

Correlation of Alpha-Klotho Levels with Biochemical Parameters in Chronic Kidney Disease Patients Undergoing Dialysis and Non-Dialysis Treatment

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ABSTRACT

Background and Aims: The Klotho protein, a coreceptor for Fibroblast Growth Factor-23 (FGF23), plays a crucial role in regulating phosphate homeostasis and vitamin D metabolism. Emerging evidence suggests that reduced α -Klotho levels are significantly associated with the development and progression of chronic kidney disease (CKD). This study aimed to evaluate α -Klotho as a potential biomarker for early detection of CKD and assess its correlation with biochemical parameters in patients with CKD undergoing dialysis and non-dialysis treatments. **Methods:** The study included 150 participants aged 20 years and older: 50 healthy controls, 50 patients with end-stage renal disease (ESRD) on hemodialysis, and 50 CKD patients not undergoing dialysis. Participants were recruited from the dialysis center at Al-Ramadi Governorate and Al-Ramadi Teaching Hospital, Iraq. Serum levels of α -Klotho, eGFR, PTH, phosphorus (P), calcium (Ca), hemoglobin (Hb), hematocrit (Hct), sodium (Na⁺), potassium (K⁺), chloride (Cl⁻), creatinine, and urea were measured. **Results:** A significant reduction in α -Klotho levels was observed in ESRD patients undergoing dialysis (83.28 ± 37.33 pg/mL) and CKD patients without dialysis (100.82 ± 42.72 pg/mL) compared to healthy controls (158.66 ± 52.46 pg/mL). Patients exhibited elevated levels of creatinine, urea, PTH, phosphorus, potassium, and uric acid, while levels of Na⁺, calcium, eGFR, and chloride were significantly reduced. Correlation analysis revealed a positive relationship between α -Klotho and eGFR, Na⁺, Hb, and Hct, whereas negative correlations were observed with creatinine and uric acid. No significant correlations were found between α -Klotho and P, K⁺, Cl⁻, or PTH. **Conclusion:** The findings highlight the potential of α -Klotho as a biomarker for kidney function in CKD patients. Its positive correlation with eGFR and other biochemical markers suggests its utility in assessing disease progression and guiding clinical management. Further studies are warranted to validate α -Klotho as a diagnostic and prognostic tool in CKD.

Keywords: Chronic Kidney Disease (CKD), Hemodialysis (HD), α -Klotho, eGFR (Estimated Glomerular Filtration Rate)

INTRODUCTION

Over two decades have passed since the discovery of α -Klotho as a gene linked to animal aging. Both the function of FGF family members and phosphate homeostasis is impacted. Phosphate equilibrium and vitamin D metabolism are influenced by the fibroblast growth factor (FGF23) receptor klotho protein [1]. The brain's choroid plexus, parathyroid glands, and proximal and distal renal tubules are where klotho is most commonly expressed [2]. Additional evidence indicates that klotho, a gene formerly thought to be anti-aging, is considerably downregulated in conjunction with the onset and progression of chronic kidney disease (CKD) [3]. The first evidence that the klotho gene suppresses aging came from this discovery. There are three different isoforms of the protein Klotho, which are all expressed by different genes: α Klotho (henceforth referred to as Klotho), β Klotho, and α Klotho [4]. Additionally, it is possible to distinguish between two kinds of Klotho: soluble or shed (sKlotho) in addition membranes (mKlotho) [5].

When mKlotho is broken down by proteases, the extracellular domain (KL1 and KL2, which collectively weigh around 130 kDa) is known as sKlotho. KL1 and KL2 repeats, which are roughly 65 kDa apiece, are found in the extensive extracellular domain of mKlotho, a transmembrane protein [6]. Interestingly, there is an additional soluble version of Klotho that is produced by an alternate method. This mRNA splicing process is called secreted Klotho (seKlotho) [7].

MATERIALS AND METHODS

Twenty-eight male and twenty-two female healthy controls, ages 20 to 60, were involved in the investigation. Of the one hundred patients, two groups had chronic renal disease: There were 50 ESRD cases with dialysis in Group 1 (twenty-seven men and twenty-three women). Group 2 consisted of twenty-one male and twenty-nine female patients with chronic renal disease who were not receiving dialysis. The age range of the subject's patients and controls was from 20 to 60. The study was conducted at the Artificial kidney unit of Al-Ramadi Teaching Hospital in Al-Anbar Province, between December 1, 2023, and June 30, 2024. Following their consent, patients with chronic renal disease had their lifestyle and treatment experience taken into account when the samples were obtained. Exclusion criteria included patients with cardiovascular disease and those who were younger than twenty or older than sixty.

Measurement of Biochemical Markers

About Six mL of venous blood were drawn from subjects (control and patients), blood was collected and divided into two tubes: two mL in a K-EDTA tube for Hb and Hct, and four mL in a gel plain tube, for obtaining serum through centrifugation after clotting within room temp. (18–25 °C) at 2050 xg for 15 min. Serum that obtained was

divided for 2 groups. First one was utilized for biochemical parameters, and CRP and PTH hormones. Second one of the serums was stored at -20 °C to measure serum α -klotho, using an ELISA kit from SunLong Biotech Company, China.

Statistical Analysis

With SPSS-28, all analyses were carried out. The standard deviation (SD) and basic mean of the data were displayed. The student t-test for differences among two independent means was used to assess the significance of discrepancies between different means. When the P-value was equal to or less than 0.05, statistical significance was established. Bivariate relationships were examined using Pearson correlation coefficients.

Results

In Table 1, the individuals' standard experimental features are shown. The eGFR levels (mL/min) of CKD in the dialysis patients' group were significantly lower than those of the control group (9.85 ± 4.8) vs (108.62 ± 11.25) also, CKD without dialysis patients'

group (22.12 ± 17.94) vs (108.62 ± 11.25) with a p-value of 0.001#. Additionally, the serum levels of Hb and Hct were significantly lower in the CKD with dialysis patients' group than the control group (8.88 vs 13.87 g/dl) and (27.91 vs 41.17), respectively. Also, in CKD without dialysis patients' group (10.15 vs 13.87 g/dl) and (31.17 vs 41.17), respectively. The study revealed a "significant decrease in serum levels" of α -Klotho (pg/mL), Na^+ (mmol/L), Iron(μ g/dl), and Calcium(mg/dl) in the CKD with dialysis patients group compared to the control group ($p < 0.05$). (83.28 vs 158.66), (134.06 vs 140.38), (92.19 vs 97.36), and (8.86 vs 9.18) respectively, Also in CKD without dialysis patients group compared to the control group ($p < 0.05$). (100.82 vs 158.66), (136.16 vs 140.38), (94.19 vs 97.36), and (8.81 vs 9.18) respectively, There was a significant increase ($p = 0.001$) in serum levels K^+ (mmol/L) in the CKD with dialysis patients group than the control group (4.63 vs 3.98). Also, in CKD without dialysis patients group compared to the control group (4.29 vs 3.98). The results of this study showed a positive correlation of α -Klotho with eGFR, Hb, Hct, and Na^+ as shown in (Table 2), While no correlations were observed between α -Klotho with Calcium, P, K^+ , and Cl^- .

Table 1: Mean $\pm$ SD for the Measured Variables in Patients and Control Group

Variable	Control Group Mean $\pm$ SD	CKD With Dialysis Patients Group Mean $\pm$ SD	P-Value	CKD Without Dialysis Patients Group Mean $\pm$ SD	P-Value
Age (years)	41.7 $\pm$ 11.6	46.4 $\pm$ 12.8	0.06	50.7 $\pm$ 10.9	0.001#
BMI (Kg/m ²)	26.05 $\pm$ 2.64	25.40 $\pm$ 4.72	0.060	26.01 $\pm$ 2.80	0.001#
eGFR (mL/min)	108.62 $\pm$ 11.25	9.85 $\pm$ 4.8	0.001#	22.12 $\pm$ 17.94	0.001#
Hb (g/dl)	13.41 $\pm$ 1.33	8.88 $\pm$	0.001#	10.15 $\pm$ 1.83	0.001#
Hct (%)	41.17 $\pm$ 2.64	27.91 $\pm$ 3.62	0.001#	31.17 $\pm$ 5.78	0.001#
α -Klotho (pg/mL)	158.66 $\pm$ 52.46	83.28 $\pm$ 37.33	0.001#	100.82 $\pm$ 42.72	0.001#
Iron(μ g/dl)	97.36 $\pm$ 40.33	92.19 $\pm$ 39.62	0.001#	94.19 $\pm$ 41.19	0.001#
P (mg/dL)	3.42 $\pm$ 0.41	4.72 $\pm$ 1.79	0.001#	4.98 $\pm$ 1.81	0.001#
Ca (mg/dL)	9.18 $\pm$ 0.46	8.86 $\pm$ 0.77	0.014#	8.61 $\pm$ 0.72	0.001#
Na^+ (mmol/L)	140.38 $\pm$ 1.78	134.06 $\pm$ 3.48	0.001#	136.16 $\pm$ 2.84	0.001#
K^+ (mmol/L)	3.98 $\pm$ 0.34	4.63 $\pm$ 0.63	0.001#	4.29 $\pm$ 0.53	0.001#
Cl^- (mmol/L)	105.14 $\pm$ 2.09	104.03 $\pm$ 2.92	0.031#	105.21 $\pm$ 2.43	0.033#

#Significant Difference between two Independent Means using Students-t-test at 0.05 level.

Table 2: The Correlation between α -Klotho and Studied Variables in Patients and Control Group

P-Value	CKD with Dialysis R (A-Klotho Pg/Ml)	P-Value	CKD Without Dialysis R (A-Klotho Pg/Ml)	P-Value
Hb (g/dl)	0.195	0.175	0.061	0.674
Hct (%)	0.214	0.135	0.100	0.488
Iron(μ g/dl)	-0.205	0.154	0.049	0.735
P (mg/dL)	-0.069	-0.069	0.053	0.717
Ca (mg/dL)	-0.052	0.720	-0.220	0.124
Na^+ (mmol/L)	-0.129	0.370	-0.103	0.476
K^+ (mmol/L)	-0.161	0.265	-0.022	0.879
Cl^- (mmol/L)	0.151	0.295	0.047	0.745
eGFR (mL/min)	0.149	0.303	0.023	0.875

*Correlation is Significant at P-Value 0.05. **Correlation is Significant at P-Value 0.01

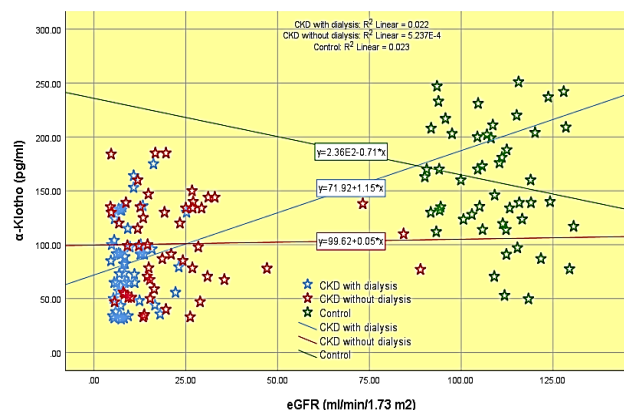


Figure 1: Correlation between α -Klotho with eGFR

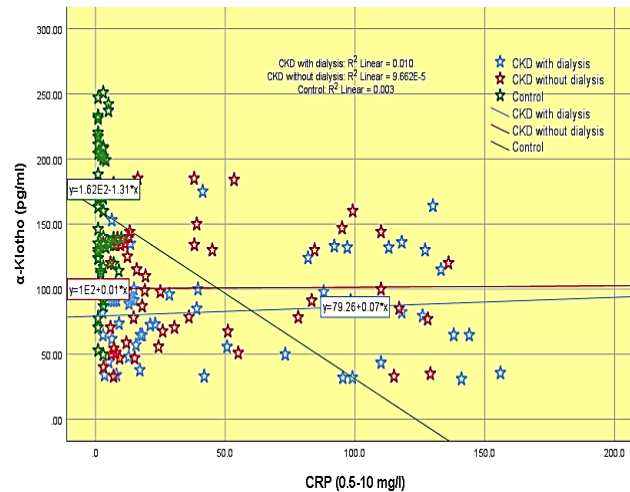


FIGURE 2: Correlation between α -Klotho with CRP

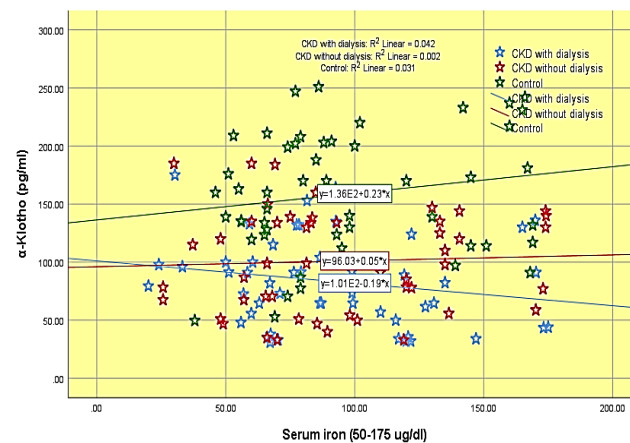


FIGURE 3: Correlation between α -Klotho with Serum Iron

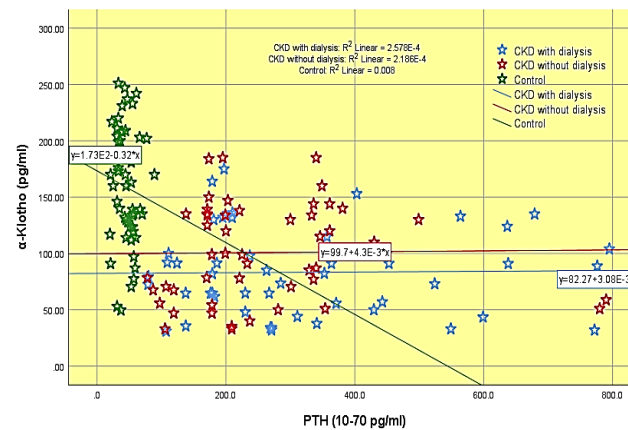


FIGURE 4: Correlation between α -Klotho with PTH

Discussion

In addition to being crucial for the urinary system, kidneys perform homeostatic roles in blood pressure regulation (preserving the balance between salt and water), electrolyte regulation, and acid-base regulation. They eliminate wastes that are directed to the bladder and act as the body's natural blood filter [8]. More data has recently emerged suggesting that klotho, once thought to be an anti-aging gene, is drastically declining as chronic kidney disease (CKD) develops and progresses. In this study, α -Klotho levels were significantly low in

patients compared to apparently healthy controls. Our findings are in agreement with another study [9]. Low levels of circulating α -klotho were linked to unfavorable outcomes for kidney disease [10]. s-Klotho levels were significantly impacted by renal function, with a discernible decline beginning at CKD stage 2. Given that s-Klotho is often eliminated by urine [11]. This reduction in serum levels along with progressive renal damage can be most probably explained by reduced renal synthesis. In fact, decreased excretion from renal injury would raise serum levels in the event of sustained renal Klotho synthesis. Alternatively, serum levels wouldn't drop in a predictable manner if

renal excretion continued to be normal [12]. Each of the two types of Klotho—the secretory and membrane versions—has a different purpose. Membrane Klotho functions as a necessary co-receptor for FGF23, a hormone generated from bone that causes the excretion of phosphate in the urine [13]. Chronic kidney disease (CKD) is a major global public health concern, yet until kidney failure sets in, the disease often progresses slowly and has few distinct symptoms. All agree that in order to approve new medications intended to decrease the progression of chronic kidney disease (CKD), biomarkers will be required[14-16]. Glomerular filtration rate (GFR) and albuminuria are the two most extensively researched biomarkers in chronic kidney disease (CKD); nonetheless, there is debate regarding their suitability as proxy endpoints for significant clinical outcomes (often called clinical endpoints) in clinical studies, particularly in the early stages of CKD[17]. GFR and albuminuria are commonly used as indicators of renal impairment and function. The definition and categorization of chronic kidney disease (CKD) are based on increased albuminuria and reduced GFR, which are also among the most powerful risk factors for renal disease consequences, such as the development of kidney failure, cardiovascular disease, and death[18]. The glomerular capillary wall's surface area, permeability to water and tiny solutes, and filtration pressure all affect GFR. The average single-nephron GFR is multiplied by the number of nephrons to determine GFR[19].

The loss in other kidney functions frequently coincides with the decline in GFR, which is widely regarded as the most helpful overall indicator of renal function in both health and sickness. Since kidney failure is defined as a significant drop in GFR, GFR decline is by definition on the path that leads to kidney failure for all renal disorders, and it has a stronger correlation with the development of kidney failure and its consequences than elevated albuminuria[20]. In the current study, the level of e GFR was significantly lower in patients compared to the healthy control group. This study agrees with the results of another study would suggest that Patients with established chronic kidney disease may have better risk prognoses if

the drop in eGFR is identified over time[21]. The data of this study showed a significant decrease in the levels of Hb in CKD patients as compared with control group. The study agrees with A. S. Go *et al.*, [22]. decreased hemoglobin levels in kidney illness that is persistent. Anemia is a frequent side effect of chronic kidney disease (CKD) that is linked to worsening kidney function, lower quality of life, an increased risk of sickness, a higher death rate, and higher medical expenses[23]. One of the most prevalent electrolyte issues in patients with end-stage renal failure is sodium imbalance. Dialysis patients are susceptible to hyponatremia through a variety of pathways, so determining the extracellular volume (ECV) status is a crucial first step in identifying potential causes. Furthermore, a number of large population-based studies have discovered that residual renal function degradation, low BMI, and levels of S. albumin and S. creatinine are important predictors of hyponatremia in hemodialysis patients [24]. The present study shows a decrease in serum Na⁺ concentrations between the patients group and control group. The result is consistent with the study done by Kadhim *et al.*, [25]. Our study showed that α -Klotho has a positive correlation with eGFR ($r=0.149$), Na⁺ ($r=0.129$), Hct ($r=0.214$), and Hb ($r=0.195$) in CKD with dialysis Patient group also, α -Klotho has shown a weak positive correlation with Hct ($r=0.100$), Hb ($r=0.195$) in CKD without dialysis Patient group. The findings of this research are consistent with these results[26-28]. Serum α -Klotho levels were linearly associated with eGFR, Na⁺, Hb, and Hct, this result is in agreement with the research findings[29-31]. There was no correlation between PTH, P, K⁺, and Cl⁻.

Conclusion

The α -Klotho was lower in patients compared to healthy controls. The results indicate a positive correlation between α -Klotho and eGFR, Na⁺, Hb, and Hct. Additionally, our study found no statistical correlation to suggest an effect of α -Klotho on these parameters (PTH, P, K⁺, Cl⁻).

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