Al-Qadisiyah Journal of Pure Science
ISSN(Printed): 1997-2490 ISSN(Online): 2411-3514
DOI: 10.29350/jops
Al-Qadisiyah Journal of Pure Science

Association between Alpha-Klotho level and some biochemical parameters in dialysis and non-dialysis-treated chronic kidney disease patients

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Abstract

Background /Aims:

Klotho protein is the receptor for Fibroblast Growth Factor-23 (FGF23), regulating phosphate homeostasis and vitamin D metabolism. Recently, more evidence has suggested that the development and progression of CKD are significantly associated with a decline in klotho. Therefore, this study aims to investigate the controversy on the potential role of α- Klotho as an early biomarker in Chronic Kidney Disease (CKD), to assess whether α-Klotho is a reliable marker of kidney.

Methods: The study included 50 control groups, 50 with end-stage renal disease undergoing hemodialysis and 50 CKD without dialysis patients, in the dialysis center at Al-Ramadi Governorate and Al-Ramadi Teaching Hospital, Al-Anbar, Iraq. They were aged 20 years or over. α- Klotho, eGFR, PTH, P, Ca, Hb, Hct, Na^+, K^+, Cl^-, Creatinine, and Urea were measured in both patients and the control group.

Results: This study demonstrated a substantial decrease in the mean concentration rate of α- Klotho (83.28±37.33 pg/mL) in ESRD with dialysis patients and (100.82±42.72) pg/mL CKD without dialysis patients compared to the mean concentration rate in the control group (158.66±52.46 pg/mL). Additionally, the study showed a significant increase in the concentration of creatinine, urea, PTH, P, K+, and uric acid while a significant decrease was observed in the concentration of Na+, calcium, eGFR, and Cl in patients.

Conclusion: The results showed that α- Klotho has a positive correlation with eGFR, Na+, Hb, and Hct. A negative correlation was noticed between α-Klotho with creatinine and Uric acid. Moreover, there were no correlations with Cl^- P, K^+, and PTH.

Keywords: Chronic kidney disease (CKD), Hemodialysis (HD), α-Klotho, eGFR (Estimated Glomerular Filtration Rate).

1.Introduction

For almost 30 years, α-Klotho has been identified as a gene involved in the aging process in animals. It influences phosphate homeostasis and the activity of FGF family members. Klotho protein is a fibroblast growth factor (FGF23) receptor that influences phosphate balance and vitamin D metabolism [1]. Klotho is mostly expressed in the proximal and distal renal tubules, parathyroid glands, and the choroid plexus in the brain [2]. More data suggest that the development and progression of CKD are significantly associated with a drop in klotho, which was formerly described as an anti-aging gene [3].

The klotho gene was first discovered to be involved in the suppression of aging. The protein Klotho has three isoforms: αKlotho (hereinafter referred to as Klotho), βKlotho, and γKlotho, each encoded by distinct genes [4]. Furthermore, Klotho can be separated into two forms: membrane (mKlotho) and soluble or shed (sKlotho) [5]. While sKlotho is the extracellular domain alone (KL1 and KL2, which together are roughly 130 kDa) and is the result of proteolytic cleavage of mKlotho. mKlotho is a transmembrane protein with a large extracellular domain containing KL1 and KL2 repetitions (each approximately 65 kDa) [6]. Notably, there is another soluble form of Klotho, which is generated by alternative. This is known as secreted Klotho (seKlotho) mRNA splicing [7].

2. Materials and Methods

The study included fifty healthy controls (28 males and 22 females) aged 20-60 years and one hundred patients included two groups of chronic kidney disease patients:
Group 1 included 50 cases of ESRD with dialysis (27 males and 23 females). Group 2 included 50 cases of chronic kidney disease without dialysis (21 males and 29 females). 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 and Al-Ramadi Teaching Hospital in Al-Anbar Province, between December 1, 2023, and June 30, 2024. The lifestyle and treatment history were taken into consideration when the samples were collected after approval from the patients with chronic kidney disease. Patients with cardiovascular disease and patients aged less than 20 years and older than 60 years were excluded.

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 at room temperature (18–25 °C) at 2050 xg for 15 min. The obtained serum was divided into two parts; the first part was utilized for biochemical parameters, and CRP and PTH hormones. The second part of the serum was stored at -20 °C to measure serum α-klotho, using an ELISA kit from SunLong Biotech Company, China.

Statistical analysis

All analyses were performed using SPSS-28. The data was presented as basic mean and standard deviation (SD) values. The significance of discrepancies between various means was determined using the student t-test for differences between two independent means. Statistical significance was determined when the P-value was equal to or lower than 0.05. Pearson correlation coefficients were used to investigate bivariate connections.

3. 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(µg/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).

Table 1: Mean ± SD for the measured variables in patients and control group

Variable	Control group Mean±SD	CKD with dialysis Patients group Mean±SD	P-Value	CKD without dialysis Patients group Mean±SD	P-Value
Age (years)	41.7±11.6	46.4±12.8	0.06	50.7±10.9	0.001#
BMI (Kg/m2)	26.05±2.64	25.40±4.72	0.060	26.01±2.80	0.001#
eGFR (mL/min)	108.62±11.25	9.85±4.8	0.001#	22.12±17.94	0.001#
Hb (g/dl)	13.41±1.33	8.88±	0.001#	10.15±1.83	0.001#
Hct (%)	41.17±2.64	27.91±3.62	0.001#	31.17±5.78	0.001#
α-Klotho (pg/mL)	158.66±52.46	83.28±37.33	0.001#	100.82±42.72	0.001#
Iron(µg/dl)	97.36±40.33	92.19±39.62	0.001#	94.19±41.19	0.001#
P (mg/dL)	3.42±0.41	4.72±1.79	0.001#	4.98±1.81	0.001#
Ca (mg/dL)	9.18±0.46	8.86±0.77	0.014#	8.61±0.72	0.001#
Na^+ (mmol/L)	140.38±1.78	134.06±3.48	0.001#	136.16±2.84	0.001#
K^+ (mmol/L)	3.98±0.34	4.63±0.63	0.001#	4.29±0.53	0.001#
Cl^- (mmol/L)	105.14±2.09	104.03±2.92	0.031#	105.21±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 (α-Klotho pg/mL)	P-Value	CKD without dialysis r (α-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(µg/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

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^- .

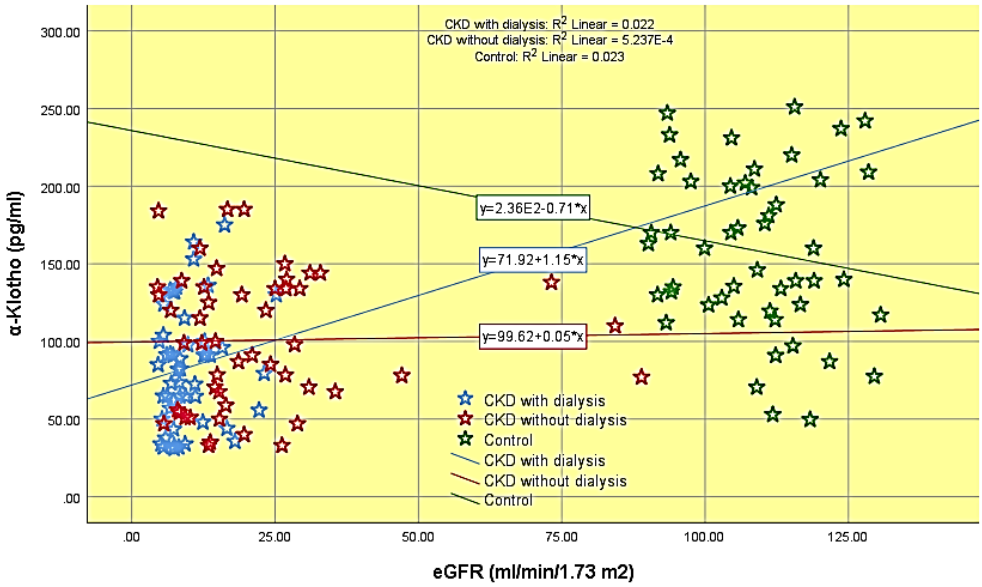

Figure 1. Correlation between α-Klotho with eGFR

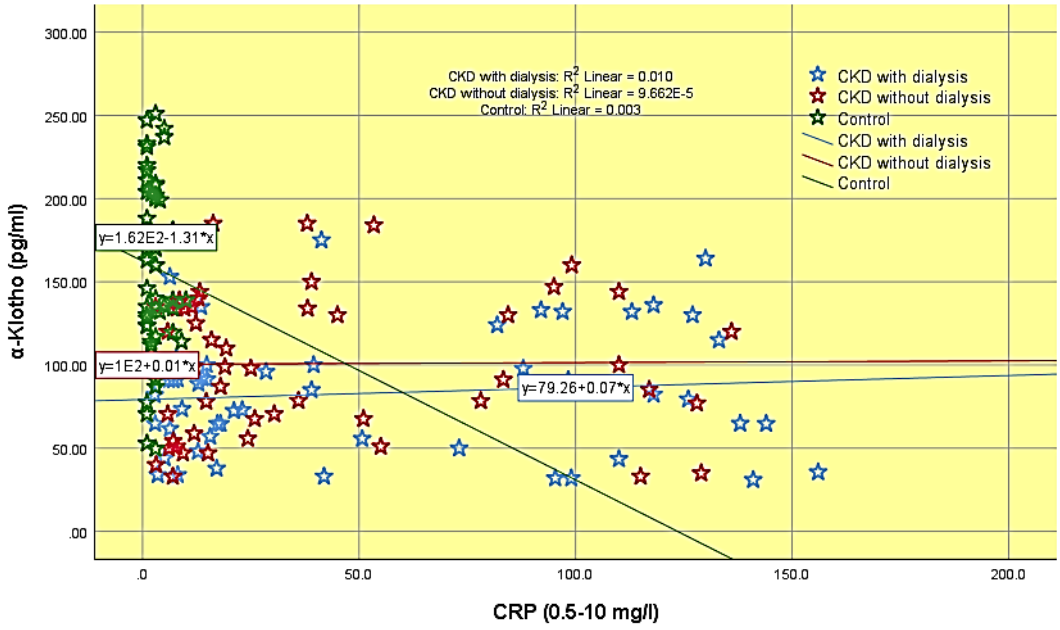

Figure 2. Correlation between α-Klotho with CRP

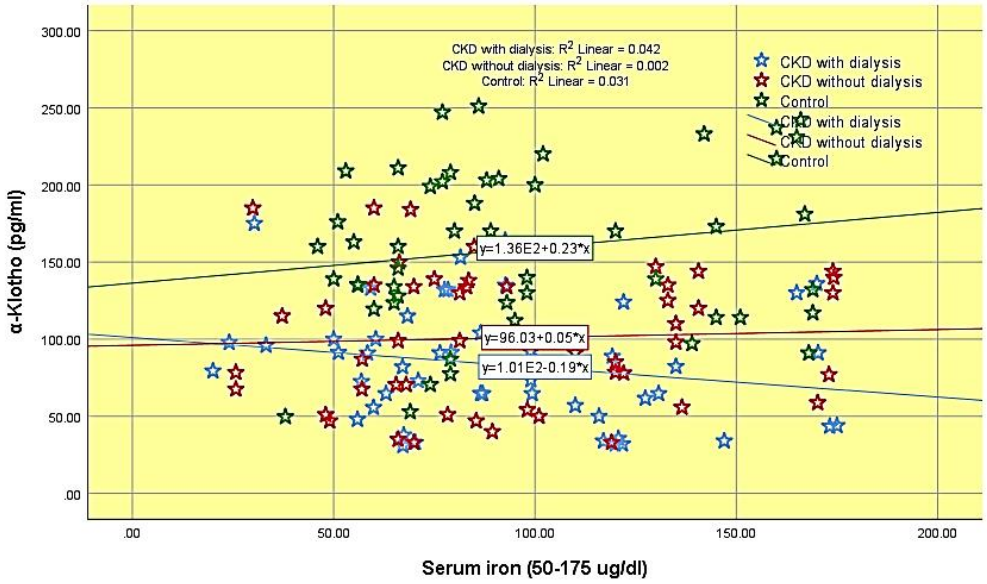

Figure 3. Correlation between α-Klotho with serum Iron

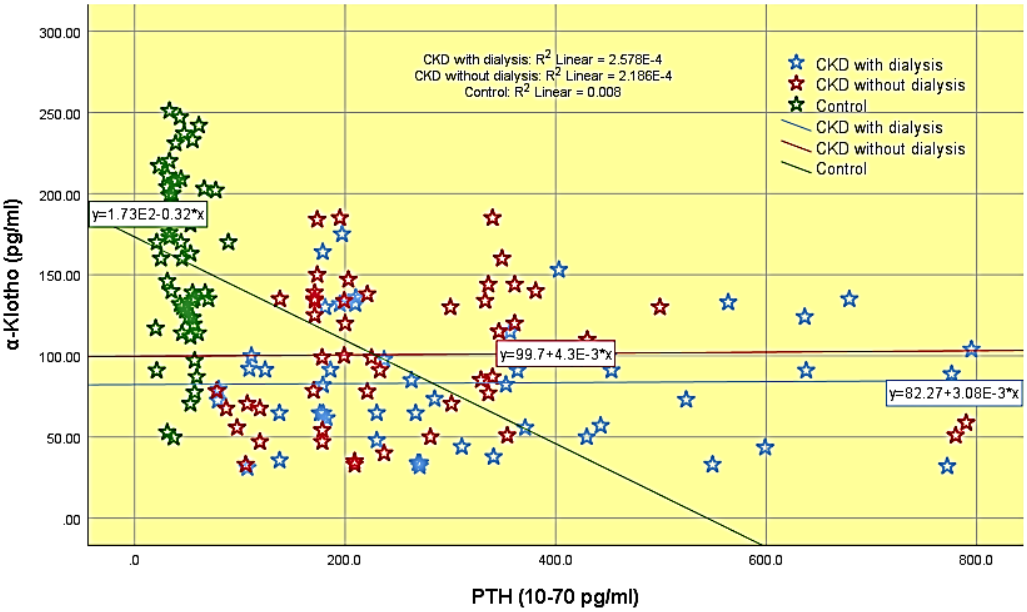

Figure 4. Correlation between α-Klotho with PTH

4. 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. [3]. 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], [15] , [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], [27], [28]. Serum α-Klotho levels were linearly associated with eGFR, Na^+, Hb, and Hct , this result is in agreement with the research findings [29], [30], [31]. There was no correlation between PTH, P, K^+, and Cl-.

5. 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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