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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

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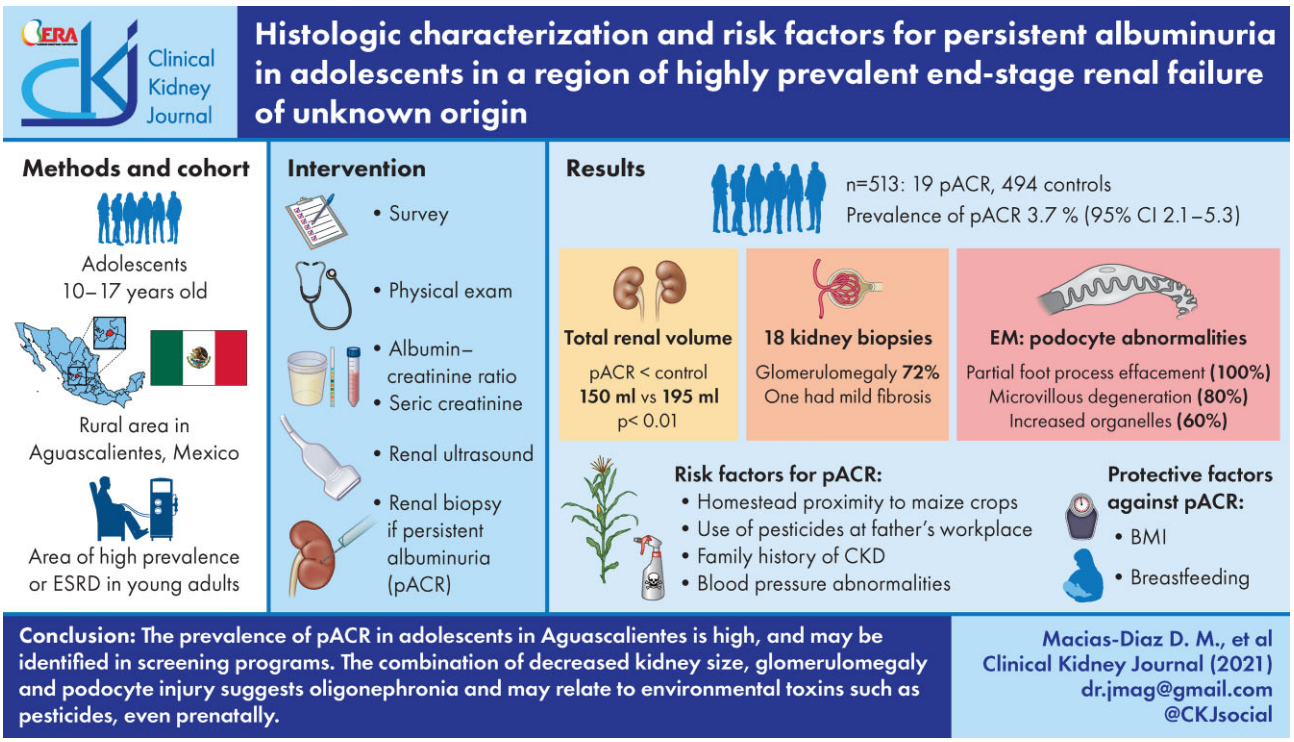
survey were conducted. Patients with an abnormal ACR (≥30 mg/g) or a low glomerular filtration rate (GFR) (≤75 mL/min/1.73 m^2) were re-evaluated and a renal ultrasound (US) was obtained. A kidney biopsy was performed in patients with persistent albuminuria.

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%; $P \leq 0.01$), 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.

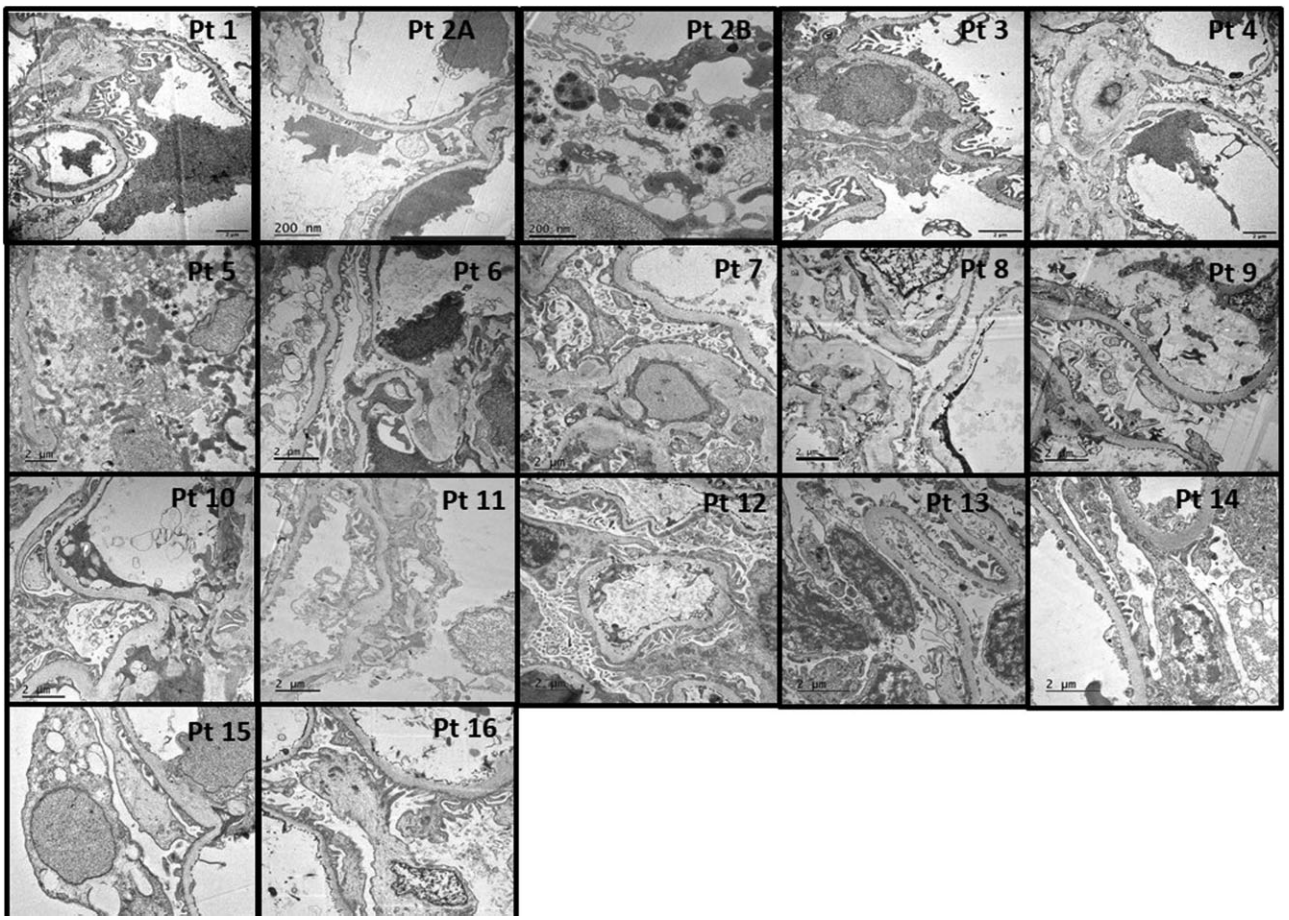


FIGURE 1: Electron microscopy histology images of 16 patients with persistent albuminuria.

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.

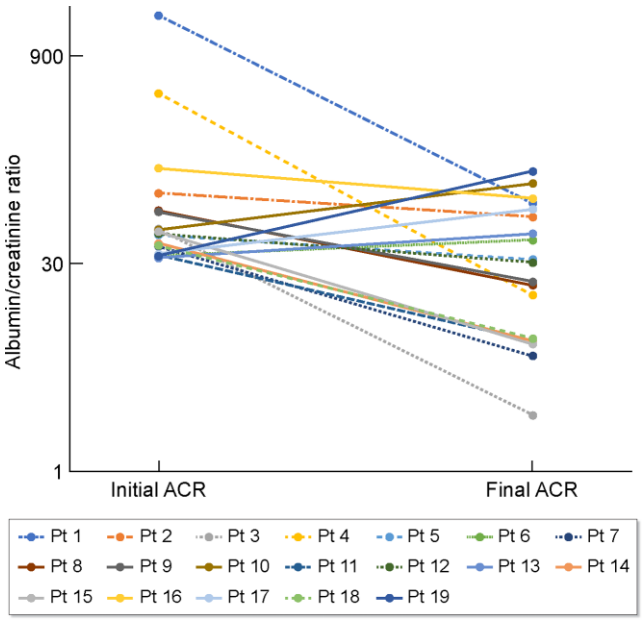


FIGURE 3: Comparison of initial and final ACR in patients with persistent albuminuria (at the time of detection and the end of follow-up) (166 versus 42.7 mg/g; $P = 0.03$).

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.

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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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