Subject Area:

Role of sclerostin, fibroblast growth factor-23, and Klotho in hemodialysis patients

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Role of sclerostin, fibroblast growth factor-23, and Klotho in hemodialysis patients

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Abstract

Introduction
Chronic kidney disease–mineral bone disorder leads to decreasing bone health. Sclerostin is produced by osteocytes similar to fibroblast growth factor-23 (FGF-23) and might upregulate the FGF-23 production by the osteocyte. FGF-23 may act as a mineralization inhibitor. Klotho serves as an obligate coreceptor for the FGF-23 and represents a marker of chronic kidney disease–mineral bone disorder.

Aim
We examined the association between serum sclerostin, FGF-23, Klotho, parathyroid hormone, high-sensitivity C-reactive protein (hsCRP), and phosphorus levels in hemodialysis (HD) patients.

Patients and methods
A total of 63 HD patients in the National Institute of Urology and Nephrology and 20 controls were enrolled in the study. Serum sclerostin, FGF-23, Klotho, parathyroid hormone, hsCRP, and phosphorus levels were assayed.

Results
We found that serum sclerostin, FGF-23, Klotho, and hsCRP levels of the HD patients were higher than controls [median and interquartile range are 1.379 (0.217–11.680) ng/ml, 61.71 (9.821–565.5) pg/ml, 201.74 (90.53–402.73) pg/ml, and 25.79 (12.10–41.92) mg/l vs. 0.535 (0.169–0.830) ng/ml, 33.077 (10.45–67.342) pg/ml, 349.49 (201.23–721.5) pg/ml, and 4.375 (2.27–6.21), respectively; $P < 0.001$). The sclerostin level was significantly correlated with the serum phosphorus, FGF-23, Klotho, and hsCRP ($r = 0.296$, $P < 0.007$; $r = 0.239$, $P = 0.03$; $r = -0.336$, $P = 0.002$; and $r = 0.469$, $P < 0.001$, respectively). Moreover, FGF-23 was significantly correlated with the serum phosphorus and hsCRP ($r = 0.335$, $P = 0.002$, and $r = 0.379$, $P < 0.001$, respectively). There were a significant negative correlation between serum Klotho and serum phosphorus, FGF-23, and hsCRP ($r = -0.363$, $P < 0.001$; $r = -0.220$, $P = 0.046$; and $r = -0.881$, $P < 0.001$, respectively).

Conclusion
There is an association between serum levels of sclerostin, phosphorus, and FGF-23 in HD patients, and Klotho may represent an early marker of renal damage.

Keywords: Fiboblast growth factor-23, hemodialysis, klotho, sclerostin

INTRODUCTION

Chronic kidney disease–mineral bone disorder (CKD-MBD) is defined by abnormalities in mineral and hormone metabolism leading to decreasing bone health with the presence of soft tissue calcification [1]. CKD-MBD is initiated early in the course of kidney disease and is associated with changes in the morphology and functions of osteocytes [2].

Sclerostin, a glycoprotein encoded by the SOST gene and secreted by osteocytes, is considered a new marker for osteoblastic activity. It increases with declining renal function and is elevated in hemodialysis (HD) patients [3]. Sclerostin is

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a part of the parathyroid hormone (PTH)–calcium–phosphate–vitamin D axis and has been suggested to be a marker of the bone disease, occurring in HD patients [4]. Moreover, this marker may be associated with the inflammatory vessel disease and calcification of vessels. In the skeleton, Wnt/β-catenin signaling is predominantly an anabolic pathway. Loss-of-function human mutations that weaken Wnt/β-catenin signaling are generally associated with decreased bone mass [5]. It has been found that sclerostin is an inhibitor of the Wnt/β-catenin metabolic pathway in bone cells, and when osteocytes reduce the release of sclerostin in response to any mechanical stimuli acting on bone, this would promote the activation of osteogenic pathway Wnt/β-catenin in osteoblasts. This signaling pathway plays an important role in osteogenesis and bone turnover [6].

As sclerostin is produced by osteocytes similar to fibroblast growth factor-23 (FGF-23), it might upregulate the FGF-23 production. In turn, FGF-23 may act as a mineralization inhibitor [7].

In the kidney, FGF-23 acts on the FGF-receptor/α-Klotho coreceptor complex to reduce apical expression of key sodium–phosphate cotransporters within the renal proximal tubules, inhibiting tubular reabsorption of phosphate to induce phosphaturia. It also decreases 1,25-dihydroxyvitamin D synthesis to lower 1,25-dihydroxyvitamin D-stimulated intestinal absorption of phosphate [8].

α-Klotho (Klotho) is a transmembrane (TM) protein manifested in the distal tubular cells of the kidney. It serves as an obligate coreceptor for the FGF-23 protein derived from the bone [9].

Circulating soluble Klotho (s-Klotho) results from TM-Klotho shedding and acts on phosphate and calcium tubular transport. Decreased TM-Klotho prevents actions of FGF-23 and decreases s-Klotho. Thus, levels of s-Klotho could represent a marker of CKD-MBD. Both forms of Klotho (TM-Klotho and s-Klotho) are concerned with the physiologic regulation of mineral metabolism [10].

Reduction of renal Klotho mRNA expression and of both TM-Klotho and s-Klotho is responsible for the development of kidney tubular cell resistance to FGF-23 [11].

In this study, we measured the serum sclerostin, FGF-23, and Klotho levels in HD patients and controls and examined the association between serum sclerostin, FGF-23, Klotho, PTH, high-sensitivity C-reactive protein (hsCRP), and phosphorus levels in these patients.

PATIENTS AND METHODS

Patients

A total of 63 outpatients receiving maintenance HD treatment for at least 6 months (three times per week for 4–5 h per session) in the National Institute of Nephrology and Urology and 20 apparently healthy controls with normal kidney function were enrolled in the study between May and October 2017. The HD patient group ($N = 63$) consisted of 33 male and 30 female,

with mean age of 50.4 ± 11.8 years. The control group ($N = 20$) consisted of 13 male and seven female, with mean age of 41 ± 9.5 years. The exclusion criteria were primary or metastatic bone disease; bone fracture; acute bacterial or viral infection; malignant disease; history of renal transplantation; and diseases known to affect bone metabolism, such as rheumatoid arthritis, hypercortisolism, and hyperthyroidism.

The HD patients were classified according to the PTH level: subgroup I (<150 pg/ml, $n = 52$), subgroup II (150–300 pg/ml, $n = 6$), and subgroup III (≥300 pg/ml, $n = 5$).

Written informed consent was obtained from all participants, and approval for the study was obtained by the ethics committee of National Institute of Urology and Nephrology.

Methods

Blood samples were collected from HD patients after an overnight fasting before the midweek HD session and from control participants after overnight fasting. Samples were centrifuged at 3000 rpm for 15 min, and serum was collected in aliquots and stored at $-20°C$ (within 30 min of blood sampling) until needed for the measurement of serum parameters. Serum creatinine, urea, and phosphorus levels were measured using an automated analyzer (VITROS 5.1 FS, Ortho Clinical Diagnostics, Raritan, NJ 08869). hsCRP was done using Turbox apparatus (Orion Diagnostica, Espoo, Finland).

Serum PTH level was measured by human PTH, enzyme linked-immunosorbent assay (ELISA) kit (Wuhan ElAab Science Co. Ltd, Wuhan, China), with a lower limit of detection of 5.86 pg/ml, and intra-assay and interassay coefficient of variations of 5.6 and 9.3%, respectively.

Serum levels of sclerostin, FGF-23, and Klotho were assayed by human sclerostin, FGF-23, and Klotho ELISA kit (Wuhan EiAab Science Co., Ltd, Wuhan, China), with a lower limit of detection of 0.1 ng/ml, 3 pg/ml, and 2.4 pg/ml, respectively. The intra-assay and interassay coefficient of variations were 5.3 and 8.4%, respectively, for sclerostin; 4 and 6.4%, respectively, for FGF-23; and 5.3 and 9.6%, respectively, for Klotho.

Statistical analysis

Data were analyzed using statistical program for social science, version 23. Quantitative data were expressed as mean ± SD. Qualitative data were expressed as percentage. Results are significant when P value less than or equal to 0.05.

The following tests were done:
(1) Independent-samples t-test of significance was used when comparing between two means.
(2) Mann–Whitney U-test was used when comparing two means of not normally distributed data.
(3) Kruskal–Wallis test used when the normality and homogeneity of variances are not met.
(4) Spearman's correlation was used for correlating not normally distributed continuous data. (+) sign indicates direct correlation and (−) sign indicates inverse correlation, and also values near to 1 indicate strong correlation and values near 0 indicate weak correlation.

RESULTS

Table 1 shows comparison between the control and HD groups regarding the laboratory data. Table 2 shows comparison between the control and HD groups regarding sclerostin, FGF-23, Klotho, and hsCRP. Serum sclerostin, FGF-23, Klotho, and hsCRP levels of the HD patients were high compared with those of the controls [median and interquartile range are 1.379 (0.217–11.680) ng/ml, 61.71 (9.821–565.5) pg/ml, 201.74 (90.53–402.73) pg/ml, and 25.79 (12.10–41.92) mg/l vs. 0.535 (0.169–0.830) ng/ml, 33.077 (10.45–67.342) pg/ml, 349.49 (201.23–721.5) pg/ml, and 4.375 (2.27–6.21) mg/l, respectively; $P < 0.001$] (Figs. 1–4).

We found that both serum sclerostin and Klotho were significantly increased in males (2.398 ± 2.12 ng/ml and 233.34 ± 91.53 pg/ml, respectively) than females (1.378 ± 0.976 ng/ml and 186.06 ± 65.08 pg/ml, respectively) (Figs. 5–13).

Table 3 shows the comparison between the PTH groups within HD patients regarding the sclerostin and FGF-23. There were insignificant differences in both markers in the three groups.

In HD patients, higher age was significantly associated with the serum sclerostin and FGF-23 levels. The sclerostin level was significantly correlated with the serum phosphorus, FGF-23, Klotho, and hsCRP ($r = 0.296$, $P < 0.007$; $r = 0.239$, $P = 0.03$; $r = -0.336$, $P = 0.002$; and $r = 0.469$, $P < 0.001$, respectively). We observed that FGF-23 was significantly correlated with the serum phosphorus and hsCRP ($r = 0.335$, $P = 0.002$, and $r = 0.379$, $P < 0.001$, respectively). There was a significant negative correlation between serum Klotho with serum phosphorus, FGF-23, and hsCRP ($r = -0.363$, $P < 0.001$; $r = -0.220$, $P = 0.046$; and $r = -0.881$, $P < 0.001$,
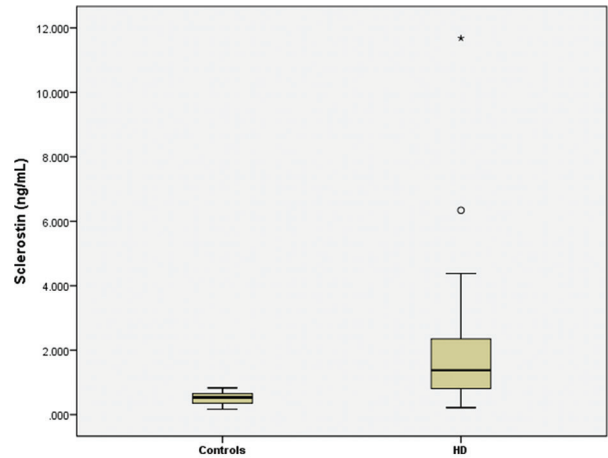
Table 1: Comparison between the control and hemodialysis groups regarding the laboratory data

Laboratory data	Control group	HD group	P (significance)
Count [n (%)]	20 (24.1)	63 (75.9)	
Age (years)			
Mean±SD	41±9.5	50.4±11.8	0.002 (S)
Median (range)	40.5 (26-55)	52 (23-75)	
Creatinine (mg/dl)			
Mean±SD	0.84±0.18	9.01±3.0	<0.001 (HS)
Median (range)	0.875 (0.51-1.1)	9.0 (3.0-17.6)	
Urea (mg/dl)			
Mean±SD	31.6±5.1	120.9±30.2	<0.001 (HS)
Median (range)	30.85 (23.9-39.0)	121.8 (57-196)	
Phosphorus (mg/dl)			
Mean±SD	3.31±0.49	5.5±1.5	<0.001 (HS)
Median (range)	3.25 (2.5-4.1)	5.3 (2.3-8.5)	
PTH (pg/ml)			
Mean±SD	73.28±20.77	133.56±168.04	0.330 (NS)
Median (range)	75.94 (39.56-122.62)	80.255 (18.41-827.25)	

HD: Hemodialysis; HS: Highly significant; PTH: Parathyroid hormone; S: Significant.

Figure 1: Comparison between the control and hemodialysis (HD) groups regarding sclerostin.

Table 2: Comparison between the control and hemodialysis groups regarding sclerostin, fibroblast growth factor-23, Klotho, and high-sensitivity C-reactive protein

Sclerostin, FGF-23, Klotho, and hsCRP	Control group	HD group	P (significance)
Count [n (%)]	20 (24.1)	63 (75.9)	
Sclerostin (ng/ml)			
Mean±SD	0.508±0.196	1.912±1.740	<0.001 (HS)
Median (range)	0.535 (0.169-0.830)	1.379 (0.217-11.680)	
FGF-23 (pg/ml)			
Mean±SD	32.898±17.435	128.218±126.38	<0.001 (HS)
Median (range)	33.077 (10.45-67.342)	61.71 (9.821-565.5)	
Klotho (pg/ml)			
Mean±SD	386.42±136.04	210.83±82.90	<0.001 (HS)
Median (range)	349.49 (201.23-721.5)	201.74 (90.53-402.73)	
hsCRP (mg/l)			
Mean±SD	4.34±1.26	26.64±8.16	<0.001 (HS)
Median (range)	4.375 (2.27-6.21)	25.79 (12.10-41.92)	

FGF-23: Fibroblast growth factor-23; HD: Hemodialysis; HS: Highly significant; hsCRP: High-sensitivity C-reactive protein.
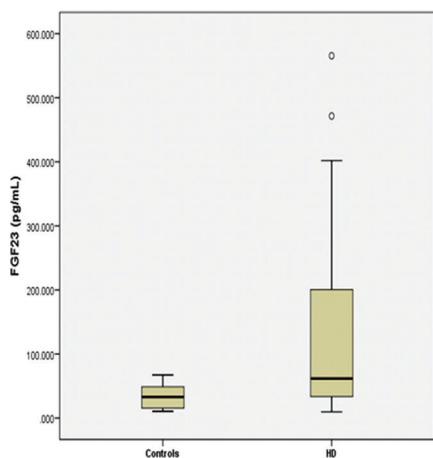
Figure 2: Comparison between the control and hemodialysis (HD) groups regarding fibroblast growth factor-23 (FGF-23).
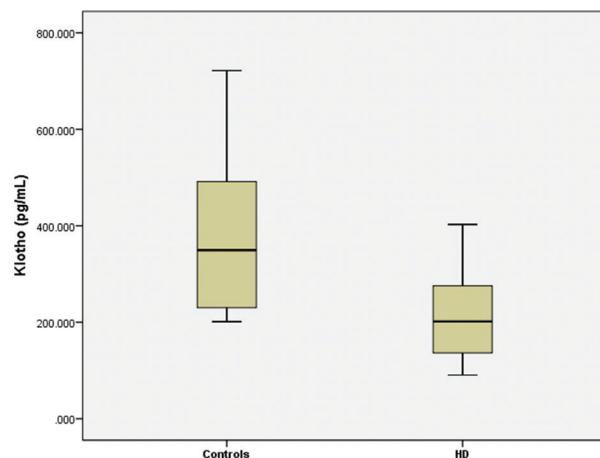

Figure 3: Comparison between the control and hemodialysis (HD) groups regarding Klotho.
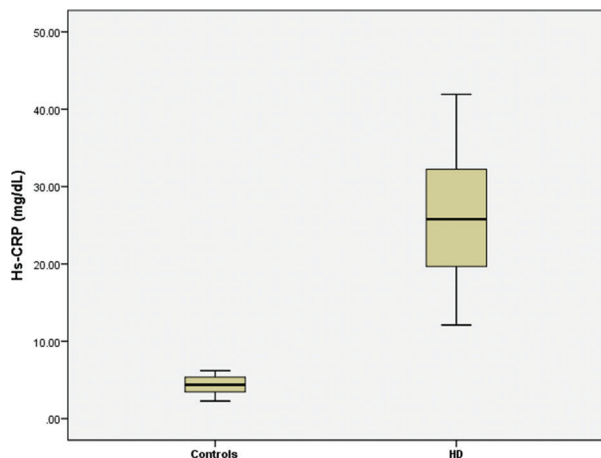

Figure 4: Comparison between the control and hemodialysis (HD) groups regarding high-sensitivity C-reactive protein (hsCRP).
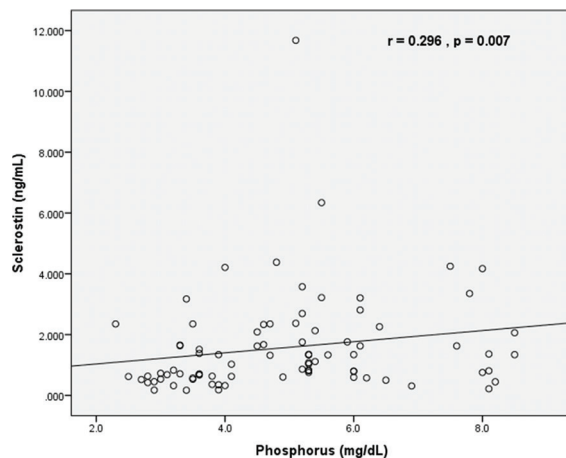

Figure 5: Correlation analysis between serum sclerostin and phosphorus.

respectively). PTH was not associated with sclerostin, FGF-23, and Klotho (Table 4).

Discussion

Sclerostin may be considered to be a new link for both bone and vessel diseases [12].

In this study, we found a high serum sclerostin level in HD patients compared with controls, which may be owing to renal failure. Pelletier et al. [3] observed that serum sclerostin level was associated with the progression of decreasing renal function.

Moreover, Evenepoel et al. [13] found that circulating sclerostin concentration is inversely correlated with the estimated glomerular filtration rate. This increase is likely the result of an increased production of sclerostin, as the study by Cejka et al. [14] in 120 patients with chronic kidney disease (CKD) showed an increased rather than a decreased urinary excretion of sclerostin with declining kidney function. Moreover, abnormal

mineral and bone metabolism and use of vitamin D treatment may be associated with serum sclerostin level [15]. The mechanism underlying signaling to the skeleton to increase the production of sclerostin in CKD is still not clear. In agreement with our study, Behets et al. [16] found that sclerostin was nearly 2.5-fold higher than controls in 100 HD patients.

This study confirmed a positive association with age and a significant increase in men than in women owing to larger skeletal mass in men. This agreed with the studies by Mödder et al. [17] and Delanaye et al. [18].

There is an evidence that FGF-23 is an earlier and more sensitive marker of disordered mineral metabolism and thus superior to serum phosphate and PTH [19]. So, routine measurement of FGF-23 in CKD and CKD-MBD is important in management of CKD. Smith concluded that lowering of FGF-23 results in better outcomes in CKD [20]. Serum FGF-23 levels were higher than normal in our HD patients. This is in agreement with Rotondi et al. [21] who observed an increase in serum FGF-23 in their 68 patients with CKD appearing from
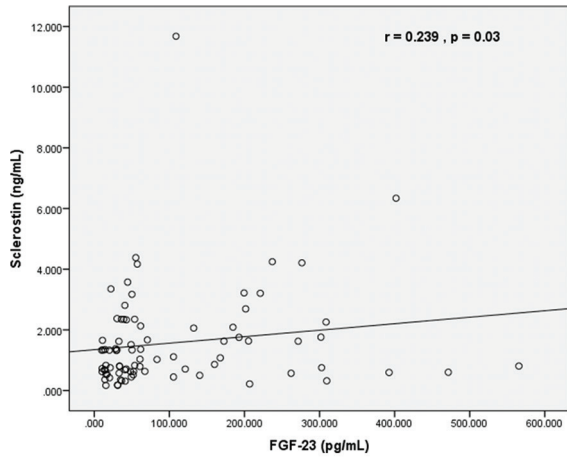
Figure 6: Correlation analysis between serum sclerostin and fibroblast growth factor-23 (FGF-23).
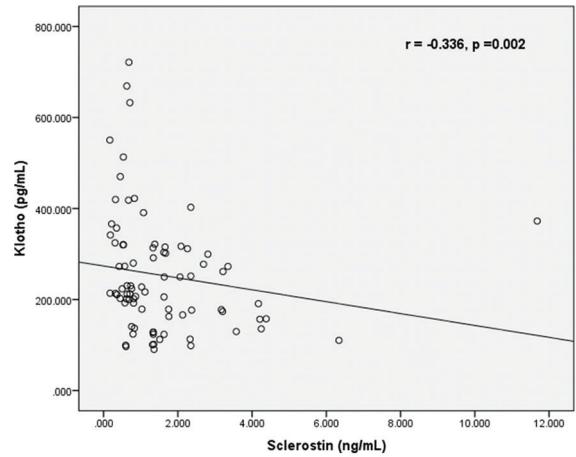

Figure 7: Correlation analysis between serum sclerostin and Klotho.
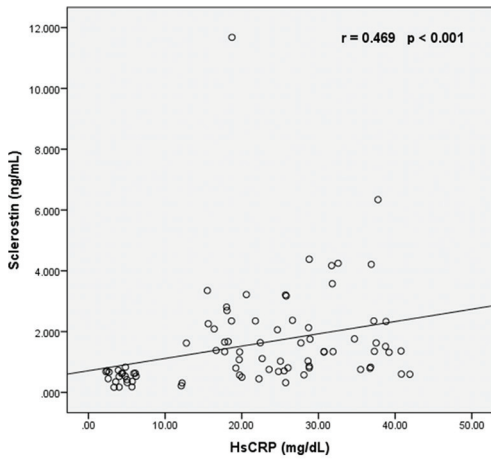

Figure 8: Correlation analysis between serum sclerostin and high-sensitivity C-reactive protein (hsCRP).

Figure 9: Correlation analysis between serum fibroblast growth factor-23 (FGF-23) and phosphorus.
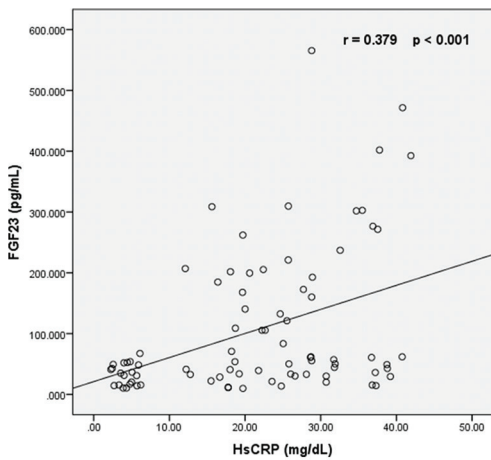
Figure 10: Correlation analysis between serum fibroblast growth factor-23 (FGF-23) and high-sensitivity C-reactive protein (hsCRP).

Figure 11: Correlation analysis between serum Klotho and phosphorus.

stage 2. In addition, the study by Almroth *et al.* [22] found FGF-23 and hsCRP in 84 HD patients compared with controls.

The risk of death during the first year on HD correlated with the FGF-23 level [23]. Bone expression of sclerostin seemed to be increased in earlier stages of CKD, whereas FGF-23 had increased expression in the late stages of CKD [24].

Table 3: Comparison between the parathyroid hormone groups within hemodialysis patients regarding the sclerostin and fibroblast growth factor-23

Laboratory data	PTH<150 (pg/ml)	PTH: 150-300 (pg/ml)	PTH>300 (pg/ml)	P (significance)
Sclerostin (ng/ml)				
Mean±SD	2.038±1.833	0.852±0.624	1.879±1.312	0.056 (NS)
Median (range)	1.625 (0.217-11.68)	0.743 (0.310-2.06)	1.32 (1.032-4.17)	
FGF-23 (pg/ml)				
Mean±SD	127.52±118.72	148.66±209.22	110.96±110.16	0.968 (NS)
Median (range)	61.72 (9.82-471.52)	73.35 (13.59-565.5)	60.9 (29.33-301.88)	

FGF-23: Fibroblast growth factor-23; PTH: Parathyroid hormone.

Table 4: Correlation analysis within hemodialysis patients

Variables	hsCRP		Sclerostin		FGF-23		Klotho	
	r	P	r	P	r	P	r	P
Age	0.300	0.006	0.282	0.010	0.286	0.009	−0.184	0.096
Creatinine	0.605	<0.001	0.438	<0.001	0.217	0.049	−0.456	<0.001
Urea	0.618	<0.001	0.483	<0.001	0.349	0.001	−0.455	<0.001
Phosphorus	0.502	<0.001	0.296	0.007	0.335	0.002	−0.363	<0.001
hsCRP	-	-	0.469	<0.001	0.379	<0.001	−0.881	<0.001
PTH	0.054	0.630	−0.077	0.491	0.056	0.315	0.010	0.928
Sclerostin	0.469	<0.001	-	-	0.239	0.030	−0.336	0.002
FGF-23	0.379	<0.001	0.239	0.030	-	-	−0.220	0.046
Klotho	−0.881	<0.001	−0.336	0.002	−0.220	0.046	-	-

FGF-23: Fibroblast growth factor-23; hsCRP, high-sensitivity C-reactive protein; PTH: Parathyroid hormone.
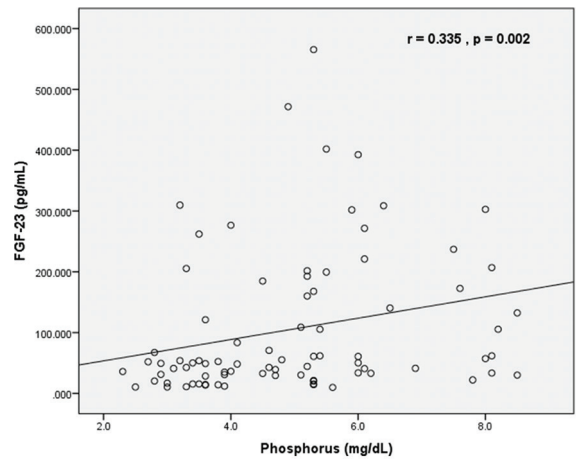

Figure 12: Correlation analysis between serum Klotho and fibroblast growth factor-23 (FGF-23).

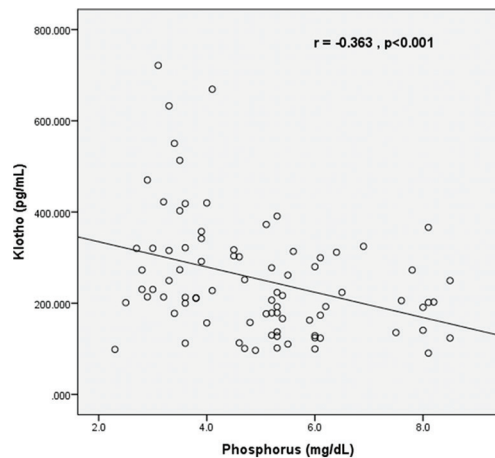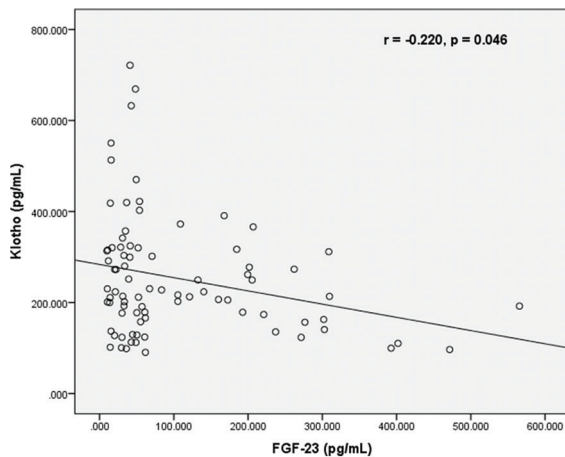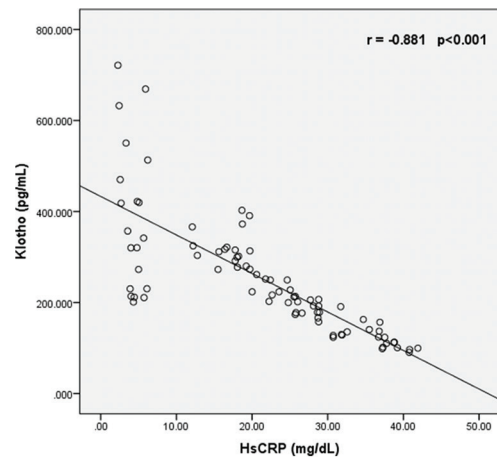**Figure 13:** Correlation analysis between serum Klotho and high-sensitivity C-reactive protein (hsCRP).

In our study, there is a significant and positive correlation between serum sclerostin and FGF-23 levels in HD patients. This is consistent with the result of a study of 90 patients with CKD not receiving dialysis therapy [3], a study on 102 HD patients [25] and the study by Moyses et al. [26]. In contrast to our results, Delanaye et al. [18] and Behets et al. [16] found no association between serum sclerostin and FGF-23 levels.

The mechanism underlying the positive association between serum sclerostin and phosphate levels remains unclear. The presence of a signaling pathway with a direct connection between sclerostin and phosphate, such as a pathway composed

of receptors of a phosphate on the surface of osteocytes that promotes the production of sclerostin, has not been identified. However, the interactions that link sclerostin and several phosphate regulators, including FGF-23, have been found. These phosphate regulators play an important role in regulating bone turnover, bone mineralization, and renal mineral homeostasis and interact closely with each other [27]. Ryan et al. [28] found that the serum intact FGF-23 level decreased in the absence of sclerostin in an animal study. Further studies are required to know if the interaction between sclerostin and the phosphate regulators including FGF-23 could help in the

understanding of the association between serum sclerostin and phosphate levels in HD patients [25].

In agreement with the study by Asamiya *et al.* [25], we observed that serum sclerostin levels did not show a significant association with PTH levels. However, Behets *et al.* [16] found an inverse relationship between sclerostin and PTH concentrations in dialysis patients. Their data are consistent with experimental data demonstrating downregulation of *SOST* by PTH [29]. Wesseling-Perry *et al.* [30] proved that high sclerostin levels coexist with high PTH levels in patients with advanced CKD. This observation suggests skeletal resistance to the action of PTH, similar to FGF-23 resistance, explaining the coexistence of high FGF-23 and PTH levels in advanced stage CKD [21,31]. These two studies contradict ours, as we found FGF-23 to have insignificant correlation with PTH. This contradiction may be owing the difference in patients, environment, and HD protocol.

Sclerostin antibody use may offer new therapeutic approaches in the therapy of mineral and bone disorders, resulting from CKD-MBD and vascular calcifications [6]. Inhibition of sclerostin activity may be an effective treatment for osteoporosis in normal participants. Suppression of serum sclerostin levels in patients with CKD may be effective for treating osteoporosis, as in normal participants. So, the control of serum phosphate level within the normal range in HD patients may have an effect on decreasing the serum sclerostin level and improving decreased bone formation [32].

Klotho, a coreceptor for FGF-23, was identified in a mouse model showing hyperphosphatemia. The FGF-23-Klotho endocrine axis is an important regulator of mineral metabolism. In CKD, early onset of Klotho deficiency contributes to renal FGF-23 resistance and an increase in circulating FGF-23. FGF-23 is an early biomarker of renal injury, and increased FGF-23 predicts adverse clinical outcomes, in particular cardiovascular disease. FGF-23 stimulates left ventricular hypertrophy, and loss of Klotho increases fibrosis, endothelial dysfunction, and vascular calcification [33].

In agreement with our observation, Rotondi *et al.* [21], Pavik *et al.* [34], Wan *et al.* [35], and Milovanova *et al.* [36] found an increase of FGF-23 and decrease of Klotho serum levels as early markers for progressive CKD.

Renal function negatively affected Klotho levels with detectable reduction starting from CKD stage 2. As Klotho is normally excreted in the urine [37], this reduction in serum levels with progressive renal damage can be explained by reduced renal synthesis. An increased renal excretion is improbable as this would contradict the presence of reduced urinary Klotho in CKD. The best predictor of Klotho was estimated glomerular filtration rate [38]. Another hypothesis is downregulation of Klotho by FGF-23 [39]. An inverse association to mortality has been reported, with higher circulating levels of Klotho associated with increased survival in the general population and in HD patients [40].

The negative relationship we found between s-Klotho and serum phosphorus may be owing to the direct effect of circulating s-Klotho on the renal expression of inorganic phosphate transporters (mainly NaPi-2a) in the proximal tubules. As s-Klotho and FGF-23 correlate negatively in CKD but exert a similar positive effect on renal expression of NaPi-2a, it is possible that the action of s-Klotho is shadowed by FGF-23 [21].

In our study, there was a negative relationship between s-Klotho and FGF-23. This supports the theory that serum Klotho are strongly related to renal function and are most probably secondary to reduced expression of TM-Klotho. This leads to early development of tubular resistance to FGF-23. So, bone synthesis of FGF-23 is increased. The results of Sakan *et al.* [41] from patients with glomerulonephritis showed parallel reduction of renal Klotho and of s-Klotho together with increase in FGF-23, which is in agreement with our data. The study by Almroth *et al.* [42] contradicts our findings in that Klotho did surprisingly not correlate with FGF-23 but agreed with ours in that it correlated negatively with the inflammatory marker hsCRP.

Limitations of our study that should be considered when reviewing the results are, first, only a small number of patients were examined, and second, the results obtained for Egyptian patients cannot be generalized to a wider population.

CONCLUSION

There is an association between serum sclerostin levels and serum phosphorus and serum FGF-23 levels in HD patients. Moreover, renal Klotho synthesis diminishes early in renal disease with increase in FGF-23 production. In addition, the FGF-23–Klotho axis is a solid candidate for a novel diagnostic and therapeutic target in CKD.

Identifying the precise mechanism underlying the signaling pathway associated with sclerostin, phosphate, and FGF-23 regulation requires further investigation.

Conflicts of interest
There are no conflicts of interest.

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