High serum soluble α-Klotho levels in patients with autosomal dominant polycystic kidney disease

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ABSTRACT

This study aims to determine fibroblast growth factor-23 and soluble α-Klotho levels in patients with autosomal dominant polycystic kidney disease. A total of 76 patients with autosomal dominant polycystic kidney disease and 32 healthy volunteers were included in the study. Serum fibroblast growth factor-23 and soluble α-Klotho levels were measured with ELISA kits. Parathyroid hormone, phosphate, calcium, creatinine, 25-hydroxyvitamin D3 levels, urinary protein to creatinine ratio and estimated glomerular filtration rate were also measured or calculated. Patients with autosomal dominant polycystic kidney disease had significantly higher serum parathyroid hormone ($p<0.001$), fibroblast growth factor-23 ($p<0.001$), soluble α-Klotho levels ($p=0.001$) and lower serum 25-hydroxyvitamin D3 levels ($p<0.001$) as compared with healthy volunteers. Serum fibroblast growth factor-23, soluble α-Klotho and 25-hydroxyvitamin D3 levels were similar in all five chronic kidney disease stages of autosomal dominant polycystic kidney disease ($p>0.05$). Fibroblast growth factor-23 ($r=-0.251$, $p=0.034$) and soluble α-Klotho levels ($r=-0.251$, $p=0.034$) were found to be negatively correlated with estimated glomerular filtration rate. This study shows increased fibroblast growth factor-23 levels in patients with autosomal dominant polycystic kidney disease which is in harmony with the general trend in patients with chronic kidney disease of other aetiologies, but, unlike them, also a significant increase in serum soluble α-Klotho levels in patients with autosomal dominant polycystic kidney disease suggesting an aberrant production or a decreased clearance of α-Klotho molecule. Considering the unique increases in erythropoietin levels due to erythropoietin production in renal cysts, we assume, patients with autosomal dominant polycystic kidney disease may potentially have different soluble α-Klotho production/clearance characteristics than the patients with other parenchymal renal diseases.

Significance of this study

What is already known about this subject?

Fibroblast growth factor-23 and its cofactor Klotho are molecules that regulate calcium and phosphate metabolism. Fibroblast growth factor-23 is markedly increased in patients with chronic kidney disease while Klotho levels are expected to be positively correlated with decreasing glomerular filtration rate.

What are the new findings?

Patients with autosomal dominant polycystic kidney disease had significantly higher fibroblast growth factor-23 ($p<0.001$) and soluble α-Klotho levels ($p=0.001$), and lower serum 25-hydroxyvitamin D3 levels ($p<0.001$) as compared with healthy volunteers. Serum fibroblast growth factor-23, soluble α-Klotho and 25-hydroxyvitamin D3 levels were similar in all 5 chronic kidney disease stages ($p>0.05$). Fibroblast growth factor-23 ($r=-0.251$, $p=0.034$) and soluble α-Klotho levels ($r=-0.251$, $p=0.034$) were found to be negatively correlated with estimated glomerular filtration rate.

How might these results change the focus of research or clinical practice?

Patients with autosomal dominant polycystic kidney disease may potentially have different soluble α-Klotho production/clearance characteristics than the patients with other parenchymal renal diseases.

INTRODUCTION

Autosomal dominant polycystic kidney disease (ADPKD) is one the most common genetic cause of end stage renal disease.[1–4] ADPKD can easily be detected in early course of the disease, but unfortunately, its course generally cannot be effectively changed by treatment.[5] Clinical presentation of ADPKD differs from the other chronic kidney disease (CKD) causes with less frequent occurrence of anaemia due to higher serum erythropoietin (EPO) levels.[6] Recent studies indicate that in ADPKD patients, proximal tubules are the main site for EPO production and EPO is significantly enriched in cysts of proximal tubular origin.[7]

Fibroblast growth factor-23 (FGF-23) contributes in the regulation of calcium (Ca) and phosphate (P) metabolism FGF-23 and is

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produced by osteocytes, osteoblasts and directly increases urinary fractional excretion of P by reducing expression of sodium-phosphate cotransporter type II (NaPi-II). It also reduces intestinal P absorption by suppressing 1--α-hydroxylase activity.[8–14] FGF-23, in order to exert its physiologic function requires its cofactor, Klotho protein. Klotho gene is reported to be expressed in the parathyroid glands, kidney and the choroid plexus. Klotho protein is translocated from the endosome to the cell membrane, extracellular domain of Klotho (soluble α-Klotho) may be disintegrated by the α-secretates ADAM 10 and 17 to generate large amounts of soluble Klotho into blood, urine and cerebrospinal fluid.[15–19] Soluble α-Klotho increases Ca reabsorption in the distal tubule and decreases P reabsorption in the proximal tubules, independently from FGF-23.[18 20–22] Serum soluble α-Klotho levels may be influenced by some factors such as age, calcium and phosphorus concentrations in healthy population.[23]

Recent studies have reported that FGF-23 is markedly increased in patients with CKD,[24 25] while soluble α-Klotho levels are decreased in parallel with lower estimated glomerular filtration rates (eGFR).[26] Although some studies in patients with ADPKD at CKD stages 1 and 2 suggest a decrease in plasma-soluble Klotho levels, there are insufficient data investigating the role and levels of FGF-23 and soluble α-Klotho and other main parameters of calcium-phosphate metabolism specifically in all stages of CKD in the ADPKD setting.[5] Therefore, in this study, we aimed to describe the role of FGF-23 and soluble α-Klotho in bone and mineral abnormalities in patients with ADPKD.

PATIENTS AND METHODS

The study was approved by the ethical committee of Antalya Training and Research Hospital in accordance with ethical standards of the Declaration of Helsinki of 1975, as revised in 2000 and each patient has signed in with an informed consent. In total, 76 patients aged 22–84 years, with ADPKD at different stages of CKD, who did not require dialysis treatment or had previous kidney transplantation history, were included in the study. Patients with CKD were classified by eGFR according to the CKD Epidemiology Collaboration formula 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), CKD stage 4 (15–29 mL/min/1.73 m^2) and CKD stage 5 (<15 mL/min/1.73 m^2 or dialysis). A total of 17, 19, 22, 12 and 6 patients were in CKD stages 1, 2, 3, 4 and 5, respectively.

A total of 31 healthy volunteers (HV), aged 40–68 years, which did not have medical history of any conditions, served as control group. Evaluation of patients and HV included past medical history, general clinical assessment, blood pressure measurement after 15 min rest, assessment of height, body weight, 8 hours fasting venous blood samples were collected in the morning. Sample sera were stored at −80°C. Samples were analysed for FGF-23, soluble α-Klotho, intact parathyroid hormone (iPTH), P, Ca, creatinine and 25-hydroxyvitamin D_3 ($25(OH)D_3$). Urinary protein to creatinine ratio was calculated in spot urine samples. Serum blood urea nitrogen, creatinine, Ca and P levels were also determined using commercially available assay kits (Beckman Coulter) and an autoanalyser (Beckman AU5800; Beckman Coulter Diagnostics, USA).

Measurement of serum $25(OH)D_3$ levels was performed using direct competitive chemiluminescence immunoassay method (DiaSorin, Stillwater, Minnesota, USA). The limit of detection was 3.5 ng/mL, and the coefficient of variation ranged between 4.8% and 11.1% $25(OH)D_3$ for this assay. Serum iPTH levels were determined using commercially available assay kits (Beckman Coulter) and an autoanalyser (Access DxI800; Beckman Coulter Diagnostics, USA). The iPTH assay is a two-site immunoenzymatic ('sandwich') assay. iPTH assay is linear up to 3500 pg/mL. The limit of detection is 1 pg/mL, and the coefficient of variation (CV) ranges between 3.5% and 6.4% for this assay. Serum soluble α-Klotho levels and FGF-23 levels were measured by using a commercially available ELISA kit (YH Biosearch, Shanghai, China) (%CV: <10 for both parameters, serum soluble α-Klotho levels assay range: 0.05–20 ng/mL, FGF-23 assay range: 5–1500 pg/mL). Those assays also employed the quantitative sandwich enzyme immunoassay technique. In order to avoid intratest variations, measurements were made in duplicate, simultaneously using the same ELISA kit.

STATISTICAL ANALYSIS

The results were statistically evaluated, using Statistical Package for the Social Sciences (SPSS; V.16.0, Chicago, Illinois, USA). Normal distribution was evaluated by Shapiro-Wilk's test. Continuous variables with normal distribution were expressed as mean±SD and continuous variables with abnormal distribution were expressed as median (minimum-maximum). Categorical variables were expressed as frequency and percentage. According to the presence of normal distribution of the parameters, for the analysis of continuous variables the Mann-Whitney U test and t-test (independent samples t-test) were used. Univariate analysis of variance was used to detect the factors that affected the serum FGF-23 and Klotho levels. Pearson and Spearman's rho tests were used for correlation analysis. With 95% CI, a p value <0.05 was considered to be statistically significant.

RESULTS

Patients with ADPKD had significantly higher serum iPTH ($p<0.001$), FGF-23 ($p<0.001$), soluble α-Klotho levels ($p=0.001$) and lower serum $25(OH)D_3$ levels compared with HV group ($p<0.001$) (table 1). When patients are classified into CKD stages, serum FGF-23, soluble α-Klotho and $25(OH)D_3$ levels were comparable in different stages of CKD ($p>0.05$). While $25(OH)D_3$, FGF-23 levels were similar in patients with CKD stage 1 and HV, FGF-23 levels were higher in CKD stages 2, 3, 4, 5 compared with HV. Soluble α-Klotho levels were higher in CKD stages 2, 3, 4 compared with HV. Soluble α-Klotho levels were similar in patients with CKD stages 1 and 5, and HV (table 2).

Soluble α-Klotho levels were in positive correlation with $25(OH)D_3$ ($r=0.27$; $p=0.025$;) and FGF-23 ($r=0.818$; $p<0.001$), and in negative correlation with eGFR ($r=-0.251$; $p=0.034$). There was no significant correlation between serum soluble α-Klotho and age ($r=0.90$; $p=0.446$) (table 3).

Table 1 Characteristics of patients with ADPKD and HV

	ADPKD (n=76)	HV (n=32)	p Value
Age (years)	50.96±15.59	49.53±7.32	0.47
Female (%)	43 (%56.57)	20 (%62.50)	0.35
Male (%)	33 (%43.43)	12 (%37.50)	0.45
BMI (kg/m^2)	28.21±5.34	27.37±4.31	0.40
SBP (mm Hg)	128.17±15.17	111.97±13.21	<0.001
DBP (mm Hg)	84.35±11.59	78.03±10.24	0.012
Creatinine (mg/dL)	1.77±1.16	0.88±0.12	<0.001
eGFR (mL/min/1.73 m^2)	57.24±33.80	90.15±20.71	<0.001
UPCR	0.15 (0.05–1.91)	0.06 (0.04–0.18)	<0.001
Calcium (mg/dL)	9.41±0.39	9.37±0.39	0.61
Phosphate (mg/dL)	3.51±0.77	3.28±0.67	0.16
Ca×P (mg^2/dL^2)	33.3±0.52	33.1±0.85	0.55
iPTH (pg/mL)	90 (30–1911)	53 (31–62)	<0.001
Albumin (g/dL)	4.27±0.34	4.26±0.28	0.89
25(OH)D3 (ng/mL)	27.02±11.56	62.13±18.37	<0.001
FGF-23 (pg/mL)	166.69 (68.60–2431.37)	82.14 (3.24–1452.86)	<0.001
Soluble α-Klotho (ng/mL)	2.92 (0.99–21.97)	2.04 (0.95–19.98)	0.001
Hb (mg/dL)	13.3±1.7	13.8±1.3	0.9
ALP (U/l)	71.45±27.23	76.25±16.36	0.35

25(OH)D3, 25-hydroxyvitamin D3; ADPKD, autosomal dominant polycystic kidney disease; ALP, alkaline phosphatase; BMI, body mass index; Ca, calcium; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate; FGF-23, fibroblast growth factor-23; Hb, haemoglobin; HV, Healthy volunteers; iPTH, intact parathyroid hormone; P, phosphate; SBP, systolic blood pressure; UPCR, urinary protein to creatinine ratio.

Analysis of correlation between serum FGF-23 levels and various renal function parameters showed that serum FGF-23 levels were in negative correlation with eGFR (r=−0.251; p=0.034) (table 3).

For determination of influence of Ca, P, 25(OH)D_3, age and eGFR on serum soluble α-Klotho levels in patients with ADPKD, regression analysis was made. Serum soluble α-Klotho levels were related with only 25(OH)D_3 (F=8.986; p=0.004) (table 4).

DISCUSSION

In this study, we reported increased serum soluble α-Klotho and FGF-23 levels in patients with CKD with ADPKD. In patients with CKD; FGF-23 levels increase with declining renal function.[27 28] Hyperphosphataemia associated with CKD, triggers FGF-23 production, and serum FGF-23 levels increase in the early stages in CKD conditions.[27] Increase of serum FGF-23 levels is recognised before the elevation of serum P and iPTH levels in patients with CKD[29] and relationship between serum FGF-23 levels and GFR is not found in earlier stages of CKD, where patients are normophosphataemic, suggesting that there may be an end-organ resistance to FGF-23 due to decreased production of its cofactor Klotho.[30 31] Pavik *et al*[5] found a significant decrease in soluble Klotho levels in ADPKD at CKD stages 1 and 2, suggesting a plasma Klotho reduction in early stages of ADPKD. Unlike Pavik's study, our patient group included patients with ADPKD from all stages of kidney insufficiency including stages 3, 4 and 5. Our results were also different with suggesting a statistically significant increase in serum soluble α-Klotho levels in patients with

Table 2 Characteristics of patients with ADPKD

	CKD1 (n=17)	CKD 2 (n=19)	CKD3 (n=22)	CKD4 (n=12)	CKD5 (n=6)	HV (n=32)	p Value
Age (years)	33.06±8.50	47.74±9.38	59.09±13.07	57.58±11.45	65.83±18.28	49.53±7.32	<0.001[a,b,c,d,e,f,g,h,l,n,o]
Creatinine (mg/dL)	0.81±0.16	1.01±0.25	1.60±0.36	2.77±0.86	4.45±1.25	0.88±0.12	<0.001[a,b,c,d,e,f,g,h,i,j,k,l,m,n,o]
eGFR (mL/min/1.73 m^2)	107.37±18.31	75.81±11.02	42.47±9.69	27.32±24.37	11.85±2.75	90.15±20.71	<0.001[a,b,c,d,e,f,g,h,i,j,k,l,m,n,o]
Calcium (mg/dL)	9.40±0.40	9.44±0.36	9.41±0.39	9.32±0.44	9.35±0.54	9.37±0.39	0.768
Phosphate (mg/dL)	3.20±0.69	3.3±0.50	3.22±0.65	4.20±0.91	4.33±0.50	3.28±0.67	<0.001[c,d,g,h,j,k,n,o]
iPTH (pg/mL)	54 (32–65)	71 (30–184)	75 (35–186)	182 (60–861)	271.5 (31–1911)	53 (31–62)	<0.001[c,g,h,i,j,k,l,n,o]
Albumin (g/dL)	4.47±0.41	4.29±0.33	4.19±0.30	4.18±0.32	4.25±0.34	4.26±0.28	0.384
25(OH)D3 (ng/mL)	23.61±8.52	26.92±13.06	32.03±13.29	24.35±9.13	22.16±3.84	62.13±18.37	<0.001[i,l,n,o]
FGF-23 (pg/mL)	148.5 (68.6–1300.0)	159.6 (106.5–2431.3)	174.9 (119.0–2256.6)	195.2 (140.9–2094.5)	172.8 (125.8–1655.9)	82.1 (3.2–1452.8)	<0.001[i,l,n,o]
Soluble α-Klotho (ng/mL)	2.96 (0.99–17.6)	2.65 (2.1–12.2)	2.87 (2.1–21.9)	3.22 (2.2–18.9)	2.86 (2.2–10.1)	2.04 (0.95–19.9)	0.025[l,n]
Hb (mg/dL)	13.6±1.7	13.5±1.5	13.5±1.7	12.4±2.0	12.6±2.1	13.8±1.3	0.45
ALP (U/L)	46.33±9.39	70.00±18.65	74.20±20.75	86.00±30.57	105.00±65.05	4.26±0.28	0.033[a,b,e]
UPCR	0.13±0.11	0.18±0.12	0.24±0.24	0.91±0.61	0.49±0.27	0.06±0.03	<0.001[c,d,e,g,h,i,j,k,l,n,o]

25(OH)D3, 25-hydroxyvitamin D3; ADPKD, autosomal dominant polycystic kidney disease; ALP, alkaline phosphatase; CKD 1–5, chronic kidney disease stages 1–5; eGFR, estimated glomerular filtration rate; FGF-23, fibroblast growth factor-23; Hb, haemoglobin; iPTH, intact parathyroid hormone; UPCR, urinary protein to creatinine ratio.
[a]CKD1 and CKD2, [b]CKD1 and CKD3, [c]CKD1 and CKD4, [d]CKD1 and CKD5, [e]CKD1 and HV, [f]CKD2 and CKD3, [g]CKD2 and CKD4, [h]CKD2 and CKD5, [i]CKD2 and HV, [j]CKD3 and CKD4, [k]CKD3 and CKD5, [l]CKD3 and HV, [m]CKD4 and CKD5, [n]CKD4 and HV, [o]CKD5 and HV.

Table 3 Association of Klotho and FGF-23 levels with serum parameters of mineral metabolism in ADPKD

	Soluble α-Klotho r (p) values	FGF-23 r (p) values
Age	0.17 (0.14)	0.17 (0.44)
Calcium	0.009 (0.94)	0.009 (0.94)
Phosphate	0.19 (0.12)	0.19 (0.12)
25(OH)D3	0.27 (0.025)	0.19 (0.11)
iPTH	0.025 (0.85)	0.025 (0.85)
ALP	0.27 (0.88)	0.27 (0.88)
FGF-23	0.818 (0.001)	
UPCR	−0.019 (0.87)	−0.019 (0.87)
eGFR	−0.251 (0.034)	−0.251 (0.034)

25(OH)D3, 25-hydroxyvitamin D3; ADPKD, autosomal dominant polycystic kidney disease; ALP, alkaline phosphatase; eGFR, estimated glomerular filtration rate; FGF-23, fibroblast growth factor-23; iPTH, intact parathyroid hormone; UPCR, urinary protein to creatinine ratio.

ADPKD. Although low Klotho levels are expected in CKD, some authors have suggested that, Klotho levels may be significantly increased in patients with CKD with higher creatinine levels (>2 mg/dL) compared with the patients with CKD with relatively lower creatinine levels (<1.2 mg/dL).[32] In our patients, we have found that serum soluble α-Klotho levels were similar in patients with CKD stages 1 and 5 with healthy control participants, and were even higher in CKD stages 2, 3, 4 compared with healthy group. In addition to unexpectedly high levels of serum soluble α-Klotho levels we have found, in our patients with ADPKD, serum soluble α-Klotho levels were not affected by neither eGFR, nor serum inorganic phosphorus levels suggesting an autonomous production of α-Klotho. Some other authors have not found any correlation between soluble α-Klotho levels and declining renal function.[33] We should remember that the possibility of autonomous production of some biologic substances is not unfamiliar in ADPKD pathophysiology. The renal cysts of ADPKD develop by overproliferation of tubular cells. While the cysts grow, cystic tubular epithelial secretion accumulates within cytes. Patients with ADPKD are known to suffer with less severe CKD-related anaemia, even in the advanced stages of their disease, due to autonomous production of EPO. As, Ito *et al*[34] have shown positive EPO staining in the cytoplasm of the cystic epithelial cells and also in cystic fluid, therefore, in case of EPO, phenomenon of tubular secretion accumulation has been demonstrated in ADPKD. Eckardt *et al*[7] have found that single interstitial cell juxtaposed to proximal tubular cysts may exert autonomous production of EPO inside the cysts, which ameliorates the anaemia during end-stage polycystic kidney disease. With respect to sodium concentrations, cysts in patients with ADPKD are mostly of proximal tubule origin, and cysts originated from proximal tubules contain the highest concentrations of EPO.[7]

Table 4 Regression analysis for level of serum soluble α-Klotho in patients with ADPKD

	Soluble α-Klotho	
	F value	p Value
Calcium	2.044	0.156
Phosphate	1.559	0.215
25(OH)D3	8.986	0.004
Age	1.897	0.171
eGFR	0.378	0.540

25(OH)D3, 25-hydroxyvitamin D3; ADPKD, autosomal dominant polycystic kidney disease; eGFR, estimated glomerular filtration rate.

On the other hand, α-Klotho mRNA is expressed strongly in the kidney, especially in renal tubules.[18] Proximal and distal convoluted tubules express Klotho protein, therefore, kidney tubules are suggested to be the main production site for soluble α-Klotho molecule. Thus, Klotho protein and EPO molecules are produced by proximal tubular epithelium, and at least in case of EPO, molecules secreted by tubular epithelial cells may built up within cystic fluid and later systemic circulation in ADPKD. We believe, same mechanism may be valid for Klotho and aberrant tubular production of Klotho in ADPKD may be an explanation for our finding of unexpectedly high plasma α-Klotho levels in patients with ADPKD. The presence of exogenously given soluble α-Klotho in proximal lumen, suggests that α-Klotho traffics across renal tubules from basolateral membrane to luminal side, and tubules may also be the clearing site for α-Klotho molecule.[18] The other explanations of high levels α-Klotho may be impaired renal clearance of soluble α-Klotho in patients with ADPKD and maybe some other forms of CKD.[33] Therefore, renal cysts of ADPKD may be an aberrant source for α-Klotho and, additionally, decreased clearance of soluble α-Klotho due to cyst-induced tubular damage may contribute in unexpectedly increased levels of circulating α-Klotho we have found in our patients.

But unfortunately, our study was designed to be a clinical one and has not aimed to perform advanced procedures such as measuring α-Klotho contents in aspirated cyst fluids. Therefore, at this point, our speculations about the pathophysiologic explanations for the cause/causes of our extraordinary findings suggesting increased circulating α-Klotho levels in ADPKD, needs to be supported by further experimental and clinical studies.

On the other hand, besides measuring α-Klotho levels in cystic fluids, urine and membrane-bound Klotho were beyond the scope of our study; we had some other limitations, such as relatively small size of the study population, potential effects of treatments, for example, RAS inhibitors, active vitamin D analogues, have all been disregarded. But we believe, if it is true, possibility of high α-Klotho levels in ADPKD deserves special attention and may have some clinical and pathophysiological impacts.

In conclusion, our data show that elevated FGF-23 and soluble α-Klotho levels were present in patients with ADPKD. Further research is needed to identify and explain the cause of paradoxical outcomes related to FGF-23 and soluble α-Klotho levels in patients with ADPKD.

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Competing interests None declared.

Patient consent Obtained.

Ethics approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

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REFERENCES

1 Mekahli D, Bacchetta J. From bone abnormalities to mineral metabolism dysregulation in autosomal dominant polycystic kidney disease. *Pediatr Nephrol* 2013;28:2089–96.
2 Gabow PA. Autosomal dominant polycystic kidney disease. *N Engl J Med* 1993;329:332–42.
3 Harris PC, Torres VE. Polycystic kidney disease. *Annu Rev Med* 2009;60:321–37.
4 Torres VE, Harris PC. Autosomal dominant polycystic kidney disease: the last 3 years. *Kidney Int* 2009;76:149–68.
5 Pavik I, Jaeger P, Ebner L, *et al*. Soluble Klotho and autosomal dominant polycystic kidney disease. *Clin J Am Soc Nephrol* 2012;7:248–57.
6 de Almeida EA, Alho I, Marques F, *et al*. Haemoglobin and erythropoietin levels in polycystic kidney disease. *Nephrol Dial Transplant* 2008;23:412–3.
7 Eckardt KU, Möllmann M, Neumann R, *et al*. Erythropoietin in polycystic kidneys. *J Clin Invest* 1989;84:1160–6.
8 Nitta K, Nagano N, Tsuchiya K. Fibroblast growth factor 23/Klotho axis in chronic kidney disease. *Nephron Clin Pract* 2014;128:1–10.
9 Yamashita T, Yoshioka M, Itoh N. Identification of a novel fibroblast growth factor, FGF-23, preferentially expressed in the ventrolateral thalamic nucleus of the brain. *Biochem Biophys Res Commun* 2000;277:494–8.
10 Pereira RC, Juppner H, Azucena-Serrano CE, *et al*. Patterns of FGF-23, DMP1, and MEPE expression in patients with chronic kidney disease. *Bone* 2009;45:1161–8.
11 Bai XY, Miao D, Goltzman D, *et al*. The autosomal dominant hypophosphatemic rats R176Q mutation in fibroblast growth factor 23 resists proteolytic cleavage and enhances in vivo biological potency. *J Biol Chem* 2003;278:9843–9.
12 Larsson T, Marsell R, Schipani E, *et al*. Transgenic mice expressing fibroblast growth factor 23 under the control of the α1(I) collagen promoter exhibit growth retardation, osteomalacia, and disturbed phosphate homeostasis. *Endocrinology* 2004;145:3087–94.
13 Shimada T, Urakawa I, Yamazaki Y, *et al*. FGF-23 transgenic mice demonstrate hypophosphatemic rickets with reduced expression of sodium phosphate cotransporter type IIa. *Biochem Biophys Res Commun* 2004;314:409–14.
14 Bai X, Miao D, Li J, *et al*. Transgenic mice overexpressing human fibroblast growth factor 23 (R176Q) delineate a putative role for parathyroid hormone in renal phosphate wasting disorders. *Endocrinology* 2004;145:5269–79.
15 Matsumura Y, Aizawa H, Shiraki-Iida T, *et al*. Identification of the human klotho gene and its two transcripts encoding membrane and secreted Klotho protein. *Biochem Biophys Res Commun* 1998;242:626–30.
16 Chen CD, Podvin S, Gillespie E, *et al*. Insulin stimulates the cleavage and release of the extracellular domain of Klotho by ADAM10 and ADAM17. *Proc Natl Acad Sci USA* 2007;104:19796–801.
17 Imura A, Iwano A, Tohyama O, *et al*. Secreted Klotho protein in sera and CSF: implication for post-translational cleavage in release of Klotho protein from cell membrane. *FEBS Lett* 2004;565:143–7.
18 Hu MC, Shi M, Zhang J, *et al*. Klotho: a novel phosphaturic substance acting as an autocrine enzyme in the renal proximal tubule. *FASEB J* 2010;24:3438–50.
19 Pavik I, Jaeger P, Ebner L, *et al*. Secreted Klotho and FGF-23 in chronic kidney disease Stage 1 to 5: a sequence suggested from a cross-sectional study. *Nephrol Dial Transplant* 2013;28:352–9.
20 Chang Q, Hoefs S, van der Kemp AW, *et al*. The beta-glucosidase Klotho hydrolyzes and activates the TRPV5 channel. *Science* 2005;310:490–3.
21 Cha SK, Ortega B, Kurosu H, *et al*. Removal of sialic acid involving Klotho causes cell-surface retention of TRPV5 channel via binding to galactin-1. *Proc Natl Acad Sci USA* 2008;105:9805–10.
22 Cha SK, Hu MC, Kurosu H, *et al*. Regulation of renal outer medullary potassium channel and renal K(+) excretion by Klotho. *Mol Pharmacol* 2009;76:38–46.
23 Yamazaki Y, Imura A, Urakawa I, *et al*. Establishment of sandwich ELISA for soluble alpha-Klotho measurement: age-dependent change of soluble alpha-Klotho levels in healthy subjects. *Biochem Biophys Res Commun* 2010;398:513–18.
24 Isakova T, Xie H, Yang W, *et al*., Chronic Renal Insufficiency Cohort (CRIC) Study Group. Fibroblast growth factor 23 and risks of mortality and endstage disease in patients with chronic kidney disease. *JAMA* 2011;305:2432–9.
25 Titan SM, Zatz R, Graciolli FG, *et al*. FGF-23 as a predictor of renal outcome in diabetic nephropathy. *J Am Soc Nephrol* 2011;6:241–7.
26 Shimamura Y, Hamada K, Inoue K, *et al*. Serum levels of soluble secreted a-Klotho are decreased in the early stages of chronic kidney disease, making it a probable novel biomarker for early diagnosis. *Clin Exp Nephrol* 2012;16:722–9.
27 Nagano N, Miyata S, Abe M, *et al*. Effect of manipulating serum phosphorus with phosphate binder on circulating PTH and FGF-23 in renal failure rats. *Kidney Int* 2006;69:531–7.
28 Fliser D, Kollerits B, Neyer U, *et al*. Fibroblast growth factor 23 (FGF-23) predicts progression of chronic kidney disease: the Mild to Moderate Kidney Disease (MMKD) Study. *J Am Soc Nephrol* 2007;18:2600–8.
29 Nakano C, Hamano T, Fujii N, *et al*. Combined use of vitamin D status and FGF-23 for risk stratification of renal outcome. *Clin J Am Soc Nephrol* 2012;7:810–19.
30 Larsson T, Nisbeth U, Ljunggren O, *et al*. Circulating concentration of FGF-23 increases as renal function declines in patients with chronic kidney disease, but does not change in response to variation in phosphate intake in healthy volunteers. *Kidney Int* 2003;64:2272–9.
31 Koh N, Fujimori T, Nishiguchi S, *et al*. Severely reduced production of Klotho in human chronic renal failure kidney. *Biochem Biophys Res Commun* 2001;280:1015–20.
32 Devaraj S, Syed B, Chien A, *et al*. Validation of an immunoassay for soluble Klotho protein decreased levels in diabetes and increased levels in chronic kidney disease. *Am J Clin Pathol* 2012;137:479–85.
33 Seiler S, Wen M, Roth HJ, *et al*. Plasma Klotho is not related to kidney function and does not predict adverse outcome in patients with chronic kidney disease. *Kidney Int* 2013;83:121–8.
34 Ito K, Asano T, Tominaga S, *et al*. Erythropoietin production in renal cell carcinoma and renal cysts in autosomal dominant polycystic kidney disease in a chronic dialysis patient with polycythemia: a case report. *Oncol Lett* 2014;8:2032–6.