The Egyptian Journal of Hospital Medicine (January 2024) Vol. 94, Page 889-895

Impact of Sodium Bicarbonate Supplementation on Serum Alpha Klotho in Chronic Kidney Disease Patients

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ABSTRACT

Background: The course of chronic kidney disease (CKD) is highly connected with the Klotho gene through multiple pathways, including renal fibrosis, vascular calcification, inflammation, and mineral bone abnormalities.

Objectives: This study aimed to assess the impact of oral sodium bicarbonate supplementation on blood levels of soluble α-Klotho in patients suffering from chronic renal disease (stage 3b:4) at Ain Shams Specialized Hospital in Cairo, Egypt, using the CKD-EPI formula.

Patients and Methods: 45 CKD patients were recruited for this interventional, prospective, open-label clinical trial from the Inpatient and Outpatient Clinics at Ain Shams Specialized Hospital in Cairo, Egypt.

Results: The cohort study had an average age of 48.13 ± 6.96 years. Serum bicarbonate (HCO_3) improved from mean 19.74 ± 1.43 mmol/L pre-study to 23.63 ± 3.23 mmol/L post-study, with a p-value of 0.000. Additionally, eGFR significantly improved with a p-value of 0.000 following the study. Serum alpha Klotho level was 596.30 ± 148.58 pg/mL before the study and 696.16 ± 172.99 pg/mL after the study, with a statistically significant difference of 0.006. Serum alpha Klotho after the study did not show a statistically significant link with serum creatinine, eGFR, potassium and phosphorus, or parathyroid. However, serum bicarbonate level and serum Klotho after the study showed a statistically significant positive correlation.

Conclusion: Serum soluble α klotho level was significantly higher post-study compared to pre-study after oral sodium bicarbonate supplementation and improved acidosis.

Keywords: Sodium bicarbonate supplementation, Serum alpha klotho, chronic kidney disease.

INTRODUCTION

Implication for health policy /practice /research /medical education:

Over 800 million people worldwide suffer from chronic renal disease, a condition that affects over ten percent of the population. Both the proximal and distal kidney tubules' cell surface membranes express the anti-aging gene klotho. Even in the initial stages of chronic renal disease, serum α klotho dropped and was associated with the pathogenic mechanism of CKD. Recently, variables of circulating klotho modulation have been found, including metabolic acidosis and proteinuria. Thus, using the CKD-EPI formula, we sought to assess the impact of oral sodium bicarbonate supplementation on blood levels of soluble α-Klotho in chronic renal disease (stage 3b: 4).

Over 16 million Americans suffer from chronic kidney disease (CKD), a serious health issue that increases mortality and morbidity. Chronic metabolic acidosis, which accelerates the course of CKD, is one of its consequences. Even in the absence of overt metabolic acidosis, acid retention can have detrimental effects [(1)].

In 1997, the Klotho gene was discovered to be an "aging suppressor" gene. If the Klotho disturbance is overexpressed, it prolongs life and accelerates aging. Although the control of circulating Klotho is mainly understood, one of its confounders has recently been discovered as metabolic acidosis and proteinuria [(2)].

Renal distal tubule cells express a transmembrane (TM) Alpha-Klotho. Fibroblast growth factor-23 (FGF23), which is in charge of the kidneys' phosphate management and calcitriol synthesis, is a coreceptor for alpha Klotho. The extracellular domain of TM-Klotho sheds, resulting in the circulating protein known as soluble α-Klotho (s-Klotho) [(3)].

The pathophysiology of CKD MBD involves vascular calcification with reduced renal klotho, hyperphosphatemia, vitamin D insufficiency, elevated FGF23, hyperparathyroidism, and renal osteodystrophy. Reduced klotho levels in CKD patients restrict FGF23 control, leaving hyperphosphatemia as the primary regulator of FGF23 synthesis [(4)].

When plasma level of bicarbonate concentrations becomes < 21–23 mmol/L in CKD patients with GFR < 40 mL/min/1.73 m^2, the rate of death and the course of the disease increase. The effects of metabolic acidosis include decreased sensitivity to calcium receptors, mobilization of bases from bones, stimulation of osteoblasts to create prostaglandins, stimulation of osteoclasts, and decreased osteoblast activity. Alkali therapy is an attempt to reduce the function of CKD progression by protecting klotho levels [(5)].

Our study aimed to evaluate the effect of oral sodium bicarbonate supplementation on soluble α-Klotho serum levels in patients with chronic kidney disease (stages 3b and 4) according to the CKD-EPI formula at Ain Shams Specialized Hospital, Cairo, Egypt.

PATIENTS AND METHODS

Study design: 45 patients with chronic kidney disease (CKD) were recruited for an interventional, nonrandomized, open-label clinical trial from the

Received:18/10/2023
Accepted:18/12/2023

Inpatient and Outpatient Clinics at Ain Shams Specialized Hospital in Cairo, Egypt.

Inclusion criteria: Patients at least 18 years old but not older than 70 years old, have an eGFR of 15:44 mL/min/1.73 m^2, and have a serum bicarbonate level of less than 22 mmol/L.

Exclusion criteria: Patients with decompensated heart failure, uncontrolled hypertension, and generalized edema.

Clinical data on patients under study was collected, including demographics and laboratory results measured before and after the study. The measured results included urine albumin/Cr ratio, serum creatinine, serum sodium, serum potassium, venous blood gas, serum phosphorus, calcium, intact PTH., and eGFR calculated with the CKD EPI 2021 Formula (GFR = 141 * min (Scr/κ,1))α * max (Scr/κ, 1)-1.209 * 0.993 Age * 1.018 [if female] * 1.159 [if black]. Serum creatinine (mg/dL) is called Scr, κ is equal to 0.7 for females and 0.9 for males, and α is equal to -0.329 for females and -0.411 for males. The numbers min and max denote the minimum and maximum values of Scr/κ and 1, respectively. The Human Soluble α-Klotho kit (IBL, Japan) was used to measure the serum levels of soluble α-Klotho (ELISA) in plasma; the normal range was 239–1266 pg/mL.

Patients were given sodium bicarbonate tablets orally 0.5 g/12 hours for 3 months and serum bicarbonate was followed every two weeks with a target level of 22-26 mmol/L.

Ethical approval: Ain Shams University Hospital Research Committee for the Department of Internal Medicine. (Ethics Committee reference number: FWA 000017585) and the 1964 Declaration of Helsinki's ethical guidelines were adhered to in all study procedures. Every patient who was enrolled in the study gave written informed permission. As a result, the writers have adhered strictly to ethical guidelines regarding plagiarism, data fabrication, and multiple publications.

Statistical analysis

The data were entered into IBM SPSS version 20, the Statistical Package for Social Science. When it was discovered that the distribution of the quantitative data was parametric, the data were expressed as percentages and figures, while the qualitative data were given as means ± standard deviations, and ranges. Two matched groups with parametric distribution and quantitative data were compared using a matched t-test. The confidence interval was set at 95% and the acceptable margin of error at 5%. As a result, whether the p-value ≤ 0.05, it was significant (S), or less than 0.001 (HS), it was considered highly significant.

RESULTS

This study included 45 CKD patients, of whom 75.6% were males. The mean age was 48.13 ± 6.96 years and their BMI ranged from 17.76 to 37.04 with a mean of 26.39 ± 3.83 kg/m^2 as shown in tables (1 and 2). As regards the etiology of CKD among the studied group, hypertension and diabetes were the most common causes with fourteen patients having hypertension and thirteen patients having diabetes as shown in figure (1).

Table (1): Demographic comparison between the groups in the study

		Number= 45
Gender	Female Male	11(24.4 percent) 34 (75.6 percent)
Age (years)	Mean ± SD Range	48.13 ± 6.96 33 – 58

SD: Standard deviation.

Table (2): Distribution of the cases under study based on BMI, height, and weight

	Number= 45	
	Mean ± SD	**Range**
Weight (kg)	75.07 ± 10.38	55 – 96
Height (meter)	1.69 ± 0.05	1.59 – 1.78
Body Mass Index (Kg/m^2)	26.39 ± 3.83	17.76 – 37.04

SD: Standard deviation.

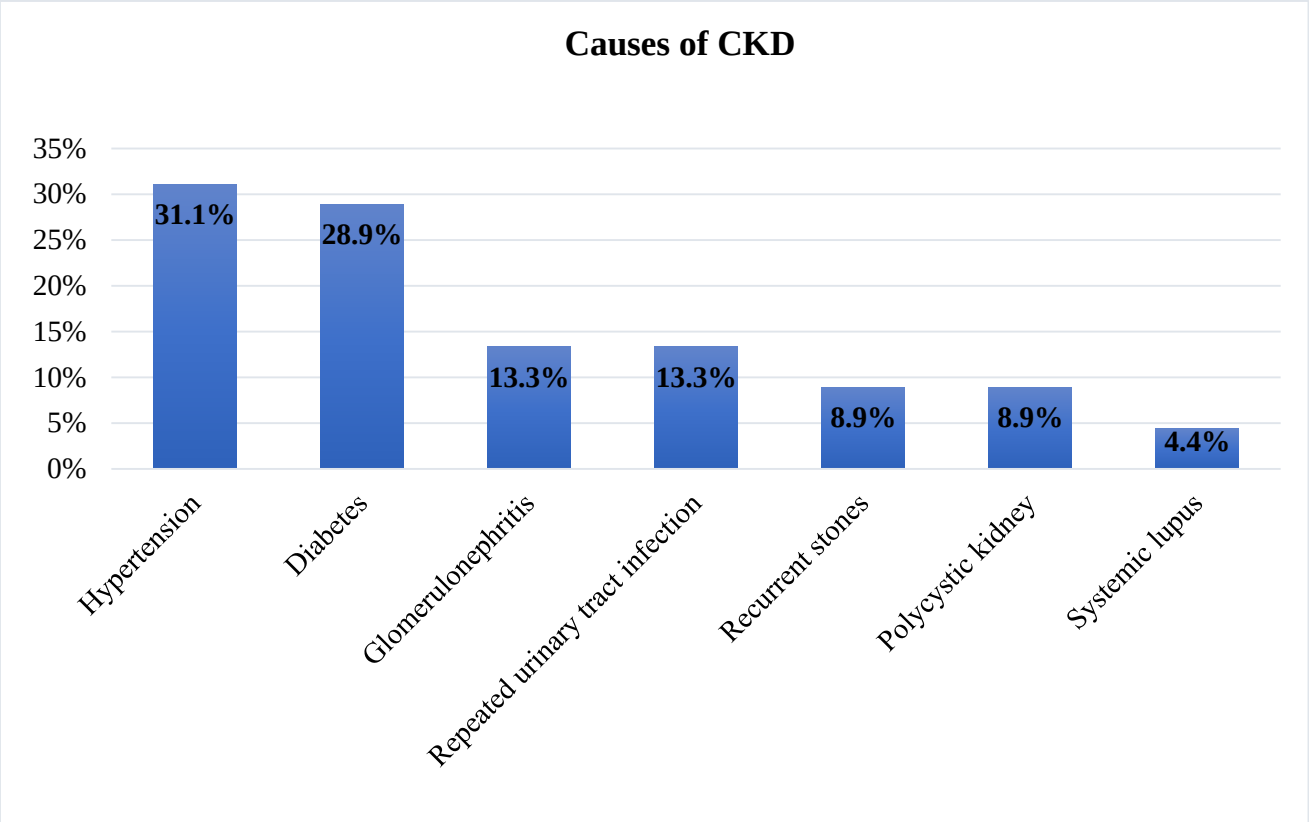

Figure (1): Distribution of the cases under study according to CKD causes.

Regarding laboratory results obtained prior to and following the study, table (3) demonstrated a highly statistically significant difference among the individuals under investigation. Bicarbonate level significantly improved after bicarbonate administration with a mean of 23.63 ± 3.23 mmol/L post-study and a p-value of 0.000. In terms of Klotho level, the mean was 596.30 ± 148.58 pg/ml prior to the study and the mean was 696.16 ± 172.99 pg/ml following the study with a P-value of 0.000. With a P-value of 0.000, the glomerular filtration rate improved following the trial compared to pre-study levels.

Table (3): Regarding Laboratory data among the studied group before and after the study

		Before the study	After the study	Test value	P-value	Significance
Klotho (pg/mL)	Mean ± SD	596.30 ± 118.58	696.16 ± 112.99	-2.516*	0.006	HS
HCO_3 (mmol/L)	Mean ± SD	19.74 ± 1.43	23.63 ± 3.23	-7.393*	0.000	HS
Serum Creatinine (mg/ dL)	Mean ± SD	3.85 ± 0.95	3.77 ± 0.92	5.249	0.000	HS
GFR (mL/ min/ 1.37 m2)	Mean ± SD	30.60 ± 7.61	31.51 ± 7.64	-5.435	0.000	HS
Potassium (mmol/L)	Mean ± SD	4.95 ± 0.63	4.98 ± 0.61	-2.472	0.017	S
Phosphorus (mg/dl)	Mean ± SD	5.04 ± 1.24	5.07 ± 1.23	-1.819	0.045	S
Calcium (mg/dl)	Mean ± SD	8.41 ± 0.69	9.21 ± 1.09	-1.212	0.005	s
iPTH (pg/ml)	Mean ± SD	93.51 ± 23.34	92.41 ± 22.98	4.171	0.000	HS

SD: Standard deviation, t: Student t-test, p: p-value for comparing between studied Groups, statistically significant at $p \leq 0.05$, Hco3: bicarbonate level, GFR: glomerular filtration rate, iPTH: intact parathyroid hormone.

Table (4) demonstrated that there was no statistically significant link between the laboratory data and Klotho (pg/mL) prior to the trial. However, following the investigation, klotho and bicarbonate levels showed a significant association with a P-value of 0.000 (Table 5 & figure 2)).

Table (4): Correlation between Klotho (pg/mL) and different laboratory data before the study

	Klotho (pg/mL) before the study	
	Correlation coefficient	**P-value**
Hemoglobin (g/dL)	-0.136	0.373
Urea (mg/dL)	0.021	0.890
Serum Creatinine (mg/ dL)	-0.064	0.676
A/C Ratio	0.261	0.083
eGFR (ml/min)	0.041	0.787
Sodium (mEq/L)	-0.264	0.080
Potassium (mmol/L)	0.041	0.791
Calcium (mg/dl)	0.214	0.159
Phosphorus (mg/dl)	-0.282	0.061
iPTH (pg/ml)	-0.274	0.068
Hco3 (mmol/L)	0.188	0.216

A/C Ratio: albumin creatinine ratio in urine, eGFR: estimated glomerular filtration rate, iPTH: intact parathyroid hormone, HCO3: bicarbonate level.

Table (5): Correlation between Klotho (pg/mL) and laboratory data after the study

	Klotho (pg/mL) after the study	
	Correlation coefficient	**P-value**
Serum Creatinine (mg/ dL)	-0.211	0.164
eGFR (ml/min)	0.084	0.585
Potassium (mmol/L)	0.052	0.737
Phosphorus (mmol/L)	-0.232	0.125
iPTH (pg/ml)	0.059	0.699
Hco3 (mmol/L)	**0.597****	**0.000**

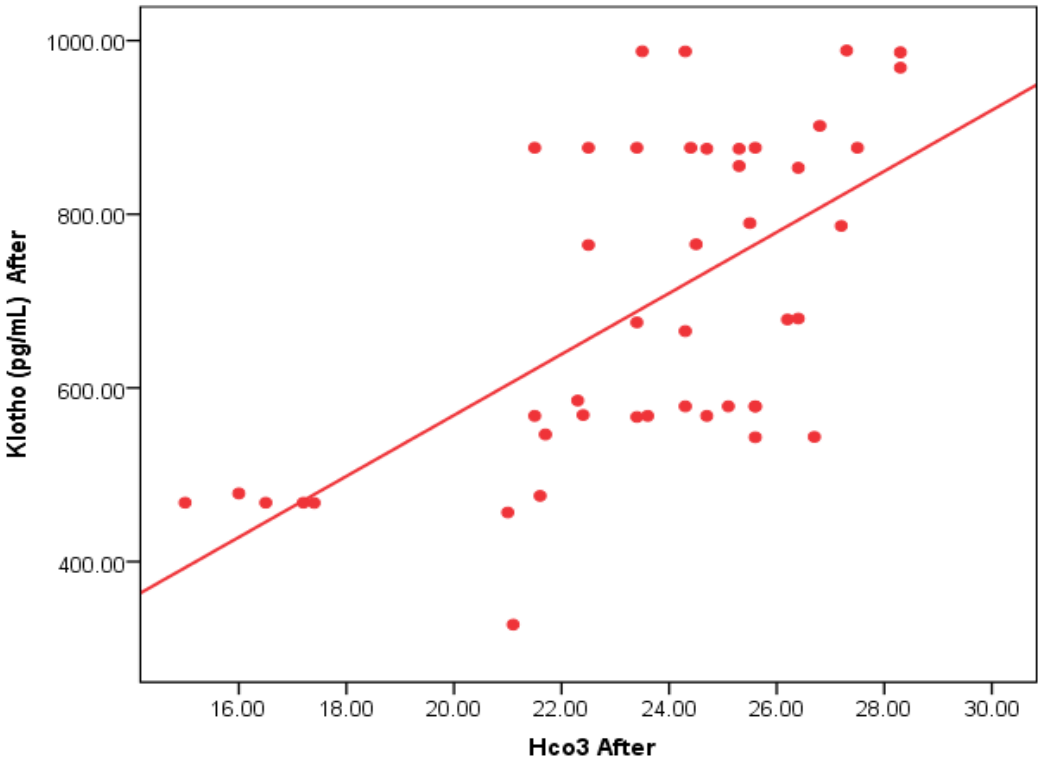

Figure (2): Klotho level and bicarbonate level correlation after the study

DISCUSSION

Individuals with chronic kidney disease incur greater social and financial expenses because of their heightened vulnerability to inflammation, heart disease, osteoporosis, and premature death. α-Klotho, a transmembrane protein with 1012 amino acids and an extracellular region that membrane proteases can break off to produce a soluble form, is one of the products of the Klotho gene. Klotho is expressed in the kidney's distal convoluted tubules, the choroid plexus, and the parathyroid glands as a co-receptor of FGF-23 [6].

Patients with CKD exhibit reduced expression of Klotho in the kidney, blood, and urine. This condition can be caused by glomerular and tubulointerstitial disorders, among other things. Uremic toxins or inflammatory cytokines facilitate the deacetylation of the Klotho gene. Numerous clinical characteristics, such as a short lifespan, cardiac remodeling, arterial calcification, bone disease, muscle atrophy, and hyperphosphatemia, are present in the setting of Klotho deficiency with CKD development [7].

Renal inflammation and fibrosis are caused by complications such as metabolic acidosis, which worsens with the progression of chronic kidney disease (CKD). This leads to an accumulation of ammonium and H^+ retention in the renal tissue, which activates the alternative complement pathway and produces hormones like endothelin, angiotensin II, and aldosterone. Alkali treatment can protect renal α-klotho levels in patients with chronic kidney disease [8].

Clinical findings indicate that lower serum a-Klotho due to prolonged metabolic acidosis may be associated with increased mortality in patients with chronic kidney disease. Patients with higher klotho levels exhibited improved survival and fewer cardiovascular events than patients with lower klotho levels in a prospective cohort study [9] of hemodialysis patients. This can be explained by different metabolisms of calcium and phosphate brought on by decreased klotho levels. Furthermore, circulating klotho enhances uremic remodeling and guards against uremic cardiomyopathy.

In the current study, the mean age was 48.13 ±6.96 years, the mean BMI was 26.39 ± 3.83 kg/m^2, and 75.6% of the participants were males. The most frequent causes of chronic kidney disease (CKD) among the cases under study were diabetes and hypertension. Regarding the Klotho level, there was a noticeable improvement with a P-value of 0.006, the mean was 596.30 ± 148.58 pg/ml prior to the study and 696.16 ± 172.99 pg/ml following it. The study found that the mean bicarbonate level was 19.74 ± 1.43 mmol/L prior to the investigation, and it increased to 23.63 ± 3.23 mmol/L following it, with a P-value of 0.000. Before the trial, the mean eGFR measurement using the CKD EPI equation was 30.60 ± 7.61 mL/min/ 1.37 m^2, and after the study, it was 31.51 ± 7.64 mL/min/ 1.37 m^2.

Regarding serum calcium level, the mean was 8.41 ± 0.69 mg/dl before the study and the mean was 9.21 ± 1.09 mg/dl after the study with a P- value of 0.005. iPTH had a mean of 93.51 ± 23.34 pg/ml before the study and a mean of 92.41 ± 22.98 pg/ml after the study with a P- value of 0.000. In line with our conclusions on the post-study improvement of eGFR, **Rizzetto *et al.*** [10] examined the effects of sodium bicarbonate treatment on creatinine clearance and electronegative LDL in patients with stages 3 and 4 of CKD. Serum bicarbonate levels rose with $p < 0.003$ and creatinine clearance improved from 40.1 ± 19.4 to 46.3 ± 19.2 mL/min/1.73 m^2, but there was no statistically significant change after 12 months of alkali therapy. Our results suggest that ammonia production was increased with increasing renal mass loss, which in turn led to complement activation and tubulointerstitial cell damage. Sodium bicarbonate supplementation reduced tubular hypercatabolism and urine ammonia output.

A systematic review of RCTs in CKD patients with GFR < 60 and studied the risk/benefit of sodium bicarbonate versus placebo on renal outcomes for ≥ 3 months follow up. It showed that sodium bicarbonate slowed the decline in renal function (eGFR or creatinine clearance) from baseline to the end of the studies. (14 studies with 2082 participants, SMD: 0.26; 95% CI: 0.13–0.40; heterogeneity I2 = 50%) [11, 12].

Regarding the post-study improvement in serum alpha Klotho level following bicarbonate therapy, there was a significant connection (R 0.597, P-value = 0.000) between bicarbonate levels and Klotho levels in the present study. **Raphael *et al.*** [13] Results showed an increase in soluble Klotho levels in the bicarbonate group compared to placebo but no significant effect on any of the other bone biomarkers, including iPTH, in a post hoc pilot study of the effect of sodium bicarbonate administration on serum Klotho and other bone markers in 194 CKD patients.

Unlike the current investigation, **Hage *et al.*** [2] recruited 20 CKD patients with stages 3-5 who were not receiving dialysis and followed them for four weeks to examine the impact of sodium bicarbonate supplementation on blood and urine alpha klotho levels. After four weeks, the mean serum bicarbonate increased to 23.4 ± 1.9 mmol/L with P < 0.001, from 19.3 ± 1.7 mmol/L at baseline. However, after four weeks there was no statistically significant change in the serum Klotho level. This may be attributed to the brief study period and the possibility of a transitional period prior to the rise in serum Klotho.

When correlating Klotho to other laboratory data, there was no statistically significant correlation between Klotho (pg/mL) before and after the trial except for bicarbonate levels following the study. This could be explained by the fact that the biochemical parameters, such as calcium, phosphorus, and PTH, are complicatedly influenced by a number of factors other than klotho, such as medication history, dietary factors, vitamin D3 level, FGF-23 level, and parathyroid

hormone. It was challenging to eliminate these factors from the current study. In conjunction with this investigation, a meta-analysis [14] was conducted on 1457 patients with CKD to determine the connections between serum soluble α-Klotho and renal function, α-klotho did not significantly correlate with serum levels of phosphorus, parathyroid, or calcium. In a group of 312 CKD patients with stages 2-4, **Seiler *et al.*** [15] investigated the relationship between blood soluble α-Klotho levels and kidney function, other biochemical indicators such as calcium, phosphorous, PTH, and unfavorable renal outcomes. Following up on patients for 2.2 ± 0.8 years, the results indicated no correlation between serum soluble α-Klotho and several biochemical indicators. Moreover, renal outcomes did not differ across groups even when patients were classified based on their serum soluble α-Klotho levels. The serum α-Klotho Spearman correlation coefficients and the calcium-phosphate metabolism parameters did not exhibit any significant level or correlations.

13,589 people were engaged in a cross-sectional study [6] to investigate the relationship between serum-soluble Klotho and chronic kidney disease. When compared to higher CKD stages, an elevated serum Klotho level was linked to an increase in eGFR and CKD stage > 3.

Soluble Klotho may have a negligible effect on the disturbance of mineral metabolism linked to different levels of chronic kidney disease. Accordingly, **Akimoto *et al.*** [16] eliminated patients receiving renal replacement treatment from their enrollment of 131 CKD patients with varying stages ranging from 1 to 5. The eGFR was found to be linked with the log-transformed serum α Klotho level, but not with albumin, calcium, phosphorus, or iPTH, according to the results.

Rotondi *et al.* [17] investigated the clinical importance of serum klotho in 68 patients with stage 2 to 4 CKD. In contrast to our findings, the s-Klotho level showed a negative association with serum phosphate (ρ = -0.28, P <.05) and PTH (ρ = -0.28, P <.05), and a positive correlation with eGFR (ρ = 0.43, P <.001) and serum calcium (ρ = 0.30, P <.01). A second prospective cohort research by **Kim *et al.*** [18] in which they examined the relationship between serum alpha klotho levels and renal progression in 243 patients with chronic kidney disease. In CKD patients, serum α klotho was independently correlated with eGFR (β=0.154; P < 0.001). Furthermore, at the median follow-up of 29.7 (6.0-62.1) months, lower serum alpha klotho levels strongly predicted the renal outcome (P<0.001).

Limitations of this study: There were certain restrictions in the ongoing investigation, such as the absence of analysis of membrane-bound Klotho, urine Klotho, and serum Klotho levels in healthy controls or CKD stage 1 patients. Furthermore, there might be additional variables, including dietary information that we were unable to account for. Our study participants may not be representative, which limits the applicability of our results as it is a single-center study.

CONCLUSION

Because Klotho has strong antiaging and cell protection properties, it is essential to avoid Klotho shortage when treating chronic kidney disease. One possible avenue to address this deficiency was acidosis correction. In CKD patients with stages 3 and 4, oral sodium bicarbonate supplementation and improved metabolic acidosis led to considerably greater serum levels of soluble α klotho, with a positive connection. To validate and strengthen our findings, we suggest doing a larger prospective cohort study over an extended period of time.

Authors' contribution

MS was involved in the development and revision of the paper for significant intellectual content, revised and analyzed the patient data, and provided final approval for the version to be published. MS made significant contributions to the conceptualization and design of this investigation. HA contributed significantly to the idea, the design, and the revision. LE contributed significant contributions to the study's idea and design, data collection, article preparation, and manuscript publication. MR participates in gathering and analysis of patient data. The final manuscript file was read and approved by all authors.

Conflicts of interest: None.
Funding/Support: No funding.

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