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Angiotensinogen and interleukin 18 in serum and urine of children with kidney cysts

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Abstract

Background: The most common disease associated with the presence of kidney cysts in the population is autosomal dominant polycystic kidney disease (ADPKD), which finally leads to end-stage renal disease.

Method: The study evaluated serum and urinary concentration of angiotensinogen (AGT) and interleukin 18 (IL-18) in a group of 39 children with renal cysts of different aetiology.

Results: Serum and urinary AGT concentration in children with renal cysts was higher compared to controls, regardless of the underlying background and gender. Serum IL-18 concentration was lower, in contrast, and the concentration of IL-18 in the urine did not differ between affected and healthy children. Negative correlation between urinary IL-18 concentration and systolic and mean arterial blood pressure was noted.

Conclusions: Higher AGT levels in serum and urine in children with renal cysts may indicate the activation of the renin–angiotensin–aldosterone system, including its intrarenal part, even before the onset of hypertension. Lower serum concentration of IL-18 in children with kidney cysts may indicate the loss of the protective role of this cytokine with the occurrence of hypertension.

Keywords

Angiotensinogen, autosomal dominant polycystic kidney disease, chronic kidney disease, interleukin 18, renal cysts

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Introduction

Renal cysts are fluid spaces of heterogeneous size, located within the kidney parenchyma, resulting from the widening and disintegration of various parts of the renal tubules. Renal cysts, according to their aetiology, can be divided into acquired and genetically determined cysts.[1,2] A positive family history, clinical picture, location and morphology of the cyst, the presence of extra-renal symptoms are important clues in the diagnostic process.[2] The most common kidney cystic disease is autosomal dominant polycystic kidney disease (ADPKD), which occurs at a frequency of 1/500–1000 of live births and affects about 12.5 million people worldwide.[2–5] Initial diagnosis of ADPKD is based on imaging.[6–8] Ultrasonographic criteria for the diagnosis of ADPKD according to Revine and Pei are currently used.[8,9] Moreover, according to a current consensus on imaging in children with cystic kidney diseases, the presence of one cyst is highly suggestive for ADPKD in children below 15 years with positive family history.[10] ADPKD is the most common genetic cause of end-stage renal failure in adults.[5,11–13] Due to the progression of the disease along with the age of patients and the accompanying complications, resulting in the development of end-stage renal failure and renal replacement therapy,[14–18] markers of early kidney damage are of interest. This could

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help identify children at risk of rapid disease progression and the risk of accompanying complications of the kidneys and other organs. So far there is no data confirming the usefulness of the evaluation of kidney injury markers in children with renal cysts.

Angiotensinogen (AGT) is a substrate produced mainly in the liver but also in the heart, brain, adipose tissue and kidneys. It is a part of the endocrine renin–angiotensin–aldosterone system (RAAS), which in pathologic conditions plays an important role in the development of cystic kidney diseases and hypertension. AGT in the kidneys is synthesized in proximal tubule cells.[19,20] The renal production of AGT is stimulated in response to angiotensin II and is regulated by positive feedback.[21,22] The high concentration of AGT in the urine induces angiotensin II-dependent sodium reabsorption and the development of sodium-dependent hypertension.[23] Ramanathan et al. demonstrated that the activation of intrarenal RAAS plays an important role in the pathogenesis of hypertension in ADPKD and chronic kidney disease (CKD) progression in adults.[12] Increased urinary excretion of AGT in hypertension was also described by Park et al.,[24] Isobe et al. in adults,[19] and by Suzue et al. in CKD in children with diabetes type 1.[25] Determination of AGT in the urine may be a useful biomarker for intrarenal activity of the RAAS.[20] In ADPKD polycystines with abnormal structure resulting from genetic mutations contribute to the growth and proliferation of renal tubule cells and increase the concentration of intracellular cAMP (cyclic adenosine monophosphate), which leads to the formation of cysts, then their enlargement and the secretion and accumulation of fluid inside them.[26–28] Expanding renal cysts by exerting pressure on neighbouring blood vessels cause local ischemia and activates the intrarenal RAAS.[29] Activation of renal RAAS in addition to the effect on blood pressure (BP), also plays a role in cyst enlargement and accelerates renal fibrosis.[14,30,31] In patients with ADPKD with coexisting hypertension, the left ventricle hypertrophy and diastolic dysfunction followed by systolic cardiac dysfunction could occur early.[32] Saigusa et al. documented that suppressing AGT synthesis using Gen 2 antisense oligonucleotide, which blocks intrarenal RAAS slows the progression of PKD in mice.[33]

IL-18 belongs to the IL-1 family. IL-18 is produced by monocytes, macrophages, dendritic cells and osteoblasts, and the active form of IL-18 is released during cell apoptosis. Receptors for IL-18 are found, among others, on endothelial cells.[34–36] This cytokine plays an important role in the regulation of both innate and acquired immunity.[37] The concentration of IL-18 in the urine is increased in acute kidney injury (AKI), which is associated with apoptosis and necrosis of the proximal tubule cells.[36,38,39] Therefore, research has focused on the utility of urinary IL-18 concentration measurement as a biomarker for the early detection of renal damage, especially in children.[40] However, there are limitations to the routine use of urinary IL-18 as a biomarker. In multi-organ diseases the concentration of IL-18 in the urine may be not only be the consequence of renal tubular necrosis but also the result from injury to other tissues and the apoptosis of lung cells or ischemic injury of the myocardium. Therefore, further studies are needed to determine the diagnostic value of IL-18 in both AKI and CKD including the relationship of IL-18 concentration with the progression of kidney injury.[12,25,36,41] There are no data on IL-18 levels in children with kidney cysts.

Objectives

As previously reported, since in kidney cysts the key alteration is RAAS activation and tubular destruction, we tested the hypothesis that levels of serum and urinary AGT and IL-18 are higher in polycystic kidneys and correlate to increased BP and decline in kidney function. In order to test this hypothesis, we assessed AGT and IL-18 concentration in serum and urine in children with kidney cysts of various aetiology and their relationship to the underlying kidney disease, gender and the presence of arterial hypertension.

Material and methods

Included into the study were 39 children and adolescents (23 girls and 16 boys) aged from 1.5 to 20 years (mean 10.9 years) with cysts of the kidneys. In all subjects the presence of cysts in the renal parenchyma was confirmed by ultrasound examination. Based on family history and ultrasound examination according to Pei et al.'s criteria for positive diagnosis,[9] a subgroup of children with ADPKD (20 children, 51%) was identified. From the study group, 15 children had a positive family history of ADPKD. The rest were named 'a non-ADPKD subgroup' (19 children, 49%). We excluded children with ARPKD, tuberous sclerosis and known syndromic ciliopathies, such as Bardet-Biedl and Joubert syndromes, from the non-ADPKD group. Of the non-ADPKD group, 18 children had single cysts, one child having more than 10 cysts, corresponding to cystic dysplasia.

Most of the children from the study group were not treated pharmacologically. Only four children with arterial hypertension from the ADPKD subgroup were administered antihypertensive drugs without angiotensin-converting enzyme inhibitors.

Body mass, height and BP as well as routine biochemical tests were performed on the subjects. Hypertension was diagnosed when mean arterial BP recordings were equal to or exceeding 95.cc according to nationally valid percentile charts.[42–45] Estimated glomerular filtration rate (eGFR (ml/min/1.73 m^2)) was calculated using the Schwartz formula.

Table 1. Characteristics of examined children with renal cysts and controls.

Parameter	Children with kidney cysts					Control group ($n = 20$)
	Whole group ($n = 39$)	ADPKD ($n = 20$)	Non-ADPKD ($n = 19$)	Girls ($n = 23$)	Boys ($n = 16$)	
Age (year)	10.9 ± 4.9 (1.9–19.8)	10.9 ± 5.1 (3.4–19.8)	11.0 ± 5.0 (1.9–18.7)	10.8 ± 4.6 (3.4–18.7)	11.1 ± 5.6 (1.9–19.8)	8.8 ± 3.9 (1.8–17.2)
Height (cm)	142.3 ± 24.9 (80–184.5)	141.7 ± 26.0 (97–184.5)	142.9 ± 24.3 (80–176)	142.1 ± 22.5 (97–173)	142.5 ± 28.7 (80–184.5)	130.8 ± 22.7 (82–172)
SDS for height	–0.03 ± 1.12 (–2.97–2.24)	–0.05 ± 1.0 (–2.97–1.8)	–0.02 ± 1.2 (–1.9–2.2)	0.2 ± 1.2 (–2.97–1.81)	–0.3 ± 1.0 (–1.9–2.2)	–0.4 ± 0.9 (–1.9–2.0)
BW (kg)	40.7 ± 17.8 (11–78)	41.7 ± 19.8 (13–78)	39.6 ± 16.0 (11–69)	40.5 ± 17.6 (13–78)	41.0 ± 18.8 (11–70)	33 ± 16.1 (9.7–63)
SDS for BW	0.13 ± 1.09 (–3.2–2.07)	0.2 ± 1.2 (–3.2–1.9)	0.05 ± 1.0 (–1.3–2.1)	0.2 ± 1.2 (–3.2–1.91)	–0.06 ± 1.0 (–1.3–2.07)	–0.003 ± 1.0 (–1.6–1.8)
BMI (kg/m²)	18.9 ± 3.29 (13.5–28.7)	19.3 ± 4.0 (13.5–28.7)	18.4 ± 2.4 (14.4–22.3)	18.9 ± 3.7 (13.5–28.7)	18.9 ± 2.7 (14.8–23.4)	18.0 ± 3.4 (14.2–26.6)
SDS for BMI	0.23 ± 0.93 (–1.38–2.02)	0.3 ± 1.1 (–1.4–2.02)	0.2 ± 0.8 (–1.3–1.7)	0.3 ± 1.0 (–1.38–2.02)	0.2 ± 0.9 (–1.3–1.7)	0.7 ± 2.1 (–1.5–8.8)
SYS	109.8 ± 13.3 (90–137)	111.6 ± 14.0 (90–137)	107.9 ± 12.6 (90–130)	111.4 ± 13.3 (90–137)	107.4 ± 13.4 (90–130)	
DIA	64.9 ± 8.9 (45–84)	66.7 ± 10.5 (45–84)	63.1 ± 6.8 (55–75)	66.5 ± 9.2 (50–84)	62.8 ± 8.3 (45–77)	
MAP	79.9 ± 9.8 (61.7–98.3)	81.7 ± 11.1 (61.7–98.3)	78.0 ± 8.2 (66.7–93.3)	81.5 ± 10.0 (63.3–98.3)	77.7 ± 9.3 (61.7–93.3)	
SDS for SYS	0.58 ± 1.0 (–1.22–2.63)	0.8 ± 1.1 (–0.8–2.6)	0.4 ± 0.9 (–1.2–1.6)	0.8 ± 1.0 (–0.84–2.63)	0.3 ± 0.9 (–1.2–1.8)	
SDS for DIA	0.39 ± 0.7 (–0.95–2.07)	0.5 ± 0.8 (–0.9–2.07)	0.2 ± 0.7 (–0.8–2.0)	0.5 ± 0.7 (–0.6–2.07)	0.2 ± 0.8 (–0.9–2.0)	

Data are presented as mean ± standard deviation (minimum–maximum).
BW: body weight; SDS: standard deviation score; BMI: Body Mass Index; SYS: systolic arterial pressure; DIA: diastolic arterial pressure; MAP: mean arterial pressure; ADPKD: autosomal dominant polycystic kidney disease.

The control group consisted of 20 healthy children (10 girls and 10 boys) between 1.5 and 17.5 years of age (mean 8.8 years). They were outpatients diagnosed for bedwetting or qualified for 'one day' surgical procedures, and their parents/caregivers agreed to additional tests. All children in the study were in good clinical condition and had no symptoms of acute infection. Table 1 presents the characteristics of the studied groups of children with renal cysts and the control group.

The study was approved by the Bioethics Committee of the Medical University of Silesia in Katowice (Resolution No. KNW/0022/KB1/21/15) and, before the examination, written consent from parents or legal guardians and from children over 16 years of age was obtained.

Laboratory tests

Blood samples (3–5 ml) for laboratory tests were drawn in the morning (8.00–9.00) during the scheduled control in the outpatient clinic. After centrifugation 1000× for 15 min at 4°C, the serum was stored at –20°C until assayed. Urine samples (50–100 ml) were collected at the same time and was also frozen at –20°C until evaluated. Determination of concentrations of IL-18 and AGT was performed in the Chair and Department of Medical and Molecular Biology, SMDZ in Zabrze, SUM in Katowice.

The concentration of IL-18 in serum and urine was performed by ELISA (enzyme-linked immunosorbent assay) using a set from e-Bioscience (USA) no. BMF267/2, according to the manufacturer's protocol. Determination of concentrations of AGT was carried out using a kit from Cloud-Clone (USA) no. SEA797 Hu, according to the manufacturer's protocol.

Statistical analysis

The database was prepared with Excel, Microsoft software. All statistical calculations were done using the licensed version 10.0 software Statistica (StatSoft Inc., USA). The results were considered significant at a p value below 0.05. The following parameters of descriptive statistics were presented: arithmetic mean, median, minimum and maximum value, lower and upper quartile values, and standard deviation. The presence of normal distribution was checked using the Shapiro–Wilk test. Levene test was chosen to check the homogeneity of variations. Parametric

tests were used for variables with normal distribution. Mann–Whitney nonparametric test was used for comparisons of variables deviating from the normal distribution. Pearson's test or Spearman's rank correlation test were conducted for the analysis of correlation, adequately to the distribution of the analysed variables.

Results

The values of laboratory tests in the group of children with kidney cysts, divided into patients with ADPKD, non-ADPKD and gender are presented in Table 2. The mean values of basic laboratory tests in the study group and in the subgroups did not differ significantly. The mean value of eGFR in the group of children with kidney cysts was 119.8 ± 32.7 ml/min/1.73 m^2. Only in 5 children in the study group was the value of eGFR reduced (2 children with ADPKD and 3 children from non-ADPKD subgroup).

The concentrations of the investigated markers (AGT, IL-18) in serum and urine in children with renal cysts and in the control group are presented in Figures 1 and 2. Serum and urinary AGT concentration in children with renal cysts was significantly higher compared to the control group, regardless of the underlying aetiology of the cysts and gender. The AGT/creatinine index in urine showed comparable results as urine AGT concentration (data not shown). The concentration of IL-18 in the serum was significantly lower in children with kidney cysts in comparison with healthy children. In contrast, the concentration of IL-18 in the urine did not differ significantly between sick and healthy children.

In the analysis of correlation of serum and urine AGT concentrations we found that in children with ADPKD:

- a negative correlation between serum AGT and diastolic BP (DBP) values ($r = –0.520$, $p = 0.019$) and mean arterial pressure (MAP) ($r = –0.460$, $p = 0.041$)
- a positive correlation between urinary excretion of AGT and eGFR ($r = 0.559$, $p = 0.010$).

After normalizing AGT serum concentrations to BMI in the subgroup of ADPKD (similarly as in whole group of children with kidney cysts – data not shown) we observed:

- a negative correlation between AGT/BMI and the age of the subjects ($r = –0.620$, $p = 0.004$), systolic BP (SBP) ($r = –0.621$, $p = 0.003$), DBP ($r = –0.625$, $p = 0.003$), MAP ($r = –0.655$, $p = 0.002$)
- a positive correlation of the AGT/creatinine ratio in the urine with age of examined children ($r = 0.489$, $p = 0.029$) and eGFR ($r = 0.505$, $p = 0.023$).

Table 2. Laboratory tests and eGFR value in children with renal cysts.

Parameter	Wholegroup ($n = 39$)	ADPKD ($n = 20$)	Non-ADPKD ($n = 19$)	Girls ($n = 23$)	Boys ($n = 16$)
Na (mmol/l)	140.1 ± 2.2 (134–143)	140.0 ± 2.5 (134–143)	140.2 ± 1.7 (136–143)	140.3 ± 1.7 (136–143)	139.8 ± 2.8 (134–143)
K (mmol/l)	4.5 ± 0.4 (4.0–5.4)	4.4 ± 0.4 (3.98–5.1)	4.5 ± 0.3 (4.0–5.4)	4.4 ± 0.3 (3.98–5.1)	4.5 ± 0.4 (4.0–5.4)
Creatinine (µmol/l)	48.0 ± 15.6 (22–77)	48.3 ± 17.5 (22–77)	47.8 ± 13.7 (29–73)	45.8 ± 13.7 (22–69)	51.25 ± 17.9 (27–77)
Uric acid (µmol/l)	266.2 ± 67.8 (134–370)	267.4 ± 66.5 (150–368)	264.9 ± 71.0 (134–370)	253.4 ± 65.0 (134–351)	284.4 ± 69.7 (151–370)
Urea (mmol/l)	4.3 ± 1.3 (2.4–6.8)	4.4 ± 1.4 (2.4–6.8)	4.2 ± 1.2 (2.5–6.1)	4.16 ± 1.25 (2.4–6.6)	4.4 ± 1.3 (2.5–6.8)
eGFR (ml/min/1.73m²)	119.8 ± 32.7 (38.2–179.3)	121.5 ± 35.0 (38.2–176.0)	117.9 ± 30.8 (47.8–179.3)	115.9 ± 28.25 (38.2–156.2)	125.3 ± 38.5 (47.8–179.3)

Data are presented as mean ± standard deviation (minimum–maximum).
For all comparisons $p > 0.05$.
eGFR: glomerular filtration rate; ADPKD: autosomal dominant polycystic kidney disease.

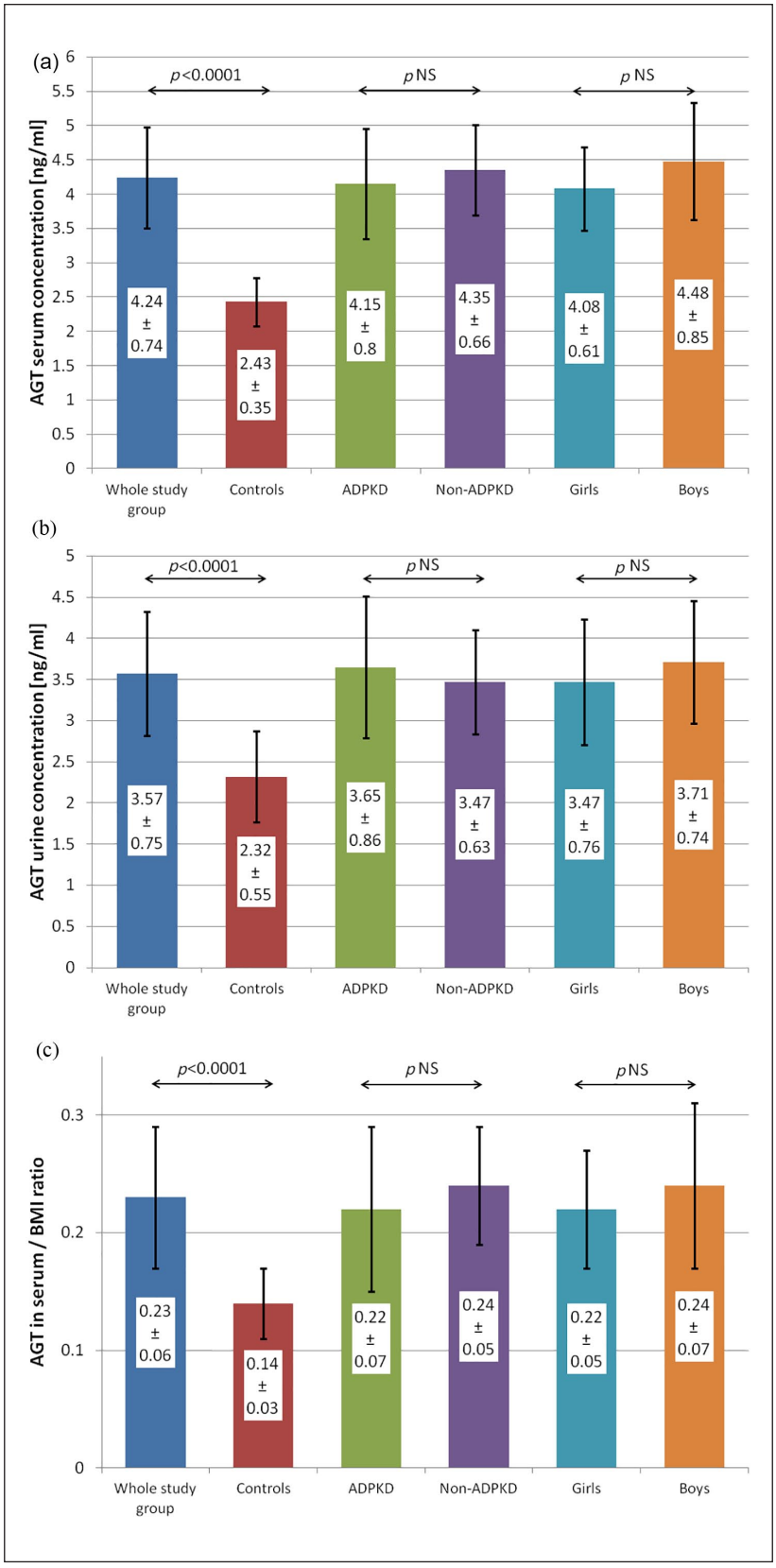

Figure 1. Concentration of AGT in (a) serum and (b) urine, and (c) AGT (serum)/BMI ratio in children from the whole study group, control group and subgroups of the study group.
AGT: angiotensinogen; ADPKD: autosomal dominant polycystic kidney disease; BMI: body mass index.

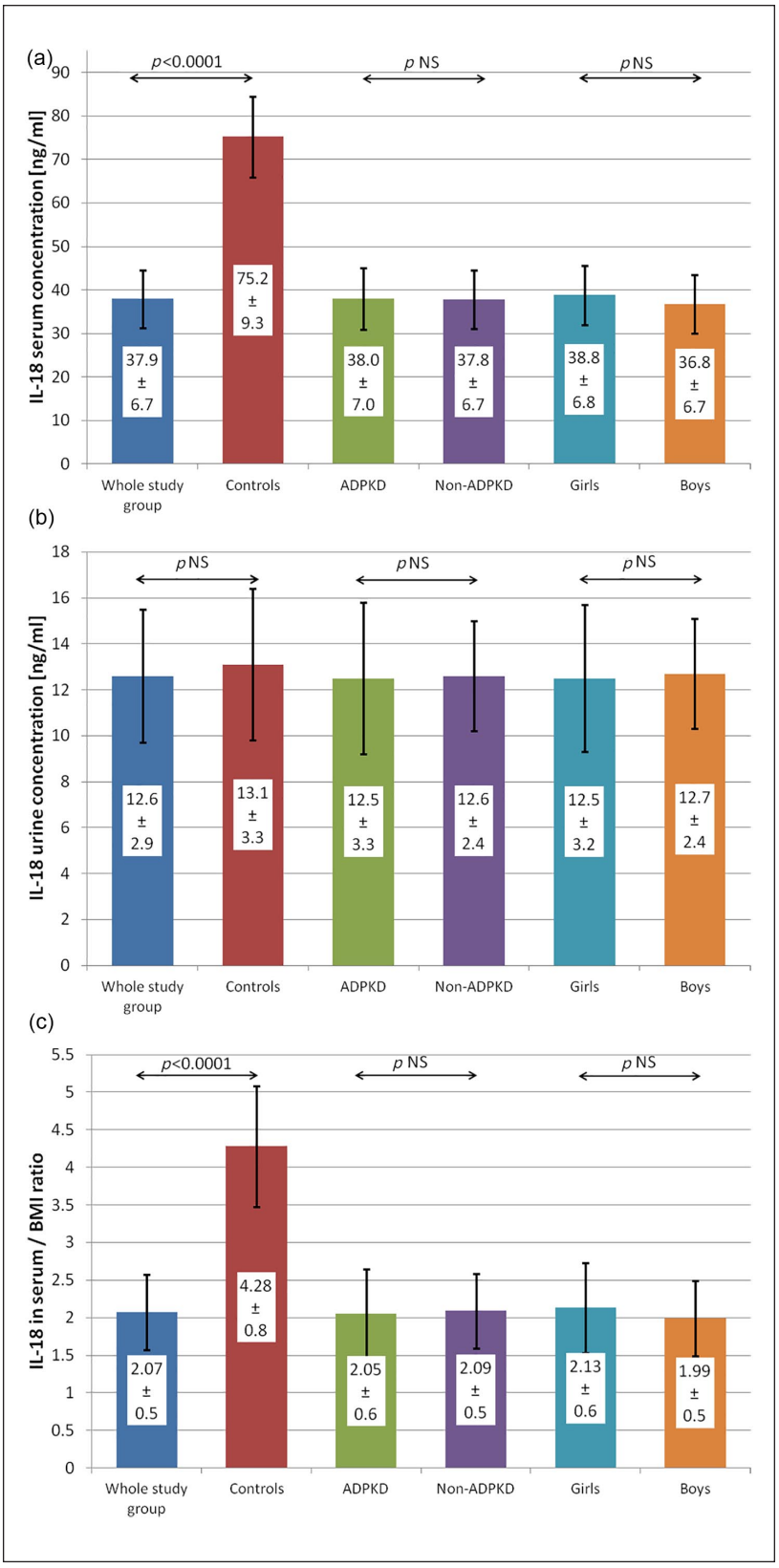

Figure 2. Concentration of IL-18 in (a) serum and (b) urine, and (c) IL-18 (serum)/BMI ratio in children from the whole study group, control group and subgroups of the study group.
IL-18: interleukin 18; ADPKD: autosomal dominant polycystic kidney disease; BMI: body mass index.

Table 3. Evaluation of the correlation between the tested markers and the results of anthropometric measurements, biochemical parameters and BP values.

Parameter	Children with kidney cysts			
	AGT (S)	AGT (U)	IL-18 (S)	IL-18 (U)
Age (year)	$r = –0.174$ $p = 0.291$	$r = –0.002$ $p = 0.990$	$r = –0.155$ $p = 0.347$	$r = –0.246$ $p = 0.131$
Height (cm)	$r = –0.154$ $p = 0.349$	$r = 0.015$ $p = 0.928$	$r = –0.093$ $p = 0.574$	$r = –0.244$ $p = 0.134$
SDS for height	$r = 0.147$ $p = 0.372$	$r = 0.086$ $p = 0.603$	$r = 0.218$ $p = 0.183$	$r = 0.052$ $p = 0.754$
BW (kg)	$r = –0.222$ $p = 0.175$	$r = 0.028$ $p = 0.865$	$r = –0.124$ $p = 0.453$	$r = –0.24$ $p = 0.143$
SDS for BW	$r = –0.022$ $p = 0.893$	$r = 0.141$ $p = 0.391$	$r = 0.214$ $p = 0.191$	$r = 0.02$ $p = 0.911$
BMI (kg/m²)	$r = –0.276$ $p = 0.089$	$r = 0.086$ $p = 0.601$	$r = –0.025$ $p = 0.882$	$r = –0.157$ $p = 0.339$
SDS for BMI	$r = –0.158$ $p = 0.337$	$r = 0.087$ $p = 0.599$	$r = 0.126$ $p = 0.443$	$r = –0.055$ $p = 0.740$
SYS	$r = –0.18$ $p = 0.274$	$r = –0.162$ $p = 0.325$	$r = –0.163$ $p = 0.322$	$r = –0.325$ $p = 0.044$
DIA	$r = –0.230$ $p = 0.158$	$r = –0.029$ $p = 0.860$	$r = –0.078$ $p = 0.637$	$r = –0.294$ $p = 0.069$
MAP	$r = –0.221$ $p = 0.176$	$r = –0.091$ $p = 0.582$	$r = –0.121$ $p = 0.464$	$r = –0.325$ $p = 0.043$
SDS for SYS	$r = –0.144$ $p = 0.488$	$r = –0.228$ $p = 0.162$	$r = –0.091$ $p = 0.582$	$r = –0.208$ $p = 0.204$
SDS for DIA	$r = –0.01$ $p = 0.549$	$r = 0.001$ $p = 0.956$	$r = –0.029$ $p = 0.863$	$r = –0.176$ $p = 0.285$
Creatinine (µmol/l)	$r = –0.153$ $p = 0.354$	$r = –0.232$ $p = 0.155$	$r = 0.01$ $p = 0.951$	$r = –0.229$ $p = 0.160$
Urea (mmol/l)	$r = 0.104$ $p = 0.530$	$r = –0.152$ $p = 0.357$	$r = –0.185$ $p = 0.261$	$r = 0.229$ $p = 0.161$
Uric acid (µmol/l)	$r = –0.207$ $p = 0.205$	$r = –0.112$ $p = 0.498$	$r = 0.073$ $p = 0.657$	$r = 0.009$ $p = 0.957$
Na (mmol/l)	$r = –0.139$ $p = 0.397$	$r = –0.1$ $p = 0.544$	$r = –0.081$ $p = 0.623$	$r = –0.17$ $p = 0.301$
K (mmol/l)	$r = 0.191$ $p = 0.245$	$r = 0.232$ $p = 0.155$	$r = –0.083$ $p = 0.614$	$r = –0.003$ $p = 0.987$
eGFR (ml/min/1.73m²)	$r = –0.127$ $p = 0.443$	$r = 0.297$ $p = 0.066$	$r = –0.143$ $p = 0.386$	$r = –0.092$ $p = 0.576$

BP: blood pressure; BW: body weight; SDS: standard deviation score; BMI: Body Mass Index; SYS: systolic arterial pressure; DIA: diastolic arterial pressure; MAP: mean arterial pressure; AGT: angiotensinogen; IL-18: interleukin 18; S: serum; U: urine.

In the non-ADPKD subgroup, we showed a significant negative correlation between AGT/BMI and eGFR ($r = –0.598$, $p = 0.007$).

In the analysis of the correlation of serum and urinary IL-18 concentrations in the whole group of children with renal cysts a significant negative correlation was found between urinary IL-18 concentration and SBP and MAP values (Table 3).

For the IL-18/creatinine ratio in the urine the above correlations were non-significant. Analysing the subgroup with ADPKD, for serum IL-18/BMI ratio, we observed a significant negative correlation with age ($r = –0,546$, $p = 0.013$), SBP ($r = –0.560$, $p = 0.010$) and eGFR ($r = –0.516$, $p = 0.020$).

In the subgroup with non-ADPKD, we showed a significant negative correlation between IL-18/BMI ratio and the age of the subjects ($r = –0.464$, $p = 0.045$), SBP ($r = –0.515$, $p = 0.024$), MAP ($r = –0.490$, $p = 0.033$) and eGFR ($r = –0.523$, $p = 0.022$).

Discussion

In our study we showed a higher concentration of AGT in both serum and urine, in children with various variants of kidney cysts, which may reflect the expression of RAAS activation regardless of the aetiology, before the clinical onset of hypertension. However, we did not show a direct relationship between AGT serum concentrations and its urinary output with absolute values of arterial pressure, as well as with SDSs for SBP, DBP and MAP. This might be connected with the absence of other metabolic disorders in studied children and the relatively low number of examined children. Rebholz et al. showed in adults on a high-sodium diet the increase of AGT urine concentration with rising BP. These authors postulate that the concentration of AGT in urine may be an indirect marker of BP sodium susceptibility.[22] In the group of children with kidney cysts examined in our study, AGT concentration did not show dependence with natremia, however, in the studied group of children the serum sodium concentration remained at the average level 140 mmol/l. The direct relationship between urinary excretion of AGT and disease severity indices, including high BP and eGFR, has been confirmed by Luyckx et al.,[46] Park et al.[24] and Padma et al.[47] Kocyigit et al. analysed AGT concentrations in urine in adult patients with ADPKD. They showed the highest concentration of AGT in the urine of patients with

ADPKD with associated hypertension, including patients on RAAS blockade compared to patients with ADPKD and normal BP.[48] Also, Kurultak et al. documented significantly higher urinary AGT concentrations in patients with ADPKD without hypertension as compared to healthy controls, but they did not examine patients with cysts of other origins.[49]

Absolute serum AGT level and AGT/BMI ratio showed a negative correlation with age in the ADPKD subgroup. We also noted a similar correlation of AGT/BMI ratio and age in a group of children with renal cysts and lack of it in healthy controls. It can be explained by the possible presence of several other factors (not estimated in this study) which interact with RAAS activity and BMI value (e.g. sympathetic nervous system, natriuretic peptide system). Studies by Matsusaka et al. suggested that the tubular uptake of the precursor of liver origin contributes to intrarenal ANG II expression. They demonstrated that knockdown of liver AGT stopped the proximal tubule accumulation of AGT and angiotensin II.[50]

The level of many substances produced at rates that are proportional to body size may be normalized by using BMI or creatinine excretion as an index of body size. Urinary biomarkers, such as albumin as markers of kidney injury, are frequently reported as a normalized ratio to urinary creatinine concentration. Biomarker to creatinine ratio is an accepted method for explaining variations, for example, in albumin concentration resulting from variations in urine volume due to hydration, diuresis, or concentration changes induced by antidiuretic hormone or tubular injury,[51] so we decided also to present actual values of AGT and IL-18 and normalized values considering them equally important. They reflect different attitudes. The normalization enables more precise evaluation, especially in smaller groups of patients and allows to highlight the differences. Park et al. also used AGT/creatinine index, which represents the local RAAS and confirmed its activation in adult hypertensive patients with ADPKD.[24] Ahmed et al. found that a rising BMI is associated with a steadily increasing level of angiotensin-dependent control of the renal circulation, which they concluded has significant public health considerations.[52]

When analysing subgroups of children with renal cysts of different aetiology, our results did not show differences in serum and urinary AGT concentrations in the subgroup of children with ADPKD as compared to the non-ADPKD subgroup, thus indicating no increased activation of the RAAS system in children with ADPKD. Also, when describing the study group by gender, no increased serum and urine concentrations of AGT were found in boys compared to girls. Also, in our study, the analysis of any correlation of AGT concentrations, both in serum and in urine, with anthropometric measurements, showed no significant relationships in the whole group of children with kidney cysts. The negative correlation between serum AGT and DBP and MAP values found in ADPKD children in the absence of such a correlation for urinary AGT and AGT/creatinine in urine, may indicate only a slight effect of RAAS intrarenal activation in ADPKD on the consolidation of hypertension in children with kidney cysts. This may be explained by taking into account that in studied children the values of DBP and MAP were comparable, and hypertension was noted only in four children and treated pharmacologically. The above mentioned negative correlation could be possibly explained by the lack of other metabolic disorders and accompanying diseases in the group of studied children and the relatively low number of examined children.

In addition, in the subgroup with ADPKD we found a positive correlation between urinary AGT concentration and AGT/creatinine ratio in urine and eGFR values. Interestingly, similar relationships were not observed in the non-ADPKD subgroup. These results demonstrate the effect of RAAS on the regulation of glomerular filtration with initial hyperfiltration and indicate the need for further studies in a larger group of children with renal cysts. Salih et al. compared the group of adults with ADPKD with a group with CKD without ADPKD and showed significantly higher urinary AGT concentrations in the ADPKD group.[53] The limitation of our study is the lack of analysis of the relationship between urinary AGT and total kidney volume adjusted to height (htTKV), other cystic burden parameters (cyst volume, cystic index and cyst number and sizes), albuminuria or proteinuria. TKV was not determined in all patients, that's why we had no possibility of performing comparisons, although total length of the kidneys in most of the cases did not exceed 95th percentile for age. Also, albuminuria was present in only eight children from the examined group since we had patients in early stages of kidney cystic disease.

IL-18 is involved in the pathogenesis of many chronic diseases, such as inflammatory bowel diseases, obesity, heart disease, chronic obstructive pulmonary disease, systemic lupus erythematosus and is usually detected as elevated levels.[37,54] Some researchers believe that in the metabolic syndrome it is a secondary mechanism in order to compensate insulin resistance induced by other pro-inflammatory agents.[55,56] According to others, the primary deficiency of IL-18 causes the development of metabolic syndrome, colitis and macular degeneration in experimental animals. IL-18 deficient mice were shown to have an increased appetite, obesity and exhibit the symptoms of metabolic syndrome including atherosclerosis and insulin resistance.[57] In the available literature, reports on the role of IL-18 in kidney damage associated with the formation of cysts in children are lacking. Parikh et al. in a study in ADPKD in adults documented that IL-18 excretion in urine was slightly and stably elevated over the three years of observation, but without correlation of total kidney

volume changes or kidney function deterioration.[38] In the experimental part of the study these authors confirmed that epithelial cells, which are lining the cyst, show strong expression of IL-18 but they did not, however, evaluate serum IL-18 levels. In our study, we found significantly lower serum IL-18 levels in children with renal cysts compared to healthy children. This may reflect the loss of the protective role of the above-mentioned cytokine in the course of cystic kidney degeneration. The presence of IL-18 in epithelial cells suggests a role for IL-18 in local barrier defences which may be broken in some chronic diseases.[37] In contrast, the concentration of IL-18 in the urine in the group of children with renal cysts did not differ significantly compared to healthy controls. Perhaps this is due to the slight involvement of cystic changes in the kidneys in children compared to the adult population or that cytokine is not excreted into the urine but is accumulated inside the cysts.

The concentration of IL-18 in serum and urine in subgroups with ADPKD and non-ADPKD did not differ. In addition, the concentration of IL-18 in the serum showed no dependence on gender. In the correlation analysis of serum and urinary IL-18 concentrations and anthropometric measurements, BMI did not show significant correlations. Vilarrasa et al. demonstrated that IL-18 concentrations were also similar in men and women in the healthy adult population. Also, they did not find any differences between obese and normal-weight subjects.[58] Significantly higher IL-18 concentrations were found in subjects with arterial hypertension, which corresponds with our observations of the negative relationship of low urine IL-18 concentration and serum IL-18/BMI with SBP and MAP values, and may reflect the loss of the protective role of this cytokine in children with ADPKD. We have shown that the presence of kidney cysts is important in the pathogenesis of hypertension in patients with cystic renal degeneration.

Alexander et al. confirmed that monocytes showed enhanced pro-inflammatory gene expression in adult subjects with hypertension and identified IL18 receptor accessory protein as a potential novel mediator of arterial hypertension.[59]

In conclusion, it should be noted that higher levels of AGT in both blood and urine in children with various aetiology of renal cysts may suggest the activation of the RAAS system including its intrarenal part, even before the onset of clinically detected arterial hypertension. The AGT concentration seems to be a useful biomarker for an increased risk of hypertension in that group of patients. Serum IL-18 concentration in children with renal cysts was significantly lower than in healthy children, which may reflect the loss of the protective role of IL-18 in the course of cystic kidney degeneration and also indicate for the association of this cytokine with the occurrence of hypertension related to that renal condition.

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