

Article

Evaluation of Klotho, Vitamin D3, and Uric Acid Levels in Patients with Chronic Kidney Disease in Al-Sharqat City

Arkan Tawfiq Muhammad ¹

1. Tikrit University, College of Basic Education / Al-Sharqat, Department of Science

*Correspondence: maanqqqq@gmail.com

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Abstract: Compare the levels of Klotho protein in individuals with chronic kidney disease (CKD) to those in healthy individuals. Ninety individuals took part in the study: sixty patients with chronic renal disease and thirty healthy individuals as a control group.

With a mean level of 340 ± 95 pg/mL compared to 720 ± 110 pg/mL in the control group, the levels of Klotho protein were considerably lower in CKD patients than in healthy individuals ($P < 0.001$). Additionally, the mean level of vitamin D3 was substantially lower in patients (14.9 ± 3.7 ng/mL) than in healthy individuals (52.4 ± 3.8 ng/mL) ($P < 0.001$).

In contrast, the study showed a significant increase in uric acid levels in patients with chronic kidney failure, with an average of (8.70 ± 1.30) mg/dL compared to (5.00 ± 0.80) mg/dL in the control group ($P < 0.001$).

Keywords: Kidney failure; Klotho; Vitamin D3; Uric acid;

1. Introduction

The kidney is a vital organ that resembles the shape of a bean, and the kidneys are located on both sides of the spine in the middle of the lower back. One kidney in an adult human weighs about 150 g and contains approximately one million filtration units called nephrons. (1) The nephron consists of the glomerulus, the collecting duct, the proximal and distal tubules, and the loop of Henle. (2) The main function of the kidney is to filter and eliminate waste and poisons; it also controls the pH, ions, and fluid balance. (3) Renal failure is the inability of the kidneys to perform the excretory function, which results in the retention of nitrogenous waste from the circulation. There are two types of kidney failure: acute kidney failure (ARF) and chronic kidney failure (CRF). A decline in kidney function, or what is called a decrease in the estimated glomerular filtration rate (eGFR) to less than 60 ml/min/1.73 m² for at least three months, is the hallmark of chronic kidney failure (CRF), regardless of the cause. (4).

Three types of disorders can cause chronic kidney disease: extrarenal (low renal perfusion pressure), intrinsic renal (vascular, glomerular, or tubulointerstitial) diseases, or postrenal anuria (obstruction) (5).

One of the most significant bioactive substances that is essential to cells is glutathione. Glutathione is the scientific name for this endogenous antioxidant that the body spontaneously produces. Glutamate, cysteine, and glycine are its three constituent amino acids. Glutathione is essential for immune system support, detoxification, and shielding cells from oxidative stress and free radical damage. (6)

Sunlight (ultraviolet rays) is a major source of vitamin D, which is linked to bone health. Vitamin D is a fat-soluble steroid hormone that plays a vital role in regulating calcium and phosphorus balance in many tissues and organs (7). There are two types of vitamin D, sometimes called calciferols: vitamin D₂ (ergocalciferol), which is usually given orally, and vitamin D₃ (cholecalciferol), which is produced by the body. (8) Vitamin D₃ is produced by exposure to ultraviolet radiation and is synthesized internally in humans or obtained from animals (liver, fish, and egg yolks), while vitamin D₂ is produced by plants and fungi (9)

The UA is a heterocyclic organic compound comprising of carbon, hydrogen nitrogen, and oxygen, with the formula C₅H₄N₄O₃, with a molecular weight of 168Da. It is the major result of purine nucleoside catabolism in humans. Xanthine oxidase converts adenosine and guanosine to xanthine, a common intermediate molecule that is oxidized to UA (10)

Objectives of the Study

1. Measurement of serum Klotho protein levels in patients with chronic kidney disease and healthy individuals.
2. Determination of serum vitamin D₃ levels in both study groups.
3. Assessing uric acid levels in the studied samples
4. Comparison of these biomarkers between patients and healthy individuals.

2. Materials and Methods

2.1 Study Design and Sample Collection

This case-control study was conducted from January 2025 to March 2025 at the dialysis unit of Al-Sharqat Hospital in Salah Al-Din Governorate. The study included 90 participants: 60 patients diagnosed with chronic kidney disease by nephrologists based on established clinical and laboratory criteria, and 30 Apparently healthy individuals were selected as a control group. Prior to sample collection, all participants provided written informed consent,

and the study was conducted in accordance with accepted ethical guidelines for medical research.

2.2 Blood Sample Collection

Each participant had five milliliters of venous blood extracted using sterile syringes in a hygienic setting. After being collected in tubes devoid of anticoagulants, the samples were allowed to coagulate for 15 to 20 minutes at room temperature. After that, the serum was centrifuged for ten minutes at 3000 rpm. Before being examined in a lab, the serum samples were moved to sterile Eppendorf tubes and kept at -20°C.

2.3 Laboratory Measurements

2.3.1 Serum levels of vitamin D3 (25-hydroxyvitamin D), Klotho protein, were measured using enzyme-linked immunosorbent assay (ELISA) according to the diagnostic kit manufacturer's instructions

2.3.2 Uric acid concentration was estimated using the enzymatic colorimetric method with an automated clinical chemistry analyzer, following the reagent manufacturer's standard procedures.

2.4 Statistical Analysis

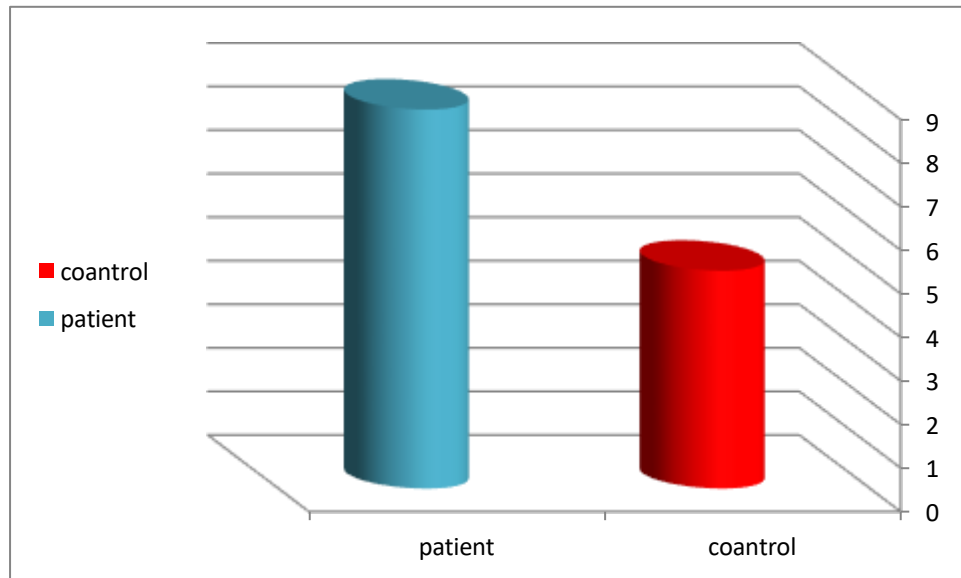
SPSS version 26 was used to analyze the data. The mean $\pm$ standard deviation (Mean $\pm$ SD) is used to express the results. Patients and healthy people were compared using the Independent Samples t-test. When necessary, qualitative variables were additionally examined using the Chi-square test. At a probability threshold of $P < 0.05$, differences were deemed statistically significant.

3. Results and discussion

Table 1. Comparison of the research parameters of patients group with control group:

Groups Parameters	Mean $\pm$ SD		P-value
	Control	Patients	
Klotho	720 $\pm$ 110	340 $\pm$ 95	0.0001**>
Vitamin D3	52.4 $\pm$ 3.8	14.9 $\pm$ 3.7	0.0001**>
uric acid	5.00 $\pm$ 0.80	8.70 $\pm$ 1.30	0.0001**>

* $P \leq 0.05$, ** $P \leq 0.01$,



1Figure 1 shows the Klotho level in the patient group compared to the control Figure group

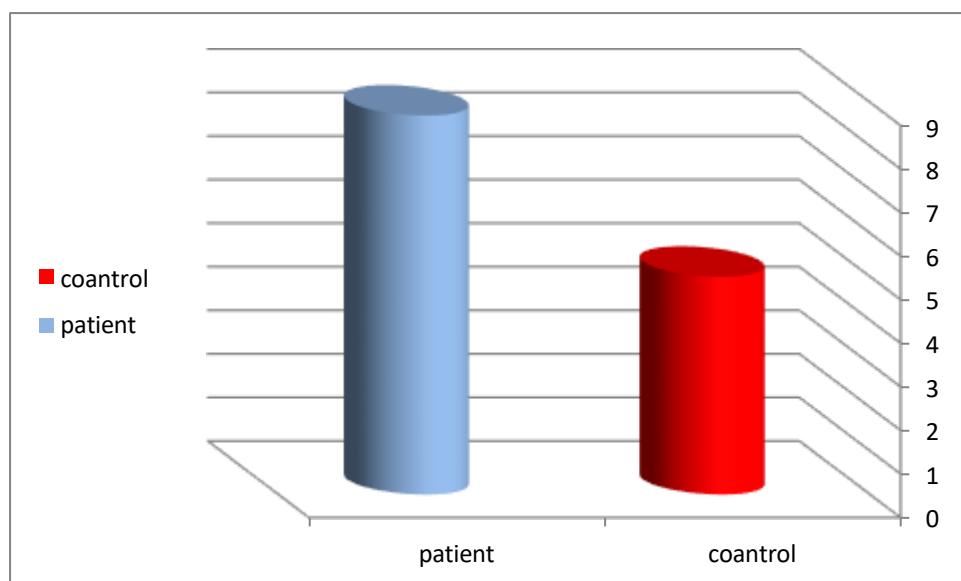


Figure 2 shows the D3 level in the patient group compared to the control group.

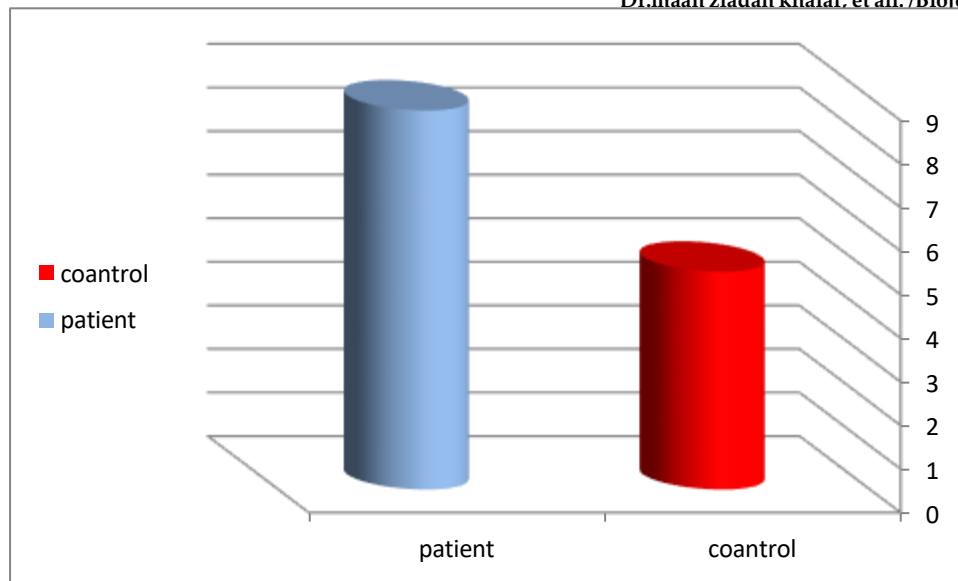


Figure 3 shows the uric acid level in the patient group compared to the control group

According to the findings, individuals with renal failure had significantly lower levels of Klotho protein (340 ± 95 pg/mL) than the healthy group (720 ± 110 pg/mL), and this difference was statistically significant ($P < 0.001$). The results of this investigation are in line with those of (Hu Ming Chang et al. 2021), who demonstrated that Klotho protein deficiency is a hallmark of chronic kidney disease and that its low levels are associated with impaired phosphate and calcium metabolism, increased inflammation and oxidative stress, both of which worsen kidney function and accelerate the course of illness. Additionally, research has demonstrated that as chronic renal disease advances, serum and urine Klotho levels progressively decrease. (11) The results of this study are consistent with those of Satoh Minoru et al. (2012), who showed that Klotho functions as an inhibitor of the Wnt/ β -catenin pathway and that its low levels cause this pathway to be activated, increasing renal fibrosis and extracellular matrix deposition, which accelerates the deterioration of kidney tissue and function. (12). According to (Kuro-o Makoto: 2011), Klotho deficiency is a precursor to chronic renal disease and is linked to increased levels of parathyroid hormone and FGF23 as well as lower levels of active vitamin D, which can result in problems related to bone and minerals (13). Klotho levels are positively connected with the glomerular filtration rate (eGFR), and they steadily decrease as kidney function deteriorates and illness advances, according to several studies. Klotho is therefore regarded as a crucial biomarker for the identification, tracking, and forecasting of the course of chronic renal disease (14).

CKD patients had considerably lower serum vitamin D3 levels (14.9 ± 3.7 ng/mL) than healthy controls (52.4 ± 3.8 ng/mL), with a very significant difference ($P < 0.001$). The results of the current study are consistent with those of Ganimusa et al. (2024) and Ravani et al. (2009), whose studies showed that vitamin D levels were lower in patients with chronic kidney disease compared to the control group. One of the most common problems in chronic kidney disease is vitamin D deficiency, as levels of both the active and stored forms of the vitamin decrease with a reduction in glomerular filtration rate (GFR) (15, 16).

In order to transform the inactive 25(OH)D form into the active 1,25(OH)₂D form (calcitriol), the kidneys are essential. Reduced synthesis of calcitriol is caused by decreased renal mass, decreased 1- α -hydroxylase enzyme activity, and increased levels of phosphorus and FGF23. Increased activation of the renin-angiotensin-aldosterone system (RAAS), which aggravates

kidney impairment and raises blood pressure, is also linked to vitamin D deficiency(17, 18) Furthermore, regulatory factors such as parathyroid hormone (PTH) and fibroblast growth factor 23 (FGF23) affect vitamin D metabolism. Elevated levels of these hormones are present in patients with chronic kidney disease, inhibiting calcitriol production and thus reducing their levels. Consequently, chronic kidney disease may develop in vitamin D deficiency and increase the risk of kidney and cardiovascular problems (19).

According to the findings, individuals with chronic renal disease had higher blood uric acid levels (8.70 ± 1.30 mg/d) than the control groups (5.00 ± 0.80 mg/dL), with a difference ($P < 0.001$). According to the current study's findings, individuals with renal failure had significantly higher levels of uric acid (UA) than the control group. This result is in line with earlier research showing increased uric acid levels in renal failure patients.

This increase is attributed to a decrease in glomerular filtration rate (GFR) and impaired renal tubular secretion, leading to uric acid accumulation in the blood. Furthermore, hyperuricemia may contribute to Dysfunction of the vascular endothelium, kidney inflammation, and increased intraglomerular pressure accelerate the progression of chronic kidney disease and problems that lead to kidney damage

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5. Conclusion

The results of this study revealed clear and significant differences in the levels of gluten, vitamin D3, and uric acid among patients with kidney failure. Our results showed a marked decrease in the levels of both gluten protein and vitamin D3, while conversely, The uric acid concentration was low in the control group.

6. Recommendations

1. The studied biomarkers, including Klotho protein, vitamin D3, and uric acid, may be useful in patients with chronic kidney disease to aid in early detection and monitoring of disease progression.
2. Future studies with larger sample sizes and longer timeframes are needed to verify and determine the effective predictive role of these biomarkers in disease progression and complications.
3. Evaluate the relationship between these biomarkers and kidney function parameters, particularly estimated glomerular filtration rate (eGFR), serum creatinine levels, and serum urea levels.

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