

ORIGINAL PAPER

# Fibroblast growth factor 23 and $\alpha$ -Klotho serum concentration did not differ between children with autosomal dominant polycystic kidney disease and healthy controls

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## ABSTRACT

**Introduction:** Autosomal dominant polycystic kidney disease (ADPKD) is the most common inherited monogenous kidney disease that can also be seen in children. One of the indicators of changes in metabolism in ADPKD children according to the novel knowledge are fibroblast growth factor 23 (FGF-23) and  $\alpha$ -Klotho protein.

**Aim of the study:** To observe whether children who suffer from ADPKD whilst preserving normal kidney function (eGFR > 90 ml/min/1.73 m<sup>2</sup>) have any changes in the concentration of FGF-23 and Klotho protein along with the growth of renal volume.

**Material and methods:** The study was a cross-sectional analysis of 70 children aged from 1 to 18.8 years. FGF-23 and Klotho were measured by the ELISA tests. Clinical and laboratory analysis comprised the following: ultrasound measurement of the total kidney volume (TKV), eGFR calculation, serum calcium, phosphate, and urinary albumin excretion.

**Results:** Serum concentration of FGF-23 did not differ between children suffering from ADPKD and the control group (27.77 pg/ml vs. 24.15 pg/ml;  $p = 0.96$ ).  $\alpha$ -Klotho concentrations were also similar (1650 pg/ml vs. 1440 pg/ml;  $p = 0.14$ ) between both groups. Klotho correlated significantly with FGF-23 ( $R_s = 0.55$ ;  $p = 0.000002$ ) and eGFR ( $R_s = 0.25$ ;  $p = 0.039566$ ). No significant relation was detected between FGF-23 or Klotho concentration and albumin excretion, TKV, age, or anthropometric measures.

**Conclusions:** FGF-23 and  $\alpha$ -Klotho concentration did not differ significantly between children suffering from ADPKD and the control group.

## KEY WORDS:

ADPKD, children, FGF-23,  $\alpha$ -Klotho, progression.

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## INTRODUCTION

Autosomal dominant polycystic kidney disease (ADPKD) is the most common hereditary type of monogenic kidney disease, affecting 1 out of 1000 births [1]. ADPKD is caused by a mutation in the *PKD1* or *PKD2* genes (responsible for 85% and 15% of the cases of ADPKD, respectively), the products of which are polycystin 1 and 2, which act as transmembrane proteins involved in the transportation of calcium [2]. The main manifestation of ADPKD is a bilateral progressive cystic dilation of the renal tubules with an eventual compression of normal renal parenchyma, kidney enlargement, and loss of renal function. At ages between 20 and 40 years, 50% of the normal parenchyma is replaced by cysts. Fifty per cent of patients reach end-stage renal disease by the age of 60 years. An early manifestation of ADPKD includes the following: hypertension, albuminuria, proteinuria, and impaired renal function, and it occurs before the age of 15 years [3, 4].

One of the abnormalities that develop as kidney function declines is the disturbance of calcium and phosphate metabolism. Fibroblast growth factor 23 (FGF-23) is secreted by osteocytes and osteoblasts and regulates bone mineralisation. In the kidney, FGF-23 binds to the canonical FGF receptor in the presence of the Klotho cofactor and suppresses renal tubular phosphate reabsorption and  $1\alpha$ -hydroxylase activity, which exerts effects on the bone/kidney/parathyroid axis. In conclusion, it leads to phosphaturia and hypophosphataemia and low serum levels of 1,25 dihydroxy vitamin D [2, 5, 6]. FGF-23 increases in response to serum phosphorus level increase in patients with chronic kidney disease (CKD); it occurs from early stages of CKD and helps maintain the serum phosphate level in its normal range. During the CKD progression, FGF-23 concentration increases and Klotho protein concentration decreases. However, it has not been confirmed from which stage of CKD during ADPKD in children the changes in concentration occur [7, 8]. Decrease in glomerular filtration rate in ADPKD correlates with an increase in total kidney volume (TKV), defined as the sum volume of both kidneys [9, 10]. It seems that the increase of FGF-23 concentration is one of the earliest biochemical markers of decreased glomerular filtration rate [11].

The aim of this study is to investigate the serum levels of FGF-23 and soluble Klotho in children with ADPKD, who retain normal renal function (eGFR > 90 ml/min/1.73 m<sup>2</sup>), and whether those level changes depend on classic indicators of disease progression.

## MATERIAL AND METHODS

The study was designed as a cross-sectional analysis amongst ADPKD children and healthy controls. The Local Ethical Committee approved the protocol. Informed consent was obtained before inclusion of each patient.

## STUDY GROUP

The study was a cross-sectional analysis of 70 children (45 patients with ADPKD – 29 females and 16 males; and 25 patients comprised the healthy control group – 15 females and 10 males), aged from 1 to 18.8 years (average age 10.7 years), who were recruited from Paediatric and General Nephrology Outpatient Clinics.

Clinical characteristics of the study group are described in Table 1. Patients were not hypertensive. Also, they did not receive renin-angiotensin system inhibition.

The group of ADPKD patients consisted of both children who fulfilled the ultrasound criteria of Ravine *et al.* for the diagnosis of disease (bilateral cysts in the kidneys, including a minimum of three cysts with diameter  $\geq 10$  mm), as well as children who did not meet the criteria, who were diagnosed with at least one cyst and a positive family history for ADPKD. A similar method of qualification was used in the available population studies of children with ADPKD [12]. Children with significant comorbidities not related to ADPKD were excluded.

## METHODS

Blood pressure and anthropometric measurements were performed in a standardised setting during routine control visits. Urine and fasting serum samples were taken in the morning. Serum for FGF-23 and Klotho was carefully centrifuged and frozen at  $-80^{\circ}\text{C}$  until analysis.

TKV was assessed by an ultrasound performed by an experienced physician using ProSound Alpha 7 Aloka apparatus with a convex transducer. It was calculated from the formula:  $KV = 0.52 \times D \times S \times G$ , where: KV – kidney volume, D – length of the kidney (cm), S – width of the kidney (cm), G – kidney thickness (cm). Total kidney volume was defined as the sum of the volume of both kidneys and was related to the patient's body surface (TKV/BSA [cm<sup>3</sup>/m<sup>2</sup>]). The number of cysts was also calculated.

Routine biochemical analysis was performed and comprised the following: Biochemical testing, which included renal parameters, electrolytes, and parameters of calcium-phosphate metabolism by a standard laboratory procedure. Evaluation of kidney function was assessed by measuring the serum creatinine concentration (mg/dl) and creatinine clearance (ml/min/1.73 m<sup>2</sup>). The latter was calculated using the "Bedside Schwartz" formula for children. The albumin/creatinine ratio was established from morning urine.

FGF-23 concentration levels were measured using the Human Fibroblast Growth Factor 23 Kit Elisa Kit from Wuhan EIAab Science Co. Ltd, while the Klotho protein was measured using the Cloud-Clone Corp Enzyme-linked Immunosorbent Assay Kit.

The data collected during the examination was subjected to statistical analysis. The normality of the distribution of continuous variables was assessed using the

TABLE 1. Clinical and laboratory data in the study group and healthy controls

Characteristic	Total cohort <i>n</i> = 70	ADPKD group <i>n</i> = 45	Healthy controls <i>n</i> = 25	<i>p</i> -value
FGF-23 (pg/ml)	25.96 (9.24–138.05)	27.77 (12.49–134.29)	24.15 (8.46–154.78)	0.96
Klotho (pg/ml)	1530 (795–2400)	1650 (950–2580)	1440 (680–1950)	0.14
Age (months)	136.5 (80–184)	138 (82–187)	135 (77–181)	0.68
Body mass (kg)	39 (20.5–58) SDS 0.38	41 (22–59) SDS 0.39	37 (20.5–56) SDS 0.38	0.83
Height (cm)	152.25 (122–172) SDS 0.83	155.5 (122–172) SDS 0.82	149 (126–166.5) SDS 0.86	0.67
uACR	10.2 (4.2–24.69)	17.59 (5.38–45.96)	5.21 (3.26–6.57)	< 0.001
eGFR (ml/min/1.73 m <sup>2</sup> )	149.5 (133.65–171.05)	150.1 (137–171.9)	140.8 (114.5–160.6)	0.065
Serum calcium (mg/dl)	2.65 (2.54–2.68)	2.65 (2.54–2.67)	2.63 (2.6–2.68)	0.63
Serum phosphate (mg/dl)	1.705 (1.5–1.81)	1.66 (1.44–1.81)	1.71 (1.51–1.83)	0.48
Number of cysts (> 10 mm in diameter)	1 (0–4.25)	3 (2–7)	0 (0–0)	< 0.001
TKV (cm <sup>3</sup> )	222.31 (126.57–315.52)	248.40 (148.18–459.53)	178.14 (109.33–223.94)	< 0.001
TKV/BSA (cm <sup>3</sup> /m <sup>2</sup> )	135.34 (105.91–187.3)	155.24 (120.55–224.54)	115.43 (98.34–126.07)	< 0.001

ADPKD – autosomal dominant polycystic kidney disease, FGF-23 – fibroblast growth factor 23, uACR – urine albumin–creatinine ratio, eGFR – estimated glomerular filtration rate, TKV – total kidney volume, BSA – body surface area; data are presented as median value and 27–75 interquartile range

Shapiro-Wilk test. Data are presented as median value and 25–75 interquartile range. Due to distribution other than normal, comparisons of continuous variables between two different groups were performed using the Mann-Whitney test. The Spearman correlation coefficient was used to assess the correlation of variables. A *p*-value below 0.05 was considered statistically significant. STATISTICA 10.0 and PQStat v1.6.6 programs were used for statistical analysis.

## RESULTS

The median serum level of FGF-23 was 27.77 pg/ml (interquartile range 12.49–134.29 pg/ml) in children

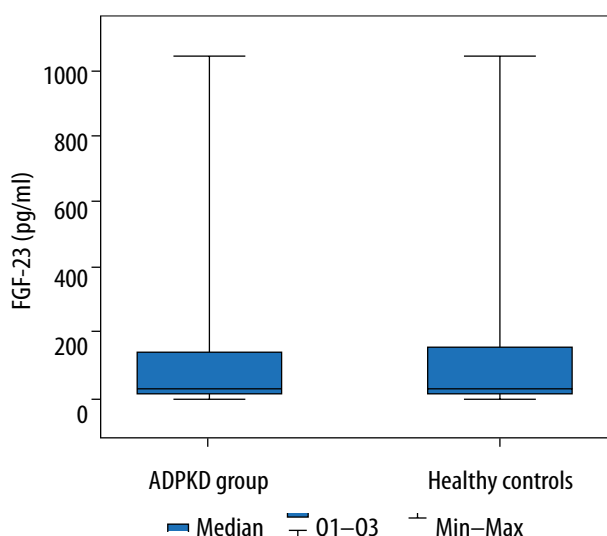


FIGURE 1. Fibroblast growth factor 23 (FGF-23) serum concentration in the study autosomal dominant polycystic kidney disease (ADPKD) group and healthy controls

with ADPKD and 24.15 pg/ml (interquartile range 8.46–154.78 pg/ml) in the healthy group (Fig. 1). The median serum  $\alpha$ -Klotho level was 1650 pg/ml (interquartile range 950–2580 pg/ml) in children with ADPKD and 1440 pg/ml (interquartile range 680–1950 pg/ml) in the healthy group (Fig. 2).

There were no differences in FGF-23 levels as well as  $\alpha$ -Klotho protein levels between children with ADPKD and the healthy control group. Also, no differences were detected between male and female subjects.

Both FGF-23 and  $\alpha$ -Klotho had a few large outlier cases; removing these cases did not change the result. There was no relation for these cases.

Children with ADPKD compared to the control group manifested a statistically significant difference in TKV (mean value 248.40 cm<sup>3</sup> vs. 178.14 cm<sup>3</sup>; *p* < 0.001).

ADPKD patients mean cysts number was 3, and there was a statistically significant positive correlation between the number of cysts and TKV (*p* < 0.001). There was also positive correlation between the number of cysts and the patients' age (*R*<sub>s</sub> = 0.41; *p* = 0.000751), body mass (*R*<sub>s</sub> 0.38; *p* = 0.001731), and the height (*R*<sub>s</sub> = 0.42; *p* = 0.000605).

Between patients with ADPKD and the control group the value of TKV/BSA and ACR (Table 1) presented a statistically significant difference (*p* < 0.05).

The analysis showed a statistically significant positive correlation between the serum levels of FGF-23 and the serum  $\alpha$ -Klotho protein level (*R*<sub>s</sub> = 0.55; *p* = 0.000002) (Fig. 3). Additionally, the total group of patients had a significantly positive correlation between Klotho protein levels in the serum and eGFR (*R*<sub>s</sub> = 0.25; *p* = 0.039566) (Fig. 4). However, FGF-23 and Klotho protein concentra-

tion did not show a similar correlation with ACR, TKV, TKV/BSA, body weight (kg), height (cm), age (months), phosphate concentration, and calcium concentration in the Spearman's rank correlation coefficient. In the healthy control group there was a significant negative correlation between Klotho protein levels and the age of children (months) ( $R_s = -0.45$ ;  $p = 0.033131$ ). Such a relationship was not observed in the group of ADPKD patients.

The Spearman's rank correlation coefficient showed positive correlation between ACR and TKV/BSA ( $R_s = 0.45$ ;  $p = 0.000152$ ), as well as ACR and TKV ( $R_s = 0.35$ ;  $p = 0.003349$ ) and ACR and the number of cysts ( $R_s = 0.4$ ;  $p = 0.001192$ ). In addition, there was negative correlation between the level of phosphate and TKV ( $R_s = -0.46$ ;  $p = 0.006852$ ) in the total group of patients as well as the healthy control group ( $R_s = -0.81$ ;  $p = 0.000839$ ). Such a correlation was not observed in the group of patients with ADPKD. Also, the level of phosphate concentration negatively correlated with height ( $R_s = -0.60$ ;  $p = 0.000159$ ), weight ( $R_s = -0.66$ ;  $p = 0.000022$ ), and age ( $R_s = -0.65$ ;  $p = 0.000035$ ) of the children.

## DISCUSSION

The main purpose of this study was the assessment of FGF-23 and Klotho protein concentration among the paediatric patients with ADPKD and in a control group of healthy children. We did not find a significant difference in the level of FGF-23 in the serum between patients and the healthy control group.

Most of the research states that the eGFR decrease is correlated with FGF-23 concentration increase in ADPKD and CKD patients in both adults [13–16] and children [8, 17–21]. The highest concentration of FGF-23 was found among patients with end-stage renal disease [19, 22]. FGF-23 can be recognised as an independent predictor of CKD progression in children [23]. In this study we did not find any correlation between FGF-23 serum and eGFR levels. This could be due to the fact that all of the patients had normal eGFR levels. Additionally, there was no difference in the eGFR levels between ADPKD patients and healthy children. It is hard to detect the correlation between eGFR and FGF-23 serum concentration during the early stages of CKD because of the difficulty to precisely assess eGFR. In ADPKD patients the GFR threshold beyond which FGF-23 concentration increases has not been determined [24]. There are studies showing that patients with APDKD have higher serum FGF-23 levels than patients with CKD with a different aetiology [25]. Our research shows that children with ADPKD and eGFR > 90 ml/min/1.73 m<sup>2</sup> do not have elevated FGF-23 serum levels.

Our results showed that children with ADPKD had comparable Klotho protein concentrations to patients from the healthy control group. The mean serum Klotho values were similar to the results achieved by

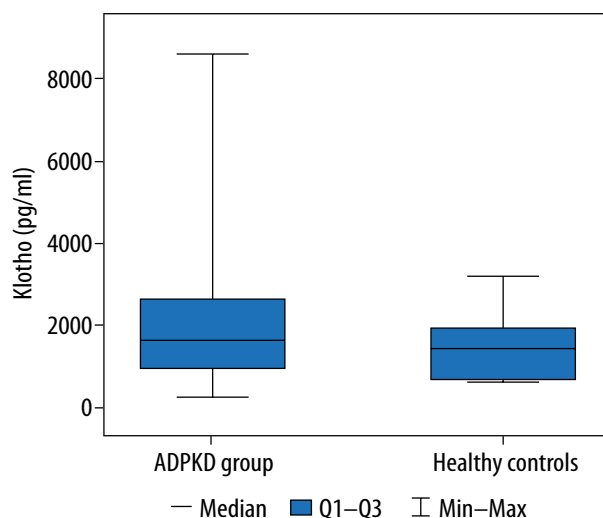


FIGURE 2. Klotho serum concentration in the study autosomal dominant polycystic kidney disease (ADPKD) group and healthy controls

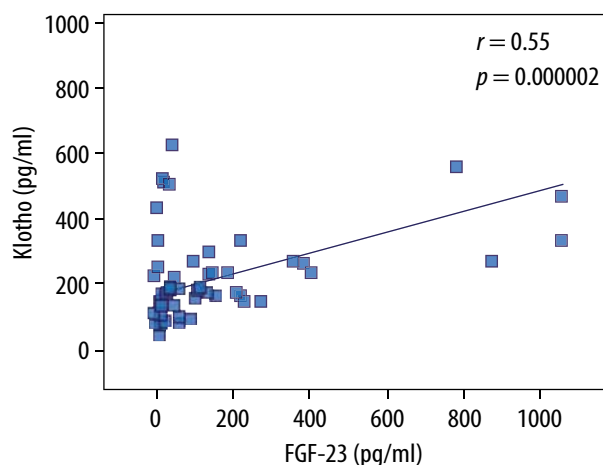


FIGURE 3. Spearman positive correlation between the serum levels of fibroblast growth factor 23 (FGF-23) and Klotho concentrations in the study group

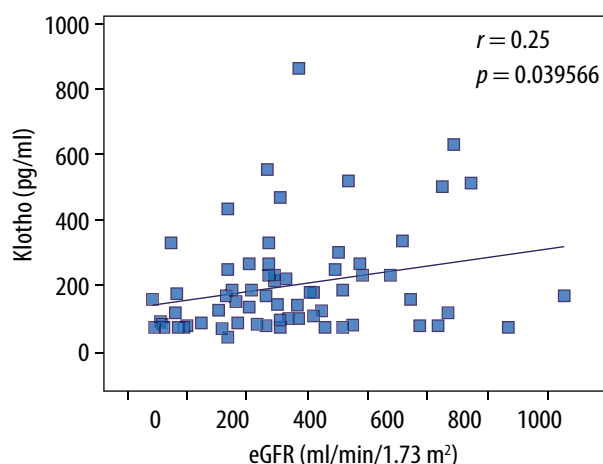


FIGURE 4. Positive correlation between serum levels of Klotho and estimated glomerular filtration rate (eGFR) in the study group calculated using the Spearman's rank correlation coefficient

Gkentzi *et al.* during studies conducted on a group of 159 healthy children [25].

Klotho protein concentration increased as the eGFR increased, which was observed in the total cohort group. Information about Klotho protein fluctuations during CKD progression are disparate [13–19, 26–29]. Most of the available research says that adult patients with ADPKD experience serum Klotho concentration decrease with the progression of CKD [13–15, 26, 27]; the same can also be observed in children [17–19]. According to Akiyama *et al.*, Klotho concentration is significantly lower in patients with CKD caused by ADPKD than in patients with CKD of a different aetiology [30]. This is explained by abnormalities localised in renal tubules that occur during ADPKD [25]. On the other hand, Sari *et al.* suggest autonomic Klotho protein production as the cause of its increased concentration during ADPKD. It could be connected with excessive tubule cell proliferation and cyst growth, and thus increased secretion of some substances [16]. A parallel situation has been suggested in which erythropoietin accumulates in renal cysts of patients suffering from ADPKD [31]. Both EPO as well as Klotho protein could be produced by the epithelium of the proximal tubule cells [32].

Our study confirms that children with ADPKD have larger kidney volume than healthy children. We did not observe any correlation between total kidney volume and Klotho protein or FGF-23 concentration, which is in contradiction to the above-mentioned hypotheses. However, it is possible that in some ADPKD patients there could be “productive cysts” that can produce excessive Klotho protein, for example because of the existence of extra tubule cells, and cysts without them in other patients. It could be the reason for our large number of outlier cases. Further histopathological studies are needed.

With the kidney volume increase, ACR values are also increased, and this value was higher among children suffering from ADPKD compared to the healthy ones. It could be caused by kidney damage due to the ADPKD. The ACR value was not correlated with Klotho protein concentration or with FGF-23 concentration. In that respect ACR seems to be a better early-stage kidney damage marker compared to Klotho protein or FGF-23.

ADPKD children at early stages of chronic kidney disease (stage 1) do not show changes in the FGF-23-Klotho axis, which could be dependent rather on further eGFR decrease than on increase of kidney volume or cyst number.

Our study revealed that Klotho concentration decreases depending on the age of healthy children. The group of patients suffering from ADPKD did not exhibit such a dependency. Similar correlation was found in a group of healthy adults [33] and children with CKD of a varied aetiology [18]. According to several sources, both FGF-23 and Klotho do not correlate with age [25].

During the research it was observed that with the increase of FGF-23 concentration Klotho protein concentration also increased. Scientists from Greece also obtained the same results during their research on healthy children [25]. Similar results were also published by Sari *et al.* [16]. In our research we observed a positive correlation between FGF-23 and Klotho concentration both in children with ADPKD and the children from the healthy control group. Other publications regarding CKD present different results [18, 34], or in some cases no correlation was observed [28, 29, 33, 35–37]. It would seem that because Klotho is a co-receptor for FGF-23 an increase of FGF-23 should decrease the level of Klotho [19]. Because the ADPKD patients in our study did not have elevated FGF-23 concentration and did not have decreased glomerular filtration, no such correlation was observed.

In the study, no disorders in calcium-phosphate metabolism in children with ADPKD were observed. Both calcium and phosphate concentrations were not correlated with FGF-23 or Klotho concentrations. Data regarding the relation between FGF-23 and Klotho protein with calcium and phosphate concentration are contradictory [8, 18–21, 25, 38–40].

The discovery of FGF-23 and Klotho gave hope for its use as a kidney disease progression marker. However, the literature on this topic is scarce. Most of the authors studied changes in Klotho and FGF-23 changes in CKD patients including many other causes of disease than ADPKD, which can blur the view of the matter.

## CONCLUSIONS

According to the study, FGF-23 and Klotho concentration does not differ significantly between children suffering from ADPKD and the control group. At this stage of the disease, its concentration is not related to the classical markers of progression of the disease. Our study had a number of limitations, including its cross-sectional design and low number of children in the ADPKD group. This is clearly the result of the rather rare diagnosis of this disease before 18 years of age. Hence, further research on this topic requires a larger study group in which more variables should be taken into consideration.

## DISCLOSURE

The authors declare no conflict of interest.

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