



Kidney Research and Clinical Practice

journal homepage: <http://www.krcp-ksn.com>
Contents lists available at [ScienceDirect](http://www.sciencedirect.com)

Original Article

Assessment of the relationship between serum soluble Klotho and carotid intima–media thickness and left ventricular dysfunction in hemodialysis patients

Emad Abdallah ^{1,*}, Osama Mosbah ¹, Ghada Khalifa ¹, Amna Metwaly ²,
Omnia El-Bendary ³¹ Department of Nephrology, Theodor Bilharz Research Institute, Giza, Egypt² Department of Intensive Care Unit, Theodor Bilharz Research Institute, Giza, Egypt³ Department of Clinical Chemistry, Theodor Bilharz Research Institute, Giza, Egypt

A B S T R A C T

Article history:

Received 23 September 2015

Received in revised form

27 November 2015

Accepted 16 December 2015

Available online 21 January 2016

Keywords:

Cardiovascular diseases

End-stage renal disease

Fibroblast growth factor-23

Soluble Klotho

Background: The aim of our study was to assess the relationship between soluble Klotho (s-Klotho) and carotid intima–media thickness (CIMT) and left ventricular (LV) dysfunction in hemodialysis (HD) patients.**Methods:** This is a cross-sectional study conducted on 88 patients with end-stage renal disease on regular HD. Serum levels of calcium, phosphorus, parathyroid hormone, and C-reactive protein were measured. The serum levels of s-Klotho and fibroblast growth factor-23 (FGF-23) were measured using an Enzyme linked immunosorbent assay (ELISA) kit. Echocardiography and measurement of CIMT were also conducted. The studied patients were divided according to the median s-Klotho level into 2 groups: patients with low s-Klotho (Group I) and patients with high s-Klotho (Group II).**Results:** Mean value of s-Klotho was significantly low in HD patients compared to controls ($P = 0.001$), and mean value of FGF-23 was significantly high in HD patients compared to controls ($P = 0.001$). The mean values of parathyroid hormone, FGF-23, and phosphorus were significantly high in Group I compared to Group II, whereas the mean value of serum calcium was significantly low in Group I compared to Group II. The mean values of CIMT, LV mass (LVM), LVM index, and LV ejection fraction (LVEF) were high in Group I compared to Group II. Patients with low s-Klotho had significantly more coronary artery disease (CAD). In a regression analysis of s-Klotho with different markers of cardiovascular diseases, s-Klotho showed significant association with CIMT, LVEF, and CAD, but not with LVM and LVM index.**Conclusion:** The present study showed that patients with a low s-Klotho were more often associated with increased CIMT, LV dysfunction, and CAD, and it seems that there was independent association between s-Klotho and CIMT, LVEF, and CAD.Copyright © 2016. The Korean Society of Nephrology. Published by Elsevier. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

* Corresponding author. Theodor Bilharz Research Institute, El-Nile Street, Waraq El-Hadar, P.O. Box 30, Imbaba, Giza 12411, Egypt.

E-mail address: drabdallah96@gmail.com (E Abdallah).<http://dx.doi.org/10.1016/j.krcp.2015.12.006>2211-9132/Copyright © 2016. The Korean Society of Nephrology. Published by Elsevier. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

Introduction

Chronic kidney disease (CKD) is associated with increased levels of parathyroid hormone (PTH) and fibroblast growth factor-23 (FGF-23) and hypocalcemia, hyperphosphatemia, bone disease, vascular calcification, and cardiovascular morbidities collectively referred to as CKD-mineral and bone disorder [1–3]. Recent reports suggested that increased levels of FGF-23 and decreased levels of soluble Klotho (s-Klotho) are common manifestations of CKD that develop earlier than increased levels of phosphate (Ps) or PTH [4,5].

A transmembrane (TM) protein known as soluble α -Klotho (s-Klotho) is primarily produced in the kidney distal tubular cells [6]. Soluble α -Klotho acts as a coreceptor for the bone-derived protein FGF-23 [7,8]. Regulation of both renal handling of Ps and renal synthesis of calcitriol needs a cofunction of both FGF-23 and TM-Klotho [9]. Soluble α -Klotho is the circulating protein resulting from the shedding of the extracellular domain of TM-Klotho operated by 2 metalloproteinases of the A disintegrin and metalloproteinase domain-containing protein (ADAM) family: ADAM10 and ADAM17 [10]. In particular, s-Klotho inhibits the sodium-Ps cotransporter NaPi2a expression in the proximal tubules, thus generating a phosphaturic effect additive to and independent of FGF-23 [11–13], and activates the ion channel TRPV5 in the distal tubules, thus increasing tubular reabsorption of calcium (Ca) [14]. Therefore, α -Klotho, with its TM and soluble forms, is deeply involved with the physiological regulation of mineral metabolism [15].

CKD is a common risk factor for cardiovascular diseases (CVD) such as coronary artery disease (CAD), cerebrovascular stroke, peripheral vascular disease, and heart failure [16]. Although part of CVD burden in patients with CKD is related to traditional risk factors, CKD-associated disturbance in Ca–Ps homeostasis plays a crucial role as well [2]. Recent studies showed that FGF-23 and its coreceptor s-Klotho have an important role in Ca–Ps homeostasis, and they could be the missing link in the detrimental relationship between CKD and CVD [17].

Given the strong cardioprotective effects of Klotho demonstrated in preclinical studies, the present study aimed to assess the association between s-Klotho and carotid intima–media thickness (CIMT) and left ventricular (LV) dysfunction in patients with end-stage renal disease (ESRD) on regular hemodialysis (HD). It was hypothesized that low levels of s-Klotho are associated with a larger burden of CVD in these patients.

Methods

Study population

This is a cross-sectional study conducted on 88 patients with ESRD on regular HD of at least 6-month duration. All patients have been attending the nephrology dialysis unit in the Theodor Bilharz Research Institute, Giza, Egypt. All patients received 3 sessions of HD/wk, each of 4-hour duration using a polysulfone dialyzer (Fresenius, St. Wendel, Germany) with surface area of 1.4–1.6. Patients with advanced congestive heart failure [defined clinically by elevated jugular venous pressure, ascites, peripheral edema, and shortness of breath at rest and LV ejection fraction (LVEF) of <30% in echocardiography requiring hospital-based support, a heart transplant, or palliative care according to guidelines of the American College of Cardiology/

American Heart Association] [18], sepsis, or malignancy were excluded from the study. Informed written consent was obtained from all participants. The study protocol was approved by the institute ethics committee, and the study was performed in accordance with the Declaration of Helsinki.

The studied patients were divided according to the median s-Klotho level (cut point, 476 pg/mL) into 2 groups: patients with low s-Klotho <476 pg/mL (Group I, $n = 44$) and patients with high s-Klotho >476 pg/mL (Group II, $n = 44$).

A control group consisting of 28 normal individuals was used to give our reference values for s-Klotho, FGF-23, CIMT, and echocardiographic findings. There were 17 men and 11 women with a mean age of 53.3 ± 16.2 years, with normal renal function and no evidence of acute or chronic underlying disease.

Each patient underwent a thorough history and clinical examination. Demographic characteristics and coexisting conditions such as atherosclerotic CAD diagnosed by electrocardiography and coronary angiography within 3 months of enrollment in the study were collected.

Fasting blood samples were collected from the patients before the HD session, immediately centrifuged, aliquoted in vials, and stored at -60°C until the time of analysis. Thawing the test samples was carried out at a low temperature by mixing them completely before measurement. Routine examinations included complete blood analysis, kidney function tests (serum urea, creatinine, sodium and potassium, and uric acid), random blood sugar, serum electrolytes, lipid profile, and serum albumin. Serum levels of Ca, Ps, PTH, and C-reactive protein were measured.

Serum levels of s-Klotho and FGF-23 measurement

The serum levels of s-Klotho were measured using an Enzyme linked immunosorbent assay (ELISA) system (Immuno-Biological Laboratories, Gunma, Japan) [19], and this assay detects circulating s-Klotho using 2 monoclonal antibodies that specifically recognize the extracellular domain of Klotho, with a lower limit of detection of 6.15 pg/mL and the intra-assay and interassay coefficients of variation of <10%. The serum levels of FGF-23 were measured using a commercial sandwich ELISA kit (Kainos Laboratories, Inc., Tokyo, Japan) [20] that uses a 2-site ELISA for the full-length molecule. Two specific murine monoclonal antibodies recognize the biologically active FGF-23, with a lower limit of detection of 3 pg/mL and interassay and intra-assay coefficients of variation of <5%.

Residual renal function estimation

Residual renal function (RRF) was estimated by calculating glomerular filtration rate expressed in mL/min/1.73 m². Glomerular filtration rate was estimated as the mean of urea and creatinine clearance using 24-hour urine collections and the mean of the post- and pre-HD plasma urea and creatinine. RRF was considered zero in patients with a urinary output <100 mL/24 h [21]. Kt/V was used to assess dialysis adequacy [22].

Echocardiography

After the HD session, each patient underwent echocardiography to determine LV mass (LVM), LVM index (LVMI), and LVEF.

For determination of LVM, the Devereux formula was used [23]. LVM (g): $1.04 [(LVID + PWT + IVST)^3 - LVID^3] - 14$, where LVID indicates LV internal dimension, PWT indicates posterior wall thickness, and IVST indicates interventricular septal thickness. LVM was divided by body surface area to measure LVMI. According to the current guidelines, LV dysfunction was defined as an LVEF < 45%.

Measurement of CIMT

After the HD session, each patient underwent ultrasonography of the carotid arteries using a high-resolution real-time scanner with a 7.5-MHz transducer [24]. The examination was done with the patient in the supine position, and the common carotid artery and carotid bifurcation were scanned on both sides. The carotid artery was scanned in the longitudinal and transverse directions. The site of the most advanced atherosclerotic lesion that showed the greatest distance between the lumen–intima interface and the media–adventitia interface was the maximum intima–media thickness (IMT) value. When plaque was detected on ultrasonography, it was observed as localized thickening rather than a circumferential change in the vessel wall. The greatest thickness of the intima–media complex (including plaque) was used for the maximum IMT value. We identified patients having atherosclerosis based on atheromatous plaques of focal increases in IMT ≥ 1.1 mm in accordance with a prior study that showed the normal limit of IMT to be ≤ 1.0 mm [25].

Statistical analysis

Data are presented as means \pm SD or number (%). The 2-tailed Student's *t* test was used to compare the mean values of 2 groups (GraphPad Quick calc; Graphpad Software Inc., San Diego, CA, USA). Categorical data were compared using the chi-square test. The Spearman rank correlation test was used to analyze the correlations between s-Klotho levels and markers of mineral metabolism. Multivariate regression analysis (using MedCalc software; MedCalc software bvba, Acaciaaan, Ostend, Belgium) was used to analyze the association between s-Klotho and markers of CVD, with a linear regression analysis model with unstandardized betas and a 95% confidence interval (CI) for CIMT, LVM, LVMI, and LVEF and a logistic regression analysis model was used for CAD. The linear regression analysis model was also used to determine the association between FGF-23 and CIMT, LVM, LVMI, and LVEF and the logistic regression analysis model was used for CAD. Statistical analyses of data were performed using SPSS for Windows, version 16.0 (SPSS Inc., Chicago, IL, USA). A *P* value < 0.05 was considered to be significant, and *P* < 0.01 was considered to be highly significant.

Results

Demographic characteristics of HD patients and controls included in this study are shown in Table 1, where the mean age of studied patients was 58.6 ± 19.3 years. Fifty-one (58.0%) patients were men and 37 (42.1%) were women. The most common causes of ESRD were diabetes mellitus, hypertension, and glomerulonephritis (Table 1).

Mean value of s-Klotho was significantly low in HD patients compared to the control group (477.9 ± 76.2 vs. 863.7 ± 261.8 , *P* = 0.001), and mean value of FGF-23 was significantly high in

HD patients compared to the control group (60.5 ± 17.6 vs. 35.8 ± 13.9 , *P* = 0.001; Table 2).

Patients with a low s-Klotho (<476 pg/mL) were older (63.2 ± 9.4 vs. 53.8 ± 16.2 , *P* = 0.001) and had high FGF-23, PTH, Ps, and C-reactive protein and low Ca and RRF (Table 2) compared to Group II. There were no significant differences in the mean values of serum creatinine, lipid profile, hemoglobin, serum glucose, serum albumin, serum uric acid, and Kt/V between the 2 groups (Table 2).

s-Klotho and markers of mineral metabolism

The mean values of serum levels of PTH, FGF-23, and Ps were significantly high in patients of Group I compared to patients of Group II, whereas the mean value of serum levels of Ca was significantly low in Group I compared to Group II (Table 2).

Using the Spearman rank correlation test, we found that there was significant negative correlation between s-Klotho and FGF-23 ($r = -0.717$, *P* = 0.001, 95% CI for $r = -0.806$ to -0.598 ; Fig. 1), PTH ($r = -0.484$, *P* = 0.001, 95% CI for $r = -0.630$ to -0.306 ; Fig. 2), and Ps ($r = -0.548$, *P* = 0.001, 95% CI -0.680 to -0.383 ; Fig. 3), whereas there was significant positive correlation between s-Klotho and Ca ($r = 0.294$, *P* = 0.005, 95% CI 0.0903–0.474; Fig. 4).

s-Klotho and markers of CVD

The mean values of CIMT, LVM, LVMI, and LVEF were high in patients of Group I compared to patients of Group II. The prevalence of CAD was higher among patients with low s-Klotho [36 (81.1%) vs. 22 (50.0%), *P* = 0.004; Table 3].

Using the Spearman rank correlation test, we found that there was significant positive correlation between s-Klotho and LVEF ($r = 0.392$, 95% CI 0.199–0.556, *P* = 0.001; Fig. 5).

Table 4 shows the results of the multivariate regression analysis of s-Klotho with CIMT, LVM, LVMI, LVEF, and CAD as different markers for CVD. After adjustments for age, gender, mean blood pressure, and duration of HD, s-Klotho showed

Table 1. Demographic characteristics of the studied patients and control group

Variable	Studied patients (n = 88)	Control group (n = 28)	<i>P</i>
Age (y)	58.6 \pm 19.3	53.3 \pm 16.2	0.192
Gender (M/F)	51/37 (58.0/42.1)	17/11 (60.7/39.3)	1.000
BMI (g/m ²)	24.6 \pm 5.2	26.2 \pm 5.6	0.167
SBP (mmHg)	124.6 \pm 15.5	119.2 \pm 11.3	0.091
DBP (mmHg)	85.7 \pm 25.4	80.4 \pm 19.1	0.312
Mean BP (mmHg)	98.7 \pm 28.8	93.4 \pm 22.6	0.376
Duration of dialysis (mo)	59.5 \pm 35.4	–	–
Anuria	30 (34.1)	–	–
Etiology of ESRD			
DM	33 (37.5)		
HTN	27 (30.7)		
GN	15 (17.0)		
CIN	5 (5.7)		
SLE	3 (3.4)		
APKD	2 (2.3)		
Unknown	3 (3.4)		

Data are presented as means \pm SD or number (%).

APKD, adult polycystic kidney disease; BMI, body mass index; BP, blood pressure; CIN, chronic interstitial nephritis; DBP, diastolic blood pressure; DM, diabetes mellitus; ESRD, end-stage renal disease; GN, glomerulonephritis; HTN, hypertension; SBP, systolic blood pressure; SLE, systemic lupus erythematosus.

Table 2. Laboratory parameters of the studied patients and control group

Variables	All patients (n = 88)	Control group (n = 28)	P	Group I (s-Klotho < 476 pg/mL, n = 44)	Group II (s-Klotho > 476 pg/mL, n = 44)	P
sCr (mg/dL)	7.9 ± 1.8	0.8 ± 0.3	0.001	7.5 ± 1.2	8.3 ± 3.1	0.114
S. Ca (mg/dL)	8.4 ± 1.0	8.5 ± 0.6	0.618	8.1 ± 0.8	8.7 ± 1.3	0.014
S. Ps (mg/dL)	1.9 ± 1.1	0.9 ± 0.2	0.001	2.1 ± 0.5	1.8 ± 0.6	0.013
S. PTH (pg/mL)	71.7 ± 25.1	31.2 ± 23.4	0.001	76.9 ± 21.3	67.1 ± 24.5	0.048
RRF (mL/min/1.73 m ²)	2.1 ± 1.5			2.4 ± 1.8	3.1 ± 1.3	0.040
Kt/V	1.6 ± 0.6			1.5 ± 0.4	1.6 ± 0.5	0.101
T. cholesterol (mg/dL)	154.4 ± 16.4	122.3 ± 14.6	0.001	151.4 ± 17.4	148.3 ± 17.3	0.404
LDL-C (mg/dL)	123.3 ± 37.3	101.5 ± 27.8	0.005	124.4 ± 36.8	122.2 ± 36.6	0.779
TGs (mg/dL)	116.5 ± 20.5	91.7 ± 16.3	0.001	117.7 ± 22.3	115.3 ± 19.2	0.590
Hb (g/dL)	11.2 ± 1.5	13.8 ± 1.2	0.001	10.8 ± 1.5	11.2 ± 1.4	0.199
Bl. glucose (mg/dL)	136.7 ± 26.4	118.6 ± 21.3	0.001	132.1 ± 27.3	141.4 ± 25.8	0.102
C-RP (mg/L)	16.1 ± 4.2	0.71 ± 0.4	0.001	20.9 ± 5.1	17.3 ± 6.2	0.004
S. albumin (g/dL)	3.8 ± 0.7	4.2 ± 0.6	0.008	3.7 ± 0.4	3.9 ± 0.9	0.182
S. uric acid (mg/dL)	6.8 ± 1.0	5.7 ± 1.2	0.001	6.7 ± 0.7	7.1 ± 1.4	0.094
S-Klotho (pg/mL)	477.9 ± 76.2	863.7 ± 261.8	0.001	427.8 ± 46.2	535.4 ± 61.4	0.001
S. FGF-23 (pg/mL)	60.5 ± 17.6	35.8 ± 13.9	0.001	67.8 ± 24.2	50.3 ± 11.2	0.001

Data are presented as mean ± SD.

Bl., blood; Ca, calcium; C-RP, C-reactive protein; FGF-23, fibroblast growth factor-23; Hb, hemoglobin; LDL-C, low-density lipoprotein cholesterol; Ps, phosphorus; PTH, parathyroid hormone; RRF, residual renal function; S., serum; sCr, serum creatinine; s-Klotho, soluble Klotho; T., total; TG, triglyceride.

significant association with CIMT, LVEF, and CAD, but not with LVM and LVMI.

FGF-23 and markers of mineral metabolism

Using the Spearman rank correlation test, we found that there was positive correlation between FGF-23 and Ps ($r = 0.497$, $P = 0.001$, 95% CI 0.321–0.640) and PTH ($r = 0.405$, $P = 0.001$, 95% CI 0.214–0.567), whereas there was negative correlation between FGF-23 and Ca ($r = -0.237$, $P = 0.026$, 95% CI -0.426 to -0.029).

FGF-23 and CVD markers

Table 4 shows the results of multivariate regression models of FGF-23, total cholesterol, triglycerides, and blood glucose with CIMT, LVM, LVMI, LVEF, and CAD as different markers for CVD. After adjustments for age, gender, mean blood pressure, and duration of HD, FGF-23, total cholesterol, triglycerides, and

blood glucose were not independently associated with CIMT, LVM, LVMI, LVEF, and CAD.

Discussion

In the present study, we measured the s-Klotho levels and determined the association between s-Klotho levels and markers of mineral metabolism (FGF-23, PTH, Ca, and Ps) and CVD (CIMT, CAD, LVM, LVMI, and LVEF) in patients with ESRD on regular HD. Our study showed that there was significant negative correlation between s-Klotho levels and serum levels of FGF-23, PTH, and Ps and positive correlation between s-Klotho and serum levels of Ca. In addition, the present study showed that lower levels of s-Klotho are significantly associated with signs of CVD such as increased CIMT, more CAD, and LV dysfunction.

Our study showed that serum levels of s-Klotho were significantly decreased in patients with ESRD on regular HD compared to the control group, as previously reported in CKD patients [26] and patients on HD [27–29]. It has been reported

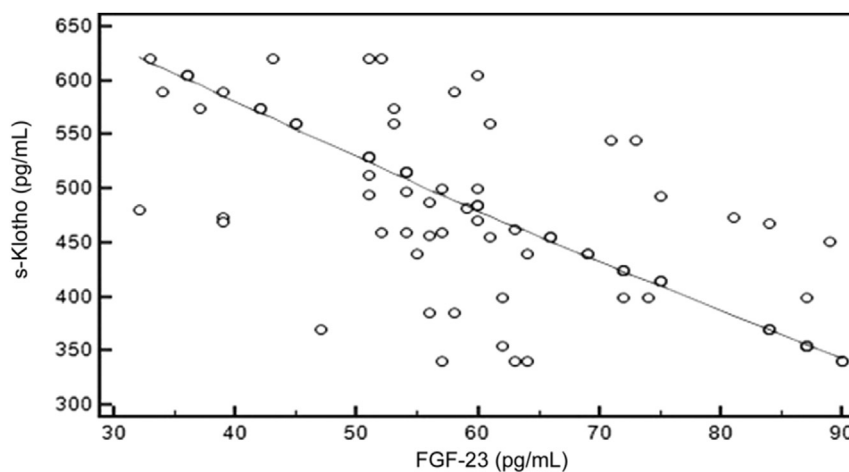


Figure 1. Correlation coefficient between the serum level of s-Klotho and FGF-23. $r = -0.717$, 95% confidence interval, -0.806 to -0.598, $P = 0.001$. FGF-23, fibroblast growth factor-23; s-Klotho, soluble Klotho.

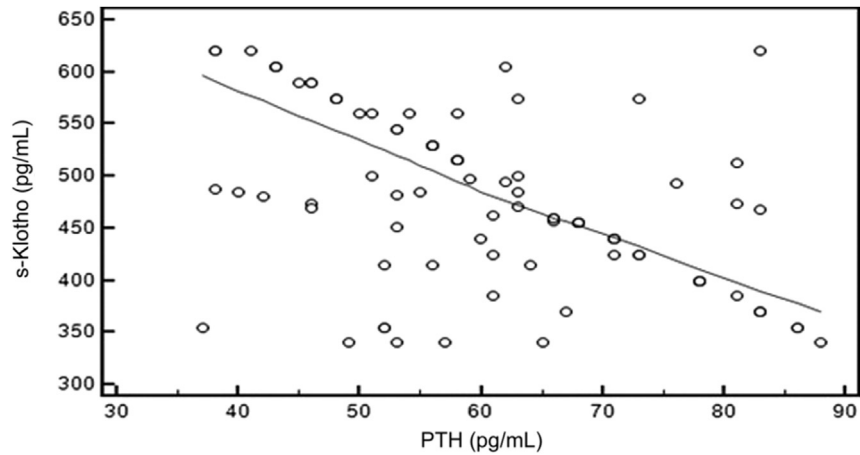


Figure 2. Correlation coefficient between the serum level of s-Klotho and PTH. $r = -0.484$, 95% confidence interval -0.630 to -0.306 , $P = 0.001$. PTH, parathyroid hormone; s-Klotho, soluble Klotho.

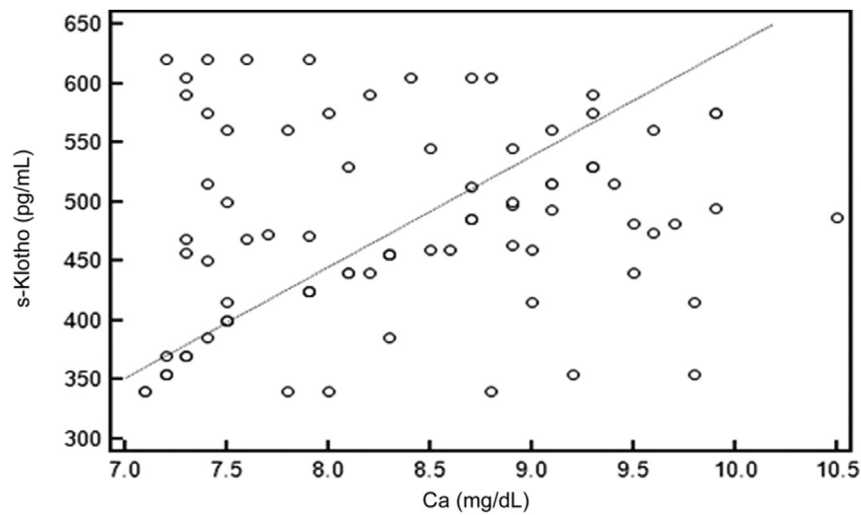


Figure 3. Correlation coefficient between the serum levels of s-Klotho and Ca. $r = 0.294$, 95% confidence interval 0.0903 – 0.474 , $P = 0.005$. Ca, calcium; s-Klotho, soluble Klotho.

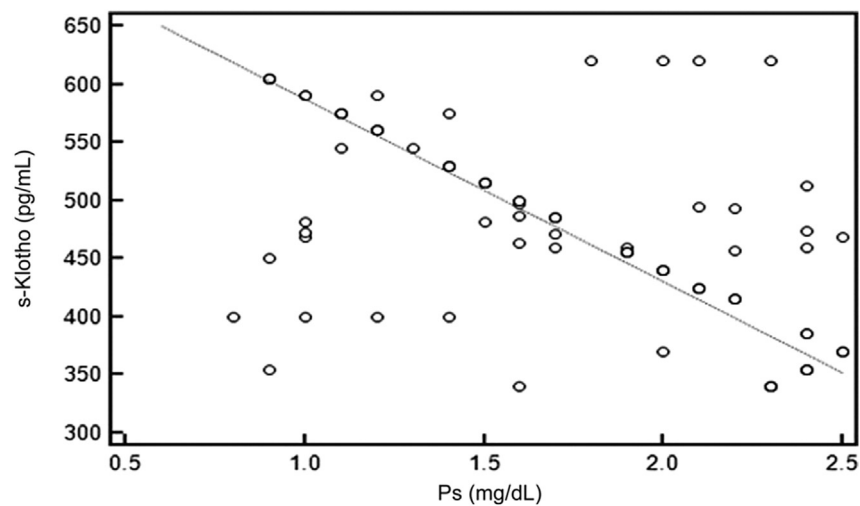


Figure 4. Correlation coefficient between the serum levels of s-Klotho and Ps. $r = -0.548$, 95% confidence interval -0.680 to -0.383 , $P = 0.001$. Ps, phosphate; s-Klotho, soluble Klotho.

Table 3. Echocardiographic findings, CIMT, and CAD of the studied patients and control group

Variable	All patients (n = 88)	Control group (n = 28)	P	Group I (s-Klotho < 474, n = 44)	Group II (s-Klotho > 474, n = 44)	P
LVM (g)	193.4 ± 22.3	112.7 ± 23.6	0.001	198.6 ± 26.3	188.2 ± 18.3	0.034
LVMI (g/m ²)	110.4 ± 21.5	66.4 ± 21.3	0.001	120.7 ± 23.2	110.2 ± 18.6	0.022
LVEF (%)	49.6 ± 10.2	58.6 ± 6.3	0.001	45.9 ± 10.3	55.2 ± 9.3	0.001
CIMT (mm)	1.0 ± 0.2	0.8 ± 0.2	0.001	1.2 ± 0.2	0.9 ± 0.1	0.001
CAD	58 (65.9)	—		36 (81.1)	22 (50.0)	0.004

Data are presented as means ± SD or number (%).

CAD, coronary artery disease; CIMT, carotid intima-media thickness; LVEF, left ventricular ejection fraction; LVM, left ventricular mass; LVMI, left ventricular mass index; s-Klotho, soluble Klotho.

that s-Klotho levels are severely reduced in patients with CKD compared to control subjects [30]. However, it appears that s-Klotho levels are not completely depleted, even in patients with ESRD on HD [27–29]. This finding suggests that in humans, a basal level of s-Klotho may be produced from other organs than the kidneys, such as the brain and parathyroid glands, as previously reported in mice [15,31,32]. Recent studies revealed that s-Klotho has been decreased in the early stage of CKD, even before rising of Ps or PTH and emerged as a powerful player in Ca–Ps homeostasis that is thought to contribute to the high burden of CVD in CKD patients [5,17]. This study showed that patients with a low s-Klotho (< 476 pg/mL) had a high age and low RRF compared to Group II, and there was no significant differences in the mean values of Kt/V between the 2 groups. Our results are in agreement with the results of Lindberg et al [28], who reported that patients with high s-Klotho were more with good RRF, and Sawires et al [29], who reported that there was no significant correlation between s-Klotho and Kt/V and there was significant negative correlation between s-Klotho and age.

The present study showed that patients with a low s-Klotho were more often associated with CIMT, LV dysfunction, and CAD, and it seems that there was independent association between s-Klotho and CIMT, LVEF, and CAD using multivariate regression analysis. Our results are in agreement with results of Kitagawa et al [33] who reported that the serum Klotho level was found to significantly correlate with markers of CKD-mineral and bone disorder and is an independent biomarker of arterial stiffness in patients with

CKD. In contrary to our results, Buiten et al [34] reported that s-Klotho was not independently associated with CVD in dialysis patients and did not support a direct cardioprotective effect of s-Klotho.

Many clinical studies have suggested that s-Klotho exerts strong cardioprotective effects. For instance, s-Klotho has been shown to protect against vascular calcifications in rodent models of CKD, whereas in humans without CKD, higher s-Klotho levels have been related to a lower incidence of mortality and CVD [28,35–37]. Moreover, low s-Klotho levels have been associated with increased arterial stiffness in CKD patients [33]. Further support for a direct role of Klotho in vascular homeostasis comes from *in vitro* studies showing endogenous expression of s-Klotho in human vascular smooth muscle cells [38]. Interestingly, inhibition of s-Klotho expression in aortic vascular smooth muscle cells resulted in accelerated calcification of these cells [38]. However, the exact role of s-Klotho in the progression of CVD in dialysis patients remains to be elucidated.

There many reports that CVD starts to develop in early stages of CKD and that s-Klotho starts to decrease also in early stages of CKD. Therefore, patients with ESRD on regular HD have been exposed to low s-Klotho levels for a prolonged period, predisposing them to vascular calcifications and atherosclerosis.

The role of s-Klotho in the development of atherosclerotic disease in dialysis patients might be overshadowed by the large amount of other pathophysiological stimuli for CVD prevalent in these patients, such as obesity, diabetes, dyslipidemia, and

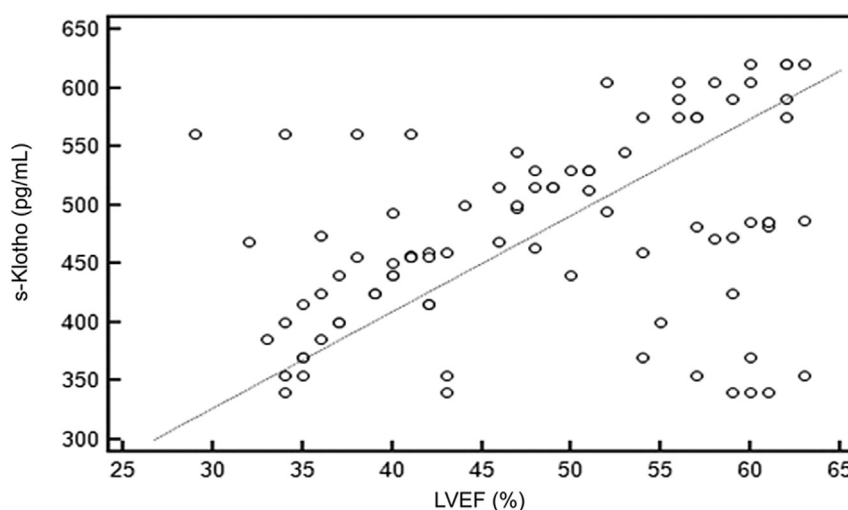


Figure 5. Correlation coefficient between s-Klotho and LVEF. $r = 0.392$, 95% confidence interval 0.199–0.556, $P = 0.001$. LVEF, left ventricular ejection fraction; s-Klotho, soluble Klotho.

Table 4. Multivariate linear regression analysis of the predictive factors for CIMT, LVM, LVMI, LVEF, and CAD

Variables	CIMT		LVM		LVMI		LVEF		CAD	
	β	<i>P</i>	β	<i>P</i>	β	<i>P</i>	β	<i>P</i>	OR	<i>P</i>
s-Klotho	−0.213	0.040	−0.184	0.070	−0.123	0.080	0.251	0.040	0.342	0.050
FGF-23	0.132	0.060	0.114	0.070	0.134	0.070	−0.162	0.060	0.451	0.060
T. cholesterol	0.075	0.130	0.033	0.160	0.124	0.080	0.004	0.310	0.312	0.090
Triglycerides	0.046	0.150	0.113	0.090	0.031	0.210	0.006	0.110	0.123	0.110
Bl. glucose	0.012	0.230	0.026	0.140	0.017	0.260	0.015	0.090	0.412	0.060

Adjusted for age, gender, mean blood pressure, and duration of dialysis.

Bl., blood; CAD, coronary artery disease; CIMT, carotid intima–media thickness; FGF-23, fibroblast growth factor-23; LVEF, left ventricular ejection fraction; LVM, left ventricular mass; LVMI, left ventricular mass index; OR, odds ratio; s-Klotho, soluble Klotho; T., total.

hypertension. This might explain the disparity between data found in rodents with only Klotho deficiency and patients suffering from a wide variety of comorbidities. However, our study showed that there were no significant differences in the mean values of lipid profile, blood glucose, mean blood pressure, body mass index, and duration of dialysis between the 2 groups. Using multivariate regression analysis, we found that there was no independent association between FGF-23, blood glucose, total cholesterol, and triglycerides and CVD markers. These findings may explain that s-Klotho seems to be independently associated with CIMT, LVEF, and CAD as markers of CVD.

In our study, s-Klotho is significantly correlated with markers of mineral metabolism, which is consistent with many studies in CKD patients [5,33] that reported that s-Klotho correlated negatively with PTH and Ps and positively with Ca, whereas no correlation was found between s-Klotho and 1,25 dihydroxycholecalciferol and fractional excretion of Ca. Also our study is consistent with studies in HD patients [34] that reported that the serum s-Klotho level was significantly associated with a lower plasma 25 hydroxy vitamin D [25(OH)D] and lower PTH [29].

In addition, in our study, FGF-23 was significantly associated with markers of mineral metabolism, and FGF-23 was not independently associated with markers of CVD. Our results are consistent with studies in HD patients [34] that reported that FGF-23 showed a strong positive association with Ps and PTH and FGF-23 was not independently associated with CVD. In addition, our results are comparable with the results from studies in CKD patients, where FGF-23 levels correlated positively with PTH and Ps and negatively with 1,25 dihydroxycholecalciferol and no correlation existed with Ca and fractional excretion of Ca [5].

This study has some limitations. We studied only a small sample of patients. Data on dietary Ca, Ps, and medication intake, which may affect the levels of serum Ps, Ca, s-Klotho, and FGF-23 were not collected. Ps and PTH were not statistically analyzed as risk factors for CVD.

In conclusion, the present study showed that patients with a low s-Klotho were more often associated with increased CIMT, LV dysfunction, and CAD, and it seems that there was an independent association between s-Klotho and CIMT, LVEF, and CAD.

Conflicts of interest

All authors have no conflicts of interest to declare.

References

- [1] Moe S, Drüeke T, Cunningham J, Goodman W, Martin K, Olgaard K, Ott S, Sprague S, Lameire N, Eknoyan G; Kidney Disease: Improving Global Outcomes (KDIGO): Definition evaluation, and classification of renal osteodystrophy: a position statement from Kidney Disease: Improving Global Outcomes (KDIGO). *Kidney Int* 69: 1945–1953, 2006
- [2] Kidney Disease: Improving Global Outcomes (KDIGO) CKD-MBD Work Group: KDIGO clinical practice guideline for the diagnosis, evaluation, prevention, and treatment of Chronic Kidney Disease–Mineral and Bone Disorder (CKD-MBD). *Kidney Int Suppl* 76 (Suppl 113):S1–S130, 2009
- [3] Moe SM, Drueke T: Improving global outcomes in mineral and bone disorders. *Clin J Am Soc Nephrol* 3 (Suppl 3):S127–S130, 2008
- [4] Isakova T, Wahl P, Vargas GS, Gutiérrez OM, Scialla J, Xie H, Appleby D, Nessel L, Bellorich K, Chen J, Hamm L, Gadegbeku C, Horwitz E, Townsend RR, Anderson CA, Lash JP, Hsu CY, Leonard MB, Wolf M: Fibroblast growth factor 23 is elevated before parathyroid hormone and phosphate in chronic kidney disease. *Kidney Int* 79:1370–1378, 2011
- [5] Rotondi S, Pasquali M, Tartaglione L, Muci ML, Mandanici G, Leonangeli C, Sales S, Farcomeni A, Mazzaferro S: Soluble α -klotho serum levels in chronic kidney disease. *Int J Endocrinol* 2015: 872193, 2015. Published online 2015 Mar 19. doi:10.1155/2015/872193
- [6] Mian IS: Sequence, structural, functional, and phylogenetic analyses of three glycosidase families. *Blood Cells Mol Dis* 24:83–100, 1998
- [7] Kurosu H, Ogawa Y, Miyoshi M, Yamamoto M, Nandi A, Rosenblatt KP, Baum MG, Schiavi S, Hu MC, Moe OW, Kuro-o M: Regulation of fibroblast growth factor-23 signaling by klotho. *J Biol Chem* 281:6120–6123, 2006
- [8] Urakawa I, Yamazaki Y, Shimada T, Iijima K, Hasegawa H, Okawa K, Fujita T, Fukumoto S, Yamashita T: Klotho converts canonical FGF receptor into a specific receptor for FGF23. *Nature* 444:770–774, 2006
- [9] Liu S, Tang W, Zhou J, Stubbs JR, Luo Q, Pi M, Quarles LD: Fibroblast growth factor 23 is a counter-regulatory phosphaturic hormone for vitamin D. *J Am Soc Nephrol* 17:1305–1315, 2006
- [10] Chen CD, Podvin S, Gillespie E, Leeman SE, Abraham CR: Insulin stimulates the cleavage and release of the extracellular domain of klotho by ADAM10 and ADAM17. *Proc Natl Acad Sci USA* 104: 19796–19801, 2007
- [11] Hu MC, Kuro-o M, Moe OW: Klotho and chronic kidney disease. *Contrib Nephrol* 180:47–63, 2013
- [12] Hu MC, Kuro-o M, Moe OW: Renal and extrarenal actions of klotho. *Semin Nephrol* 33:118–129, 2013
- [13] Hu MC, Shi M, Zhang J, Pastor J, Nakatani T, Lanske B, Razaque MS, Rosenblatt KP, Baum MG, Kuro-o M, Moe OW: Klotho: a novel phosphaturic substance acting as an autocrine enzyme in the renal proximal tubule. *FASEB J* 24:3438–3450, 2010
- [14] Cha SK, Ortega B, Kurosu H, Rosenblatt KP, Kuro-o M, Huang CL: Removal of sialic acid involving klotho causes cell-surface

- retention of TRPV5 channel via binding to galectin-1. *Proc Natl Acad Sci USA* 105:9805–9810, 2008
- [15] Kuro-o M: Phosphate and klotho. *Kidney Int* 79 (Suppl 121): S20–S23, 2011
- [16] Go AS, Chertow GM, Fan D, McCulloch CE, Hsu CY: Chronic kidney disease and the risks of death, cardiovascular events, and hospitalization. *N Engl J Med* 351:1296–1305, 2004
- [17] Shimada T, Hasegawa H, Yamazaki Y, Muto T, Hino R, Takeuchi Y, Fujita T, Nakahara K, Fukumoto S, Yamashita T: FGF-23 is a potent regulator of vitamin D metabolism and phosphate homeostasis. *J Bone Miner Res* 19:429–435, 2004
- [18] Hunt SA, Abraham WT, Chin MH, Feldman AM, Francis GS, Ganiats TG, Jessup M, Konstam MA, Mancini DM, Michl K, Oates JA, Rahko PS, Silver MA, Stevenson LW, Yancy CW, Antman EM, Smith Jr SC, Adams CD, Anderson JL, Faxon DP, Fuster V, Halperin JL, Hiratzka LF, Jacobs AK, Nishimura R, Ornato JP, Page RL, Riegel B; American College of Cardiology; American Heart Association Task Force on Practice Guidelines American College of Chest Physicians International Society for Heart and Lung Transplantation Heart Rhythm Society: ACC/AHA 2005 Guideline Update for the Diagnosis and Management of Chronic Heart Failure in the Adult: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines (Writing Committee to Update the 2001 Guidelines for the Evaluation and Management of Heart Failure): developed in collaboration with the American College of Chest Physicians and the International Society for Heart and Lung Transplantation: endorsed by the Heart Rhythm Society. *Circulation* 2005;112:e154–e235. Published online 2005 Sep 13. doi:10.1161/CIRCULATIONAHA.105.167586
- [19] Yamazaki Y, Imura A, Urakawa I, Shimada T, Murakami J, Aono Y, Hasegawa H, Yamashita T, Nakatani K, Saito Y, Okamoto N, Kurumatani N, Namba N, Kitaoka T, Ozono K, Sakai T, Hataya H, Ichikawa S, Imel EA, Econs MJ, Nabeshima Y: Establishment of sandwich ELISA for soluble alpha-klotho measurement: age-dependent change of soluble alpha-klotho levels in healthy subjects. *Biochem Biophys Res Commun* 398:513–518, 2010
- [20] Imel EA, Peacock M, Pitukcheewanont P, Heller HJ, Ward LM, Shulman D, Kassem M, Rackoff P, Zimering M, Dalkin A, Drobny E, Colussi G, Shaker JL, Hoogendoorn EH, Hui SL, Econs MJ: Sensitivity of fibroblast growth factor 23 measurements in tumor-induced osteomalacia. *J Clin Endocrinol Metab* 91:2055–2061, 2006
- [21] European Best Practice Guidelines Expert Group on Hemodialysis-European Renal Association: Section I. Measurement of renal function, when to refer and when to start dialysis. *Nephrol Dial Transplant* 17:7–9, 2002
- [22] Daugirdas JT: Second generation logarithmic estimates of single-pool variable volume Kt/V: an analysis of error. *J Am Soc Nephrol* 4:1205–1213, 1993
- [23] Devereux RB, Reichek N: Echocardiographic determination of left ventricular mass in man. Anatomic validation of the method. *Circulation* 55:613–618, 1977
- [24] Nakamura A, Shikata K, Hiramatsu M, Nakatou T, Kitamura T, Wada J, Itoshima T, Makino H: Serum interleukin-18 levels are associated with nephropathy and atherosclerosis in Japanese patients with type 2 diabetes. *Diabetes Care* 28:2890–2895, 2005
- [25] Handa N, Matsumoto M, Maeda H, Hougaku H, Ogawa S, Fukunaga R, Yoneda S, Kimura K, Kamada T: Ultrasonic evaluation of early carotid atherosclerosis. *Stroke* 21:1567–1572, 1990
- [26] Shimamura Y, Hamada K, Inoue K, Ogata K, Ishihara M, Kagawa T, Inoue M, Fujimoto S, Ikebe M, Yuasa K, Yamanaka S, Sugiura T, Terada Y: Serum levels of soluble secreted alpha-klotho are decreased in the early stages of CKD, making it a probable novel biomarker for early diagnosis. *Clin Exp Nephrol* 16:722–729, 2012
- [27] Yokoyama K, Imura A, Ohkido I, Maruyama Y, Yamazaki Y, Hasegawa H, Urae J, Sekino H, Nabeshima Y, Hosoya T: Serum soluble alpha-klotho in hemodialysis patients. *Clin Nephrol* 77: 347–351, 2012
- [28] Lindberg K, Amin R, Moe OW, Hu MC, Erben RG, Östman Wernerson A, Lanske B, Olsson H, Larsson TE: The kidney is the principal organ mediating klotho effects. *J Am Soc Nephrol* 25: 2169–2175, 2014
- [29] Sawires HK, Essam RM, Morgan MF, Mahmoud RA: Serum klotho: relation to fibroblast growth factor-23 and other regulators of phosphate metabolism in children with chronic kidney disease. *Nephron* 129:293–299, 2015
- [30] Koh N, Fujimori T, Nishiguchi S, Tamori A, Shiomi S, Nakatani T, Sugimura K, Kishimoto T, Kinoshita S, Kuroki T, Nabeshima Y: Severely reduced production of klotho in human chronic renal failure kidney. *Biochem Biophys Res Commun* 280:1015–1020, 2001
- [31] Kuro-o M, Matsumura Y, Aizawa H, Kawaguchi H, Suga T, Utsugi T, Ohyama Y, Kurabayashi M, Kaname T, Kume E, Iwasaki H, Iida A, Shiraki-Iida T, Nishikawa S, Nagai R, Nabeshima YI: Mutation of the mouse klotho gene leads to a syndrome resembling ageing. *Nature* 390:45–51, 1997
- [32] John GB, Cheng CY, Kuro-o M: Role of klotho in aging, phosphate metabolism, and CKD. *Am J Kidney Dis* 58:127–134, 2011
- [33] Kitagawa M, Sugiyama H, Morinaga H, Inoue T, Takiue K, Ogawa A, Yamanari T, Kikumoto Y, Uchida HA, Kitamura S, Maeshima Y, Nakamura K, Ito H, Makino H: A decreased level of serum soluble klotho is an independent biomarker associated with arterial stiffness in patients with chronic kidney disease. *PLoS One* 2013;8:e56695. Published online 2013 Feb 19. doi:10.1371/journal.pone.0056695
- [34] Buiten MS, de Bie MK, Bouma-de Krijger A, van Dam B, Dekker FW, Jukema JW, Rabelink TJ, Rotmans JI: Soluble klotho is not independently associated with cardiovascular disease in a population of dialysis patients. *BMC Nephrol* 15:197, 2014
- [35] Hu MC, Shiizaki K, Kuro-o M, Moe OW: Fibroblast growth factor 23 and Klotho: physiology and pathophysiology of an endocrine network of mineral metabolism. *Annu Rev Physiol* 75:503–533, 2013
- [36] Semba RD, Cappola AR, Sun K, Bandinelli S, Dalal M, Crasto C, Guralnik JM, Ferrucci L: Plasma klotho and cardiovascular disease in adults. *J Am Geriatr Soc* 59:1596–1601, 2011
- [37] Navarro-Gonzalez JF, Donate-Correa J, Muros de Fuentes M, Perez-Hernandez H, Martinez-Sanz R, Mora-Fernandez C: Reduced klotho is associated with the presence and severity of coronary artery disease. *Heart* 100:34–40, 2014
- [38] Lim K, Lu TS, Molostvov G, Lee C, Lam FT, Zehnder D, Hsiao LL: Vascular klotho deficiency potentiates the development of human artery calcification and mediates resistance to fibroblast growth factor 23. *Circulation* 125:2243–2255, 2012