Soluble Klotho and Fibroblast Growth Factor 23 Levels in Diabetic Nephropathy: Relationship with Arterial Stiffness

1 Antalya Training and Research Hospital, Internal Medicine, Division of Nephrology 2 Akdeniz University, Faculty of Medicine, Internal Medicine, Division of Nephrology 3 Antalya Training and Research Hospital, Biochemistry

Ayca Inci,[1] Funda Sari,[2] Refik Olmaz,[1] Melahat Coban,[1] Suleyman Dolu,[1] Metin Sarikaya,[1] Hamit Yasar Ellidag,[3]

OBJECTIVES

Diabetic nephropathy has become the leading cause of end-stage kidney disease in recent years [1]. In this cross-sectional study, we investigate the relationship between soluble Klotho (s-Klotho) levels, fibroblast growth factor 23 (FGF23) levels, markers of chronic kidney disease (CKD), bone-mineral metabolism and arterial stiffness in 109 diabetic nephropathy patients (mean age 61.63 ± 9.77 years) and 32 healthy controls (mean age 49.53 ± 7.32 years).

METHODS

For this cross-sectional study, we included 109 diabetic nephropathy patients (mean age 61.63 ± 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 (mean age 49.53 ± 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.Blood samples were collected to measure the levels of s-Klotho, FGF23, serum creatinine, Calcium, Phosphorus, 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 no correlation was found with other bone mineral metabolism, blood pressure or arterial stiffness parameters. Creatinine levels significantly differed ($p = 0.009$) between three s-Klotho-level sub-groups, with the high creatinine levels in the sub-group with the lowest s-Klotho levels and estimated glomerular filtration rate (eGFR).

Table 1: Characteristics of patients with diabetic nephropathy

Parameters	*CKD1 (n = 8)*	*CKD 2 (n = 28)*	*CKD3 (n = 55)*	*CKD4 (n = 18)*	*Control (n = 32)*	*p*
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.

Table 2: Association of s-Klotho and FGF23 levels with serum parameters of mineral metabolism in diabetic nephropathy patients

	s-Klotho	FGF23
Age (years)	$r = 0.080$ $p = 0.410$	$r = 0.081$ $p = 0.400$
Calcium (mg/dL)	$r = 0.169$ $p = 0.083$	$r = 0.013$ $p = 0.895$
Phosphate (mg/dL)	$r = 0.032$ $p = 0.745$	$r = 0.062$ $p = 0.531$
25hD (ng/mL)	$r = -0.014$ $p = 0.892$	$r = -0.081$ $p = 0.416$
PTH (pg/mL)	$r = -0.055$ $p = 0.586$	$r = 0.070$ $p = 0.489$
ALP (U/L)	$r = -0.099$ $p = 0.410$	$r = -0.020$ $p = 0.866$
FGF23 (pg/mL)	$r =$ **0.768** $p =$ **<0.001**	-
UPCR(mg/d)	$r = -0.060$ $p = 0.541$	$r = 0.028$ $p = 0.776$
eGFR (mL/min/1.73 m²)	$r = 0.160$ $p = 0.097$	$r = -0.018$ $p = 0.851$
SBP (mmHg)	$r = 0.121$ $p = 0.241$	$r = 0.154$ $p = 0.135$
DBP (mmHg)	$r = 0.144$ $p = 0.161$	$r = 0.126$ $p = 0.220$
PP (mmHg)	$r = 0.100$ $p = 0.334$	$r = 0.137$ $p = 0.184$
PWV (m/s)	$r = 0.090$ $p = 0.384$	$r = 0.129$ $p = 0.212$
AIx	$r = 0.076$ $p = 0.462$	$r = 0.037$ $p = 0.721$

Table 3: 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

Table 4: 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

CONCLUSIONS

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. 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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