Original article

Central Eur J Paed 2017;13(2):107-116
DOI 10.5457/p2005-114.178

# RENAL FUNCTIONAL RESERVE IN CHILDREN WITH A SOLITARY FUNCTIONING KIDNEY AND CHRONIC KIDNEY DISEASE

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**Received**: May 11, 2017
**Accepted**: July 31, 2017

**Key words:** Unilateral renal agenesis ▪ Multicystic dysplastic kidney ▪ Renal function ▪ Cystatin C ▪ Chronic renal failure.

**Objective** – To examine renal functional reserve (RFR) and blood pressure (BP) in children with a solitary functioning kidney (SFK) and stage 1-3 chronic kidney disease (CKD). **Method** – RFR was measured in 48 children with SFK and in 10 healthy children, as the difference between un-stimulated and stimulated clearance of endogenous creatinine by a meat-free oral protein load (OPL). Cimetidine was given 48 h prior to the measurement when the study subjects were on a diet free of meat, fish and poultry. Serum cystatin C and urinary protein (UPRT)/urinary creatinine (UCr) were examined before and 2 hours after OPL. BP was determined by office and by 24-h ambulatory BP monitoring (ABPM). **Results** – The majority of the patients (79.6%) had congenital SFK, while the remaining had acquired SFK due to unilateral nephrectomy. Sixteen patients had CKD1, 19 patients had CKD2 and 13 had CKD3. The patients and controls did not differ in terms of age, gender, body size, office and 24-h blood pressure readings and basal GFR. Kidney size was greater and serum cystatin C was higher in patients than in controls. Increased proteinuria and arterial hypertension were found in 24.3% and 18.9% of the patients, respectively. Nocturnal hypertension was more common than that during the daytime. After OPL, GFR significantly increased, more in controls than in patients. Among the patients, the RFR was the highest in the CKD3 group. **Conclusion** – OPL induced an increase in GFR above its basal value. This response was higher in healthy children than in those with SFK. The positive relationship between RFR and CKD stage and the highest RFR in CKD3 patients suggests well preserved renal functional reserve in patients with moderate renal failure. ABPM is necessary for BP evaluation in children with SFK.

## Introduction

A solitary functioning kidney (SFK) is prone to hemodynamic dysfunction and glomerular hyperfiltration due to the lower nephron number. The smaller the number of the nephrons, the likelihood is greater of developing hyperfiltration injury, which contributes to the progression of chronic kidney disease (CKD) (1-5). The KIMONO study found that nearly a third of 206 children with a SFK at a mean age of 9.5 years had renal injury, defined as the presence of hypertension and/or albuminuria, and/or use of renoprotective medication (6). Children with an additional urinary tract anomaly had

a higher percentage of renal injury compared to children who only had SFK. However, an increased risk of slow progressive decline in GFR was demonstrated in both groups (6). Baudoin et al., in a cohort of 111 patients with a SFK after unilateral nephrectomy demonstrated a rising trend of reduced GFR, hypertension and increased proteinuria over 25 years of follow-up (7).

Modification of glomerular hyperfiltration by dietary protein intake may have a diagnostic and therapeutic impact on CKD progression (8, 9). Glomerular hemodynamic response to an acute oral protein load (OPL), or to amino acid infusion, known as renal functional reserve (RFR), has been proposed to be a reliable diagnostic test to assess *in vivo* hyperfiltration in various forms of renal disorders (10-14). Normal RFR is defined as the capacity of the kidney to increase its basal GFR by at least 20% after short-term protein overload (15). The reduction or absence of RFR could imply that the number of functioning residual nephrons is reduced, and therefore they are already in a state of permanent glomerular hyperfiltration (10).

The present study was designed to assess RFR in children with a SFK, and normal or mild to moderate chronic reduction of GFR. The hypothesis was that RFR is not preserved in patients with moderate GFR reduction.

## Subjects and methods

### *Study population*

Patients with a SFK documented by renal ultrasound and a dimercaptosuccinic acid (DMSA) scan were invited to enter the study. For inclusion they were required to meet the following criteria: 1. To be aged between 2 and 18 years, 2. To have congenital or acquired SFK, 3. To have Schwartz et al.'s estimated GFR (eGFR) (16) above 30 ml/min/1.73 $m^2$ and 4. To be without infection and without any treatment that could influence renal function or blood pressure (BP). This means that patients were not receiving renin-angiotensin-system inhibitors or any other antihypertensive drugs. Patients with vesicoureteral reflux were given antibacterial prophylaxis, and patients with CKD stage 3 received vitamin D, calcium carbonate and iron preparations.

Patients who had neurogenic or functional bladder dysfunction as well as those with multisystemic malformations were excluded from the study. Healthy children who were relatives of the patients with end stage renal disease caused by non-hereditary nephropathy were included in the control group. They were considered 'healthy' if the results of physical and comprehensive laboratory examinations were normal as well as their eGFR and renal ultrasound. The study was approved by the Ethics Committee of the University Children's Hospital. Written informed consent from the parents and written assent from the children was obtained.

## Method

The size of the SFK was determined using the maximal bipolar ultrasound measurement obtained in either prone- or supine-positioned patients using a 3.5 or 5-MHz probe. Renal size percentiles were evaluated according to a standard length-versus height-normogram (17), and by the normogram of renal length versus body surface area (BSA) for a single kidney (18). Compensatory hypertrophy was determined when the measured renal size was greater than the 95th percentile for the pair of kidneys (17), and SFK hypertrophy when the renal size was greater than the 90 percentile for a single kidney (18).

Blood pressure was examined by casual (office) BP measurement using validated mercury manometers, and by ambulatory blood pressure monitoring (ABPM) over 24 h; every 15 minutes during the daytime (6:00 to 22:00) and every 30 minutes during

the night time (22:00 to 6:00) using a validated automatic oscillometric device (Model 90207 SpaceLabs). Hypertension was defined as systolic and/or diastolic BP above the 95th percentile according to sex and height (19, 20). Comparison of blood pressure values was also performed using SDS values for blood pressure which were calculated using the Pediatric Percentile Calculator for Height, Weight, BMI, and Blood Pressure (http://www.quesgen.com/BMIPedsCalc.php). Non-dipping BP was defined as the absence of a nocturnal mean arterial pressure fall of at least 10%.

### *Renal function*

Renal function was analyzed by 24-h and 2-h creatinine clearance, estimated GFR (eGFR) (16), and serum cystatin C. The 24-h timed-urine collection for basal endogenous creatinine clearance was obtained before treatment with cimetidine ($GFR_0$), while 2-h creatinine clearances were determined pre ($GFR_1$) and post ($GFR_2$) OPL test. The OPL test was performed according to the method of Hellerstain et al. (10). Cimetidine was given for 48 h prior to the study at a total daily dose of 12.1±4.9 mg/kg while on a diet free of meat, fish and poultry. The test commenced at 8 am. The study subjects were asked to empty their urinary bladder completely which was documented by less than 10 ml of residual urine according to an ultrasound bladder scan. The first urine collection period lasted 2 hours and was stimulated by 5 ml/kg of water intake per 30 minutes. At the end of this period the study subjects completely emptied their bladder, which was again confirmed by ultrasound scan. Thereafter, the study subjects were given a meal containing milk, cheese, eggs and baked food which contained 1 g/kg body weight of protein. Fifty minutes after completing the meal, the second 2-hour urine period collection started which was performed exactly as the first one. Blood specimens were obtained after both urine collection periods.

Serum cystatin C was measured before (cystatin $C_1$) and 2 hours after OPL (cystatin $C_2$) by enzyme-linked immunoassay (ELISA). Urinary protein excretion (UPRT) was determined from accurate 2-h timed urine collection before and after OPL, using pyrogallol red and molybdate on a Selecta Bioanalyser. Serum (sCr) and urinary creatinine (UCr) were determined using Jaffe's method using a Dimension Autoanalyser (Date Behring). The renal clearance of creatinine (GFR) was calculated using the formula GFR=UCr × V × 1.73/sCr × t × BSA, where UCr=creatinine concentration in urine, sCr=creatinine concentration in serum, V=volume of urine over a given time period, t=period of urine collection in minutes, BSA=body surface area expressed in $m^2$. The Rfd was obtained as the difference between the afterload ($GFR_2$) and preload creatinine clearance ($GFR_1$) using the formula: Rfd(in ml/min/1.73 m2)=$GFR_2$-$GFR_1$. Renal functional reserve (RFR) was calculated using the following formula: RFR(%)=($GFR_2$-$GFR_1$) ·100/$GFR_1$. Patient groups were classified based on $GFR_0$ as CKD stage 1 to stage 3 according to the Kidney Disease Outcomes Quality Initiative (K/DOQI) CKD criteria (21).

### *Statistical analysis*

All data were stored and analysed using SPSS 12.0 for Windows (SPSS Chicago, IL, USA). Data are presented as the mean ±SD, or as median and interquartile range (IQR), where appropriate. Comparisons of normally distributed variables within and between groups were performed using *t* test. In cases of non-normal distribution, a non-parametric Mann-Whitney U and Wilcoxon matched-pairs tests were performed, while the proportions were compared with the $\chi^2$ test. The relationships between variables were analyzed by Pearson's correlation test. A val-

ue of $p<0.05$ was considered to be statistically significant.

## Results

A total of 48 patients (32 males) mean age 9.8±4.2 years and 10 healthy children (6 males) mean age 11.7±4 years were included in the study. The majority of the patients (79.6%) had a congenital SFK due to unilateral renal agenesis or multicystic dysplastic kidney, while 20.4% patients had acquired a SFK due to unilateral nephrectomy of the severe dysplastic and/or scarred kidney at a median of 5 (IQR 2.2-10) years before the study period. Nephrectomy was generally performed when renal uptake on scintigraphy was <10%. Both congenital and acquired SFK were more frequent (58.5%) on the right side. In patients with unilateral renal agenesis without additional anomalies of the urinary tract, SFK on the right side was even more frequent (70.6%) (Table 1). Vesicoureteric reflux was found in 11 (22.7%) patients. Ipsilateral (i.e. the side of the SFK only) ureterovesical junction obstruction was identified in 4 (8.2%) patients. Basal GFR ($GFR_0$) was decreased in 32 (65.3%) patients; 19 patients (38.8%) had CKD2 and 13 patients (26.5%) had CKD3. The clinical characteristics of the patients and children from the control group are presented in Table 1.

There were no significant differences in age, gender, body height, body mass index, casual and 24 h BPs, $GFR_0$, basal serum creatinine ($sCr_0$), and proteinuria (UPRT/$UCr_0$) between patients and controls. However serum cystatin C was higher and renal size was larger in patients than in controls. In addition, increased proteinuria and arterial hypertension were only found in the patients. An unexpected result was a loss of normal circadian BP rhythm (night drop in blood pressure less than 10%) in 50% of the children from control group. Most of these children reported poor sleep during the night of ABPM, but refused to check this by repeated 24h ABPM. Compensatory renal hypertrophy and single kidney hypertrophy were found in 61.2% and 26.5% of the patients, respectively. Renal size, as well as compensatory hypertrophy, decreased with increasing CKD stage (Table 2). In addition, renal size was negatively correlated with the stage of CKD ($r=-0.432$; $p=0.001$), but was positively correlated with $GFR_0$ ($r=0.381$; $p=0.0.009$), $GFR_1$ ($r=0.370$; $p=0.005$), casual systolic ($r=0.443$; $p=0.001$) and diastolic BP ($r=0.469$; $p=0.000$) and 24 h systolic ambulatory BP ($r=0.396$; $p=0.009$). Renal size, casual systolic and diastolic BP, as well as 24-systolic ambulatory BP, positively correlated ($p<0.001$) with age, body height and BMI.

Although Rfd was higher in healthy children than in patients (Table 1), the post-load increase in glomerular filtration ($GFR_2$) and urinary creatinine excretion ($UCr_2$) were statistically significant only in the patients (Table 3). Furthermore, RFR was higher in CKD3 patients compared to CKD1 and CKD2 (Table 3). Rfd positively correlated with RFR ($r=0.876$; $p=0.000$), CKD stage ($r=0.500$; $p=0.000$) and $GFR_2$ ($r=0.588$; $p=0.000$). In contrast, negative correlations of Rfd were found with $GFR_1$ ($r=-0.287$, $p=0.030$) and cystatin $C_2$ ($r=-0.293$, $p=0.041$). $GFR_2$ negatively correlated with cystatin $C_1$ ($r=-0.402$; $p=0.004$) and cystatin $C_2$ ($r=-0.344$, $p=0.015$) while positively correlating with Rfd ($r=0.588$, $p=0.000$), $GFR_1$ ($r=0.545$, $p=0.000$) and $GFR_0$ ($r=0.0.299$, $p=0.039$).

Table 1 Characteristics of the patients and the controls

| Parameters | Patients | Controls | p |
|---|---|---|---|
| Number of children | 49 | 10 | - |
| Age (years) | 9.8± 4.2 | 11.7±4 | ns |
| Male (%) | 32 (65.3) | 6 (60) | ns |
| BH (cm) | 138.8±25.1 | 149.4±25.9 | ns |
| BMI (kg/m$^2$) | 18.5±3.9 | 18.4±2 | ns |
| *Renal disease* (%) | | | |
| URA or MCDK | 39 (79.6) | - | |
| UN | 10 (20.4) | - | |
| SFK on the right side | 58.5 | - | |
| VUR | 11 (22.7) | - | |
| Ipsilateral obstruction | 4 (8.2) | - | |
| *Blood pressure* (mmHg) | | | |
| Casual SBP | 106.9±10.2 | 107.2±6.7 | ns |
| Casual DBP | 68±8.7 | 68.2±7.0 | ns |
| 24-h SBP | 106.9±10.2 | 107.2±6.7 | ns |
| 24-h DBP | 68±8.7 | 68.2±7 | ns |
| Prevalence of daytime systolic hypertension (%) | 8.1 | 0 | ns |
| Prevalence of daytime diastolic hypertension (%) | 10.8 | 0 | ns |
| Prevalence of night time systolic hypertension (%) | 18.9 | 0 | ns |
| Prevalence of night time diastolic hypertension (%) | 17.9 | 0 | ns |
| Prevalence of non-dipping (%) | 30.0 | 50.0 | ns |
| *Renal size* | | | |
| Renal length (mm) | 104.6±19.9 | 92.7±15.6 | 0.06 |
| Patients with renal length/BH >95 percentile for two kidneys | 30 (61.2%) | 0 | 0.001 |
| Patients with renal length/BSA >90 percentile for solitary kidney | 13 (26.5%) | - | - |
| *Renal function* | | | |
| Basal serum creatinine ($sCr_0$) (µmol/l) | 70 (60-88) | 70 (59-91) | ns |
| Serum Cystatin C (mg/l) | 1.22 (0.91-1.46) | 0.69 (0.58-0.75) | 0.01 |
| $GFR_0$ (ml/min/1.73 m$^2$BSA) | 83.2 (61.8-106.9) | 73 (68-86) | ns |
| eGFR (ml/min/1.73 m$^2$BSA) | 107.1 (85.5-123.9) | 102.1 (93.9-110.8) | ns |
| Urinary creatinine (mg/kg/h) | 0.74 (0.62-0.89) | 0.71 (0.60-0.89) | ns |
| Urinary protein/ urine creatinine (mg/mg) | 0.11 (0.08-0.23) | 0.09 (0.08-0.13) | ns |
| Prevalence of increased basal proteinuria (%) | 24.3 | - | 0.08 |
| Rfd (ml/min/1.73 m$^2$BSA) | 9.5 (-4.8 to 24.6) | 56.5 (21.7 to71.2) | 0.02 |
| RFR (%) | 13 (-5.6 to 31.8) | 18.2 (-1.4 to 52.6) | ns |
| Cimetidine dose (mg/kg) | 12.1±4.8 | 18±3.8 | ns |

Values are expressed as mean ±SD, or as median (interquartile range); BH= body height; BMI=Body mass index; URA=Unilateral renal agenesis; MCDK=Multicystic dysplastic kidney; UN=Unilateral nephrectomy; SFK=Solitary functioning kidney; VUR=Vesicoureteral reflux; SBP=Systolic blood pressure; DBP=Diastolic blood pressure; BSA=Body surface area; $GFR_0$=Basal 24 h endogenous creatinine clearance; eGFR=Estimated creatinine clearance; Rfd=Renal functional difference (Rfd=creatinine clearance after oral protein load-creatinine clearance pre-oral protein load); RFR=Renal functional reserve [$RFR(\%)=(GFR_2-GFR_1)x100/GFR_1$].

Table 2 Comparative analysis of the tested parameters between the three groups of the patients

| Parameters | Groups of the patients | | | Comparisons | | |
|---|---|---|---|---|---|---|
| | CKD1 (n=16) | CKD2 (n=19) | CKD3 (n=13) | CKD1 vs CKD2 | CKD1 vs CKD3 | CKD2 vs CKD3 |
| Male (%) | 10 (62.5) | 12 (63.2) | 9 (69.2) | ns | ns | ns |
| Age (years) | 10.5±5 | 9.6±2.5 | 8.9 ± 3.8 | ns | ns | ns |
| BH (cm) | 144.2±28.2 | 138.9±25.1 | 130±23.3 | ns | ns | ns |
| BMI (kg/m$^2$) | 19±14.5 | 18.1±4.1 | 18.4±4.2 | ns | ns | ns |
| Congenital SFK (%) | 13 (81.3) | 18 (94.7) | 9 (69.2) | ns | ns | ns |
| Renal length (mm) | 118±19.8 | 100.6±1 | 90.2±13.4 | 0.005 | 0.000 | 0.049 |
| Patients with renal length/BH percentile >95 for one pair kidneys (%) | 15 (93.8) | 9 (47.4) | 3 (23.1) | 0.045 | 0.001 | ns |
| Patients with renal length/BSA >90 percentile for solitary kidney (%) | 7 (43.8) | 3 (15.8) | 1 (8.3) | ns | ns | ns |
| $GFR_1$ (ml/min/1.73m$^2$) | 106.3 (95.3-115.2) | 74.3 (69.8-79.2) | 50.2 (43.7-58.3) | 0.000 | 0.000 | 0.000 |
| $GFR_2$ (ml/min/1.73m$^2$) | 106.3 (87.8-137.7) | 80.5 (62.8-98.2) | 59.4 (49.3-110.6) | 0.003 | 0.010 | ns |
| Cystatin $C_1$ | 1.32 (0.99-1.49) | 1.32 (0.99-1.49) | 1.42 (0.93-1.54) | ns | ns | ns |
| Cystatin $C_2$ | 1.39 (0.89-1.51) | 1.39 (0.96-1.64) | 1.26 (0.85-1.58) | ns | ns | ns |
| Rfd (ml/min/1.73m$^2$) | 8 (-24.2-23.4) | 6.2 (-9.8-22.1) | 12.2 (1.7-55) | ns | ns | ns |
| RFR (%) | 7 (-24.8 to 21.8) | 8 (-14.1 to 32.4) | 29.4 (3.3 to 96.4) | ns | 0.020 | 0.032 |
| Patients with negative RFR (%) | 6 (37.5) | 8 (42.1) | 0 | ns | 0.019 | 0.010 |

Values are expressed as mean ±SD, or as median (interquartile range); BH=Body height; BMI=Body mass index; SFK=Solitary functioning kidney; BSA=Body surface area; $GFR_1$=Clearance of endogenous creatinine before oral protein load; $GFR_2$=Clearance of endogenous creatinine after oral protein load; Rfd=Renal functional difference (Rfd=$GFR_2$-$GFR_1$), RFR=Renal functional reserve; RFR=($GFR_2$-$GFR_1$)x100/$GFR_1$; Cystatin C1=Cistatin before oral protein load; Cystatin C2=Cistatin post oral protein load.

Table 3 Renal function pre- and post-OPL in the patients and the controls

| Parameters | Patients (n=49) | | | Control (n=10) | | | Patients vs Controls | |
|---|---|---|---|---|---|---|---|---|
| | Pre OPL | Post OPL | Pre vs post OPL | Pre OPL | Post OPL | Pre vs post OPL | Pre OPL | Post OPL |
| sCr (µmol/l) | 73 (57.5-86) | 74 (58-87) | ns | 74 (59-90.5) | 75 (62-85.5) | ns | ns | ns |
| UCr (mg/kg/h) | 0.68 (0.59-0.66) | 0.74 (0.65-0.94) | 0.026 | 0.51 (0.43-0.68) | 0.77 (0.51-1.03) | ns | ns | ns |
| GFR (ml/min/1.73 m$^2$) | 75.9 (59.5-97.4) | 86.6 (62.3-112.3) | 0.017 | 103.2 (85.2-122.7) | 129 (92-166.8) | ns | 0.018 | 0.009 |
| UPRT/UCr (mg/mg) | 0.18 (0.13-0.28) | 0.24 (0.17-0.34) | ns | 0.16 (0.02-0.41) | 0.15 (0.04-0.31) | ns | ns | ns |
| Cystatin C (mg/l) | 1.22 (0.91-1.46) | 1.32 (0.94-1.55) | ns | 0.69 (0.58-0.75) | 0.70 (0.60-0.77) | ns | 0.000 | 0.000 |

OPL=Oral protein load; sCr=Serum creatinine; UCr=Urine creatinine; GFR=Endogenous creatinine clearance; UPRT=Urinary protein.

## Discussion

Mechanism of CKD progression is very complex and has not been sufficiently clarified. Presently, at least two mechanisms are discussed: (a) the loss of nephrons leads to compensatory mechanisms in the remaining nephrons (glomerular hypertension, hyperfiltration, hypertrophy) which increase their vulnerability to any further challenge (overload hypothesis); and (b) proteinuric glomerular disease leads, in one way or another, to tubulointerstitial inflammation and fibrosis, accounting for the further deterioration in renal function (fibrosis hypothesis) (22). The extent of tubulointerstitial fibrosis is the best predictor of kidney survival, irrespective of the underlying disease (23).

Early therapeutic intervention during renal impairment offers the best chance of preventing progression of CKD to its terminal stage. However, evaluating early derangement of kidney function, such as glomerular hyperfiltration, is a very difficult task. It is impossible to measure in a human *in vivo* single nephron GFR (hyperfiltration) or to estimate total nephron number *in vivo.* Furthermore, early phase glomerular dysfunction cannot be detected by baseline GFR alone (24-26). Moreover, there are many problems when evaluating GFR in children (25). Renal inulin clearance is the gold standard for GFR but it is compromised by lack of availability, technically difficult assays and problems in collecting timed urine samples, especially in children (27). Its replacement in clinical practice may be creatinine clearance following a meat-free protein meal in children pre-treated with cimetidine (10). In addition, subtle decreases in GFR are more readily detected by changes in serum cystatin C than by serum creatinine (28), in part because of the shorter half-life of cystatin C.

In this study, we examined GFR by measuring creatinine clearance and serum cystatin C. In addition, eGFR was used to determine basal GFR, i.e before treatment with cimetidine. We found that serum cystatin C was a better marker for baseline GFR reduction in the patients with a SFK than sCr, eGFR and 24-h $GFR_0$ (Table 1). A similar observation was reported by Wasilewska et al. (29) who found increased concentrations of serum cystatin C in 44% of children with a SFK and normal eGFR. In accordance with Hellerstain et al. (10) we found that measured creatinine clearance, following a meat-free protein meal and pre-treatment with cimetidine ($GFR_1$), better identified the difference in pre-load as well as in post-load GFR between patients and controls (Table 3). Therefore, in children with a SFK we recommend that GFR should be determined by measuring serum Cystatin C and/or by creatinine clearance following a meat-free protein meal and pre-treatment with cimetidine.

Renal functional reserve (RFR) has long been accepted as an early marker of glomerular hyperfiltration (10, 30). Our hypothesis that RFR is not preserved in patients with moderate GFR reduction was not confirmed by the results of this study. Patients with CKD3 had lower renal mass but higher RFR than patients with CKD1 and CKD2. This means that a SFK with a moderately decreased number of residual nephrons preserves its capacity to respond to OPL. At the same time, it might be that patients with lower RFR maintain their enhanced GFR by glomerular hyperfiltration which is unmasked by OPL. Previous studies on RFR in patients with low baseline GFR have shown a preserved and a partially or completely blunted renal response to protein load (31-34). Like our results, de Santo et al. showed that there is increased RFR capacity with renal disease progression (31). It was observed that most of the time, even in an advanced stage of failure, kidneys partially retain their ability to increase filtration when increased demand is placed upon them (32). Furthermore, En-

glund et al. demonstrated a preserved RFR despite a low baseline GFR in transplanted children (33). Contrary to our findings, Barai et al. demonstrated that there was progressive decline in RFR with disease progression (23.4% in healthy controls to 6.7% in stage 4 CKD) (34). The large differences found in the literature concerning RFR in CKD, as well as in SFK patients in general (35-39), are difficult to explain and may be related to the heterogeneity of patients included in the studies, and differences in the techniques used to asses renal function. Englund et al. (33) and Laville et al. (39) found that a RFR measured with inulin and creatinine clearance gave different results. They concluded that RFR determined by creatinine clearance was not reliable as it did not depend on changes in GFR alone, but also on secondary increased tubular secretion of creatinine caused by increased serum creatinine. We measured RFR by creatinine clearance modified according to Hellerstain et al.'s protocol, which provides GFR measurements that are similar to the clearance of inulin (12, 40).

In line with other studies (6, 7, 29) we found that a SFK in children may cause renal failure, arterial hypertension and increased proteinuria. The majority of our patients had decreased renal function that may not be just a consequence of having only one kidney. According to the experimental studies, a congenital SFK has extra nephrons as a consequence of intrauterine adaptation of nephrogenesis (41-43). An acquired SFK after unilateral nephrectomy is not necessarily prone to chronic renal failure, as has been well demonstrated in donors undergoing unilateral nephrectomy (44). Nevertheless, the high prevalence of an ipsilateral anomaly in our patients with a SFK may explain the reduction of renal function; 22.7% had documented VUR and an additional 8% had ipsilateral obstructive nephropathy. In a large study including 206 children with a SFK, ipsilateral malformation of the urinary tract was identified in 26% children with a congenital SFK and in 30% children with an acquired SFK (6).

Our patients with a SFK in general had larger renal size than healthy children, due to compensatory hypertrophy. Nevertheless, patients with more severe renal impairment had smaller renal size and less common compensatory renal hypertrophy compared with those with better renal function. In addition, renal size inversely correlated with CKD stage, but positively correlated with basal and preload GFR, as well as with casual and 24-h systolic BP.

Although casual and ambulatory BP did not differ between patients and controls, arterial hypertension was found only in patients. Nocturnal hypertension was identified in nearly 19% of the patients, being more common than during daytime. Therefore, we recommend 24-h ABPM as an obligatory method for evaluating BP in children with a SFK. Other authors have also identified a higher rate of arterial hypertension in patients with SFK, compared with the normal paediatric population (6, 45). We found BP to be positively related with CKD stage, thus being higher in patients with more severe renal impairment. In addition, we identified increased proteinuria in a quarter of the patients, which is similar to that reported in the KIMONO study (6).

Our study has limitations that should be considered. They are related to the non-homogeneity of the group of patients with regard to the origin of their SFK (congenital or acquired), and to the small number of subjects in the control group. An additional and very important limitation of this study refers to the disturbed circadian BP profile in half of the children from the control group that could be explained by technical problems (poor sleep during the night period).

## Conclusion

OPL induced an increase in GFR. This response (Rfd) was higher in healthy children

than in those with a SFK. The observed positive relationship between RFR and CKD stage, and the highest RFR in CKD3 patients suggest well preserved RFR in patients with moderate renal failure. ABPM is necessary for BP evaluation in children with a SFK. Further studies of RFR, and its long-term follow-up, should be carried out on a larger number of children with CKD.

**Acknowledgments:** The study was supported by the Ministry of Science and Environmental Protection of the Government of Serbia, grant no: 175079.

**Authors' contributions:** Conception and design: APA and JKS; Acquisition, analysis and interpretation of data: APA, DP, GŠ and AS; Drafting the article: APA and GML; Revising the article critically for intellectual content: APA, BSD, and BM; Approved final version of the manuscript: APA, and DP.

**Conflict of interest:** The authors declare that they have no conflict of interest.

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