Clinical Kidney Journal, 2022, vol. 15, no. 7, 1300–1311

https://doi.org/10.1093/ckj/sfac018
Advance Access Publication Date: 14 January 2022
Original Article

ORIGINAL ARTICLE

Histologic characterization and risk factors for persistent albuminuria in adolescents in a region of highly prevalent end-stage renal failure of unknown origin

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ABSTRACT

Background. End-stage renal failure of unknown origin (ESRD-UO) is a public health problem in Mexico and many regions of the world. The prevalence of ESRD-UO in Aguascalientes, Mexico, is one of the highest worldwide, particularly in adults between 20 and 40 years of age. Our aim was to screen adolescents for chronic kidney disease (CKD) to identify risk factors and histologically characterize adolescents with persistent albuminuria.

Methods. This was a cross-sectional, observational and comparative study of adolescents in whom serum creatinine and the albumin:creatinine ratio (ACR) were determined when screening for CKD. A clinical evaluation and risk factor

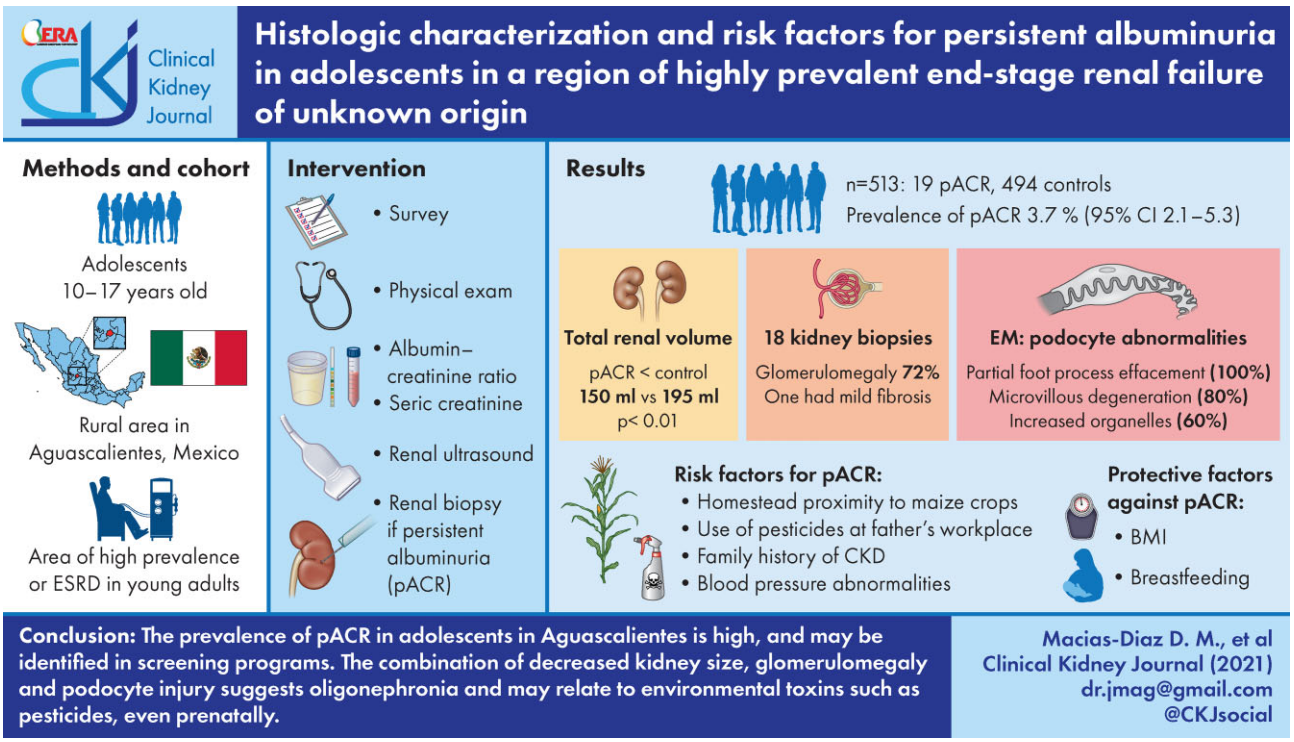
Received: 16.12.2021; Editorial decision: 4.1.2022

survey were conducted. Patients with an abnormal ACR (≥30 mg/g) or a low glomerular filtration rate (GFR) (≤75 mL/min/1.73 m^2) were re-evaluated and a renal ultrasound (US) was obtained. A kidney biopsy was performed in patients with persistent albuminuria.

Results. A total of 513 students were included; 19 had persistent albuminuria and 494 were controls. The prevalence of persistent albuminuria was 3.7% [95% confidence interval (CI) 2.1–5.3]. Only one patient had a decreased GFR. None of the patients with persistent albuminuria had anatomical abnormalities of the urinary tract by renal US. Patients with persistent albuminuria had a decreased total renal volume compared with the control group (150 versus 195 mL/m^2; $P < 0.01$). Eighteen kidney biopsies were performed; 72% had glomerulomegaly and only one patient had mild fibrosis. Podocyte abnormalities were evident on electron microscopy, including partial fusion (100%), microvillous degeneration (80%) and increased organelles (60%). Risk factors for persistent albuminuria were: homestead proximity to maize crops, the use of pesticides at the father's workplace, a family history of CKD and blood pressure abnormalities. The body mass index and breastfeeding were protective factors.

Conclusions. The prevalence of persistent albuminuria in adolescents in Aguascalientes is high and histologic compromise is characterized by podocyte injury in the absence of fibrosis. The renal volume of persistent albuminuria patients was decreased, suggesting oligonephronia. Exposure to environmental toxins such as pesticides, even prenatally, may be responsible for this pathological entity. Screening programs in adolescents by determining ACR are necessary in this setting.

GRAPHICAL ABSTRACT



Keywords: chronic kidney disease of unknown origin, persistent albuminuria, pesticides, renal disease in adolescents, renal hypoplasia

BACKGROUND

End-stage renal disease (ESRD) of unknown origin (ESRD-UO) is one of the main causes of chronic kidney disease (CKD). In Mexico, ESRD-UO accounts for the greatest global impact in terms of disability-adjusted life years {DALY; 448/100 000 [95% confidence interval (CI) 356–556]} and had the greatest increase in the world between 1990 and 2019 (3.35%) [1].

We recently reported in Aguascalientes, Mexico, the initial results of the state CKD and kidney biopsy registry. In this state, ESRD-UO was the main cause of ESRD (54%), with a predominance among adults 20–40 years old. Males were more often affected (60.9%) and cases were mainly found in two municipalities (Calvillo and Aguascalientes). Between the years 2012 and 2019, the group that underwent the greatest number of kidney biopsies was 20–30 years of age (23.2%) and the most prevalent histologic finding in that age group was focal and segmental glomerulosclerosis with subnephrotic proteinuria [2].

Based on that study's findings, and specifically due to the high prevalence of ESRD-UO among the 20- to 40-year-old population, we conducted a screening study and obtained a prospective cohort of middle school students.

The aim of this study was to screen middle school students for CKD in the municipality of Calvillo, Aguascalientes, identify students with persistent proteinuria and characterize them with imaging studies and a kidney biopsy if warranted. Secondarily, we describe the course of CKD patients during the first year of follow-up.

MATERIALS AND METHODS

This was a cross-sectional study of middle school students from the municipality of Calvillo, Aguascalientes, with no previous personal history of CKD.

Selection of participants

Middle school students between 10 and 17 years old, with no history of CKD were included. Students with a history of intense exercise the day before (≥2 h), presence of fever, medication intake or data of active infection were excluded. Similarly, women in menstrual periods were excluded. One week prior to conducting the studies, an informative talk was given to the parents. Only those who signed the informed consent and who met the inclusion criteria were accepted for the study. Between 1 February 2020 and 25 April 2021, six middle schools were visited in six communities in the municipality of Calvillo. During the first stage of the study, between 1 February 2020 and 30 September 2020, 480 students were evaluated, representing 65% of the student population in those schools. In March and April 2021, we visited two middle schools with a greater number of students, but only 30 students in a population of 1013 were evaluated.

We obtained their first morning urine to determine the albumin:creatinine ratio (ACR) and serum was obtained by peripheral venipuncture to measure serum creatinine. Patients with an ACR ≥30 mg/g or an estimated glomerular filtration rate (eGFR) ≤75 mL/min/1.7 3 m^2 (Schwartz formula) were scheduled for an appointment for repeat labs a median of 5 months [interquartile range (IQR) 0.36–5.3] after the first assessment and a renal ultrasound (US). Those with persistent albuminuria (defined as an ACR ≥30 mg/g in both assessments) or a GFR ≤75 min/1.73 m^2 and a US without anatomical abnormalities were recommended to undergo a percutaneous kidney biopsy.

Patients with a history of fever, who exercised >2 h on the previous day, and menstruating females were excluded. They were scheduled at a later date for sample collection.

Survey and initial evaluation

We conducted a survey of risk factors that included demographic and environmental factors and habits potentially associated with CKD (Supplementary data, Material 1). This survey had been previously applied and validated in similar CKD screenings in Mexico [3]. In most cases it was applied on the same day the sample was obtained, provided the participant's parents were available. The initial physical examination included measuring weight and height. The standard deviation (SD) score (z score) was used to determine the body mass index (BMI) and height and weight were calculated with the app STAT GrowthCharts version 3.2 used by the National Center for Health Statistics (Hyattsville, MD, USA).

Blood pressure

Blood pressure (BP) was measured by trained personnel (nurses and physicians) with a manual arm manometer (Welch Allyn, Skaneateles Falls, NY, USA) and the appropriate cuff. Elevated BP and hypertension were defined on the basis of international consensus. For the preadolescent, elevated BP was defined as systolic blood pressure (SBP) and/or diastolic blood pressure (DBP) ≥90th–<95th percentile and hypertension as SBP and/or DBP ≥95th–<95th percentile + 12 mmHg or ≥130/80 mmHg (whichever is lower). For adolescents, elevated BP was defined as 120/<80 to 129/<80 mmHg and hypertension as BP ≥130/80 mmHg [4]. Only one BP determination was used for this study's purposes.

Biochemical assessment

The urine samples were collected from the first morning urine in sterile polypropylene containers. Blood samples were collected by peripheral venipuncture, obtaining 5 mL of blood in a dry tube.

Urinalysis

Urinary albumin was measured by nephelometry (Vitros 4600; Ortho Clinical Diagnostics, Raritan, NJ, USA).

Determination of urinary and serum creatinine

A blood sample was obtained from the adolescents to measure serum creatinine; the sample was refrigerated and processed in a central reference laboratory. Urinary and serum creatinine were determined by standardized dry chemistry (Vitros 4600; Ortho Clinical Diagnostics). Creatinine calibration was based on the isotope dilution mass spectrometry (IDMS) reference method. The eGFR was calculated with the bedside IDMS-traceable Schwartz equation for children: eGFR (in mL/min/1.73 m^2) = 41.3 × [height (in m)/serum creatinine (in mg/dL)] [5].

Imaging evaluation

A renal US was obtained in all patients with an isolated or persistent ACR ≥30 mg/g and in 20 patients with an ACR <30 mg/g. Each kidney was measured in the transverse and anteroposterior axis (at the renal sinus midpoint), as well as longitudinally. In the absence of national percentile charts, they were described on the basis of recent publications [6]. The renal volume was estimated with the ellipsoid formula: [volume = π/6(longitudinal × anteroposterior × transverse)] [7, 8]. The renal volume was corrected according to the body surface area (BSA) with the Dubois formula: [BSA (m^2)= body weight (kg)$^{0.425}$ × body height (cm)$^{0.725}$ × 0.007 184]. The percentiles were described according to the total volume and then corrected for BSA [9, 10]. The US was performed by a trained radiologist who was blinded to the patient's clinical subtype using real-time high-resolution DP-22000 equipment and a 5-mHz convex transducer (Phillips Healthcare, Andover, MA, USA).

Kidney biopsy

The patients were hospitalized for the renal biopsy and it was performed with US guidance. A Magnum instrument with a 18 Fr, 20 cm needle was used (Bard, Covington, GA, USA).

All biopsies were analyzed by a certified nephropathologist. One biopsy core was dissected into two portions, one for light microscopy and the other for immunofluorescence. The other core was used for electron microscopy (EM). The core for immunofluorescence was placed in Michel's fixative and that for light microscopy in 10% neutral buffered formalin. For light microscopy, the sample was embedded in paraffin. Sections were cut serially every 3 µm and stained with hematoxylin and eosin, periodic acid–Schiff (PAS), silver methenamine, and Masson trichrome. For immunofluorescence, sections of snap-frozen biopsies were cut every 2 µm in a Cryostat set at −30°C and stained with fluorescein-tagged polyclonal rabbit antibodies against immunoglobulin A (IgA), immunoglobulin G (IgG) and immunoglobulin M (IgM), complement (C1q, C3c, and C4d), kappa and lambda light chains and fibrinogen (GeneTex VR; GeneTex, Irvine, CA, USA). Segmental glomerulosclerosis was diagnosed if synechiae from the Bowman's capsule to the glomerular basement membrane and/or segmental glomerulosclerosis were detected. These findings were classified based on the Columbia classification of focal segmental glomerulosclerosis (FSGS). Glomerulomegaly was diagnosed if the average glomerular diameter was greater than the average reported in tables adjusted for age, height and sex [11]. Interstitial fibrosis, tubular atrophy and interstitial inflammation were classified based on qualitative observations, and expressed in relative and absolute frequencies (mild, 10–25%; moderate, >25–50%; severe, >50%). Tubulitis was defined as the presence of inflammatory cell infiltration (lymphocytes or polymorphonuclear cells) in at least one transverse tubule and categorized as mild (0–5 infiltrating cells), moderate (5–10 cells) or severe (>10 cells). Mesangial proliferation was defined as more than three cells in an individual glomerular mesangial region, away from the vascular pole. Vascular changes were described in medium-size arteries.

Processing of EM samples is described in Supplementary data, Material 2. The EM samples were visualized using a JEM-2100 transmission electron microscope (JEOL, Tokyo, Japan) at 200 kV with the objective lens number 2, and the images were recorded with a 4K OneView camera (Gatan, Pleasanton, CA, USA). The digital micrographs were processed with ImageJ. Foot process effacement was quantified as follows (average of the percentage of foot process effacement observed in at least five complete capillary loops in a glomerulus).

Follow-up

Patients with isolated and persistent albuminuria were subsequently followed. Complementary laboratory studies were requested, including a lipid profile, glucose and serum electrolytes. Patients scheduled for biopsy were screened for hepatitis B, hepatitis C and human immunodeficiency virus (HIV) serology. If warranted, patients who were being followed were tested for acid alpha-galactosidase A enzymatic activity by fluorometry to exclude Fabry's disease.

Statistical analysis

The descriptive analysis depended on the type of variable and distribution was evaluated with the Kolmogorov–Smirnov test. Continuous variables with a normal distribution were expressed as means and standard deviations (SDs). Variables with an abnormal distribution were evaluated as medians and interquartile ranges (IQRs). Dichotomic and ordinal variables were expressed as relative and absolute frequencies. Between-group comparisons were made according to the type of variable. Continuous variables with a normal distribution were analyzed with Student's *t*-test and those with a nonparametric distribution were analyzed with the Mann–Whitney *U* test. Ordinal and dichotomic variables were compared with Fisher's exact test or the chi-squared test, as appropriate. Multivariate analysis by logistic regression was performed by selecting variables with a P-value <0.1 and biological significance. Risk factors were expressed as odds ratios (ORs) and 95% confidence intervals (CIs). A P-value <0.05 was considered significant. Stata version 16.0 (StataCorp, College Station, TX, USA) was used for the analyses.

Ethical considerations

This study fulfills all the principles of the Declaration of Helsinki. It was submitted to and approved by the ethics committee of the Centenario Hospital Miguel Hidalgo on 14 February 2019 (approval 2019-A-03). An addendum to the initial submission and an additional informed consent form were provided to patients in whom an acid alpha-galactosidase A enzymatic test was performed.

RESULTS

Among the 535 students who were evaluated, 20 were eliminated because they were >17 years of age and 2 were 10-year-old minors. The final analysis included 513 students with an average age of 13.3 years (SD 1.5) and males were slightly predominant (53.6%). In the first evaluation, 40 students were found to have albuminuria ≥30 mg/g [7.7% (95% CI 5.5–10.1)] and 1 had a concomitant decrease in GFR. After the second evaluation, a median of 5 months (IQR 0.36–5.3) after the first assessment, 19 patients were found to harbor persistent albuminuria [3.7% (95% CI 2.1–5.3)]. The κ index between the first and second evaluations was 0.62.

The median ACR of the 19 patients was 48.9 mg/g (interqurtile range (IQR) 40–71). The eGFR did not differ between patients with persistent albuminuria and the remaining study population (112 versus 115 mL/min/1.73 m^2; P = 0.84). One female patient had an initial GFR of 42 mL/min/1.73 m^2 and her ACR was 1747 mg/g.

General characteristics and physical examination

The group with persistent albuminuria ($n = 19$) and the remaining control group students ($n = 494$) were compared. Between groups, a difference in age <1 year was detected [12.6 versus 13.3 (P = 0.05). Weight and BMI were lower in the persistent albuminuria group [47 versus 53 kg (P = 0.01) and 19.1 versus 20.8 kg/m^2 (P = 0.02)], but in terms of percentiles and Z-value, were not statistically significant (Table S1 of Supplementary data, Material 3).

SBP and DBP values did not differ between groups. Among the total population, 22 patients (4.2%) had isolated systolic hypertension and 84 (16.3%) had isolated diastolic hypertension. There was a greater proportion of cases of isolated elevated BP in the persistent albuminuria group [elevated SBP 31.5% versus 12.3% (P = 0.01) and elevated DBP 31.5% versus 12.5% (P = 0.01)] (Table 1).

Survey

Among the 513 students, 320 surveys were completed: 19 in the persistent albuminuria group and 301 in the control group.

Table 1. General and somatometric characteristics

Variables	All patients ($N = 513$)	Persistent albuminuria ($n = 19$)	Controls ($n = 494$)	P-value
Female, *n* (%)	238 (46.4)	10 (52.6)	228 (46.1)	0.64
Age (years), mean (SD)	13.3 (1.5)	12.6 (1.05)	13.3 (1.6)	0.05
GFR (mL/min/1.73 m^2), median (IQR)	115 (100–126)	112 (103–126)	115 (100–126)	0.84
GFR <90 mL/min/1.73 m^2, *n* (%)	36 (7.4)	2 (11.1)	34 (7.3)	0.63
Albuminuria (mg/g), median (IQR)	8.6 (6.2–13.2)	48.9 (40–71)	8.4 (6.2–12.4)	<0.01
Weight (kg), median (IQR)	53 (45–62)	47 (37.5–56.5)	53 (45.4–62)	0.01
Height (m), median (IQR)	1.57 (1.53–1.64)	1.55 (1.48–1.60)	1.57 (1.53–1.64)	0.26
BMI, median (IQR)	20.8 (18.3–24.7)	19.1 (16.5–21.7)	20.8 (18.3–24.7)	0.02
SAP (mmHg), median (IQR)	110 (100–116)	110 (100–120	110 (100–115)	0.71
SAP percentile, median (IQR)	50 (50–90)	50 (50–90)	50 (50–90)	0.50
Elevated SBP, *n* (%)	67 (13)	6 (31.5)	61 (12.3)	0.01
Systolic HT, *n* (%)	22 (4.2)	1 (21)	21 (4.2)	0.57
DAP (mmHg), median (IQR)	70 (60–73)	71 (60–77)	70 (70–73)	0.36
DAP percentile, median (IQR)	90 (50– 90)	90 (50–90)	90 (50–90)	0.42
Elevated DBP, *n* (%)	68 (13.2)	6 (31.5)	62 (12.5)	0.01
Diastolic HT, *n* (%)	84 (16.3)	4 (21)	80 (16.1)	0.57
Elevated BP, *n* (%)	80 (15.5)	5 (26.3)	75 (15.1)	0.18
SAH, *n* (%)	90 (17.5)	5 (26.3)	85 (17.2)	0.35
Elevated BP or SAH, *n* (%)	170 (33.1)	10 (52.6)	160 (32.3)	0.06

SAP: systolic arterial pressure; DAP: diastolic arterial pressure; HT: hypertension; SAH: systemic arterial hypertension.
BP was measured once.

Home characteristics

The median number of individuals in each household was 5 (IQR 4–6) and 23.9% were overcrowded (>3 individuals per room). Water is provided to most houses by public service, with indoor plumbing (95.3%), and 44.6% have running water on a daily basis. Only 20 (6.2%) obtained water to cook from a well, 79 (24.6%) from the faucet, and 223 (70%) used bottled water. A total of 89% drank bottled water. The average household income was 4800 pesos [IQR 4000–6000 (equivalent to US$ 200) (IQR 200–300)] (Table 2 and Supplementary data, Material 3).

A total of 51% of houses were near a river and there was no difference between groups, and 21.8% were located at an average distance of 100 m from crop fields (IQR 15–200). The homes of cases with persistent albuminuria were more frequently located near crop fields (47.3% versus 20.2%; P = 0.01). Maize crops were significantly associated (36.8% versus 10.9%; P ≤ 0.01), while closeness to guava crops had a tendency towards statistical significance (21% versus 8.3%; P = 0.08). The remaining variables are shown in Table 2 and Supplementary data, Material 3.

Characteristics of pregnancy

The median number of pregnancies among the students' mothers was 4 (IQR 3–5). The persistent albuminuria group reported fewer pregnancies than the control group (3 versus 4; P < 0.01). Maternal age at the time of the student's gestation was not different (24 versus 26.5 years; P = 0.61). No prenatal characteristics differed between groups. The average gestational age was 38.8 weeks and only 7.5% had low birth weight. There were no differences in birth weight. A total of 52.6% of patients with persistent albuminuria were breastfed, as were 82% in the control group (P < 0.01), for a median lactation duration of 6 months (IQR 3–12) (Table 2 and Supplementary data, Material 3).

Family history

A history of CKD in the entire population was 24.2% and was more frequent in the persistent albuminuria group (42.1 versus 19.9; P = 0.03) (Table 2).

Parental characteristics

The parents' age did not differ between groups. The fathers' contact with pesticides was greater in the persistent albuminuria group (63.1% versus 37.3%; P = 0.02). This contact was at work (63.1% versus 36.1%; P = 0.01). The remaining characteristics did not differ between groups (Table 3). The families of patients being followed were contacted by phone to obtain the names of the pesticides used. A total of 28 families were contacted, of which 14 provided appropriate answers and reported the use of the following pesticides: malathion 9 (64.2%), cypermethrin 5 (35.7%), glyphosate 4 (28.5%) and parathion 3 (21.4%).

Multivariate analysis

Two multivariate analysis models were created. The first took into account the physical clinical characteristics and included the greatest number of patients ($n = 513$). This model revealed that BP abnormalities were a risk factor [OR 2.6 (95% CI 1.02–6.5), P = 0.043], while the BMI was protective [OR 0.86 (95% CI 0.75–0.98), P = 0.029]. The second model analyzed sociodemographic factors and included 320 patients. That model revealed that proximity of the house to maize crops, the father's contact with pesticides at work and a family history of CKD were significant risk factors, while breastfeeding was protective (Table 3).

Imaging characteristics

A renal US was performed in a total of 61 patients: 19 in the persistent albuminuria group, 21 patients with isolated albuminuria and 21 without albuminuria. The group of patients without

Table 2. Environmental, personal and family factors

Variables	All patients ($n = 320$)	Persistent albuminuria ($n = 19$)	Controls ($n = 301$)	P-value
Inhabitants per home, median (IQR)	5 (4–6)	5 (4–7)	5 (4–6)	0.42
Overcrowding >3, *n* (%)	77 (23.9)	5 (26.3)	72 (23.9)	0.78
Water supply, *n* (%)				0.09
Indoor plumbing	305 (95.3)	17 (89.4)	288 (95.6)	
Well	11 (3.4)	1 (5.5)	10 (3.3)	
Water for cooking, *n* (%)				
Well	20 (6.2)	2 (10.5)	18 (5.9)	0.33
Tap water	79 (24.6)	6 (31.5)	73 (24.2)	0.42
Bottled	224 (70)	12 (63.1)	212 (70.4)	0.60
Water to drink, *n* (%)				
Bottled	285 (89)	17 (89.4)	268 (89)	1.0
Sewer system, *n* (%)				0.16
Public	281 (87.8)	16 (84.2)	266 (88.3)	
Tubed outside	12 (3.7)	2 (11.1)	10 (3.3)	
Septic tank	27 (8.4)	1 (5.5)	26 (8.6)	
Endogamy, *n* (%)	6 (1.9)	0	6 (1.9)	1.0
Income (US$), median (IQR)	240 (200–300)	240 (200–360)	240 (200–300)	0.91
≤200 USD/month, *n* (%)	58 (18.1)	4 (21)	54 (17.9)	0.75
Insecticide use, *n* (%)	180 (56.2)	11 (57.8)	169 (56.1)	1.0
Proximity to a river, *n* (%)	180 (56.1)	12 (63.1)	168 (55.8)	0.63
Proximity to crops, *n* (%)	70 (21.8)	9 (47.3)	61 (20.2)	0.01
Guava	29 (9)	4 (21)	25 (8.3)	0.08
Maize	40 (12.4)	6 (36.8)	33(10.9)	<0.01
Distance, median (IQR)	100 (15–300)	100 (50–200)	100 (12.5–350)	0.89
Drugs, *n* (%)	58 (18.1)	3 (15.7)	55 (18.2)	1.0
NSAID	27 (8.4)	0	27 (8.9)	0.37
Any disease, *n* (%)	48 (15)	2 (10.5)	46 (15.2)	1.0
Migraine	4 (1.2)	0	4 (1.3)	1.0
Sting or bite, *n* (%)	192 (60)	9 (47.3)	183 (60.7)	0.33
Scorpion	110 (34.3)	7 (36.8)	103 (34.2)	0.81
Physical activity, *n* (%)	176 (55)	12 (63.1)	164 (54.1)	0.48
Supplements, *n* (%)	33 (10.3)	3 (15.8)	30 (9.9)	0.4
Pregnancy characteristics				
Pregnancies, median (IQR)	4 (3–5)	3 (3–4)	4 (3–5)	<0.01
Abortions, *n* (%)	0.51 (0.7)	0.18 (0.4)	0.53 (0.74)	0.08
Gestational age (weeks), median (IQR)	26 (22–31)	24 (22–32)	26.5 (22–31)	0.61
Gestation number, median (IQR)	2 (1–4)	2 (1–3.5)	2 (1–4)	0.43
Complications, *n* (%)	92 (28.7)	5 (26.3)	87 (28.9)	1.0
Exposure to chemicals, *n* (%)	54 (16.6)	2 (10.5)	52 (17.2)	0.74
Chloride	44 (13.7)	0	44 (14.6)	0.08
Smoke	54 (16.8)	3 (15.8)	51 (16.9)	1.0
Drugs, *n* (%)	42 (13.1)	2 (10.5)	40 (13.2)	1.0
Antibiotic	19 (5.9)	0	19 (6.3)	0.61
Anti-HT	4 (1.25)	0	4 (1.3)	1.0
Neuro	3 (0.9)	0	3 (0.9)	1.0
Radiation, *n* (%)	2 (0.6)	0	2 (0.6)	1.0
Cesarean, *n* (%)	144 (45)	9 (47.3)	135 (44.8)	0.51
Gestational age (weeks), mean (SD)	38.8 (2)	39.6 (1.3)	38.8 (2)	0.02
Prematurity, *n* (%)	46 (14.3)	1 (5.2)	45 (14.9)	0.48
Weight (kg), mean (SD)	3.234 (0.58)	3 248 (0.46)	3 232 (0.58)	0.57
Low birth weight, *n* (%)	24 (7.5)	1 (5.2)	23 (7.6)	1.0
Lactation, *n* (%)	257 (80)	9 (52.6)	247 (82)	0.005
Duration (months), median (IQR)	6 (3–12)	6 (3–9)	6 (3–12)	0.67
Diabetes family, *n* (%)	193 (60.3)	9 (47.4)	184 (61.1)	0.33
SAH family, *n* (%)	204 (63.7)	12 (63.1)	192 (63.7)	0.71
Cancer family, *n* (%)	114 (35.6)	10 (52.6)	104 (34.5)	0.13
CKD family, *n* (%)	68 (24.2)	8 (42.1)	60 (19.9)	0.03
First-degree, *n* (%)	18 (5.6)	3 (15.7)	15 (4.9)	0.08
Glasses of water, median (IQR)	4 (3–8)	4 (3–7)	4 (3–8)	0.52
Sugary beverages, *n* (%)	290 (90.6)	18 (94.7)	272 (90.4)	1.0
Beverages/week, median (IQR)	1 (1–2)	2 (1–3)	1 (1–2)	0.08
Smoking, *n* (%)	40 (12.7)	0	40 (13.6)	0.14
Alcohol, *n* (%)	114 (35.6)	3 (15.7)	111 (36.8)	0.08
Drugs, *n* (%)	6 (1.8)	0	6 (1.9)	1.0
Characteristics of parents				
Father's age (years), median (IQR)	44 (39–49)	45 (34–49)	44 (39–49)	0.21
Fieldwork, *n* (%)	199 (62.5)	13 (68.4)	186 (61.7)	0.80
Pesticides, *n* (%)	124 (38.7)	12 (63.1)	112 (37.3)	0.02
Work, *n* (%)	120 (37.5)	12 (63.1)	108 (36.1)	0.018

SAH: systemic arterial hypertension.
IQR expressed as quartile 1–quartile 3.

Table 3. Multivariate analysis by logistic regression on persistent albuminuria

Model 1	OR (95% CI)	P-value	Model 2[a]	OR (95%CI)	P-value
BP abnormalities	2.6 (1.02–6.5)	0.045	Proximity of home to maize crops	4.1 (1.39–11.9)	0.01
BMI	0.86 (0.75–0.98)	0.029	Pesticides at father's work	3.5 (1.24–10.1)	0.017
			CKD in family	3.3 (1.14–9.6)	0.028
			Breastfeeding	0.16 (0.05–0.49)	0.001

Model 1, $n = 513$; Model 2, $n = 320$.
[a]Model adjusted for age and sex.
BP abnormalities: elevated BP and hypertension.

Table 4. Imaging characteristics of patients with persistent albuminuria and control group

Variables	All patients ($N = 61$)	Persistent albuminuria ($n = 19$)	Without albuminuria ($n = 42$)	P-value
Longitudinal RK (mm), mean (SD)	93.9 (9.2)	89.9 (9.2)	95.6 (8.7)	0.02
Anteroposterior RK (mm), mean (SD)	43.3 (7.4)	40.6 (8.6)	44.7 (6.5)	0.05
Transverse RK (mm), mean (SD)	43.1 (6.3)	40.3 (7.1)	44.6 (5.8)	0.01
Percentile RK, median (IQR)	10 (5–50)	10 (2.5–50)	10 (5–50)	0.64
Volume RK (mL), median (IQR)	94.6 (75–114)	71.4 (59.1–96.8)	97.8 (84–110)	<0.01
Volume RK/BSA (mL/m^2), median (IQR)	61.4 (51.6–70)	51.6 (40.2–70.1)	62.2 (56.2–70)	0.02
Volume RK <10th percentile, *n* (%)	7 (11.4)	6 (31.5)	1 (2.3)	<0.01
Longitudinal LK (mm), mean (SD)	93.9 (9.6)	92.1 (9.1)	94.7 (9.9)	0.32
Anteroposterior LK (mm), mean (SD)	44 (6.4)	42.9 (8.2)	44.6 (5.5)	0.34
Transverse LK (mm), mean (SD)	42.5 (5.2)	40.8 (5.5)	43.2 (4.9)	0.09
Percentile LK, median (IQR)	10 (10–50)	10 (10–50)	30 (10–50)	0.78
Volume LK (mL), mean (SD)	94.2 (29.1)	86.7 (33.5)	97.6 (26.6)	0.17
Volume/BSA (mL/m^2), median (IQR)	59 (51.7–69.33)	56.1 (47.9–72.3)	60.8 (52.7–67.8)	0.46
Volume LK <10th percentile, mean (SD)	4 (6.5)	3 (15.7)	1 (2.3)	0.085
Total kidney volume (mL/m^2), median (IQR)	179 (155–215)	150.5 (134.4–184.1)	195 (167.7–220.8)	<0.01

RK: right kidney; LR: left kidney.
IQR expressed as quartile 1–quartile 3.

albuminuria was obtained in the last two visits to schools in the town of Calvillo and their inclusion depended only on informed consent and the number of students who attended on those days. The 61 patients were grouped as persistent albuminuria patients ($n = 19$) and the rest were assigned to the control group ($n = 42$). Echogenic abnormalities were reported in eight patients, although there was no difference between groups (2 versus 6; $P = 1.0$). None of the patients had urinary tract abnormalities.

All evaluated measurements were lower in the persistent albuminuria group. The right kidney (RK) specifically had an average 4.7 mm difference in the longitudinal, anteroposterior and transverse axes, which was significant (Table 4). The RK volume was lower in the persistent albuminuria group, in both absolute terms (71.4 versus 97.8 mL; $P < 0.01$) and after adjustment for BSA (51.6 versus 62.2 mL/m^2; $P < 0.01$). The proportion of RKs below the 10th percentile was also greater in the persistent albuminuria group (31.5 versus 2.1; $P < 0.01$). All measurements of the left kidney (LK) were also lower in the persistent albuminuria group but were not significant. The proportion of patients below the 10th percentile in terms of adjusted volume to BSA revealed a tendency towards significance (15.7 versus 2.3; $P = 0.08$). The total volume (RK volume + LK volume) was lower in the persistent albuminuria group (150.5 versus 195 mL/m^2; $P < 0.01$).

Kidney biopsy

Of the 19 patients with persistent albuminuria, 18 agreed to the biopsy. The median number of glomeruli per biopsy was 16 (IQR 13–20). The median glomerular diameter was 170 µm (IQR 163–177). The average of the largest glomerulus was 200 µm (IQR 190–200). Thirteen patients (72.2%) had glomerulomegaly (defined as the average glomerular diameter above the average reported in tables and adjusted for age, height and sex) and all had at least one glomerulus with glomerulomegaly. Six patients had mesangial proliferation and two had glomerulosclerosis. One patient had 15% interstitial fibrosis and the rest had no fibrosis. Sixteen patients (84.2%) had reabsorption proteins and were PAS and Jones positive. On light microscopy, the diagnosis of FSGS not otherwise specified was reached in two patients. One patient had IgA mesangial deposits.

Electron microscopy was performed in 17 patients. One patient had no glomeruli and only the tubulointerstitium could be evaluated. All patients ($n = 15$) showed 30% foot process effacement (IQR 30–35, minimum 20–maximum 60), 12/15 (80%) had microvillous degeneration, 9/15 (60%) showed increased organelles in the podocytes, 5/15 (33.3%) had an increase in mesangial matrix and 5/15 (33.3%) had scant electron-dense deposits (Figures 1 and 2 and Supplementary data, Material 3)

Follow-up

All 19 patients with persistent albuminuria are currently being followed. The median follow-up duration is 11 months (IQR 8–15). Hepatitis B, hepatitis C and HIV serologies were negative in the 18 cases with a kidney biopsy. Fabry disease screening was conducted in 17 patients with persistent albuminuria and was negative in all cases.

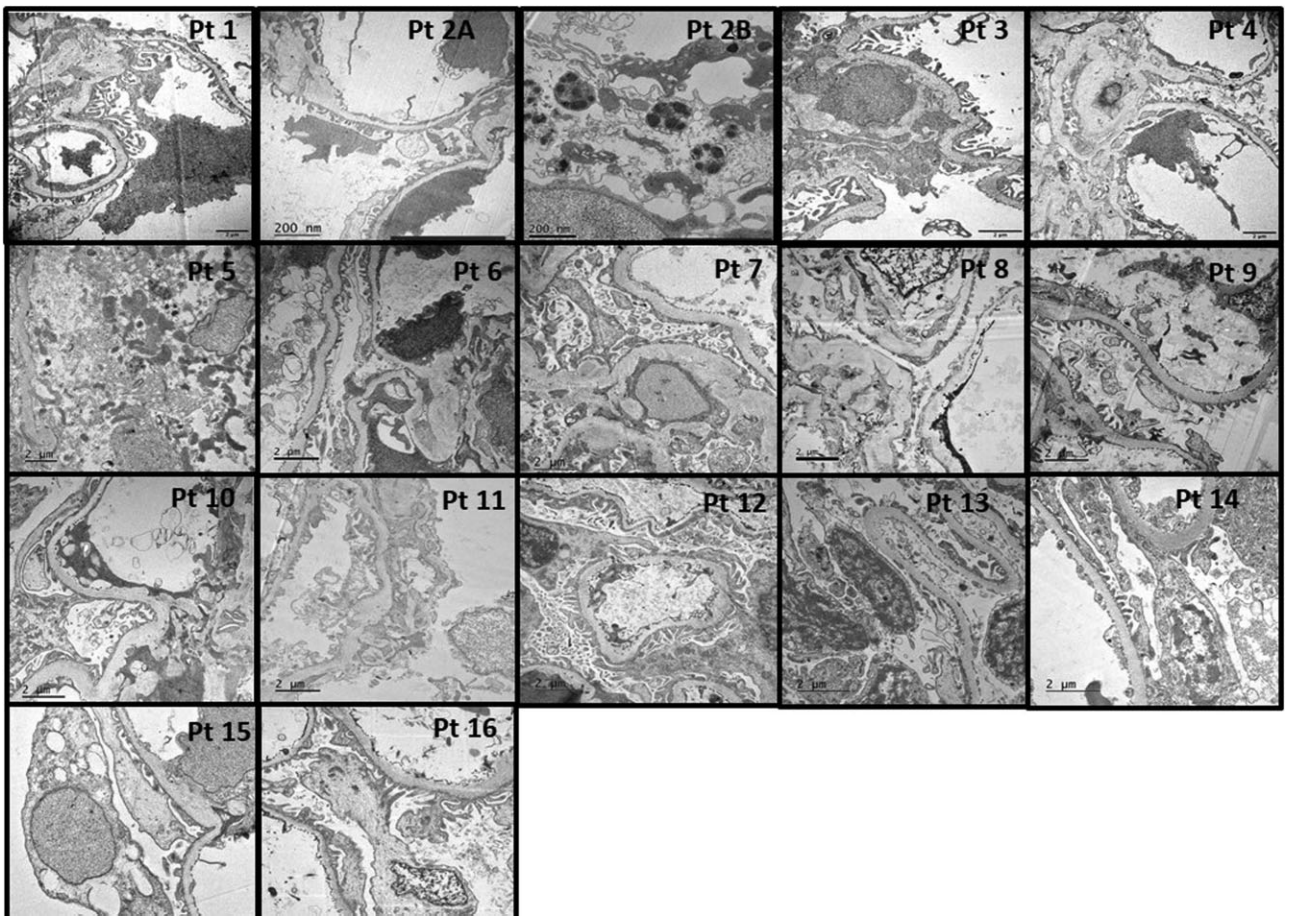


FIGURE 1: Electron microscopy histology images of 16 patients with persistent albuminuria.

Complementary laboratory studies were obtained in 33 patients (18 in the albuminuria group and 15 in the control group). Twelve patients (44%) had an abnormal fasting glucose, but there was no significant difference between groups (Supplementary data, Material 3). Losartan, 12.5 mg every 24 h, was initiated in patients with persistent albuminuria, and it was gradually increased to 50 mg every 24 h depending on tolerance and the persistence of albuminuria. The median ACR during follow-up decreased significantly (49 versus 30.3 mg/g; P = 0.03), while the GFR showed no significant differences (114 versus 115 mL/min/1.72 m^2; P = 0.78), with an average delta of +2 mL/min (range −3–7.8) (Figure 3).

DISCUSSION

This study was conducted in a region with a high prevalence of ESRD-UO and in which we found a high prevalence of persistent albuminuria [3.7% (95% CI 2.1–5.3)] in adolescents. Isolated albuminuria was detected in 7.7% (95% CI 5.5–10.1), with a index of 0.62 κ between the first and second determinations. Previous studies in adolescents performed mostly in the US population reported a persistent albuminuria prevalence between 0.06% and 3% [12–18]. The causes of CKD in adolescents differ from those in adults throughout the world, as adolescents have a greater tendency to harbor urological abnormalities or primary glomerulopathies [19]. In this study, there were no patients with urologic abnormalities and in the renal biopsy, only one patient showed IgA nephropathy. Histologic abnormalities in 18 patients with a kidney biopsy were very consistent, whereby glomerulomegaly was evident on light microscopy and only one case presented mild interstitial nephritis. On EM, all patients had podocyte abnormalities characterized by segmental foot process effacement, microvillous degeneration and an increase in podocyte organelles. Thickened basement membranes, mesangial expansion and scant electron-dense deposits were also common findings. The decrease in renal volume in the persistent albuminuria group detected by US is striking. Nine patients had a kidney volume <10th percentile (<45 mL/m^2). When grouped, these patients had a decreased GFR (103.5 versus 127 mL/min; P = 0.01), suggesting a significant degree of oligonephronia. The relationship between the calculated kidney volume by US and kidney function has been validated in many populations and can be a surrogate in the diagnosis of renal hypoplasia and oligonephronia [20, 21]. Prematurity and low birthweight are among the best-known risk factors; in the persistent albuminuria group, only one case of low birthweight was reported and none of the patients were born before week 36.

The low kidney volume in conjunction with the histologic finding of segmental podocyte process simplification in the absence of fibrosis suggests that renal injury may be secondary to oligonephronia. Since none of the patients had comorbidities that could account for the loss of nephrons, and in the absence of fibrosis in the biopsy, it appears that the injury could have developed in the prenatal stage. It is known that the initial structural

Pts	Age	Gender	GFR	ACR	Right kidney volume/BS	Left kidney volume/BS	Mean glomerular diameter	Major glomerular diameter	% IFTA	% fusion	Microvillous degeneration	Increase of podocyte organelles	Thickening of the basement membrane	Mesangial matrix increase	Sparse electron-dense mesangial deposits	Para-mesangial deposits	Mesangium intersection	Lipid lysosomes in tubules	Tubule protein lysosomes	Intracapillary lymphocytes
1	13	M	114	52.3	85.1	73.5	168	200	No	20	+	No	No	No	+	No	No	+	No	+
2	14	F	42	1747.1	28.3	36.5	323	350	15	20	No	No	No	No	+	No	No	No	+	No
3	14	M	108	39.8	83.1	94	155	170	No	20	+	No	No	No	+	No	No	+	No	No
4	14	F	110	71.3	46.4	69.3	184	200	No	35	No	+	No	+	+	No	No	+	No	No
5	12	M	85	94.9	30.5	35.9	224	280	No	NA	NA	NA	NA	NA	NA	NA	NA	No	+	No
6	14	M	91	52.7	40.2	47.8	177	220	No	30	+	+	+	+	No	+	+	No	+	No
7	14	F	127	142.4	30.6	47.9	176	200	No	30	+	No	No	+	+	No	+	No	No	No
8	12	M	117	34.5	53.4	73.7	152	170	No	30	+	No	No	+	+	No	No	No	No	No
9	12	F	111	69.7	69.1	50.5	154	190	No	30	+	No	No	No	No	No	No	No	No	No
10	14	M	98	488.2	54.9	48.4	163	170	No	30	+	+	+	No	No	No	No	No	No	No
11	11	M	104	34.2	47.5	43.3	170	200	No	35	No	+	+	+	No	No	No	No	No	No
12	13	F	103	47.8	78.7	79.2	166	180	No	60	+	+	+	No	No	No	No	No	No	No
13	11	M	126	50.4	70.1	72.3	166	190	No	45	+	+	+	No	No	No	No	No	No	No
14	12	F	125	41.9	40.7	55.8	175	200	No	45	+	+	+	No	No	No	No	No	No	No
15	12	F	155	40.3	69.9	64.1	180	200	No	35	+	+	No	No	No	No	No	+	No	No
16	12	F	193	34	51.6	67.8	150	190	No	35	+	+	No	No	No	No	No	No	No	No
17	12	F	132	35.2	49.4	51.7	181	200	No	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
18	12	M	123	33	71	56.9	196	220	No	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
19	13	F	118	48.9	38.8	56.1	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA

Abnormal finding Normal finding

FIGURE 2: Characteristics of demographic findings, renal volume adjusted to BSA, glomerular diameter and EM findings of 19 patients with persistent albuminuria. Pts: patients; IFTA: interstitial fibrosis and tubular atrophy.

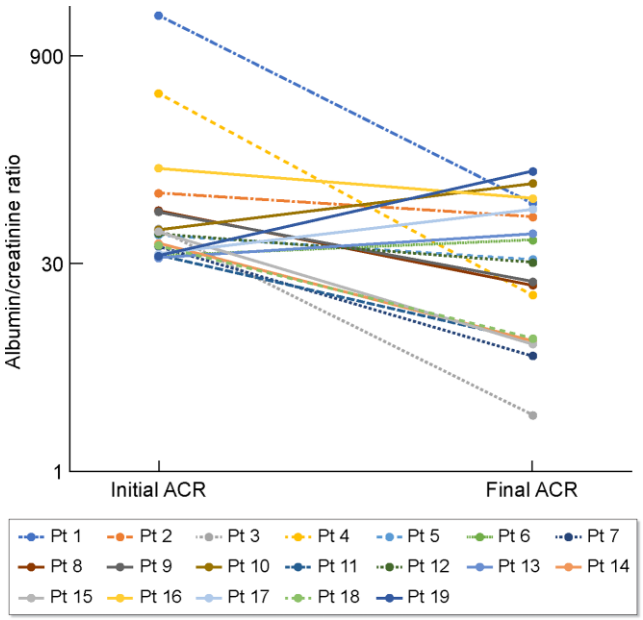


FIGURE 3: Comparison of initial and final ACR in patients with persistent albuminuria (at the time of detection and the end of follow-up) (166 versus 42.7 mg/g; $P = 0.03$).

injury in the context of oligonephronia is at the podocyte level and culminates in focal and segmental sclerosis injuries [22–24]. These abnormalities agree with the findings reported in the Aguascalientes CKD and renal biopsy registry, where almost 50% of diagnoses established by kidney biopsy in 10- to 30-year-olds have been FSGS with subnephrotic proteinuria [2]. Likewise, the average time until patients with renal hypoplasia require substitution therapy in a large European cohort was 32 years, which is similar to the age in which ESRD-UO patients begin substitution therapy in Aguascalientes [2, 25].

Among the analyzed risk factors, a greater proportion of patients with persistent albuminuria had isolated systolic and diastolic prehypertension. This increase in BP may be associated with incipient abnormalities in kidney function and oligonephronia. The inverse relation with the BMI is an unexpected finding, but is consistent with usual clinical practice in our area since patients between the ages of 20 and 40 years with ESRD of unknown cause are not usually overweight.

A positive family history of CKD was more frequent in the persistent albuminuria group, although the cause was not specified. Also, a history of type 2 diabetes mellitus and hypertension did not differ between groups. The association of CKD and a positive history has been reported in many studies [26]. Other entities such as Fabry's disease were excluded, as well as Alport's syndrome, due to the absence of hematuria and EM with no supporting findings. There are multiple genetic abnormalities associated with the findings suggesting renal hypoplasia and that will have to be subsequently explored. Known genes explain only 20% of cases and suggest the existence of other genes and mechanisms such as epigenetic and environmentally induced abnormalities [27, 28].

The direct relationship of persistent albuminuria and the home's proximity to crop fields, as well as the father's contact with pesticides, could represent an approximation of this entity's underlying cause. The uniformity of the histological findings suggests the presence of a strong toxic component in the genesis of this entity. Mexico is a country with severe environmental control problems, and specifically with the use of pesticides, the indices of acute intoxication remain very high and there is no surveillance registry on their long-term chronic effects and injuries [29, 30 and Supplementary data, Material 4]. The use of highly dangerous pesticides is generalized and commonplace and their control is minimal. In this region, organophosphates (parathion and malathion), glyphosate and cypermethrin are some of the pesticides used, and their association with deterioration of kidney function and nephrotoxicity has been suggested in epidemiological studies and in animal models [31–33].

As in this report, countries such as El Salvador, Nicaragua, Sri Lanka and other regions in Mexico have reported a high prevalence of ESRD of unknown cause, particularly in males of reproductive age [34–37]. However, there are some features that differentiate them from our study, particularly the histologic findings. Most of the reported involvement in those regions is tubulointerstitial, while in our area it is predominantly glomerular, and specific to podocytes [38–42]. Another differentiating feature in

other countries is its association with heat-induced stress and dehydration due to the high temperatures reported in those regions and less than optimal hydration [43, 44]. In the case of the Calvillo region, the annual average maximum temperature is 24°C, so it is improbable that this factor exerts the same degree of influence [45].

Another potential environmental factor is exposure to heavy metals and their synergies with other environmental elements [46, 47]. The Calvillo region has water wells with fluoride concentrations above international recommendations; this has been previously documented [2].

Exposure to environmental toxins, and specifically to pesticides and metals such as fluoride, suggests that they may play a significant role in the genesis of renal disease in our area. Again, the absence of fibrosis in the biopsies and US findings detecting renal hypoplasia suggest that this exposure and injury developed in the prenatal period.

The protection provided by breastfeeding has not been previously reported. However, the intestine of breastfed infants has a superior qualitative intestinal immune system. In the context of an environmental disease, this suggests better elimination of ingested toxins at the intestinal level [48, 49]. Nephrogenesis is known to develop in the first 4 weeks of gestation, and to a lesser extent, until week 40, although there is evidence that, at least in premature individuals, nephrogenesis and maturation persist in the extrauterine period [50–52]. In models of premature animals, breastfeeding has been shown to improve kidney function, perhaps playing a role in maturation and concluding in adequate nephrogenesis [53].

This study has several weaknesses, perhaps the result of specifying the pesticides used in our region, since there are many, and their current use may not reflect those that were prevalent one or two decades ago. We lack a comparator outside the municipality of Calvillo, which renders contrasting features such as exposure to heavy metals in the water difficult. However, in the face of an enormous ESRD-UO problem in Mexico, this is the first study that characterizes this pathology, which most probably is present in many parts of Mexico and other countries with a high prevalence of ESRD-UO. As is evident, this study can lead to various research lines such as exposure to environmental toxins since the prenatal period and the genetic study of patients with persistent albuminuria [54].

Clearly, screening adolescents with albuminuria is a necessary measure (at least in our area), since timely detection may provide the opportunity to initiate treatment and lifestyle changes that may hinder the progression of renal disease.

Furthermore, and with no more required evidence, it is simultaneously imperative to improve the epidemiological surveillance systems in terms of the use of pesticides and improvement in water and food quality.

CONCLUSION

In a population with a high prevalence of ESRD of unknown origin, screening for CKD revealed a high prevalence of persistent albuminuria in adolescents. It is histologically characterized by glomerulomegaly, podocyte involvement and the lack of fibrosis. Kidney volume by US was lower in the persistent albuminuria group. Risk factors for persistent albuminuria were abnormalities in BP, a family history of CKD, the proximity of housing to crop fields and the father working with pesticides. The BMI and having been breastfed are protective factors. Overall, these findings confirm podocyte injury as the cause of CKD in this population. Triggering factors such as oligonephronia or specific podocyte toxins must be approached in future studies.

SUPPLEMENTARY DATA

Supplementary data are available at *ckj* online.

FUNDING

The present study was funded by the State Government of Aguascalientes.

CONFLICT OF INTEREST STATEMENT

The results presented in this article have not been published previously in whole or part, except in abstract format. The authors declare no conflicts of interest with respect to the information in the present article.

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Clinical Kidney Journal

Clinical Kidney Journal, 2022, vol. 15, no. 7, 1253–1274

https://doi.org/10.1093/ckj/sfac017
Advance Access Publication Date: 14 January 2022
CKJ Review

CKJ REVIEW

Detecting, preventing and treating non-adherence to immunosuppression after kidney transplantation

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ABSTRACT

Medication non-adherence (MNA) is a major issue in kidney transplantation and it is associated with increased risk of rejection, allograft loss, patients' death and higher healthcare costs. Despite its crucial importance, it is still unclear what are the best strategies to diagnose, prevent and treat MNA. MNA can be intentional (deliberate refusal to take the medication as prescribed) or unintentional (non-deliberate missing the prescribed medication). Its diagnosis may rely on direct methods, aiming at measuring drug ingestions, or indirect methods that analyse the habits of patients to adhere to correct drug dose (taking adherence) and interval (time adherence). Identifying individual risk factors for MNA may provide the basis for a personalized approach to the treatment of MNA. Randomized control trials performed so far have tested a combination of strategies, such as enhancing medication adherence through the commitment of healthcare personnel involved in drug distribution, the use of electronic reminders, therapy simplification or various multidisciplinary approaches to maximize the correction of individual risk factors. Although most of these approaches reduced MNA in the short-term, the long-term effects on MNA and, more importantly, on clinical outcomes remain unclear. In this review, we provide a critical appraisal of traditional and newer methods for detecting, preventing and treating non-adherence to immunosuppression after kidney transplantation from the perspective of the practising physician.

Keywords: behaviour therapy, drug monitoring, graft rejection, immunosuppressive agents, medication adherence, organ transplantation, patient education, risk factors

INTRODUCTION

Kidney transplantation is the treatment of choice for end-stage kidney disease. However, despite advances in short-term outcomes, long-term renal allograft loss remains a significant issue [1–3]. One of the most important and often underestimated modifiable factors that strongly affects graft fate is medication non-adherence (MNA) [4]. It has been reported that MNA is responsible for nearly 20% of antibody-mediated rejections [5] and 16% of early graft losses [6]. This is a matter of concern, as rejection-induced graft loss is associated with an increased risk of sensitization, which reduces the chances of being re-transplanted [7]. Finally, rejection and the associated increased immunosuppression burden increase hospitalization

Received: 14.11.2021; Editorial decision: 11.1.2022

Box 1. Take home messages on MNA

MNA is associated with increased risk of rejection, allograft loss, patients' death and higher healthcare costs.

The degree of MNA, which can influence the clinical outcomes and that requires a specific treatment strategy, is not defined

MNA risk factors are associated with patients, therapy, disease characteristics, healthcare organization, and socioeconomic and cultural characteristics. Some of these factors can be modifiable and represent the corner of treatment strategies.

Because MNA can appear/worsen during extremely stressful moments or anytime, it must be constantly monitored. Since risk factors can vary at any moment, different strategies may need to be adopted in the same patient.

Intentional MNA, which is characterized by a—usually unrecognized—deliberate refusal to comply with the prescribed therapy, is hard to diagnose and treat. These patients are hardly included in clinical trials. Constant motivational-behavioural interventions may represent the only viable resource to prevent and treat intentional MNA.

Unintentional MNA is characterized by non-deliberate reduced adherence to the prescribed therapy. It is easier to diagnose and to treat. Unintentional MNA diagnostic tools might occasionally be oversensitive.

Strategies that have been assessed for the prevention and treatment of MNA include:

- the commitment of healthcare personnel involved in drug distribution (i.e. pharmacist, nurses)
- the use of electronic reminders (i.e. alarmed drug container, phone alarms and Apps)
- therapy simplification
- multidisciplinary approaches (i.e. nurses, psychologists, medical doctors and trained therapy coaches) to maximize the correction of individual risk factors.

Overall, they were shown to improve MNA, but the effect generally vanished thereafter. Moreover, no trial published so far has shown any improvement in clinical outcomes. Lack of benefit may be related to failure to include MNA patients because of the 'streetlight effect'

rates, healthcare costs [8], and the risk of dying from cardiovascular disease and cancer [9, 10].

This phenomenon is extremely common, as up to one-third of kidney transplant recipients (KTRs) may be non-adherent to immunosuppressive medications. The rate of non-adherence may also increase with time post-transplantation. Two studies reported that every 5 years after transplant, cases of MNA increase by approximately 20% [11, 12]. Despite the crucial importance of a correct intake of immunosuppressive medications, there is little guidance on how to identify MNA and promote therapy adherence [13].

Addressing MNA in routine clinical practice is challenging because MNA is often ill-defined, the diagnosis is difficult, treatment strategies are not widely available and their efficacy on clinical outcomes is not always proven.

This review aims at critically assessing the currently available evidence on MNA diagnosis, risk factors and treatment, with particular focus on those aspects that may be useful for the practising physician (Box 1).

DEFINING MNA

Adherence implies that the medication is taken at the prescribed dose and time [14]. MNA can be quantitatively assessed with the percentage of medication intake (taking adherence) or the percentage of correct inter-dose intervals (timing adherence). However, strict adherence to prescribed medications should not necessarily be regarded as an absolute requirement for every patient [15]. Many patients often practice various forms of non-adherent behaviours, not all of them carrying the risk of jeopardizing clinical outcomes [15, 16]. Most transplant physicians would agree that minor deviations from a prescribed treatment schedule (e.g. occasionally taking tacrolimus (Tac) 1–3 later than the prescribed time) are acceptable [15]. In contrast, establishing the degree of non-adherence that impairs clinical outcomes is not an easy task [15, 17].

A useful distinction is the one between intentional and non-intentional MNA [18]. Intentional MNA represents a deliberate refusal to take the recommended medications properly. This attitude seems to involve almost 14% of the KTRs [18]. It may take place shortly after transplantation, or later over the course of follow-up [19]. Late-onset intentional MNA may follow stressful events. Intentional MNA is almost universally mis-diagnosed and does not usually respond to the standard treatment strategies.

Non-intentional MNA, which refers to a non-deliberate attitude to missing the prescribed drugs, can involve up to 62% of KTRs [18]. Among unintentional MNA, we can distinguish two further subgroups: the unintentional non-adherent patients who seek medical advice after having realized they have missed the dose. They are usually prone to follow healthcare suggestions to improve MNA. This attitude, which can be enhanced by various factors such as hectic lifestyle, low health literacy, immigration/ethnical background, is the least dangerous and the one that may benefit the most from medication reminder interventions. The other type of unintentional MNA is represented by initially unintentionally non-adherent patients whom, however, hide their mistakes. Upon not-experiencing any evident immediate adverse consequences, they eventually become intentional non-adherent patients. These patients suffer more commonly from timing rather than dose adherence. A typical setting is represented by the so-called 'drug holidays' [20, 21], an interval of time when a chronically medicated patient temporarily stops taking the medication. This may happen during weekends, vacations or at any unpredictable time [22]. This category of patients may be easier to treat at earlier stages, but eventually presents similar problems in identification and treatment as the genuine intentional MNA patients. One relatively common manifestation of the development of this condition, which should alert the transplant team, is the frequent missing of outpatient clinic visits (Box 2).

MEASURING MNA

Every strategy for measuring MNA has its own pros and cons and no approach can be regarded as a gold standard. Herein, we summarize the most common strategies that can be distinguished between direct and indirect strategies (Table 1).

Direct methods

Direct methods are aimed at directly measuring patient drug ingestion. Ideally, such methods should be easy, cheap, non-time-consuming and should not represent an excessive bur-

Box 2. Patients' sentences suggestive of intentional and non-intentional MNA

Intentional MNA	Non-intentional MNA
After missing clinic visit: 'Sorry, I forgot to come to the clinic, but I am very busy for the moment.'	'Sorry, I realize that yesterday I forgot my medication, what should I do?'
'I feel intoxicated with all these drugs.'	'I wrongly took twice my medication and now I am worried.'
'What?? Are you asking me if I am properly taking my medication? You are offending me!'	'My wife is out for the weekend and I am not sure about my medication!'
'I read that vitamins can counteract the toxic effect of immunosuppressive medication, can I take them?'	'Sorry, it's a hard time, I realize that I started forgetting my pills, can you help me?'

Table 1. Diagnostic methods to measure MNA; definition, advantages and disadvantages of direct and indirect methods for diagnosis of MNA

Methods	Definition	Advantages	Disadvantages
Direct			
Directly observed therapy	Sightly supervised drug administration by healthcare personnel or caregiver	High reliability	Expensive Time-consuming Loss of independence
Wireless observed therapy [23]	Ingestible sensor system embedded in pills.	High reliability	Expensive Gastrointestinal discomfort Skin reaction to ingestion detector
Therapeutic drug monitoring [15, 27, 24–35]	Investigate the discrepancies between expected and observed drug blood levels.	Easily available at every transplant centre	Not available for every drug Reflect a short interval of time
Indirect			
Pill counts [36]	Healthcare personnel, caregivers and pharmacists can count pill and monitor drug refills	Inexpensive	Patients can hide pills Requires a single distribution system Time-consuming
Electronic monitoring [37–42]	Use of microprocessors embedded in the medication container		Do not assure drug ingestion Uncomfortable device Expensive
Self-reported questionnaire [14, 43, 44]	Questions to determine whether and how often the patients did not correctly take the prescribed medication	Easy, inexpensive and can be done during routine visits	Can underestimate intentional MNA

den for the patients. Unfortunately, none of the available strategies fulfils all these requirements. Moreover, despite being the most efficacious strategies to identify MNA, they may not always be effective in strongly intentionally non-adherent patients.

Direct observed therapy consists of a sight view supervised drug administration by a healthcare personnel or a caregiver. This strategy, which is cost- and time-consuming, has never been tested via clinical trials. Moreover, most patients, particularly the most obstinate intentional non-adherent ones, would hardly be willing to accept such a close direct supervision.

Recently, wireless observed therapy (WOT) has been proposed to diagnose MNA. WOT is based on an ingestible sensor system, which is embedded in pills or capsules. Upon encountering gastric fluid, a signal is released that is recorded by an adhesive personal monitor (APM). This theoretically allows 100% certainty to be achieved concerning the actual number and timing of drug intake [23]. A pilot study on 20 stable adult KTRs used ingestible event marker-enteric coated mycophenolate sodium (IEM-ECMPS) [23]. This study showed that the detection rate was 99%. After 9 weeks of mean follow-up, patients did not experience any serious adverse event or acute rejection. However, eight patients prematurely discontinued treatment due to IEM-ECMPS gastrointestinal symptoms ($n = 2$), skin intolerance to APM ($n = 2$) or insufficient system usability ($n = 4$). Moreover, rash or erythema due to APM was reported in seven (37%) patients during the first month of use. Some patients have also reported feeling anxious with this type of constant surveillance [23]. An additional limitation of WOT is represented by its high cost. Therefore, despite the potential benefit, the applications of WOT may be limited to specific settings.

The most common method used to directly assess drug intake deploys the fact that, over the post-transplant follow-up, every solid organ transplant recipient undergoes regular therapeutic drug monitoring (TDM) of calcineurin inhibitors (cyclosporine and Tac) and/or of mammalian target of rapamycin (mTOR)-inhibitors (sirolimus and everolimus). The largest experience with TDM for assessing MNA comes from Tac. The presence of MNA is diagnosed based on the discrepancy between expected and observed Tac blood drug levels. The two main approaches are based on measuring the variability of Tac trough levels [intrapatient variability (IPV)], most commonly measured as medication level variability index (MLVI) or standard deviation (Tac SD) [24–26], coefficient of variation (CV) and on calculating the Tac dose to concentration ratio [27–29] (Table 2).

IPV is related to clinical outcomes. Among 356 Canadian KTRs, there was a significant 27% increase in the adjusted hazard ratio of the composite endpoint of late allograft rejection, transplant glomerulopathy or total graft loss (including death). For every 1-unit increase in Tac SD, the hazard ratio increased by

Table 2. Methods to measure MNA using TDM of tacrolimus

Method	Definition	Time interval	Tac dose	Normal range	Values correlated with MNA	Limitations	Other determinants besides MNA
MLVI[a] [24, 25]	Tac SD of at least 3 Tac trough levels	>1 year	Changed or unchanged	<2	>2	Better used as a threshold rather than a continuous number	- Dietary preferences - High metabolizers - Drug-to-drug interactions (steroids)
CV [26–28]	Tac SD/Tac mean ×100	>4–6 months (during stable phases)	Unchanged	15–30%	>50%	It is reliable only if the dose remains unchanged	- Drug–food interactions - Different laboratory assays - Different Tac formulations
Concentration/dose ratio [26]	Tac concentration (ng/mL)/Tac daily dose (mg/day) measured at steady state		Changed or unchanged	Stable within the same patients (ranging from 0.5 to 5.0 ng/mL per mg/day)	Lower than expected for each patient	Less standardized	- Clinical conditions that may increase CV independently from MNA (diarrhoea, infections, hypotension, abdominal surgery)

[a] Paediatrics liver transplant studies.
MNA, medication non-adherence; TDM, therapeutic drug monitoring; Tac, tacrolimus; SD, standard deviation.

approximately 30% [30]. A Tac SD > 2 has been associated with late acute rejection within 190 days in 379 adolescent liver transplant recipients (70% sensitivity and specificity) [25]. In another study on 297 KTRs, 71% (24/34) of the patients developing graft failure had high IPV during the first year post-transplantation [31].

Despite the fact that intentionally non-adherent patients may increase drug intake (through 'pulses') selectively at times of laboratory testing and clinic visits, they are not generally able to guess the right timing and dosage to maintain unaltered the drug trough levels; for this reason IPV can be a useful tool to uncover intentional MNA, especially in the context of unplanned or shortly planned visits [25, 32]. Unfortunately, TDM cannot be used for all immunosuppressive medications, like steroids or azathioprine, and it is challenging to use with mycophenolate (because of the limitation of TDM) and mTOR-inhibitors (because of the long half-life).

Tac fluctuations can also be observed in the context of drug-to-drug [33] and drug–food interactions and acute clinical conditions. However, in settings in which there is no alternative explanation for high IPV, especially in the context of a concomitant risky behaviour (as judged by clinical assessment, self-reporting or missed outpatient visit), then high IPV can be regarded as a valuable surrogate of MNA [15, 25, 34].

Indirect methods

Indirect methods include pill count, electronic monitoring, self-reporting questionnaire and healthcare-provided inquires. All these measures, when used individually, have poor sensitivity. However, in combination with drug monitoring, these approaches can reach a high sensitivity, although they can be cost- and time-consuming [35].

In the case of suboptimal drug levels, pill counts can help the clinician to diagnose MNA [36]. However, in cases of intentional MNA, this method can be misleading, because the patients deliberately hide the missed pills from the caregivers.

Electronic monitoring (EM) is based on the use of expensive microprocessors, which are embedded in the medication container or blister, that record the time and date of medication intake [37, 38]. In theory, EM is a highly accurate recorder of patterns of medication intake. However, the event of opening the vial does not ensure that the patient ingests the medication, especially in the case of intentional MNA. Some devices can also be uncomfortable, therefore they may lead to non-usage and to falsely categorizing patients as non-adherent [14]. EM-assessed MNA has been used in clinical trials to objectively measure the response to specific treatment [39–42].

An inexpensive measure of MNA is self-reporting questionnaires. The Basel Assessment Adherence to Immunosuppressive (BAASIS©) Medication scale, which is the most used questionnaire, includes questions to determine if and how often in the last month, the patient (1a) missed a dose immunosuppressive medication, (1b) missed more than two consecutive doses, (2) took their medication more than 2 h after the recommended dosing time and (3) changed their dose without their doctor's instruction [43]. Such measures of MNA have been associated with the rate of viral rebound in HIV patients [44]. Although self-reporting can underestimate MNA, it is helpful as an initial screening and helps identify patients worth more careful discussion regarding all medication-taking practices [14, 44].

Donor-specific antibody (DSA) formation has been linked to MNA [45]. However, this late finding gives fewer opportunities to invert the immune process. Of note, MNA can induce the appearance of non-DSA anti-HLA antibodies well before the development of full-blown anti-HLA DSA and chronic-active antibody-mediated rejection [46, 47].

INDIVIDUAL RISK FACTORS FOR MNA

The relevance of identifying risk factors in clinical practice is that they help in preventing MNA. The World Health Organization has defined five main risk factor domains: patient-related, therapy-related, disease-related, and healthcare organizational and socioeconomic factors (Figure 1).

Risk factors for non-adherence can also be divided into modifiable and non-modifiable ones [48]. Patients' physical characteristics and disease factors are generally considered unmodifiable, whereas therapy complexities and organization issues can be modified by interventions. Patients' beliefs and psychological factors can be modified as well, but this generally requires a multicomponent approach. Other risk factors include time post-transplant, health literacy, sociocultural barriers related to immigration status and ethnicities, learning and cognitive capacities, medication beliefs, overall patient lifestyle, and competing priorities. They may also be corrected, provided that *ad hoc* interventions are put in place.

Risk factors for MNA can coexist and change over time, therefore it is crucially important to continuously monitor these factors to address them as soon as they arise.

Patient-related factors

MNA in elderly patients is usually unintentional and related to factors such as forgetfulness, complex medication regimen, side effects and need for caregivers. In contrast, MNA in adolescents and young adults is related to lifestyle disruptions and progressive empowerment over caregivers to assuming responsibility for self-management [49, 50]. Overall, it has been estimated that the prevalence of MNA in adolescents and young adults is about 32% and that it accounts for 44% of all graft losses and 23% of late acute rejection episodes in this setting [43, 51, 52].

Therapy-related factors

Therapy-related factors include the number and complexity of daily medications [53], the frequent changes in dosages and drug-related side effects [54]. Identifying specific lifestyle factors that cause MNA may sometimes provide an easy way to improve medical adherence; for instance, by tailoring the timing of drug administration to the patient's working hours or to the timing of leisure activities.

Disease-related factors

Disease-related factors can be related to the history of chronic medications and the dialysis vintage. For instance, depression or cognitive impairment due to cerebrovascular disease have been linked to poor adherence in KTRs [12].

Healthcare organizational factors

The poor healthcare organization, non-private medical insurance in the USA, distance and time factors can affect MNA. Long distances from the place where patients get medications can greatly affect drug adherence. Limited time allotted by the healthcare personnel to provide patients with adequate

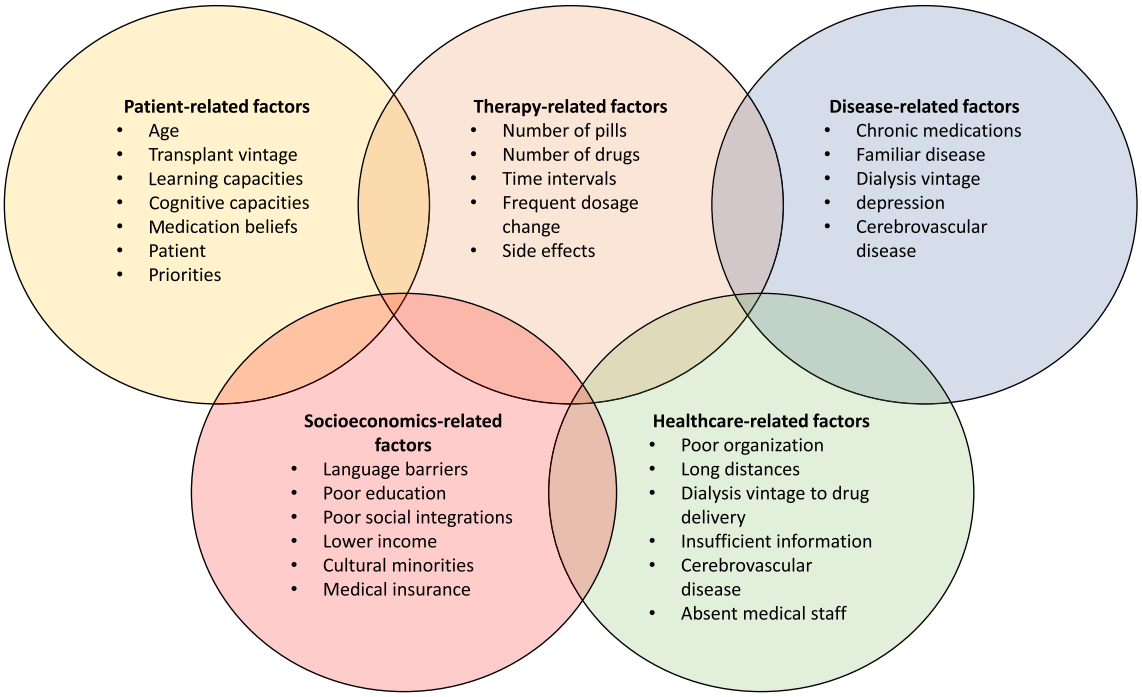

FIGURE 1: Interplay between the five different domains concerning individual risk factors for medical non adherence.

information at the time of hospital discharge, or absence of medical staff in the outpatient clinic for consultation after forgetting drug intake, all negatively impact MNA [55].

Socioeconomic and cultural factors

Socioeconomic and cultural determinants of MNA are factors such as belonging to cultural minorities with poor social integration, low socioeconomic status and lack of insurance coverage [56–58]. Constantiner *et al*., who analysed the adherence of 312 KTRs in New York City through a self-reporting questionnaire, found that younger age and lower income were significantly associated with reported MNA [56]. A recent European study found that, compared with EU-born KTRs, non-EU-born KTRs had a hazard ratio of graft failure beyond 1 year of 1.36, probably related to barriers to adherence related to recent immigration background [58].

GENERAL INTERVENTIONS FOR MNA PREVENTION AND TREATMENT

Despite the critical impact of MNA on graft survival, there are still limited interventions that comprehensively address MNA in KTRs and that have been tested in clinical trials.

Unfortunately, the randomized control trials (RCTs) performed so far on intervention strategies showed some efficacy in reducing measured MNA, but none of them was designed or be able to show any benefit on clinical outcomes [16, 59–63]. One of the additional explanations of this lack of effect on clinical outcomes could be the 'streetlight effect' in which biases in population and endpoint selection could drive misleading conclusions [63]. In fact, since non-adherent patients are less likely to accept being enrolled in monitoring and intervention trials, most of these studies probably included adherent or partially adherent patients. This might have prevented the trials from detecting the true effect of the intervention on MNA patients. Moreover, because the MNA measurements are often considered as primary endpoints, many trials have concluded that the intervention was effective, despite the lack of relevant improvement in clinical outcomes [63].

Clinical pharmacist care

Clinical pharmacists may be involved in MNA monitoring by overseeing the direct medication distribution and providing counselling [64–66]. One RCT reported that pharmacist care strategies increased measured medication adherence (mean compliance rate 95 versus 82% for intervention and controls, respectively, $P < 0.001$), but this had no impact on self-reported medication adherence and on graft outcomes [65]. Another RCT on 128 KTRs found no difference in Tac CV (31.4 versus 32.5%) or in questionnaire-based adherence rate (27% versus 25%). The main limitation of implementing pharmacist care is represented by the inaccuracy of the estimated discrepancy (i.e. the measure of MNA) between the medication collection and the actual intake. Moreover, not all patients are centralized to a single pharmacist facility. Therefore, it may be logistically challenging to track prescription refill for all KTRs [15].

Medication reminder interventions

These interventions aim at reminding the unintentionally non-adherent patients to assume their medication at the correct dose and timing, using electronic medication dispensers, freely available smartphone settings and Apps. Reese *et al.* randomized 120 KTRs to EM with customized reminders, EM with customized reminders plus provider notification or EM alone (control). Despite a significantly increased customized-EM-based adherence at 180-day assessment (78, 88 and 55%, respectively), no difference was detected in mean Tac levels

[67]. Another RCT, which randomized 80 KTRs to EM monitoring plus electronic and healthcare reminder versus standard of care, found that the intervention group had a high EM-based compliance rate (98%). However, the compliance rate in controls was not reported. Moreover, the study found no difference in mean Tac level between the intervention and control group (approximately 7ng/L in both groups). Of note, 6 of the 40 participants in the intervention group withdrew from the study prematurely, mainly due to excessive stress or feeling of being controlled. One participant died 6 months after inclusion because of a serious infection [68], this serious adverse event being apparently not related to the intervention.

Electronic reminders have been largely replaced by phone alarms and various Apps on patients' smartphones [69]. A single-centre RCT investigated the effect of using a free mobile application on Tac IPV. The authors found a marginally, albeit statistically significant, lower Tac CV at 1 month (28 versus 37%). However, the difference vanished at the 3-month assessment [70].

Remote monitoring and telemedicine

One RCT analysed the impact of telemedicine versus standard of care in 46 living-donor KTRs. The intervention arm included both chronic management process and acute-care situation support. The authors found a significantly lower questionnaire-based adherence rate (17% in the telemedicine monitoring group versus 56% in the control group). The effect persisted for up to 12 months after the end of the intervention. The authors also reported a lower incidence of hospital re-admission and shorter length of stay (median re-admission 0 versus 2; median length of stay 6 versus 13 days). It is unclear whether this resulted from the intervention itself or rather the fact that patients in the intervention groups received more intensive and close follow-up compared with the control group [60]. The practice of telemedicine has received a substantial boost from the coronavirus disease 2019 (COVID-19) epidemic [71]. Moreover, it has been reported that the COVID-19 epidemic and the related logistical problems have increased the rejection rates [72]. One recent trial including paediatric lung transplant recipients found a reduction in the Tac IPV at 6 months in 10 patients undergoing 3-weekly phone calls and regular follow-up calls (Tac SD −1.84; 95% CI: −2.95, −0.74; P = 0.004) compared with 7 controls undergoing only regular follow-up calls (Tac SD 0.59; 95% CI: −1.42, 2.60; P = 0.46) [73]. Telemedicine may be integrated with home-based dried blood spots (DBS) sampling of Tac for the purpose of therapeutic drug monitoring [74, 75]. The use of remote monitoring and telemedicine can improve patient quality of life and independence [60], limiting the patients' psychological distress, but its role in everyday clinical practice needs further validation. In our own experience during the COVID-19 pandemic, there are some patients who strongly prefer undergoing regular outpatient clinical visits rather than relying on telemedicine visits.

Therapy simplification

Studies consistently showed that medication complexity is an obstacle to medication adherence [76, 77]. Thus, regimens should be kept as simple as possible and they should be adapted to the patient's habits and lifestyle [76]. Over the last decade, several strategies to improve MNA have been focussed on therapy simplification.

Four single-arm cross over pre–post comparison studies and one RCT showed higher adherence after pill burden reduction, through switch to once-daily Tac alone [53, 78] or in combination with a full once-daily therapy [79]. Overall, these cross-over studies showed a net improvement in 10–20% of the patients. However, all these studies lack a control group. The only published RCT analysed the effect of once-daily Tac switching, based on electronic-monitored MNA. 219 KTRs were included and randomized 2:1 to once-daily ($n = 145$) and twice-daily Tac ($n = 74$) and then followed for 6 months after randomization. Medication adherence increased by 10% in the once-daily group compared with the twice-daily group (88% versus 79%) [80]. The relatively small effect of once-daily Tac in improving MNA is not unexpected, because this strategy also has its own pitfalls [81]. For instance, while young patients with an active life can benefit from a once-daily regimen, patients doing day and night shifts or elderly patients living a drug-paced life could be more comfortable with twice-daily regimens. Moreover, adherence becomes even more critical in once-daily regimens, when missing one dose has more serious consequences as opposed to cases of regular formulations [82]. In the study from Wu *et al.*, the highest coefficient of variation before switching was associated with a higher risk of reduced Tac levels after conversion (area under the receiver operating characteristic curve 0.84, sensitivity 68.3%, specificity 92%) [53].

Therapy simplification has largely experimented with Tac monotherapy. In fact, some drugs such as mycophenolate and everolimus require b.i.d. administration. Even in the case of calcineurin-free regimens, such as those based on the costimulatory drug blocker belatacept, which requires i.v. monthly administration, patients receive concomitant administration of b.i.d. mycophenolate or everolimus [83]. However, due to its long half-life, everolimus could be theoretically administered once daily, as suggested by a small recent RCT [84].

Patients should be aware that medication regimens can be personalized to meet their needs, but they should also be aware of the pros and cons of each option. Tailored therapies seem to be particularly helpful for the empowerment of patients, to reduce the feeling of overmedicalization and to lower the risk of MNA, whereas they seem to be less impactful and even harmful in the case of intentional MNA. We recommend that individual habits and lifestyle hurdles to medical adherence should be discussed with the patients and drug treatment schedules should be the result of a shared decision-making process.

Educational-behavioural intervention

The information-motivation-behavioural skills model ('IMB skills model') is a validated theoretical framework that includes three essential factors to engage and maintain a health behaviour: information, motivation and behavioural skills. The interventions provide psychoeducation, address barriers, foster motivation and discuss cultural messages on adherence behaviour. They also include electronic reminders and meetings with 'coaches'. These approaches have been effective in promoting medication adherence in other chronic diseases, such as HIV infection [39–42], and may help preventing intentional MNA in transplant recipients.

Most studies performed in transplanted patients used an RCT design and examined multicomponent interventions [85] delivered by healthcare professionals across multiple face-to-face and/or telephone sessions [59, 62, 85–90]. Four studies randomized the whole population of KTRs [59, 85, 88, 90], while four other RCTs included only non-adherent KTRs on the basis of an EM survey [62, 86, 87, 89] (Table 3).

Table 3. Clinical trial investigation to prevent or treat MNA

Strategy	First author	-Publication year -Time frame -Location	Design	-Inclusion criteria -Exclusion criteria	Patients' characteristics	-Intervention (n) -Comparator (n) -Duration	Outcome(s)	Results	Patient survival	Death-censored graft survival	Graft function	Adverse events
Clinical pharmacist care												
	Chisholm [64]	- 2001 - 1997–1999 - Augusta (USA)	RCT	- All single KTRs, aged 18–60 years, receiving the same immuno-suppressive drugs for 1 year, followed at the hospital clinic >1 year, receiving the therapy from the hospital pharmacy - Dual or combined transplant recipients, change in immunosup-pressive medications within the 1st-year post-transplant, not followed at hospital clinic, receiving drugs from other pharmacies	$N = 24$ Males 75% Caucasians 58%, African-Americans 38%, and Hispanic 4% Living-donor KTRs 33% Age 49.2 ± 10.2 CNI use: cyclosporine 88%, Tac 12%	- Pharmacist counselling and instruction ($n = 12$) - Standard of care ($n = 12$) 12 months	- Mean compliance rate (CR) for each month - Mean time of patients' compliance (CR > 80%) - % of patients reaching therapeutic target drug levels Compliance rate was calculated as dose refill/dose prescribed × 100	96.1 ± 4.7% versus 81.6 ± 11.5% ($P < 0.001$). 11 months (95% CI of 10 – 12) versus 9 (95% CI of 7 – 11) 64% versus 48% ($P < 0.05$)	NA	NA	NA	NA

Table 3. Continued

Strategy	First author	-Publication year -Time frame -Location	Design	-Inclusion criteria -Exclusion criteria	Patients' characteristics	-Intervention (*n*) -Comparator (*n*) -Duration	Outcome(s)	Results	Patient survival	Death-censored graft survival	Graft function	Adverse events
	Joost [65]	- 2014 - 2008–2010 - Erlangen (Germany)	Case–control study	- All adult German-speaking KTRs, independent of others for medication management or questionnaire completion and followed at visit to the outpatient clinic, on MMF/MPA therapy and willing to use electronic-monitored (EM) bottle for MMF/MPA	N = 67 Male 69% Living-donor KTRs 23% Age 53 (12.6) First transplant 91% CNI use: cyclosporine 82%, Tac 18%	- Pharmacist counselling and adherence support (*n* = 32) - Standard of care (*n* = 35) 12 months	- % of days with the correct dosing of MMF/MPA through EM during the 1st year post-transplant - Taking adherence (% of bottle opening/total number of doses prescribed) - Timing adherence (% of doses taken within a 6-h interval around patients' standard intake time) - Adherence rates (pill counts) -*n* of drug holidays (no MMF/MPA intake for >48 h).	(91%, CI 90.52–91.94) versus (75%, CI 74.57–76.09) P = 0.014 82% versus 95% P = 0.006 94% versus 95% P = 0.142 90% versus 97% P = 0.008 19% versus 67% P = 0.001	NA	NA	eGFR at 12 months 46 ± 15.4 mL/min versus 49 ± 14.3 mL/min P = 0.446	NA

Table 3. Continued

Strategy	First author	-Publication year -Time frame -Location	Design	-Inclusion criteria -Exclusion criteria	Patients' characteristics	-Intervention (*n*) -Comparator (*n*) -Duration	Outcome(s)	Results	Patient survival	Death-censored graft survival	Graft function	Adverse events
	Bessa [66]	- 2016 – 2014 - Sao Paulo (Brazil)	RCT	- Adult KTRs who received immunosuppressive regimens consisting of Tac, prednisone and mycophenolate sodium or azathioprine - KTRs receiving concomitant medications known to interfere with TAC pharmacokinetics	N = 128 Age 45.7 ± 11.6 versus 43.1 ± 12.5 Male 59 versus 66% Living donor 23% versus 20%	- Pharmacist counselling and adherence support (*n* = 64) - Standard of care (*n* = 64) 90 days	- % coefficient variation of Tac - Patient adherence through the BAASIS Scale at Day 28 - Patient adherence through the BAASIS at Day 90	31.4% ± 12.3% versus 32.5% ± 16.1% P = 0.673 17% versus 26% P = 0.135 27% versus 25%, P = 0.457	NA	NA	NA	NA
Medication reminder interventions												
	Reese [67]	- 2016 - 2021–2014 - Philadelphia (USA)	RCT	- Adults KTRs on twice daily Tac medication - Patients with inability to manage medications, poor English comprehension, HIV-positive serostatus, living more than 120 miles from the centre and/or discharge to an acute-care facility	N = 117 age 50 ± 11 years male 60% African-American 40% Prior transplant 12%	- Arm 1: reminders group (*n* = 40) - Arm 2: reminders-plus-notification group (*n* = 39) - Arm 3: control group. (*n* = 38) - 6 months	- Adherence at 90 days - Adherence at 14 days - CV for Tac level - Patient adherence through the BAASIS scale at Day 90	78% versus 88% versus 55% 82% versus 88% versus 58% 0.25 ± 0.14 versus 0.26 ± 0.11 versus 0.26 ± 0.13 P = 0.05 78% versus 74% versus 72% P = 0.58	1 death in arm 1	1 graft failure in the control arm	NA	No

Table 3. Continued

Strategy	First author	-Publication year -Time frame -Location	Design	-Inclusion criteria -Exclusion criteria	Patients' characteristics	-Intervention (*n*) -Comparator (*n*) -Duration	Outcome(s)	Results	Patient survival	Death-censored graft survival	Graft function	Adverse events
	Henriksson [68]	- 2016 - 2011–2013 - Stockholm (Sweden)	RCT	- all consecutive KTRs	*N* = 80 Age 44.65 (2–69) years Male 65% Living donor 45%	- Using electronic medication dispenser (EMD) (*n* = 40) - Not using EMD (*n* = 40) - 12 months	- Medication non-adherence rate - Patients with diagnosis of rejection	2% versus nonadherence 10% versus 33% (P = 0.054)	- 1 death for infection in the intervention group	NA	NA	- 3 patients felt being monitored. - stroke (*n* = 1) - 1 participant experienced extremely stressed by EMD use
	Torabi [70]	- 2017 - NA - New York City (USA)	RCT	- All KTRs or SPKTRs	*N* = 67 Age 53.7 ± 14.3 versus 51.6 ± 14.3 years Living-donor KTRs 28% versus 83%	- Use of Transplant Hero mobile App (*n* = 18) - Non-users (*n* = 18) - 3 months	- Tac CV at 1 month - Tac CV at 3 months	28% versus 37% (P = 0.014) 34% versus 35% (P = 0.63)	NA	NA	s-Creatinine reported to be not statistically different at 1 (P = 0.65) and 3 (P = 0.83) months	NA
Remote monitoring and telemedicine	Schmid [60]	- 2017 -2011–2013 - Freiburg im Breisgau (Germany)	RCT	- living-donor KTRs	*N* = 46 Age 46 (18–59) versus 51 (19–66) Male 61 versus 48% Living relate donor 39 versus 52% ABO-incompatible KT 30 versus 26%	- telemedically supported case management (*n* = 23) - Standard of care (n = 23) - 12 months	- Median unplanned hospital admission at 12 months - Median hospitalization days at 12 months questionnaire-based MNA rate	0 [(IQR) = 1] versus 1 [(IQR) = 2] U = 132.5, P = 0.002, *r* = 0.44 0 days (IQR = 6) versus 13 days (IQR = 23) U = 141.0, P = 0.005, *r* = 0.41 56.5% versus 17.4% (P = 0.013)	NA	0 versus 2 (1 rejection, 1 haemorrhage)		NA

Table 3. Continued

Strategy	First author	-Publication year -Time frame -Location	Design	-Inclusion criteria -Exclusion criteria	Patients' characteristics	-Intervention (*n*) -Comparator (*n*) -Duration	Outcome(s)	Results	Patient survival	Death-censored graft survival	Graft function	Adverse events
Therapy simplification												
	van Boekel [79]	- 2013 - 2006–2010 - Nijmegen (The Netherlands)	Cross-over study with no control group	- Adult KTRs with stable renal function on once daily Tac, with unchanged Tac dose in the previous 3 months, on potential full once daily regimen, Dutch speaking - Patients on mycophenolate regimen	$N = 75$ Age 49.6 ± 12.1 Male 61% Time after transplant 3.1 ± 3.3 years Living-donor KTR 75% Concomitant IS: prednisone 92%, azathioprine 7%, both 1% Working in shifts 24%	- After switching to fully once daily therapy ($n = 75$) - Same patients before switching to fully once daily therapy - 6 months	- Treatment convenience score at 3 weeks - Daily number of medications at 2 weeks - Daily number of tablets at 2 weeks - Self-reported adherence at 3 weeks Measured by Treatment Satisfaction Questionnaire for Medication version II	66.0 ± 14.5 versus 78.5 ± 14.5 ($P < 0.001$) 2.4 ± 0.7 versus 1.6 ± 0.7 ($P < 0.001$) 12.4 ± 3.3 versus 9.1 ± 2.6 ($P < 0.001$) 78% versus 95%	NA	NA	NA	Not registered at 6 months
	Cassuto [78]	- 2016 - NA - Multicentric (France)	Cross-over study with no control group	- Adult kidney and/or liver transplant recipients, on initial twice-daily Tac regimen - Enrolled in clinical trials	$N = 1106$ Age 52.4 ± 13.2 years Male 62% Self-reported adherence assessment at baseline: good adherence (GA) 21%, minor non-adherence (mNA) 72%, non-adherence 7% Mean general acceptance score 78%	- After switching from twice to once-daily Tac regimen ($n = 1106$) - Same patients before switching to once daily Tac 6 months	- Adherence rate at 3 months compared with baseline - Adherence rate at 6 months compared with baseline	28 versus 21% GA, 68 versus 72% nMA, 4.4 versus 7.1% non-adherence ($P < 0.001$) 26 versus 21% GA, 68 versus 72% nMA, 6.5 versus 7.1% non-adherence ($P < 0.001$)	NA	NA	NA	NA

Table 3. Continued

Strategy	First author	-Publication year -Time frame -Location	Design	-Inclusion criteria -Exclusion criteria	Patients' characteristics	-Intervention (*n*) -Comparator (*n*) -Duration	Outcome(s)	Results	Patient survival	Death-censored graft survival	Graft function	Adverse events
	Wu [53]	- 2011 - 2010 - Multicentric (Taiwan)	Cross-over study with no control group	- Adult KTRs, on twice daily Tac-based therapy for 3 months, with stable health status	N = 129 Age 51 ± 12 years Living donor 5%	- Switch to once daily Tac (*n* = 129) - Same patients before switching to once daily Tac - 6 months	- % CV of Tac	8.5 ± 5 versus 14 ± 7.5 ($P < 0.05$)				
	Fellstrom [81]	- 2018 - 2012–2015 - multicentric (Sweden)	Cross-over study with no control group	- Adult KTRs with stable renal function, on twice daily Tac regimen	N = 233 Age 50 (19–82) versus 53.5 (20–77) years Male 65% versus 76% Prior transplant 18% versus 19% MNA assessed by BAASIS questionnaire at baseline 54% versus 66%	- Switch to once daily Tac (*n* = 175) - Remain twice-daily Tac (*n* = 58) - 12 months	- Increase in adherence assessed by BAASIS questionnaire at 12 months - Reduction in through Tac levels	+2.6% versus 3.9% −0.6 ± 2.7 versus −0.2 ± 1.7 ng/mL	1 due to spleen haemorrhage in the intervention group and 1 for cardiac surgery complications in the control group	NA	No difference in eGFR at 0–12 months	8 patients in the once daily Tac group experienced AE (tumors, gastrointestinal problem, skin reaction, angina pectoris and diabetes None in the control group

Table 3. Continued

Strategy	First author	-Publication year -Time frame -Location	Design	-Inclusion criteria -Exclusion criteria	Patients' characteristics	-Intervention (*n*) -Comparator (*n*) -Duration	Outcome(s)	Results	Patient survival	Death-censored graft survival	Graft function	Adverse events
	Kuypers [80]	- 2013 - 2008–2009 - multicentric (Belgium)	RCT	- Adult KTRs, with transplant vintage 6 months–6 years, on twice daily Tac-based therapy, with stable health status	*N* = 219 Male 57% versus 62% Prior transplant 11% versus 11% Transplant vintage 3.1 ± 2.0 versus 2.9 ± 2.1 years	After 3 months of EM-based MNA assessment: - Switch to once daily Tac (*n* = 145) - Remain twice-daily Tac (*n* = 74) - 6 months	- MNA measured as % of patients who remain engaged with the same regimen at 6 months - Day-by-day % of patients with correct dosing - Difference in pre–post randomization MNA - % patients missing daily dose at 6 months	81.5 versus 71.9% (P = 0.08) 88.2 versus 78.8% (P = 0.001) +6% versus −0.7% (P < 0.001) 62% versus 40%	NA	NA	NA	Gastrointestinal 2.8% in the intervention group
Educational-behavioural intervention												
	De Geest [86]	- 2006 - NA - Basel (Switzerland)	RCT	- Adult KTRs, previously assessed as non-adherent through EM for 3 months, transplant vintage >1 year, French or German speaking - Lack of mental clarity, blindness, without a phone	*N* = 18 NA	- One home visit at the inclusion and behavioural interventions, individualized education and social support through monthly phone call for 3 months (*n* = 6) - Standard of care (*n* = 12) - 6 months	- EM-based adherence at 6 months	84% versus 81% P = 0.58	NA	NA	NA	NA

Table 3. Continued

Strategy	First author	-Publication year -Time frame -Location	Design	-Inclusion criteria -Exclusion criteria	Patients' characteristics	-Intervention (*n*) -Comparator (*n*) -Duration	Outcome(s)	Results	Patient survival	Death-censored graft survival	Graft function	Adverse events
	Russell [87]	- 2011 - NA - Columbia (USA)	RCT	- Adult KTRs, previously assessed as non-adherent through EM for 3 months, one twice daily immunosuppressive drug, English speaking, able to open EM cap, independent in medication administration, access to a telephone - No cognitive impairment, or other diagnosis who shorten lifespan	N = 15 Age 51.5 ± 7.2 years Male 47% Caucasian 80% Less than high school education 60% Living donor 27% Prior transplant 47%	- Continuous self-improvement intervention through monthly specialist nurse support (1 home visits +5 phone calls) for 6 months (*n* = 8) - Standard of care (*n* = 7) - 6 months	- EM-based MNA at 6 months	84 versus 81% P = 0.039	NA	NA	NA	NA
	Garcia [88]	- 2015 - 2011–2012 - Sao Paulo (Brazil)	RCT	- Adult KTRs	N = 108 Age 46 ± 14.1 versus 49.3 ± 12.1 years Male 56% versus 63% Living-donor KTRs 38% versus 18% (P = 0.017) Duration of dialysis 25 ± 18 versus 42 ± 31 months (P = 0.007)	- Personalized counselling by a transplant nurse through 30 consultation once a week for 3 months (*n* = 55) - Standard of care (*n* = 56) - 12 months	- % adherence assessed by Immunosuppressant Therapy Adherence Scale (ITAS) questionnaire at 3 months - Mean drug trough levels	86 versus 54% (P = 0.001) 8.7 ± 1.6 versus 8.7 ± 1.8 ng/mL	NA	NA	eGFR at 12 months 61 ± 21 versus 55 ± 24 mL/min/1.73 m^2 (P = 0.46)	NA

Table 3. Continued

Strategy	First author	-Publication year -Time frame -Location	Design	-Inclusion criteria -Exclusion criteria	Patients' characteristics	-Intervention (n) -Comparator (n) -Duration	Outcome(s)	Results	Patient survival	Death-censored graft survival	Graft function	Adverse events
	Breu-Dejean [85]	- 2016 - 2002–2003 - Toulouse (France)	RCT	- Adult stable KTRS, KT within 5 years - Cognitive or psychiatric disorders	N = 110 Age 48 ± 12 years Male 57% Related-living-donor KTs 3.6% Prior transplant 10%	- 2-h psychoeducational intervention in group of 10 persons, every week for 2 months, conducted by a multidisciplinary team (physician, psychologist, nurses, kinesiotherapist, dietitian and social worker) (n = 55) - Standard of care (n = 55) - 10 years	- Questionnaire-based adherence at 3 months	75% versus 47%	Death 12.7% versus 9.1% (P = 0.35) Death with functioning graft 8.2 versus 3.6% (P = 0.13) A log rank test not significant difference (P = 0.06)	Death-censored graft survival 69% versus 87% (P = 0.02) Duration with a functioning graft 3481 ± 894 versus 3562± 717 days (P = 0.97)	NA	NA
	Cukor [89]	- 2017 - 2011 - New York City (USA)	RCT	- KTRs on Tac regimen, aged >25 years, <98% adherence to medication prescription determined by 3 baseline pill counts and Tac trough levels - Lack of a telephone, non-English speaker	N = 33 Age 52 ± 12 years Male 40% African-American 88% Transplant vintage 37.6 ± 13.4 months	- 2 sessions of 2-h cognitive behavioural therapy in 2 weeks (n = 15) - Standard of care (n = 18) - 6 weeks	- Increased in adherence based on pill counts - Grouped Tac trough levels SD	+6% versus 0% (P = 0.04) 1.8 versus 3.5 (P < 0.05)	NA	NA	No difference	NA

Table 3. Continued

Strategy	First author	-Publication year -Time frame -Location	Design	-Inclusion criteria -Exclusion criteria	Patients' characteristics	-Intervention (*n*) -Comparator (*n*) -Duration	Outcome(s)	Results	Patient survival	Death-censored graft survival	Graft function	Adverse events
	Foster [59]	- 2018 - 2012–2016 - Multicentric (Canada and USA)	RCT	- KTRs, aged 11-24 years, transplant vintage >3 months, stable graft function - Impending graft failure, severe neurocognitive disabilities, lack of electronic pill box connectivity, use of liquid immunosuppressive medication, having a sibling participating in the study, participating in other adherence study, English or French speaking	N = 169 Age 15 (13.2–17.4) versus 16 13.3 –17.5 years Male 57 versus 61% African-American 11 versus 13% Prior transplant 9 versus 8% Living donor 46 versus 58%	- EM reminder + receive text message, email, and/or visual cue dose reminders and met with a coach at 3-month intervals (*n* = 81) - Standard of care (*n* = 88) - 12 months	- Taking adherence At 6 months - Timing adherence	78 versus 68% 73% versus 61%	NA	NA	NA	Higher number of CMV infection 0.59 versus 0% patient/month
	Low [90]	- 2019 - 2014–2015 - multicentric (Australia)	RCT	- Adult KTRs, self-manage medication, English speaking - Visually impaired patients, or unable to answer the telephone unassisted	N = 71 Age 51 ± 11 years Male 58% Living donor 20% Transplant vintage 28 (20–41) days	- Face-to-face meeting and telephone health coaching every 2 weeks for 3 months (*n* = 35) - Standard of care (*n* = 36) - 12 months	- Difference in EM-based taking adherence from 3 to 12 months - Timing adherence - SD of Tac trough levels for each patient	- 17.0 versus - 2.3% - 6.9 versus 14.0% 2.1 versus 2.3	NA	NA	NA	NA

Table 3. Continued

Strategy	First author	-Publication year -Time frame -Location	Design	-Inclusion criteria -Exclusion criteria	Patients' characteristics	-Intervention (*n*) -Comparator (*n*) -Duration	Outcome(s)	Results	Patient survival	Death-censored graft survival	Graft function	Adverse events
	Russell [62]	- 2020 - 2015–2017 - Multicentric (USA)	RCT	- Adult EM-based non-adherent KTRs, self-administered therapy, at least one twice daily immuno-suppressive medication, English speaking - No access to the telephone, unable to open an EM cap, mini-mental score < 4	$N = 89$ Age 52 ± 10 years Male 58% African-American 61% Prior transplant 15% Living donor KT 28%	- 6 months multicompo-nent adherence-promoting interventions ($n = 45$) - Standard of care ($n = 44$) - 6 months	- Average EM-base adherence at 6 months - Average EM-base adherence at 12 months	91 versus 67% ($P < 0.001$) 77 versus 60% ($P = 0.004$)	NA	NA	- S-Creatinine at 12 months 1.3 versus 2.1 mg/dL - BUN at 12 months 21 versus 28 mg/dL	No

RCT, randomized controlled trial; CNI, calcineurin inhibitor; BAASIS, Basel Assessment of Adherence to Immunosuppressive Medication Scale; EM, electronic monitoring; CI, confidential interval; NA, not applicable; MMF, mycophenolate mofetil; MPA, mycophenolic acid; eGFR, estimated glomerular filtration rate; SPKTR, simultaneous pancreas kidney transplant recipient; IQR, interquartile range; IS, immunosuppression; AE, adverse event; CMV, cytomegalovirus; BUN, blood urea nitrogen.

Two small RCTs found no difference in EM-based adherence between educational-behavioural intervention and control group [86, 90], while the other six RCTs proved that intervention significantly reduces MNA, but this effect generally vanished thereafter [59, 62, 85, 87–89]. Among these six RCTs, four of them investigated the clinical outcomes [59, 62, 85, 88], with two studies finding no difference in graft survival between groups [59, 88], one study finding a negative impact on 10-year death-censored graft survival in the intervention group [85] and the last RCT (SystemCHANGE [62]) showing a positive numerical trend, despite not statistically significant, on graft function at 12 months [62]. Interestingly, there was also a numerical trend toward an increased infection risk in the intervention groups, which needs to be further addressed in future RCT and meta-analyses. A possible explanation for this outcome is that increased adherence to antirejection drugs may result in a higher risk of overimmunosuppression.

In summary, educational-behavioural interventions are effective strategies in improving MNA, at least in the short term. Unfortunately, the extent of the long-term benefit is uncertain. Moreover, they are expensive, time-consuming and their widespread implementation may be hard to achieve in several clinical settings.

CONCLUSIONS

MNA is one of the leading causes of patient and graft loss after kidney transplantation. Unfortunately, there is no evidence to date that any single strategy for treating MNA improves the two major clinical outcomes, namely, patient death and death-censored graft failure. Therefore, every effort should be made to identify individual risk factors for MNA and to discuss with patients what are the major hurdles to adherence to the prescribed treatment schedule. Then, the plan to improve medical adherence should be personalized to the peculiar issues raised in the individual patient. While unintentional nonadherent patients can benefit from various personalized and multi-disciplinary interventions such as electronic reminders and phone Apps and therapy simplification, intentional MNA remains an Achille's heel of any transplant centre. To preventing KTRs from becoming intentional MNA patients, constant monitoring via motivational-behavioural interventions may represent the only viable resource.

CONFLICT OF INTEREST STATEMENT

None declared.

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Clinical Kidney Journal, 2022, vol. 15, no. 7, 1275–1283

https://doi.org/10.1093/ckj/sfac029
Advance Access Publication Date: 31 January 2022
CKJ Review

CKJ REVIEW

Sodium–glucose cotransporter inhibition in polycystic kidney disease: fact or fiction

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ABSTRACT

Autosomal dominant polycystic kidney disease (ADPKD) is the most prevalent hereditary kidney disease. Recent evidence suggests that the pathogenesis of ADPKD is a complex web of abnormal cellular processes including altered cell signaling, disordered cell metabolism, impaired autophagy, increased apoptosis, mitochondrial dysfunction and chronic inflammation. Sodium–glucose cotransporter (SGLT) inhibitors (SGLTi) reduce body weight, blood pressure and blood glucose levels, have kidney and cardiovascular protective activity, and have been reported to decrease inflammation, increase autophagy and improve mitochondrial dysfunction. We now review results from preclinical studies on SGLTi for ADPKD identified through a systematic search of the MEDLINE, Cochrane Library, Embase and PubMed databases. Potential underlying mechanisms for the conflicting results reported as well as implications for clinical translation are discussed, as ADPKD patients were excluded from clinical trials exploring kidney protection by SGLT2 inhibitors (SGLT2i). However, they were not excluded from cardiovascular safety trials or trials for cardiovascular conditions. A post-hoc analysis of the kidney function trajectories and safety of SGLT2i in ADPKD patients enrolled in such trials may provide additional information. In conclusion, SGLT2i are cardio- and nephroprotective in diverse clinical situations. Currently, it is unclear whether ADPKD patients may benefit from SGLT2i in terms of kidney function preservation, and their safety in this population remains unexplored. We propose a roadmap to address this unmet clinical need.

Keywords: apoptosis, autophagy, canagliflozin, dapagliflozin, polycystic kidney disease, SGLT inhibitors

Received: 12.10.2021; Editorial decision: 26.1.2022

INTRODUCTION

Autosomal dominant polycystic kidney disease (ADPKD) is the most common inherited kidney disease, which is characterized by the formation of numerous fluid-filled cysts primarily within the kidneys, often progressing to end-stage renal disease (ESRD) as renal cysts increase in number and size. Most cases are caused by mutations of either the polycystin-1 (*PKD1*) (75–85%) or polycystin-2 (*PKD2*) (15–25%) genes, the former having generally a worse prognosis [1, 2]. Sodium–glucose cotransporter-2 (SGLT2) inhibitors (SGLT2i) were introduced as antidiabetic drugs; however, more recently clinical trials have shown that they have heart and kidney protective effects in person with or without diabetes [3]. A systematic review of the literature was conducted to identify publications that detail the effect of sodium–glucose cotransporter (SGLT) inhibition in the treatment of ADPKD. The search was conducted in the following electronic databases: MEDLINE, Cochrane Library, Embase and PubMed. These electronic databases were searched on (until October 2021) using a structured search string, including the terms ADPKD and SGLT, ADPKD and SGLTi, PKD and SGLT, and PKD and SGLTi.

PATHOGENESIS OF ADPKD

The pathogenesis of ADPKD is a complex web of defective cellular processes including altered cellular signaling, increased apoptosis, impaired autophagy, mitochondrial dysfunction, increased aerobic glycolysis associated with hyperglycemia (Warburg Effect) and chronic inflammation [4]. Better understanding of these abnormal cellular processes has highlighted several treatment possibilities; however, few have fulfilled their initial promises and met the theoretical and pathophysiological expectations. The poor translation of preclinical animal studies to humans has several contributors, from animal models that do not reproduce the same molecular defect (*PKD1* or *PKD2* deficiency) to use of doses that cannot be achieved in human studies due to side effects. Thus, the treatment of ADPKD still remains a challenge revealing the unmet need for new therapeutic options, despite the availability of tolvaptan, a selective, competitive vasopressin receptor 2 (V2R) antagonist [5].

A diverse set of intracellular signaling pathways are dysregulated and contribute to the pathogenesis of ADPKD including the mammalian target of rapamycin (mTOR), AMP-activated protein kinase (AMPK), vasopressin-mediated cyclic adenosine monophosphate (cAMP) and extracellular signal-regulated kinases (ERK) pathways [6–8]. Inflammation, mitochondrial dysfunction and metabolic reprogramming have also been held responsible for the formation and expansion of cysts. Finally, as for any chronic, slowly progressive form of CKD, the reduction in functional kidney mass may result in glomerular hyperfiltration and development of focal segmental glomerular lesions. The pathogenesis of ADPKD was examined and thoroughly explained in reviews and beyond the scope of the article [4].

THERAPEUTIC APPROACHES

Targeting cAMP signaling

Vasopressin binding to V2R induces cyst formation and transepithelial fluid secretion by increasing cAMP, especially in distal tubules [9]. Thus, suppressing vasopressin is a logical approach for reducing cysts within the kidneys. This may be achieved by daily water intake of 3–4 L over 24 h, although the feasibility and efficacy on ADPKD of long-term vasopressin suppression through increased water intake has not been demonstrated [10]. In addition, tolvaptan slows cyst and total kidney volume (TKV) growth and estimated glomerular filtration rate (eGFR) loss in persons with ADPKD [11–13]. However, tolvaptan is still an expensive medication, not available widely, and has significant side-effects (hepatotoxicity, polyuria) leading to intolerance and drug withdrawal in some patients. Additionally, residual risk for CKD progression remains unacceptably high.

Targeting mTOR signaling

Sirolimus and everolimus inhibit mTOR complex 1 (mTORC1) but not mTOR complex 2 (mTORC2). Although sirolimus and everolimus showed promising results in animal models, these findings unfortunately were not replicated in clinical trials [6, 14]. A new class of drugs, mTOR kinase inhibitors, directly binds to mTOR kinase and inhibits both mTORC1 and mTORC2. It was suggested that combined mTOR1 and mTOR 2 inhibitions may be more effective than solely inhibiting mTOR1 in PKD. PP242, an mTOR kinase inhibitor, reduced renal enlargement and cyst numbers in the Han:SPRD rat model of PKD. Compared with sirolimus, PP242 has a higher anti-proliferative effect and is a more effective mTORC1 inhibitor and less toxic on bone marrow, T and B cells [15, 16].

Targeting AMPK signaling

Metformin activation of AMPK and resulting inhibition of mTORC1 signaling lead to attenuation of PKD in the *Pkd1* mutant mice [17]. However, a phase II clinical trial of metformin for ADPKD identified several limitations such as gastrointestinal side effects and reduced bioavailability following a hepatic first-pass effect [18]. In this regard, the clinically maximum tolerable oral dose of metformin (i.e. 2.0 g/day) may still not be sufficient for AMPK activation in the kidney [19, 20]. Despite this, two ongoing phase 3 clinical trials are comparing metformin with placebo [NCT04939935: Implementation of Metformin theraPy to Ease Decline of Kidney Function in Polycystic Kidney Disease (IMPEDE-PKD), expected to be completed by 2026] or tolvaptan [NCT0376460: Metformin vs Tolvaptan for Treatment of Autosomal Dominant Polycystic Kidney Disease (METROPOLIS) expected to be completed by 2022].

Targeting fat metabolism

Hepatocyte nuclear factor 4a (Hnf4a) and the peroxisome proliferator–activated receptor-a (PPARa) are two major proteins responsible for decreased Fatty Acid Oxidation (FAO) in PKD [21]. Loss of Hnf4a function in PKD results in more severe cystic disease [21], while fenofibrate, a PPARa agonist, enhanced FAO and reduced cysts in an orthologous ADPKD model [22]. However, the clinical evaluation of this drug is limited by its potential to increase serum creatinine [23]. The mechanism is unclear and interference with creatinine secretion has been suggested. In any case, the increase in serum creatinine is rapidly reversible upon stopping fenofibrate and in fact, in a post-hoc analysis of a clinical trial, fenofibrate reduced albuminuria and slowed eGFR loss over 5 years in persons with diabetes [24].

Targeting energy metabolism

In ADPKD, impaired FAO is related to decreased mitochondrial Tricarboxylic Acid (TCA) cycle efficiency [25], rendering PKD cells mostly dependent on glucose and aerobic glycolysis instead of mitochondrial oxidative phosphorylation, known as the 'Warburg effect' [26, 27]. This shift generates 4 ATP and 2 lactate

molecules instead of 36 ATP molecules, and lactate is used to build macromolecules in highly proliferative cells such as cells lining cyst tubules [28]. Also, local hypoxia with mitochondrial dysfunction including mitochondrial fragmentation, swelling and reduction in mitochondrial DNA copy number were frequently observed in PKD [29–32]. Transcription and activity of glycolytic enzymes were increased and those of gluconeogenetic enzymes decreased in kidneys of Han:SPRD Cy/+ rats. Administration of 2-deoxyglucose (2DG), a glycolytic pathway inhibitor, decreased intrarenal lactate levels, ERK1/2 phosphorylation and cell proliferation, retarded cyst progression and attenuated renal functional decline in cystic rats [33, 34]. Thus, mitochondrial dysfunction with altered metabolic arrangements such as increased glycolysis and decreased oxidative phosphorylation appeared as the main characteristic features of PKD. However, therapeutic options are highly limited for adjustment of this altered metabolic state and mitochondrial dysfunction. SGLT2i seem like a promising agent for targeting energy metabolism and mitochondrial dysfunction.

Besides pharmacological therapies, some dietary strategies were also considered to slow down the progression of PKD such as daily energy restriction, time restricted feeding, intermittent fasting and ketogenic diets [35, 36]. Time-restricted feeding, compared with isocaloric ad libitum feeding, reduced blood glucose and increased ketogenesis, which inhibited renal cyst proliferation and growth. These findings are not merely due to time-restricted feeding since ad libitum administration of a ketogenic diet similarly inhibited renal cyst growth. In addition, acute fasting in rat, mouse and feline models of PKD induced significant apoptosis in cyst-lining epithelial cells but not in normal tissue, resulting in decreased renal cystic burden. These beneficial findings were all replicated with a ketogenic diet based on β-hydroxybutyrate; therefore, the metabolic state of ketosis seemed to be the crucial mediator of beneficial effects by inhibiting cyst growth and fibrosis via altering PKD-related signaling pathways like mTOR and STAT3 [27]. A recent retrospective case series also showed the feasibility of ketogenic diet for patients with ADPKD, concluding significant weight loss, improved blood pressure management and slight improvements in eGFR, with some safety concerns such as hyperlipidemia [37]. Overall, studies suggested that dietary restriction was beneficial for PKD progression by inducing ketosis, as renal cyst cells in PKD seem to be metabolically inflexible and thus unable to adapt to alternative fuel sources apart from glucose [27]. Moreover, several studies showed that ketogenic status is associated not only with better energy metabolism, but also with improved mitochondrial function and autophagy, which eventually contributed to controlling of inflammation and fibrosis [38].

These findings are supported by several studies that showed obesity and hyperglycemia were also correlated with faster disease progression in ADPKD [39–41], as well as functional and structural kidney damage in PKD [42]. Interestingly, one of the putative mechanisms of action of SGLT2i to promote heart and kidney protection is by increasing ketones such as β-hydroxybutyrate, which may also regulate histone post-translational modifications to regulate gene expression [43]. Thus, beneficial effects of SGLT2i seem closely linked to its efficacy on metabolism similar to the metabolic state of ketosis and β-hydroxybutyrate.

Targeting autophagy

Autophagy and autophagosome fusion with lysosomes (i.e. autophagic flux) are impaired in PKD [26, 44–46]. In a Pkd1 mutant zebrafish model, knockdown of autophagy protein Atg5 promoted cystogenesis, whereas treatment with the autophagy inducer Beclin-1 reduced cyst formation and growth [45]. However, a natural autophagy enhancer trehalose was ineffective in a Pkd1 mutant model [44]. Of note, mTORC-1 inhibits autophagy, and various rapalogs enhance autophagy and reduce cystogenesis in PKD [26, 45]. Although the drug arsenal for targeting autophagy was still under investigation, dietary interventions including a ketogenic diet were shown to improve parameters related to autophagy and improved inflammatory state with less fibrosis [36].

Targeting hypoxia-inducible factor

In ADPKD, cyst growth may compromise tissue perfusion and activate hypoxia-inducible factor (HIF), as HIF-1α and HIF-2α levels are associated with cyst burden [47]. Although, HIF-1α is thought to play an active role in the process of cyst expansion and pericystic angiogenesis, the HIF-1 inhibitor 2-methoxyestradiol (2ME2) had no significant effect on kidney volume or cyst volume density [46] and the impact of HIF stabilizers in clinical use or clinical trials for uremic anemia on ADPKD progression has not been adequately explored [48]. Even though they seemed to provide crucial benefits for PKD and anemia management of CKD patients, HIF-prolyl hydroxylase inhibitors are not currently favored due to a lack of data on their potential effects on cyst development or growth [47].

In addition to the aforementioned metabolic effects of SGLT2i, they have been proposed to play a beta-blocker-like effect in the kidneys, decreasing energy demand by proximal tubule cells, and may be expected to protect proximal tubule cells from a hypoxic environment [3]. Therefore, SGLT2i decrease renal hypoxia, enhance nutrient deprivation signaling, suppress HIF-1α and activate HIF-2α, which promotes erythrocytosis [49]. In a recent review of Patel and Dahl [47], effects of SGLT2i and HIF-prolyl hydroxylase inhibitors on ADPKD were compared in detail, suggesting that SGLT2i might provide a better alternative to tolvaptan rather than HIF-prolyl hydroxylase inhibitors for patients with ADPKD.

Targeting glomerular hyperfiltration

In ADPKD, higher baseline albuminuria was associated with faster eGFR loss [50]. Indeed, tolvaptan decreased albuminuria compared with placebo, independent of blood pressure. Treatment efficacy of tolvaptan on changes in TKV and eGFR was more readily detected in patients with higher albuminuria. Together with the observation that tolvaptan causes an early, reversible dip in eGFR, followed by a slower eGFR slope [51], similar to renin–angiotensin system blockers and SGLT2i [52], this observation supports a role of glomerular hyperfiltration of remaining nephrons in the progression of ADPKD. Indeed, in long-term studies, tolvaptan slowed eGFR decline, regardless of its impact on TKV, which was initially considered to be its mechanism of action [53]. This knowledge further supports the potential benefit of SGLT2i in ADPKD.

SGLT INHIBITION

SGLTs are a family of proteins mediating the transepithelial transport of glucose in the proximal tubule of nephrons and intestinal mucosa of small intestines. SGLT2 is a high-capacity, low-affinity transporter, which mediates the remaining 90% of glucose reabsorption in the proximal renal tubule [54, 55]. SGLT inhibition decreases Na^+–glucose reabsorption in the proximal tubule, thus decreasing energy needs, induces osmotic diuresis and increases distal tubule fluid rate. This leads to an increase

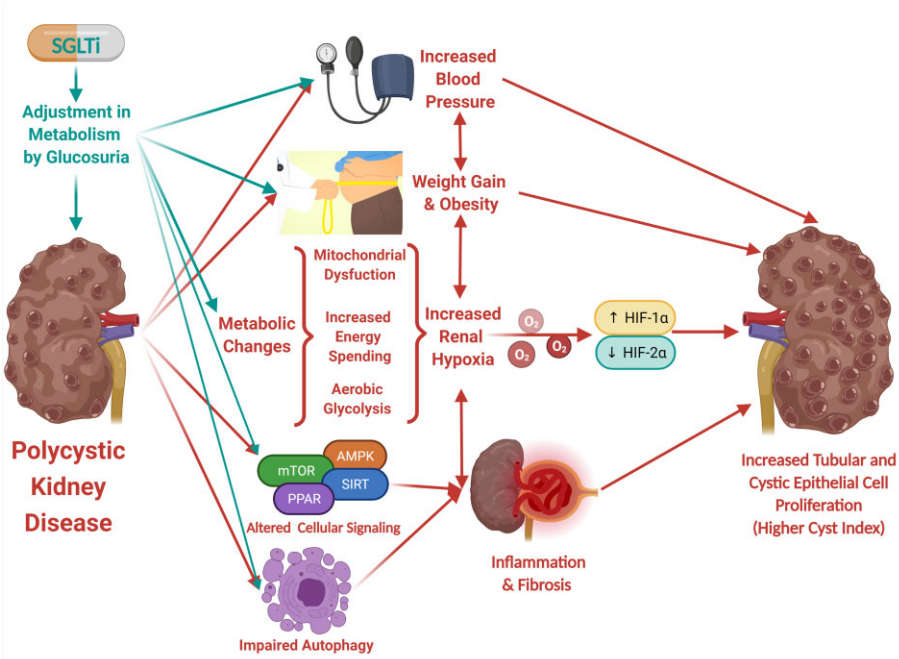

FIGURE 1: The suggested mechanisms of polycystic kidney disease (PKD) leading to higher tubular and cystic epithelial cell proliferation with higher cyst index are shown with red arrows. The possible beneficial effects of SGLTi on polycystic kidney disease (PKD) are shown with green arrows as they can interfere in multiple pathological processes of PKD by adjustments in metabolism via their glucosuric effects. SGLTi, sodium–glucose co-transporter inhibitor; HIF, hypoxia-inducible factor.

in afferent arteriolar tone because of tubuloglomerular feedback and thus corrects intra-glomerular hypertension and decreases hyperfiltration and its consequences such as albuminuria and metabolic overload of proximal tubules. Furthermore, SGLT2i have many other benefits such as reduction in HbA1c, blood pressure and weight reduction thanks to their glucosuric effect, which helps to get rid of excess glucose from body. Therefore, SGLT2 inhibition could be regarded as an antagonist of excessive carbohydrate consumption for the metabolism. Also, several studies have shown that SGLT2 inhibition has cardioprotective and nephroprotective effects in both diabetic and nondiabetic patients. Mechanisms contributing these benefits are still under investigation; however, switching metabolism to a ketotic state (e.g. increased β-hydroxybutyrate) by getting rid of excess glucose appears as the main mechanism. Importantly, an anti-inflammatory impact has been described for SLGT2i, as well as increased autophagy and improvement of mitochondrial dysfunction [38, 56]. Since PKD is associated with altered glucose metabolism, impaired autophagy, increased apoptosis and mitochondrial dysfunction, SGLT inhibition appears to be a very promising approach for the treatment (Figure 1). As the main beneficial mechanism of SGLT inhibition has similarity to a reduced carbohydrate diet, whether diet with reduced carbohydrate intake can achieve the same benefits also needs to be elucidated. While the impact of SGLT inhibition on PKD pathogenesis has been investigated over the last few years, studies are limited to preclinical models and results are conflicting. Below, we critically discuss the available and still needed studies.

SGLT INHIBITION IN PRECLINICAL PKD

SGLT inhibition has been tested in rat and murine models of PKD, but only the murine model was characterized by *Pkd1* deficiency as human ADPKD. Thus, the rat models may be suboptimal to extrapolate results to human ADPKD.

Rat PKD

Wang *et al.* studied the effect of phlorizin, an inhibitor of both SGLT1 and SGLT2, in PCKD Han:SPRD Cy rats over a 5-week period. PCKD Han:SPRD cy rats have a mutation in Anks6 (also called Pkdr1) which encodes SamCystin, a protein expressed in proximal tubules and glomeruli [57-58]. The mutation results in increased levels and mislocalization of SamCystin. These rats develop cysts exclusively in proximal tubules that become disconnected from these tubules. Phlorizin induced glycosuria and osmotic diuresis, increased creatinine clearance and decreased albuminuria. The kidney weight and cyst index were also lower in phlorizin-treated rats compared with placebo-treated rats. Moreover, phlorizin reduces cystic epithelial cell proliferation, assessed by Ki67 staining. In addition, phlorizin decreased ERK phosphorylation, which is increased in PKD, in a dose-dependent manner [58]. In another study with the same rat model, dapagliflozin for 5 weeks caused glycosuria and polyuria, increased creatinine and blood urea nitrogen (BUN) clearances, and decreased albuminuria despite failing to slow cyst growth and increasing kidney weight. Tubular epithelial cell proliferation, macrophage infiltration and interstitial fibrosis were similar in dapagliflozin- and vehicle-treated groups [54]. Kidney enlargement was attributed to widening of tubular lumen due to increased diuresis [54] and to tubular hypertrophy, thought to result from increased SGLT1-mediated glucose reabsorption in the S2 and S3 segments of proximal tubules [59]. Thus, in PCKD Han:SPRD rats with proximal tubular cysts, a dual SGLT1/SGLT2 inhibitor increased creatinine clearance, decreased kidney weight and cyst index while SGLT2i also preserved kidney function

deposit lack of an effect on cysts. Overall, the results support a protective effect of SGLT2 inhibition through mechanisms independent of cyst growth, likely dependent on interference with nonspecific mechanism of CKD progression. However, PCKD Han:SPRD Cy rats are not an optimal model of ADPKD as they involved a different protein target.

In PCK rats, dapagliflozin 10 mg/kg/day or vehicle was administered by gavage to 6-week-old male rats ($n = 9$ per group). PCK rats have a Pkhd1 genetic defect and are thus a model of autosomal recessive PKD [60]. They develop cysts in distal nephrons that remain connected to the tubule [61, 62]. Dapagliflozin increased glucosuria, urine output and creatinine clearance after 3 weeks; however, albuminuria was also increased 4-fold, suggesting that dapagliflozin induced hyperfiltration. Histological analysis and ultrasound showed a higher cyst volume and a 23% higher total kidney weight in rats treated with dapagliflozin. Renal cAMP content and Ki67 staining were similar between dapagliflozin- and vehicle-treated PCK rats [63]. Contrary to PCK rats, normal rats treated with dapagliflozin do not develop evidence of hyperfiltration and albuminuria [64, 65], so the findings in PCK rats were surprising [63]. Since PCK rats are a model of autosomal recessive PKD, the results are not directly relevant for ADPKD.

Murine PKD

Leonhard *et al.* [1] investigated the effect of salsalate (a nonsteroidal anti-inflammatory drug), metformin or canagliflozin on kidney cyst growth in an adult-onset mouse model of PKD caused by *Pkd1* deficiency. Salsalate or metformin plus salsalate increased kidney survival and reduced cystic kidney disease severity compared with untreated mutant mice. However, metformin did not add further protection to that afforded by salsalate alone and neither metformin nor canagliflozin alone was effective [1]. However, canagliflozin-treated mice were not studied in detail. Just kidney survival data were provided. Although at the end of the study kidney survival was similar to control mice, there was a lag time of 14 days (18%) between the development of kidney failure for the first control mouse and for the first canagliflozin mouse. More detailed studies are warranted. In this regard, this was an accelerated model of ADPKD in which both *Pkd1* alleles were inactivated, postnatally resulting in kidney failure at around Day 100 of disease, i.e. in very young mice, unlike human ADPKD for which only one *PKD1* allele is mutated leading to kidney failure at around age 65 years.

Understanding the results obtained in preclinical PKD

None of the preclinical studies was performed in an optimal model of human ADPKD. Overall, SGLT inhibition was protective in a rat model of proximal tubular cystogenesis but not in a rat model of human autosomal recessive PKD or in a murine model of *Pkd1* deficiency with a time course closer to the natural history of human autosomal recessive PKD than to the natural history of ADPKD. In any case, the authors of these manuscripts speculated for several potential explanations for the lack of efficacy. These include that osmotic diuresis induced by glycosuria may promote the dilation of distal tubules and linked cysts [63, 66] (Figure 1). However, human cysts are independent from tubules, and tolvaptan induced large diuresis volumes while being protective. Thus, this putative mechanism is not clinically relevant.

It has also been suggested that SGLT2 inhibition may increase vasopressin levels. In type 2 diabetic Goto-Kakizaki (GK) rats, after 8 weeks, ipragliflozin decreased body weight, serum glucose and systolic blood pressure, and increased fluid and food intake, urinary glucose and Na^+ excretion, urine volume and renal osmolar clearance, and most importantly urine vasopressin levels and solute-free water reabsorption (TcH2O). Urine vasopressin in ipragliflozin-treated rats was negatively and positively associated with fluid balance and TcH2O, respectively. Ipragliflozin increased expression of SGLT2, aquaporin 2 phosphorylated at Ser269, which is an important clue for strong vasopressin activity, and vasopressin V2 receptor. Thus, the osmotic diuresis induced by SGLT2 inhibition stimulated compensatory fluid intake and renal water reabsorption by increasing vasopressin levels to maintain body fluid volume [67]. Ho *et al.* [68] suggested that phlorizin could lead to increased endogenous vasopressin levels promoting distal tubular cyst growth, an effect that might not be seen in Han:SPRD rats as the cysts are almost exclusively of proximal tubular origin and unresponsive to vasopressin. Indeed, in type 1 diabetics, levels of copeptin, a more stable marker of vasopressin production, increased in response to empagliflozin under euglycemia and more so under hyperglycemia [69]. Under euglycemia, which would be the clinical conditions for ADPKD patients, the increase was modest (around 24%, to levels of 5.1 ± 2.8 pmol/L) and the clinical implications of this modest increase are unclear. However, in a further diabetic rat model, empagliflozin increased the expression in V2R but decreased protein and mRNA levels of AQP2, and this was associated with increased phosphorylation of AQP2 at S261, a marker of intracellular location, suggesting some blockade of V2R signaling [70]. Overall, the impact of SGLT2 inhibition on vasopressin responses and signaling under nondiabetic chronic conditions is not well understood and merits further clinical studies.

Finally, the impact of SGLT inhibition on PKD may depend on the specificity of SLGT inhibition. Cyst growth, renal function and albuminuria improved in Han:SPRD rats treated with phlorizin, which inhibits both SGLT1 and SGLT2 [58]. Thus, the result may be different with combined SGLT1 and SGLT2 inhibition as compared with SGLT2 inhibition alone, since combined complete SGLT inhibition is more powerful in terms of glycosuria [59]. In this regard, phlorizin is not in clinical use. However, diverse SGLT2i have different relative selectivity for SGLT2 and SGLT1, ranging from 20-fold (sotagliflozin) to 250-fold (canagliflozin) to over 1000-fold (in increasing selectivity order: dapagliflozin, ertugliflozin and empagliflozin, the latest with a selectivity of 2500-fold) [71].

SGLT2 INHIBITION IN CLINICAL ADPKD

Published clinical experience with SGLT2i and ADPKD remains limited or non-existent. Indeed, no publications were found in a PubMed search that also included the main SGLT2i (canagliflozin, dapagliflozin, empagliflozin, ertugliflozin and sotagliflozin) on 9 October 2021. Unfortunately, patients with ADPKD were excluded from clinical trials assessing kidney protection as primary outcome in patients with or without diabetic CKD, such as A Study to Evaluate the Effect of Dapagliflozin on Renal Outcomes and Cardiovascular Mortality in Patients With Chronic Kidney Disease (Dapa-CKD, NCT03036150), which recently demonstrated kidney protection conferred by SGLT2i in nondiabetic patients with CKD [72]. Furthermore, they were also excluded from EMPA-KIDNEY (The Study of Heart and Kidney Protection with Empagliflozin, NCT03594110) a further trial assessing kidney protection by SGLT2 inhibition in nondiabetic patients with CKD expected to be completed by 21 December 2022 [73]. Indeed, none of the ongoing 45 clinical

trials on PKD listed in ClinicalTrials.gov as of 4 October 2021 involves SGLT2 inhibition [74]. Thus, the clinical trial experience is and will remain limited for the foreseeable future. However, clinical trial NCT04680780 is exploring the feasibility of ketogenic dietary interventions and has a secondary endpoint of TKV. Promotion of ketogenesis is one of the putative kidney and cardioprotective mechanisms of action of SGLT2 inhibition [75]. However, ADPKD patients were not excluded from clinical trial assessing cardiovascular safety in persons with type 2 diabetes for assessing efficacy in cardiovascular conditions [76, 77]. Thus, a careful, targeted post-hoc analysis of several of these trials combined in search of persons with ADPKD that may have been enrolled because of coexisting diabetes or cardiovascular disease may provide further clues as to the safety and potential efficacy for kidney protection of SGLT2i in ADPKD.

SAFETY

Regarding safety, a main concern would be the risk of genitourinary infection, as an ascending infection affecting kidney cysts may have potentially severe consequences [78]. As ADPKD patients are more prone to urinary tract and cyst infections than the general population, the detrimental effects of possible infections including faster disease progression in ADPKD patients should be evaluated carefully in future studies. In any case, this information would only be hypothesis generating and would be useful to design further clinical studies as its post-hoc nature, presence of comorbidities and low patient numbers will prevent reaching definitive conclusions. In this regard, another drug with antidiabetic properties, the thiazolidinedione pioglitazone, was recently found to be safe in a phase 1b clinical trial for ADPKD, following reports of kidney protection effects in rodent PKD [79].

Another safety issue for using SGLT2i in ADPKD is their potential to exacerbation of hypovolemia, hypernatremia and acute kidney injury when combining with tolvaptan, which could prevent vasopressin-mediated water reabsorption [47]. Volume status of ADPKD patients under SGLT2i and tolvaptan treatment should be assessed carefully and their effects should be further explored in future studies.

Better understanding of the nature of ADPKD with its pathological processes is crucial for therapeutic selections in the future as some non-pharmacological dietary interventions like ketogenic diet, time-restricted diet, etc. could also provide beneficial effects to patients with ADPKD targeting altered metabolic state. Whether the usage of SGLT2i added more benefit to patients with ADPKD than other dietary interventions such as ketogenic diet or their beneficial effects are solely dependent on their glucosuria, and metabolic effects need to be elucidated in the future. Future studies and randomized controlled trials can be conducted to compare the feasibility, efficacy and the safety profile of SGLT2i and dietary intervention with a ketogenic diet based on β-hydroxybutyrate.

Table 1. Proposed roadmap to establish the safety and efficacy of SGLT2i for kidney and cardiovascular disease in persons with ADPKD

(A) Preclinical
1. Explore the efficacy and safety of SGLT2i in preclinical models of ADPKD that more closely resemble the kidney conditions: i.e. defective *Pkd1* gene and long natural history/and long-term SGLT2i treatment, leading to severe kidney disease well into adulthood.
2. Detailed characterization of the impact of SGLT2i on vasopressin and vasopressin signaling under nondiabetic conditions.

(B) Clinical. These sequential steps are proposed:
1. Create an SGLT2i-ADPKD task force.
2. Contact the leadership of all phase 3, large scale trials of cardiovascular safety or cardiovascular outcomes to extract, pool and analyze the safety and outcomes of persons with ADPKD that may have been enrolled in these trials.
3. Create a registry of ADPKD patients treated with SGLT2i for diabetes or cardiovascular conditions, assessing safety (urinary tract infections, copeptin, eGFR trajectories, TKV and albuminuria) as well as kidney efficacy (eGFR trajectories, TKV and albuminuria) outcomes.
4. Design short-term exploratory trials addressing the acute and short-term impact of SGLT2i on vasopressin, vasopressin signaling, eGFR and TKV, as well as safety.
5. Based on the result for steps 2 through 4, decide on the need, feasibility and design of larger, long-term phase 2 and phase 3 trials with endpoint efficacy on kidney protection or add warnings to drug labels regarding potential risks in ADPKD patients.

CONCLUSIONS

In conclusion, SGLT2i are currently established heart and kidney protective agents under diabetic and nondiabetic conditions. Thus, ADPKD patients might theoretically benefit from them in terms of kidney and/or heart protection and they may even be prescribed to the patients for cardiovascular conditions. However, clinical experience with SGLT2i is virtually nonexistent, as ADPKD patients were excluded from trials with kidney outcomes and whether they were enrolled in trials of cardiovascular outcomes is unclear. If ADPKD patients were enrolled in cardiovascular trials of SGLT2i, their outcomes have not been analyzed specifically. Preclinical studies had conflicting results because they were marred by the use of suboptimal animal models for a complicated human disease. In any case, there is basis for therapeutic concerns regarding the risk of infection and of increased vasopressin levels. Thus, a roadmap should be established aimed at establishing the safety of SGLT2i in persons with ADPKD, as they may need SGLT2i prescription for cardiovascular conditions, and to explore the eventual kidney benefit of this intervention (Table 1).

ACKNOWLEDGEMENTS

M.K. gratefully acknowledges the use of the services and facilities of the Koc University Research Center for Translational Medicine (KUTTAM), funded by the Presidency of Turkey, Presidency of Strategy and Budget. The content is solely the responsibility of the authors and does not necessarily represent the official views of the Presidency of Strategy and Budget.

FUNDING

This study was not funded by any grant.

AUTHORS' CONTRIBUTIONS

Contributed substantially to the conception or design of the work; or the acquisition, analysis, or interpretation of data for the work: B.A., R.E.A., S.A., A.D., H.K., A.Y. Drafted the work or revised it critically for important intellectual content: B.A., R.E.A., A.O., A.C. and M.K.

CONFLICT OF INTEREST STATEMENT

A.O. has received consultancy or speaker fees or travel support from Astellas, AstraZeneca, Amicus, Amgen, Fresenius Medical Care, Bayer, Sanofi-Genzyme, Menarini, Kyowa Kirin, Alexion, Idorsia, Chiesi, Otsuka and Vifor Fresenius Medical Care Renal Pharma, and is Director of the Catedra Mundipharma-UAM of diabetic kidney disease and the Catedra Astrazeneca-UAM of chronic kidney disease and electrolytes. A.O. is the Editor-in-Chief of *CKJ* and M.K. is member of the *CKJ* editorial board. The other authors declare that they have no conflict of interest.

ETHICAL APPROVAL

This article does not contain any studies with human participants or animals performed by any of the authors.

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