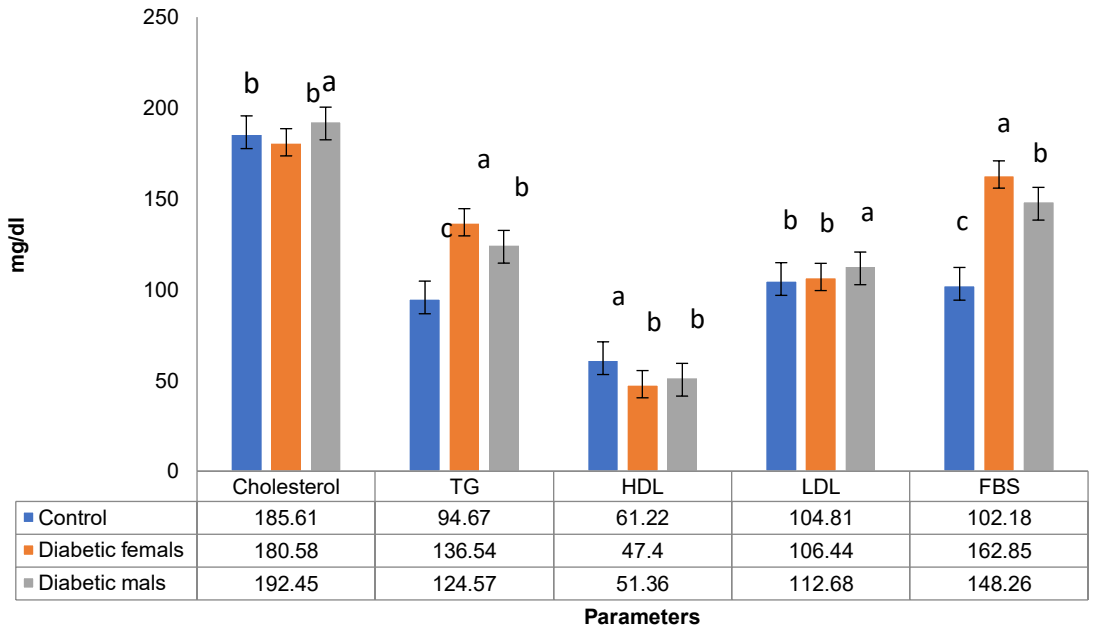

Fig 4: cholesterol, TG, HDL and LDL levels in serum of the study participants

One of the complications of diabetes is its impact on kidney function. Therefore, creatinine and urea were measured. The results showed an increase in creatinine and urea levels in patients compared to the control group, as shown in (Figs. 5, 6).

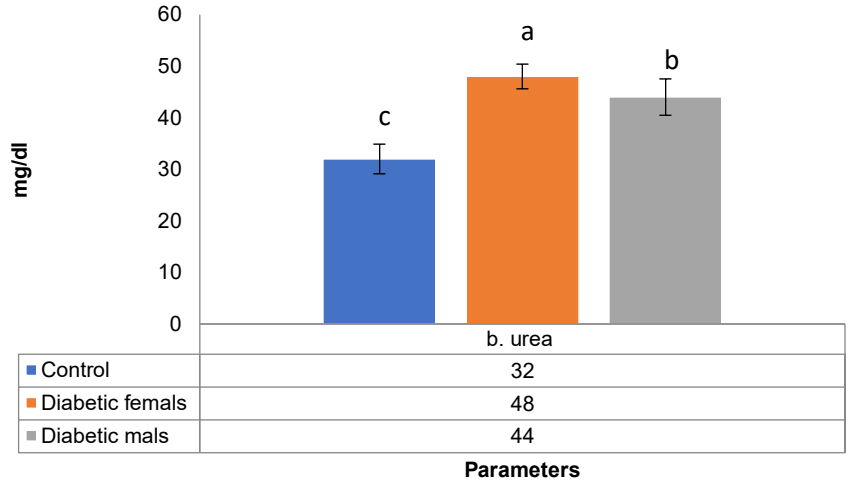

Fig. 5: B. urea levels in serum of the study participants

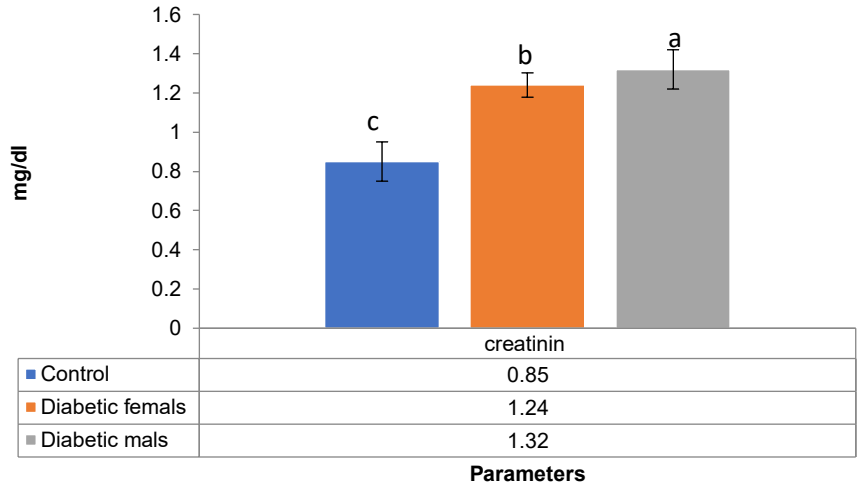

Fig. 6: Creatinine levels in serum of the study participants

In correlation analysis, the statistical analysis found a highly significant negative correlation between α-klotho expression and blood urea (r = -0.94), and α-klotho expression and creatinine (r = -0.681) at a significance level of P≤ 0.01. Nonetheless, a significant adverse relationship was observed between α-klotho expression and HbA1c (r = -0.327), as well as α-klotho expression and FBS (r = -0.421) at P≤ 0.05. Conversely, a notable positive correlation was found with LDL (r = 0.516), while no correlation was evident between α-klotho expression and the other variables examined (Table 1).

Table 1: Correlations between α-klotho Gene Expression and some biochemical parameters in patients

		T-Chol	Tg	HDL	LDL	VLDL	HbA1C	FBS	BUN	Cr
α-klotho	Pearson Correlation	-.021	.082	-.094	-.516*	.088	-.327*	.421*	-.940**	-.681**
	Sig. (2-tailed)	.076	.496	.427	.031	.467	.032	0.021	.001	.016

*. Correlation is significant at the 0.05 level (2-tailed)

**. Correlation is significant at the 0.01 level (2-tailed)

Negative mark preceding the numerical value indicates an inverse relationship

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Discussion

Table 1 shows the relation between α-klotho levels and biochemical parameters in patients. Another study also found a negative relationship between α-klotho and Urea and creatinine. A notable inverse relationship was found between the α-Klotho and the levels of HbA1c and average blood sugar. The levels of α-klotho in circulation show a negative relationship with age and plasma glucose, which reinforces its potential importance in detecting aging- and diabetes-related issues early on [22]. Recent studies has highlighted Klotho, as a potential modulator of kidney health in DM. Klotho is primarily expressed in the kidneys and has anti-inflammatory and antioxidant role, which may protect against the pathological processes that damage renal function in diabetes [23].

The ongoing research found a direct relationship between LDL and expression; yet, other lipid profile parameters showed no connection with α-klotho expression. LDL elevation, especially (oxidized LDL) may promotes oxidative stress and inflammatory signaling (NF-κB, cytokines) that have been shown to reduce Klotho expression, producing an apparent inverse relationship. Several studies support an inverse relationship between α-Klotho and indices of dyslipidemia or atherosclerotic disorders [24]. This result is not consistent with Chua HR et al. who found a correlation between reduced levels of α-klotho and decreased HDL-C as well as elevated LDL-C [25].

The findings demonstrated a significant negative association between urea, creatinine, and α-klotho expression. Research indicates a higher likelihood of diabetes in individuals with decreased α-klotho levels. The involvement of α-klotho in diabetes mellitus and diabetic nephropathy remains a topic of contention [26]. It is reported that the conflicting effects of α-klotho on insulin sensitivity, insulin secretion, and glucose metabolism complicate the interpretation of data, especially in patients with chronic renal failure and diabetes. Enhanced carbohydrate and lipid regulation were observed in diabetic α-klotho ko mice when viral α-klotho overexpression was utilized [27].

Studies found that individuals with more advanced CKD had reduced levels of α-Klotho in comparison to those with better kidney function. Individuals with diabetes who have lower levels of α-Klotho may be at increased risk for more advanced CKD and an increased burden of kidney disease. Additional research indicated that the reduction in serum α-Klotho was linked to higher levels of macroalbuminuria and proteinuria, providing more evidence for the relationship between α-Klotho and the initial phase of DN (minimal albuminuria), as well as its role in kidney function through anti-fibrotic effects, inhibiting inflammation, and increasing nitric oxide availability for greater vasodilation in a dose-dependent manner [28].

Current research is centered on synthetic and natural Klotho compounds that attach to FGFR and promote advancement rather than external Klotho. Furthermore, in comparison to physical activity, there are no noteworthy levels produced in a clinical setting. Walston highlighted the potential of oral Klotho to slow down beta cell aging and lower fasting blood glucose levels and post glucose consumption in middle-aged obese individuals. Although these items are included in the endocrinologist's guidelines for managing diabetes, the current technology makes it difficult to incorporate exogenous Klotho into human clinical and mental health practices. The Klotho benefit may be crucial in regulating mitochondrial energy metabolism, since it has not been recognized as beneficial [26].

Another research study showed that individuals with lower glycosylated Hb have decreased α-klotho levels, and Type 2 Diabetes speeds up the aging of β-cells, with α-Klotho decreasing at a rate similar to that of the normal control group. Kim and colleagues showed that elevated glucose cause deacetylation and lower transcription, leading to the activation of Klotho and its antioxidant and anti-inflammatory effects. During an in vivo study and in 24-hour urine samples, levels of FGF-23-Aldose reductase, DLL-4-associated AGE-brachial artery stiffness, biopterin, and proteinuria were elevated in the absence of Klotho [19, 29].

Conclusion

Based on our research results, we have observed a direct correlation between diabetes and low levels of α-klotho. The study found that individuals with elevated HbA1c have decreased α-Klotho compared to those with lower levels of glycosylated hemoglobin, as high glucose decrease the transcription and expression of α-Klotho. There was also an inverse relationship between urea, creatinine, and α-klotho levels.

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Authors Contributions

Yaser A.M Sulaiman: Designed and organized the research, measured the parameters and analyzed the data, wrote and reviewed the manuscript.
Moayad M. Al Anzy: Designed and organized the research, measured the parameters and analyzed the data.
Omeed Akbar Ali: Designed and organized the research, measured the parameters and analyzed the data, wrote and reviewed the manuscript.
Adnan M. Ismail: Designed and organized the research.

Ethics

The study was submitted to Tikrit University - College of Pharmacy- Scientific Research Ethical Committee (SREC) and approval was received on 04. April.2024 with number SREC 15.

Data Availability

Data are available on request from the authors.

Conflict of Interest

The authors declare that they have no conflict of interest.

Human Rights Declarations and Informed Consent

All procedures used in the study were carried out in accordance with the ethical stance of the committee responsible for human experimentation (institutional and national) and the 1964 Declaration of Helsinki. Written informed consent was obtained from all patients included in the study.

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