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Soluble Klotho levels in diabetic nephropathy: relationship with arterial stiffness

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Abstract. – OBJECTIVE: In this cross-sectional study, we investigate the relationship between soluble Klotho (s-Klotho) levels, markers of bone mineral metabolism and arterial stiffness in 109 diabetic nephropathy patients (median age 61.00± 9.77 years) and 32 healthy controls (median age 49.23 ± 7.32 years).

PATIENTS AND METHODS: Blood samples were collected to measure the levels of s-Klotho, and FGF23, serum creatinine, Calcium (Ca), Phosphorus (P), 25-hydroxyvitamin D3 (25hD) and parathyroid hormone (PTH). Pulse wave velocity (PWV) and blood pressure were also measured using a combined monitor.

RESULTS: s-Klotho, FGF23 and PTH levels were significantly higher and 25hD was significantly lower in the patients than in controls ($p < 0.001$). Systolic blood pressure, pulse pressure and PWV were also significantly higher in the patients ($p < 0.001$). s-Klotho, FGF23 and 25hD levels significantly varied between sub-groups according to CKD stages, defined according to the CKD epidemiology collaboration equation. A strong positive correlation was found between s-Klotho and FGF23 ($r = 0.768$, $p = 0.001$) levels, but not with other bone mineral metabolism, blood pressure or arterial stiffness parameters. Creatinine levels significantly differed ($p = 0.009$) between three s-Klotho-level subgroups, with the high creatinine levels in the sub-group with the lowest s-Klotho levels and estimated glomerular filtration rate (eGFR).

CONCLUSIONS: There was no correlation between eGFR and s-Klotho levels. Arterial stiffness increased in CKD but was not related to s-Klotho or FGF23 levels. Among all parameters, FGF23 levels had the greatest effect on s-Klotho levels.

Key Words:
Diabetic nephropathy, Arterial stiffness, Soluble Klotho, Chronic kidney disease, Fibroblast growth factor 23.

Introduction

Chronic kidney disease (CKD) is a major health problem worldwide. Diabetic nephropathy has become the leading cause of end-stage kidney disease in recent years[1]. Disturbances in bone mineral metabolism and bone disease are common complications of CKD and an important cause of morbidity and mortality. *Klotho* is an aging suppressor gene encoding a 130-kDa single-pass transmembrane protein with an extracellular portion; it is expressed in the distal renal tubules, parathyroid glands and the choroid plexus. Klotho is found in two forms: the membrane form and the secreted form. Membrane Klotho forms a complex with the fibroblast growth factor 23 (FGF23) receptor and is a co-receptor for FGF23[2,3]. It serves as a mediator for the actions of FGF23, namely urinary phosphate (P) excretion, inhibition of calcitriol ($1,25(OH)_2D$) secretion and inhibition of parathyroid hormone (PTH) synthesis and secretion[4]. The secreted form, soluble Klotho (s-Klotho), functions as a humoral factor and is involved in the regulation of nitric oxide production in the endothelium, the preservation of endothelial integrity and permeability, calcium (Ca^{++}) homeostasis in the kidneys and the inhibition of intracellular insulin and insulin-like growth factor-1 signalling[5]. Recent researches[6,7] have indicated that an increase in FGF23 level is the first sign of impaired CaP metabolism, well before increases in serum PTH or phosphate levels are observed.

Arterial stiffness is a powerful independent predictor of cardiovascular events and all-cause mortality[8]. It has been shown to be associated with decreased estimated glomerular filtration rate (eGFR), hypertension and diabetes mellitus[9-12]. In the general population, measurement of brachial-ankle pulse wave velocity (baPWV) is widely used as an index of arterial stiffness because of its easy execution, good reproducibility and good correlation with aortic PWV[13,14]. Arterial Klotho expression has been observed to be lower in patients with CKD than in healthy individuals[15]. Klotho is a protective factor against the

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development of vascular endothelial dysfunction. The s-Klotho receptor on the vascular endothelium has not been identified; however, it has been shown that s-Klotho protects endothelial integrity by regulating Ca entry into vascular endothelial cells[16,17]. It has been demonstrated that FGF23 levels are an independent marker of endothelial dysfunction[18].

The relationship between s-Klotho levels, FGF23 levels and kidney function in diabetic patients with CKD is yet unclear. In this study, we investigated the relationships between s-Klotho and FGF23 levels as well as parameters of CKD, bone mineral metabolism and arterial stiffness.

Patients and Methods

For this cross-sectional study, we included 109 diabetic nephropathy patients (median age 61.00 ± 9.77 years) admitted to the outpatient clinic of Antalya Research and Training Hospital Nephrology Unit between January and June 2014 and 32 healthy controls (median age 49.23 ± 7.32 years). Patients aged <18 years, pregnant women, those with clinically apparent infections, active malignancy or acute renal failure and those who used vitamin D or phosphate binders were excluded from the study. The study was conducted according to the Declaration of Helsinki and the guidelines of Good Clinical Practice and was approved by the local Ethics Committee. All patients gave written informed consent.

The CKD patients were classified according to CKD epidemiology collaboration (CKD-EPI) equation assessments of glomerular filtration rate as CKD Stage 1 (≥90 mL/min/1.73 m^2), CKD Stage 2 (60-89 mL/min/1.73 m^2), CKD Stage 3 (30-59 mL/min/1.73 m^2) and CKD Stage 4 (15-29 mL/min/1.73 m^2). The evaluation of the patients and healthy controls included the following: past medical history, clinical assessment, blood pressure measurement after 15 min of rest and measurement of height, weight and waist circumference.

Blood samples were collected in the morning after an 8-h fast. The serum was stored at -80°C. The blood was analyzed for FGF23, soluble Klotho, PTH, P, Ca, creatinine and 25-hydroxyvitamin D3 (25hD) levels. The urinary protein to creatinine ratio was calculated with spot urine protein and creatinine measurements. Serum blood urea nitrogen, creatinine, Ca and P levels were determined using commercially available assay kits (Beckman Coulter Diagnostics, Brea, CA, USA) and an autoanalyser (Beckman AU5800; Beckman Coulter Diagnostics). The serum 25hD assays were performed using the direct competitive chemiluminescence immunoassay method (DiaSorin, Stillwater, MN, USA), with a minimum threshold of 3.5 ng/mL for detection and a coefficient of the variation range of 4.8%-11.1% 25hD. Serum intact PTH levels were determined using commercially available assay kits (Beckman Coulter Diagnostics) and an autoanalyser (Access DxI800; Beckman Coulter Diagnostics). The intact PTH assay is a two-site immunoenzymatic (sandwich) assay, which is linear up to 3500 pg/mL, with a minimum threshold of 1 pg/mL for detection and a coefficient of the variation range of 3.5%-6.4%.

Serum s-Klotho and FGF23 levels were measured using a commercially available enzyme-linked immunosorbent assay (ELISA) kit (YH Biosearch, Shanghai, China) (coefficient of variation <10% for both parameters, with serum soluble α-Klotho levels assay range of 0.05-20 ng/mL and FGF23 assay range of 5-1500 pg/mL). The assays used the quantitative sandwich enzyme immunoassay technique. To avoid variability within an assay, measurements were performed simultaneously in duplicate using the same ELISA kit.

PWV and blood pressure analysis used the Mobil-O-Graph 24h PWA monitor (I.E.M. Industrielle Entwicklung Medizintechnik und Vertriebsgesellschaft mbH, Stolberg, Germany). This device's built-in algorithms were used for the central aortic and brachial blood pressure measurements by combining the pulse wave analysis. We measured baPWV, augmentation index (AIx), and pulse pressure (PP) for the assessment of arterial stiffness as well as systolic blood pressure (SBP) and diastolic blood pressure (DBP) in the supine position after 5 min of bed rest.

Statistical Analisys

Continuous variables are presented as mean ± standard deviation and categorical variables as percentages; median values are used for age. The Kolmogorov-Smirnov test was used to verify the normality of the distributions of continuous variables. Unpaired t-tests for parametric data and the Mann-Whitney U test for non-parametric data were used for statistical analysis of clinical data between the two groups; one-way analysis of variance or the Kruskal-Wallis test was used to evaluate comparisons between three or more groups. The Bonferroni correction was applied in post-hoc analyses. Correlations were assessed

Table I. Characteristics of patients with diabetic nephropathy and healthy controls

Paramater	Diabetes (n = 109)	Control (n = 32)	p
Age (years)	61.00 ± 9.77 (median)	49.23 ± 7.32 (median)	**<0.001**
Gender (F/M)	47/62	20/12	0.054
Creatinine (mg/dL)	1.57 ± 0.75	0.88 ± 0.12	**<0.001**
GFR (mL/min/1.73m2)-[CKD-EPI]	51.71 ± 23.11	90.15 ± 20.71	**<0.001**
UPCR (mg/d)	1625.43 ± 2227.62	0.06.40 ± 0.03	**<0.001**
s-Klotho (ng/mL)	5.69 ± 4.64	3.62 ± 4.27	**<0.001**
FGF23 (pg/mL)	360.29 ± 528.36	189.09 ± 293.22	**0.001**
25hD (ng/mL)	16.73 ± 13.15	62.13 ± 18.37	**<0.001**
PTH (pg/mL)	96.33 ± 111.52	57.79 ± 22.28	**<0.001**
Calcium (mg/dL)	9.36 ± 0.55	9.37 ± 0.39	0.974
Phosphorus (mg/dL)	3.43 ± 0.64	3.28 ± 0.67	0.136
ALP (U/L)	85.82 ± 33.76	76.25 ± 16.36	0.290
SBP (mmHg)	140.40 ± 25.48	111.97 ± 13.21	**<0.001**
DBP (mmHg)	82.59 ± 12.99	78.03 ± 10.24	0.212
PP (mmHg)	57.8 ± 18.55	37.9 ± 7.77	**<0.001**
baPWV (m/s)	9.32 ± 1.64	6.82 ± 1.10	**<0.001**
AIx	26.84 ± 15.87	22.03 ± 15.59	0.092

UPCR: urinary protein creatinin ratio; s-Klotho: soluble Klotho; FGF23: fibroblast growth factor 23; 25hD: 25-hydroxyvitamin D3; SBP: systolic blood pressure; DBP: diastolic blood pressure; PP: pulse pressure; baPWV: brachial artery pulse wave velocity; AIx: augmentation index.

with Pearson or Spearman correlation coefficients, and the chi-square test was used for categorical variables. Factors related to serum soluble α-Klotho levels in patients were evaluated using multiple linear regression analysis. The analyses were performed with PASW 18 software (SPSS/IBM, Chicago, IL, USA), and two-tailed *p*-values of <0.05 were considered statistically significant.

Results

The baseline characteristics of the patients (n = 109) and the healthy control group (n = 32) are presented in Table I. The levels of s-Klotho, FGF23 and PTH were significantly higher and 25hD levels were significantly lower in the patient group than in the control group ($p < 0.001$). SBP, PP and PWV levels were also significantly higher in the patient group ($p < 0.001$).

The patients were classified into CKD stages according to the CKD-EPI equation: 7.3% were in CKD Stage 1, 25.7% were in Stage 2, 50.5% were in Stage 3 and 16.5% were in Stage 4. Table II presents the main parameters for these CKD stage groups and the control group. One-way analysis of variance revealed statistically significant differences between these five groups for the levels of Ca ($p = 0.012$), P ($p = 0.08$) and albumin ($p = 0.001$). Significant differences between the groups were also detected for the FGF23 and 25hD levels ($p = 0.001$); it was determined that these differences were significant as a result of the markedly different control group values. The s-Klotho levels were found to be significantly different among the groups ($p = 0.001$), with this a result of the Stage 2 CKD group value. PWV, SBP and PP also significantly differed between the groups ($p = 0.001$). In the case of PWV, this was as a result of the Stage 1 CKD and control group values.

Correlation analyses were performed between the s-Klotho and the other bone mineral metabolism parameters, blood pressure and arterial stiffness (Table III). A statistically significant strong positive correlation was found between s-Klotho and FGF23 ($r = 0.768$, $p = 0.001$). No significant correlation was found between s-Klotho levels and eGFR, Ca levels, P levels, 25hD levels, PTH levels, SBP, DBP, PP or baPWV.

We divided the 109 patients into three groups according to their s-Klotho levels as follows: tertile 1, 0.76-3.71 ng/mL (n = 36); tertile 2, 3.77-4.65 ng/mL (n = 37) and tertile 3, 4.88-22.56 ng/mL (n = 36). A significant difference between the tertiles was found in creatinine levels ($P = 0.009$), with mean creatinine levels high in tertile 1, in which the s-Klotho and eGFR levels were lower. No difference was found between the ter-

Table II. Characteristics of patients with diabetic nephropathy.

Parameters	CKD1 (n = 8)	CKD 2 (n = 28)	CKD3 (n = 55)	CKD4 (n = 18)	Control (n = 32)	p-value
Age (years)	52.13 ± 6.68*	63.11 ± 10.87	63.42 ± 8.10	58.11 ± 11.07	49.53 ± 7.32*	<0.001[a]
Creatinine (mg/dL)	0.73 ± 0.10	0.98 ± 0.16	1.55 ± 0.29*	2.91 ± 0.70*	0.88 ± 0.12	<0.001[b]
eGFR (mL/min/1.73 m2)	103.10 ± 5.42	71.08 ± 9.88	44.06 ± 8.48	22.14 ± 4.81	90.15 ± 20.71	<0.001
Calcium (mg/dL)	9.46 ± 0.25	9.54 ± 0.42	9.38 ± 0.56	8.97 ± 0.63*	9.37 ± 0.39	0.012[c]
Phosphorus (mg/dL)	2.97 ± 0.51	3.45 ± 0.50	3.34 ± 0.58	3.90 ± 0.80*	3.28 ± 0.67	0.008[c]
PTH(pg/mL)	44.13 ± 13.31	51.41 ± 24.32	85.21 ± 47.92	206.56 ± 218.18*	57.79 ± 22.28	<0.001[c]
Albumin(mg/dL)	4.22 ± 0.21	4.10 ± 0.35	3.90 ± 0.51	3.58 ± 0.44*	4.26 ± 0.28	<0.001[c]
25hD (ng/mL)	25.09 ± 25.78	15.21 ± 9.21	17.67 ± 13.16	11.69 ± 7.20	62.13 ± 18.37*	<0.001[d]
FGF23 (pg/mL)	280.65 ± 355.11	502.493 ± 662.29	259.88 ± 381.261	481.27 ± 691.96	189.09 ± 293.22*	0.001[d]
s-Klotho (ng/mL)	5.43 ± 3.80	7.26 ± 6.16* *vs.* Control	4.95 ± 3.56	5.60 ± 4.97	3.62 ± 4.27	<0.001[e]
ALP (U/L)	84.71 ± 28.62	76.91 ± 19.46	92.15 ± 42.51	85.22 ± 27.39	4.26 ± 0.28	0.555
UPCR (mg/d)	0.38 ± 0.48	0.64 ± 1.37	1.66 ± 1.91*	3.70 ± 3.21*	0.06.40 ± 0.03	<0.001[b]
SBP (mmHg)	124.25 ± 8.64	134.00 ± 18.31	145.23 ± 29.85	144.20 ± 22.02	115.97 ± 13.21	<0.001
DBP (mmHg)	81.13 ± 8.62	79.08 ± 12.56	83.91 ± 33.66	84.87 ± 14.23	78.03 ± 10.24	0.212
PP (mmHg)	43.13 ± 6.85	54.92 ± 14.20	61.27 ± 20.98	59.33 ± 17.53	37.94 ± 7.77	<0.001
PVW (m/s)	7.37 ± 0.86*	9.32 ± 1.69	9.65 ± 1.53	9.36 ± 1.63	6.82 ± 1.10*	<0.001[d]
AIx	22.63 ± 9.56	28.52 ± 15.43	28.26 ± 17.25	21.87 ± 14.51	22.03 ± 15.59	0.295

UPCR: urinary protein creatinine ratio; s-Klotho: soluble Klotho; FGF23: fibroblast growth factor 23; 25hD: 25-hydroxyvitamin D3; SBP: systolic blood pressure; DBP: diastolic blood pressure; PP: pulse pressure; baPWV: brachial artery pulse wave velocity; AIx: augmentation index.
*,[a] Significant difference between patients with CKD 1, those in control group and others. *,[b]Significant difference between patients with CKD 3, CKD 4 and others. *,[c]Significant difference between patients with CKD 4 and others. *,[d]Significant difference between patients with CKD 1, those in control group and others. *,[e]Significant difference between patients with CKD 2 and those in control group.

tiles for P and 25hD levels. A significant difference in Ca levels was detected ($p = 0.05$), with the lowest Ca levels observed in tertile 1. No difference between the tertiles was found about proteinuria. No difference among tertiles was determined concerning SBP, DBP, PP, PWV or AIx levels (Table IV).

Regression analysis was performed to determine the impact of age, Ca levels, P levels, 25hD levels, FGF23 levels, and eGFR on serum soluble α-Klotho levels in the patients with CKD. The only significant relationship found was with FGF23 levels ($\beta = 0.894$; $p = 0.000$) (Table V).

Discussion

s-Klotho levels in CKD have been investigated in many cross-sectional studies, and the results of studies that examined the relationship between serum s-Klotho levels and e-GFR have been controversial. Sugiura et al[19] found higher s-Klotho levels in CKD patients than in a control group; Devaraj et al[20] also found the s-Klotho levels to be

Table III. Association of s-Klotho with serum parameters of mineral metabolism ,blood pressure and arterial stiffness in diabetic nephropathy patients.

	s-Klotho	
Age (years)	r = 0.080	$p = 0.410$
Calcium (mg/dL)	r = 0.169	$p = 0.083$
Phosphate (mg/dL)	r = 0.032	$p = 0.745$
25hD (ng/mL)	r = -0.014	$p = 0.892$
PTH (pg/mL)	r = -0.055	$p = 0.586$
ALP (U/L)	r = -0.099	$p = 0.410$
FGF23 (pg/mL)	r = 0.768	$p \leq 0.001$
UPCR(mg/d)	r = -0.060	$p = 0.541$
eGFR (mL/min/1.73 m2)	r = 0.160	$p = 0.097$
SBP (mmHg)	r = 0.121	$p = 0.241$
DBP (mmHg)	r = 0.144	$p = 0.161$
PP (mmHg)	r = 0.100	$p = 0.334$
PWV (m/s)	r = 0.090	$p = 0.384$
AIx	r = 0.076	$p = 0.462$

eGFR: estimated glomerular filtration rate; UPCR: urinary protein creatinine ratio; s-Klotho: soluble Klotho; FGF23: fibroblast growth factor 23; 25hD: 25-hydroxyvitamin D3; SBP: systolic blood pressure; DBP: diastolic blood pressure; PP: pulse pressure; baPWV: brachial artery pulse wave velocity; AIx: augmentation index.

higher in CKD. Another study[21] found s-Klotho levels to be significantly lower in Stage 2–5 patients than in Stage 1 CKD patients. Significantly lower s-Klotho levels have been demonstrated in hemodialysis patients[22,23]. Pavik et al[24] demonstrated a decrease in s-Klotho levels with the progression of CKD stage. In a cohort study[25], serum levels of s-Klotho were not related to kidney function in Stages 2, 3a and 4 CKD patients.

Kacso et al[26] reported that in diabetic patients with CKD, the s-Klotho levels were low in the early CKD stages and then increased with the decrease in eGFR. In contrast to these findings, our study of 109 patients with CKD secondary to diabetic nephropathy revealed higher s-Klotho levels than in healthy controls. These findings are notable as Klotho is secreted from renal tubular cells and therefore, as CKD progresses, a decrease in Klotho levels would be expected. No significant relationship was found between s-Klotho levels and eGFR in the correlation analysis. A strong, positive correlation was found between s-Klotho and FGF23 levels. Pathologically increased FGF23 levels may increase the *Klotho* gene expression[27,28], and therefore, Klotho expression may have increased in our patients because of an increase in FGF23 levels. The parallel increase in the FGF23 and s-Klotho levels between Stage 1 and Stage 2 CKD patients is notable. An increase in FGF23 also causes an increase in Klotho expression in the parathyroid gland[29]. In addition, the increase in s-Klotho levels in the patients compared with the control group could be explained by a decrease in renal clearance and possible extra-renal s-Klotho production. Another explanation for these conflicting results could be the use of different kits for measuring s-Klotho levels[30].

Table IV. Patient baseline characteristics stratified by plasma s-Klotho levels.

	1 0.76-3.71 ng/mL n = 36		2 3.77-4.65 ng/mL n = 37		3 4.88-22.56 ng/mL n = 36		
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**	**P**
Age (years)	60	11	63	10	62	8	0.328
BUN (mg/dL)	31	12	20	7	23	11	0.001
Creatinine (mg/dL)	1.89	0.92	1.26	0.37	1.57	0.75	0.009
eGFR (mL/min/1.73 m2)	43.52	23.54	58.20	20.82	53.17	23.53	0.010
Calcium (mg/dL)	9.2	0.6	9.5	0.4	9.4	0.6	0.050
Phosphorus (mg/dL)	3.6	0.8	3.3	0.6	3.4	0.5	0.649
25hD (ng/mL)	17.01	15.08	16.65	9.65	16.58	14.69	0.850
FGF23 (pg/mL)	139.13	96.03	150.03	39.51	797.56	746.25	0.001
Albumin (mg/dL)	3.8	0.5	4.0	0.3	4.0	0.5	0.065
PTH (pg/mL)	121	160	71	48	97	94	0.117
HbA1c (%)	8.2	2.0	7.2	0.9	10.4	14.2	0.051
UPCR (mg/d)	1958	2631	1083	1407	1845	2421	0.273
SBP (mmHg)	134	17	141	31	145	24	0.267
DBP (mmHg)	79	11	82	14	86	13	0.213
AIx	24	17	30	17	25	13	0.272
PWV (m/s)	9.0	1.5	9.5	1.8	9.4	1.5	0.401
Drugs n (%)							
ACEi	12 (33.3)		11 (29.7)		14 (38.8)		0.555
ARB	9 (25)		15 (40.5)		9 (25)		0.438
Metformin	6 (16.6)		23 (62.1)		14 (38.8)		**0.003**
Sulfonylureas	3 (8.3)		4 (10.8)		6 (16.6)		0.611
Insulin	18 (50)		20 (54)		21 (58.3)		0.715
Statins	9 (25)		11 (29.7)		10 (27.7)		0.914

eGFR: estimated glomerular filtration rate; UPCR: urinary protein creatinine ratio; s-Klotho: soluble Klotho; FGF23: fibroblast growth factor 23; 25hD: 25-hydroxyvitamin D3; SBP: systolic blood pressure; DBP: diastolic blood pressure; PP: pulse pressure; baPWV: brachial artery pulse wave velocity; AIx: augmentation index; ACEi: angiotensin converting enzyme inhibitors; ARB: angiotensin receptor antagonists.

Table V. Regression analysis for the serum s-Klotho levels in patients with diabetic nephropathy.

	s-Klotho	
	β value	p value
Calcium	0.058	0.367
Phosphate	0.000	0.999
25hD	-0.032	0.601
PTH	-0.009	0.889
FGF23	0.894	0.000
Age	0.033	0.709
eGFR	0.074	0.270

25hD: 25-hydroxyvitamin D3; PTH: parathyroid hormone; FGF23: fibroblast growth factor 23; eGFR: estimated glomerular filtration rate.

When the patients were divided into three groups according to their s-Klotho levels, a significant difference in creatinine levels was found among the groups, with high creatinine levels and low eGFR in the group of patients in which the s-Klotho levels were the lowest. As previously noted, we found higher s-Klotho levels in the CKD patients than in the controls; however, we observed that creatinine levels were lower in the sub-group with higher Klotho levels. FGF23 levels significantly increased with the three s-Klotho level groups, providing further evidence that an increase in s-Klotho is related to FGF23. Klotho was first discovered as an aging gene, Yamazaki et al[31] being the first to measure s-Klotho levels. s-Klotho levels have been found to be higher in children, and if children are excluded from the analysis, the age-Klotho relationship is weakened. Seiler et al[25] showed a weak relationship between age and s-Klotho. In the present work, we did not find any relationship between age and s-Klotho levels.

PTH along with FGF23 acts against hyperphosphatemia by increasing renal phosphate excretion. Studies have shown that an increase in FGF23 is the first sign of an impaired Ca-P metabolism, well before increases in serum PTH or phosphate levels are observed[6,7]. In our study, a significant difference in FGF23 levels was detected among the groups because of the markedly lower control group value. Factors potentially affecting Klotho levels were evaluated using regression analysis, but no relationship with age, C, P, 25hD, or PTH levels was found; however, a significant relationship was again found with FGF23 levels, supporting the correlation and Klotho sub-group analysis results already discussed.

The leading cause of death in patients with CKD is cardiovascular disease, regardless of whether there is a progression to Stage 5. s-Klotho has an important vasoprotective effect, and it is important for mineral homeostasis and renal protection[15,32]. The vasoprotective effect results from decreasing the oxidative stress at the cellular level and improving endothelial function[16]. Yılmaz et al[18] showed that FGF23 levels and endothelial dysfunction were independently correlated, possibly because of asymmetric dimethyl arginine, an endogenous inhibitor of nitric oxide synthase. In the study by Kitagawa et al[5] that first investigated s-Klotho and arterial stiffness in humans, serum s-Klotho levels were found to be independent biomarkers of arterial stiffness in patients with CKD. Arterial stiffness is one of the non-traditional risk factors associated with the increased cardiovascular risk due to CKD[33,34].

In the general population, the measurement of baPWV is widely used as an index of arterial stiffness because of its easy execution, good reproducibility and good correlation with aortic PWV[13,14]. In our study, baPWV, AIx, SBP, DBP and PP were measured in the CKD patients and healthy controls; baPWV was significantly higher in the patients than in the controls. Comparing the stages and the control group showed that the difference was caused by the CKD Stage 1 and control group values. However, no correlation was found between baPWV and s-Klotho or FGF23 levels. In our study, the CKD cohort mostly comprised patients with CKD at Stages 1-3 and early to middle stages of CKD rather than patients with severe renal dysfunction or uremia; this may explain this result.

Klotho expression is increased by statins and angiotensin-II and decreased by peroxisome proliferator activator receptor gamma agonists[26,35]. We studied the influence of the presence and absence of medication in our patients that could affect the serum s-Klotho levels and found no significant effect of treatment on serum s-Klotho levels in our cohort.

Our report had several limitations. It was a cross-sectional study, and we did not measure membrane-bound Klotho, urinary Klotho, or 1,25(OH)2D levels. Many of our diabetic subjects may have had diabetic complications and comorbidities, but the effect of comorbidities was not adequately addressed and the time since the diagnosis of diabetes was not known. Also, end-stage kidney failure diseases under renal replacement therapy were not included.

Conclusions

There was no correlation between eGFR and s-Klotho levels in diabetic nephropathy-related CKD patients. Arterial stiffness measured by baPWV increased in CKD patients, but it was not related to s-Klotho or FGF23 levels. The parameter with the greatest effect on s-Klotho levels was FGF23 levels.

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Conflict of Interest

The Authors declare that they have no conflict of interests.

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