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Original Investigation

Renal Morphology, Clinical Findings, and Progression Rate in Mesoamerican Nephropathy

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Background: Mesoamerican nephropathy (MeN) is a chronic kidney disease affecting rural inhabitants in Central America. We have previously described the renal morphology in 8 patients from El Salvador. To confirm the renal pathology, we have studied kidney biopsies from patients with MeN in Nicaragua. Follow-up urine and blood samples from both biopsy studies were collected to investigate the natural history.

Study Design: Case series.

Settings & Participants: In the kidney biopsy study, 19 male sugarcane workers in Nicaragua with suspected MeN were investigated with questionnaires, kidney biopsies, and blood and urine analysis. Inclusion criteria were age 20 to 65 years and plasma creatinine level of 1.13 to 2.49 mg/dL or estimated glomerular filtration rate (eGFR) of 30 to 80 mL/min/1.73 m^2. Exclusion criteria were proteinuria with protein excretion $>$ 3 g/24 h, uncontrolled hypertension, diabetes mellitus, or other known kidney disease. In the follow up-study, blood and urine from the kidney biopsy study in Nicaragua (n = 18) and our previous biopsy study of MeN cases in El Salvador (n = 7) were collected 1 to 1.5 and 2 to 2.5 years after biopsy, respectively.

Outcomes: Renal morphology, clinical, and biochemical characteristics, change in eGFR per year.

Measurements: eGFR was calculated using the CKD-EPI creatinine ($eGFR_{cr}$), cystatin C ($eGFR_{cys}$), and creatinine-cystatin C ($eGFR_{cr\text{-}cys}$) equations.

Results: In the kidney biopsy study, participants had a mean $eGFR_{cr}$ of 57 (range, 33-96) mL/min/1.73 m^2. 47% had low plasma sodium and 21% had low plasma potassium levels. 16 kidney biopsies were representative and showed glomerulosclerosis (mean, 38%), glomerular hypertrophy, and signs of chronic glomerular ischemia. Mild to moderate tubulointerstitial damage and mostly mild vascular changes were seen. In the follow up-study, median duration of follow-up was 13 (range, 13-27) months. Mean change in $eGFR_{cr}$ was -4.4 ± 8.4 (range, -27.7 to 10.2) mL/min/1.73 m^2 per year. Most patients had stopped working with sugarcane cultivation.

Limitations: 3 biopsy specimens had 4 or fewer glomeruli.

Conclusions: This study confirms the renal morphology of MeN: chronic glomerular and tubulointerstitial damage with glomerulosclerosis and chronic glomerular ischemia. Follow-up data show that eGFRs, on average, deteriorated.

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INDEX WORDS: Mesoamerican nephropathy (MeN); chronic kidney disease (CKD); renal pathology; renal morphology; kidney biopsy; Central America; heat stress; dehydration; sugarcane; environmental exposure; CKD of unknown etiology (CKDu); endemic nephropathy; disease progression; Nicaragua; El Salvador.

Mesoamerican nephropathy (MeN), an endemic form of chronic kidney disease (CKD), affects rural inhabitants in Central America.[1-3] Cross-sectional studies in Nicaragua and El Salvador have shown a high prevalence of CKD in villages near the Pacific coast, where agricultural work and other types of manual labor are the main source of employment.[4,5] Men working in sugarcane fields are often

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affected and the disease may progress to chronic kidney failure, a devastating diagnosis in most Central American countries, where the resources for renal replacement therapy are limited. The cause and pathogenesis of MeN are still not fully known, but the extreme occupational conditions of the sugarcane workers have made repeated dehydration the leading hypothesis.[6,7]

Patients with MeN present with elevated creatinine levels, no hypertension, and urine albumin levels are normal or of non-nephrotic range. In 2012, we performed the first study on kidney biopsies and clinical presentations of 8 agricultural workers in El Salvador with MeN.[8] We found a distinctive renal morphology with widespread glomerulosclerosis and signs of chronic glomerular ischemia, but only mild to moderate tubulointerstitial changes. To confirm these findings in a larger cohort and in another area, we have designed the present study in Nicaragua. To examine the progression rate and possible prognostic factors in MeN, we have collected follow-up blood and urine samples from patients in the present biopsy study and also from our previous biopsy study in El Salvador.[8]

METHODS

Nicaragua Kidney Biopsy Study

Men with CKD of unknown cause and a history of sugarcane work who had been to a medical visit at the Research Center of Health, Work and Environment, National Autonomous University of Nicaragua at León during a 12-month period were selected as possible participants. Inclusion criteria were age 20 to 65 years and plasma creatinine level of 100 to 220 μmol/L (1.13-2.49 mg/dL) or estimated glomerular filtration rate (eGFR) of 30 to 80 mL/min/1.73 m^2. Exclusion criteria were proteinuria with protein excretion $>$ 3 g/24 h, uncontrolled hypertension (blood pressure $>$ 140/90 mm Hg or $>$1 antihypertensive drug), diabetes mellitus (blood glucose $>$ 126 mg/dL), or other known kidney disease.

The study was performed in May 2014 (ie, after sugarcane harvest season). Kidney biopsies and blood, urine, and clinical data were collected. Ultrasound examinations of kidneys were carried out before the biopsy. Participants answered questionnaires (both open- and closed-ended questions) about employment (years in different occupations); exposure to chemicals or pesticides (yes/no); fluid intake (volume and type of fluid consumed); current medicine use and estimation of weekly intake of nonsteroidal anti-inflammatory drugs (NSAIDs), acetaminophen, and other analgesics during different periods in their lifetime; smoking habits (past or current smoking); and if they had ever fainted at work (yes/no).

Blood and Urine Samples

Within 1 week prior to the kidney biopsy procedure, blood and urine samples were collected and analyzed at the laboratory of the faculty of medicine at National Autonomous University of Nicaragua at León for hepatitis B and C virus, hematocrit, white blood cell count, international normalized ratio, activated partial thromboplastin time, and urine dipstick, sediment, culture, and 24-hour protein.

At the morning of the biopsy, new blood and urine samples were collected and stored at −20°C for 1 to 4 days, then shipped on dry ice to Karolinska Institutet and stored at −70°C. At Karolinska University Hospital Laboratory (KUHL), plasma was analyzed for creatinine, cystatin C, potassium, sodium, uric acid, calcium, albumin, alanine aminotransferase, antineutrophil cytoplasmic antibody and antinuclear antibody screening, anti−glomerular basement membrane (GBM) antibodies, and complement. Urine was analyzed for albumin, creatinine, uric acid, sodium, potassium, *N*-acetyl-β-D-glucosaminidase (NAG), and α_1-microglobulin. All analyses were performed according to standard protocols. The creatinine and cystatin C assays used were isotope-dilution mass spectrometry (IDMS) traceable. Urine arsenic, cadmium, mercury, and lead were analyzed with inductively coupled plasma mass spectrometry according to US Environmental Protection Agency methods 200.8[9] at ALS Scandinavia AB Laboratory, Luleå, Sweden. When calculating mean values, urinary values below the lower limits of detection were imputed as one-half the lower limit of detection value; albumin $<$ 3.0 mg/L was converted to 1.5 mg/L; NAG $<$ 0.5 nkat/L, to 0.25 nkat/L; α_1-microglobulin $<$ 6 mg/L, to 3 mg/L; cadmium $<$ 0.05 μg/L, to 0.025 μg/L; mercury $<$ 0.2 μg/L, to 0.1 μg/L, and lead $<$ 0.5 μg/L, to 0.25 μg/L. eGFR was calculated using the CKD-EPI (CKD Epidemiology Collaboration) equations for creatinine ($eGFR_{cr}$), cystatin C ($eGFR_{cys}$), and creatinine−cystatin C ($eGFR_{cr\text{-}cys}$).[10,11] Reference values are presented in the tables and were according to KUHL, except for plasma potassium and uric acid (from the Nicaragua laboratory). For urine heavy metal reference range, see references 12 and 13.

Kidney Biopsies

Percutaneous ultrasound-guided kidney biopsies were performed at Hospital La Fraternidad, filial San José, using a spring-loaded needle (16 Ga Biocore II MG/DANA 2.2 MG; Histo). After the biopsy, patients stayed for 24-hour observation. Biopsy specimens were divided and prepared according to standard protocols for light microscopy, immunofluorescence, and electron microscopy (for details see Item S1, available as online supplementary material). De Galantha staining was performed on paraffin-embedded tissue to detect urate crystals.[14]

Two senior consultants in renal pathology (A.W. and M.S.) did individual evaluations of the renal morphology followed by a discussion to reach consensus.

Biopsy specimens with 10 or more glomeruli were rated as representative; 7 to 9 glomeruli, as marginally representative; and fewer than 7 glomeruli, as not representative. Grading of tubular atrophy, interstitial inflammation, and interstitial fibrosis in the cortical area were defined as follows: mild, affecting $<$25% of the area; moderate, affecting 26% to 50%; and severe, affecting $>$50%.[15] Glomerular hypertrophy and vascular pathology were graded semiquantitatively as no/normal, mild, moderate, or severe.

Podocyte foot-process effacement was semiquantified by calculating the number of slits per micron of GBM in 5 random capillaries per glomerulus. Effacement was defined as widespread, ≥80% of measured areas with $<$1.0 slit/μm GBM, or segmental, 21% to 79% of evaluated areas with $<$1.0 slit/μm GBM.

Follow-up of El Salvador Biopsy Study

Seven of 8 patients from our previous kidney biopsy study in El Salvador[8] were followed up with blood and urine sampling 19 to 26 months after kidney biopsy. Samples were collected in May through July (ie, after harvest season). Four samples were transported to KUHL and analyzed for plasma creatinine (IDMS traceable), sodium, and potassium. Three samples were analyzed at Hospital Rosales, San Salvador, with a creatinine assay not traceable to IDMS. $eGFR_{cr}$ was calculated. When calculating change in $eGFR_{cr}$, baseline samples from the same laboratory were used. One patient had initiated hemodialysis therapy; creatinine analyzed before dialysis therapy initiation was used as follow-up. One patient was lost to follow-up.

Follow-up of Nicaragua Kidney Biopsy Study

Of 19 patients, 18 were followed up with blood and urine sampling 13 to 17 months after the biopsy. Samples were collected in June or October (ie, outside of harvest season). Samples were analyzed at KUHL for plasma creatinine (IDMS traceable), sodium, potassium, uric acid, and cystatin C (IDMS traceable) and urine albumin, creatinine, and α_1-microglobulin. $eGFR_{cr}$, $eGFR_{cys}$, and $eGFR_{cr\text{-}cys}$ were calculated. One patient (patient 14) was lost to follow-up.

Ethics Statement

Ethics committees in Stockholm, Sweden (2012/441-31/3; 2013/1225-32; 2015/849-32), at the National Autonomous University of Nicaragua at León (ACTA No 83, 2013; ACTA No 31, 2015), and at the Hospital Nacional Rosales, San Salvador, El Salvador (ACTA No 03, 2012; ACTA exp No 11, 2016) approved the study. All participants have given informed consent.

Statistical Analysis

In the follow-up study, simple linear regression was calculated to predict change in $eGFR_{cr}$ per year based on biochemical data at baseline. Morphology associations were calculated using unpaired *t* test comparing mean change in $eGFR_{cr}$ per year between groups with different morphology; patients with not representative biopsies (Nicaragua patients 4 and 11) were excluded.

RESULTS

Nicaragua Kidney Biopsy Study

There were 100 possible participants identified, 50 of whom matched the inclusion/exclusion criteria and were invited to participate. Twenty patients did not answer and 11 declined. Thus, 19 patients agreed and were included in the study. All participating patients had normal blood pressure 1 week prior to the biopsy.

Questionnaires

Exposure and clinical data are presented in Table 1. All patients had previously been working with sugarcane cultivation for 2.5 to 38 years. Work tasks were mainly cane cutting, irrigation, and seeding. Regular NSAID and acetaminophen use (defined as weekly use for ≥2 years) was reported from 32% and 53% of patients, respectively. There were 47% who reported no regular use of analgesic. Mean self-reported liquid intake for a workday was 9.0 (range, 3.4-15.1) L, and mean current daily liquid intake was 5.3 (range, 1-10) L. Water was the main liquid consumed (77% workdays, 72% of current liquids). Soda and fruit juice were 11% of the intake for workdays and 25% of the current intake. All but 2 patients used electrolyte solutions during the workday (mean, 11% of liquids consumed).

Blood Tests

Results of most analyses are presented in Table 2. Alanine aminotransferase level was elevated in 1 patient. Serum osmolality was elevated in 3 patients, at 304, 304, and 316 mOsm/L. Renin level was elevated in 8 patients; in these patients, mean sodium level was 136 (range, 135-137) mEq/L, 3 had hypokalemia, and all 8 had aldosterone levels within the reference range. In patient 1, aldosterone and aldosterone/renin levels were elevated, at 1,080 pmol/L and 60 pmol/mIU, respectively. All patients had plasma glucose, calcium, and phosphate levels within the reference ranges and had negative screening results for hepatitis B and C virus and human immunodeficiency virus (HIV). No antineutrophil cytoplasmic or anti-GBM antibodies were found. Complement levels showed no signs of activation. Antinuclear antibody screening with immunofluorescence was positive in patient 13; subsequent analysis for double-stranded DNA antibodies, centromere antibodies, and extractable nuclear antigens gave negative results.

Table 1. Nicaragua Biopsy Study: Clinical Characteristics and Questionnaire Data at Time of Kidney Biopsy

Characteristic	Value
Age, y	33 ± 8 (24-54)
BMI, kg/m^2	25 ± 4 (19-37)
Systolic BP, mm Hg	121 ± 8 (105-133)
Diastolic BP, mm Hg	75 ± 7 (56-88)
Kidney length, mm	91 ± 8 (74-104)
Duration of sugarcane work,[a] y	12 ± 8 (2.5-38)
Duration as sugarcane cutter, y	7 ± 7 (0-26)
Duration of agricultural work,[b] y	15 ± 10 (3-40)
Antihypertensive medicine	0 (0)
Antibiotics past 6 mo	0 (0)
Regular long-time NSAID use[c]	6 (32)
Current NSAID use	4 (21)
Current acetaminophen use	7 (37)
Herbal medicine use	1 (5)
Ever fainted at work	7 (37)
Exposure to chemicals/pesticides	7 (37)
Current smoking	0 (0)
Past smoking	6 (32)
Alcohol consumption	4 (21)

Note: n = 19. Values for categorical variables are given as count (percentage); for continuous variables, as mean ± standard deviation (range).

Abbreviations: BMI, body mass index; BP, blood pressure; NSAID, nonsteroidal anti-inflammatory drug.

[a]Defined as work at sugarcane plantation.

[b]Agricultural work including sugarcane work and other agricultural work.

[c]Use of 1 to 2 or more tablets per week during 2 years or longer.

Urine Tests

Results regarding urinary biomarkers and heavy metals are presented in Table 3. Two of the 4 patients with hypokalemia had urine potassium excretion > 40 mEq/L and all 4 hypokalemic patients had urine potassium-creatinine ratios > 1.5 mEq/mmol, indicating renal potassium losses.[16] In the 9 patients with low plasma sodium levels, mean urine sodium excretion was 88.6 ± 51.6 (range, 21-172) mEq/L. Dipstick showed no hematuria, leukocytes, or

Table 2. Nicaragua Biopsy Study: Blood Tests at Time of Biopsy

Variable	Mean ± SD (Range)
$eGFR_{cr-cys}$, mL/min/1.73 m^2	56 ± 18 (36-96)
$eGFR_{cr}$, mL/min/1.73 m^2	57 ± 18 (33-96)
$eGFR_{cys}$, mL/min/1.73 m^2	57 ± 20 (38-109)
Plasma	
Creatinine, mg/dL	1.66 ± 0.38 (1.04-2.51)
Cystatin C, mg/L	1.47 ± 0.31 (0.85-1.89)
Potassium, mEq/L	3.8 ± 0.6 (2.2-4.9)[a]
Sodium, mEq/L	137 ± 2 (133-139)[b]
Magnesium, mEq/L	1.41 ± 0.22 (1.02-1.64)[c]
Uric acid, mg/dL	7.45 ± 2.02 (4.44-12.02)[d]
Renin, mIU/L	68 ± 134 (18-650)
Aldosterone, pmol/L	265 ± 217 (86-1,080)
Aldosterone-renin ratio, pmol/mIU	9.5 ± 13.0 (0.3-60)
Albumin, g/dL	4.4 ± 0.3 (3.8-5.1)
Alanine aminotransferase, U/L	31 ± 33 (3-151)
Serum osmolality, mOsm/kg	296 ± 7 (288-316)

Note: n = 19. Reference values: eGFR, >90 mL/min/1.73 m^2; creatinine, <1.13 mg/dL; cystatin C, <0.99 mg/L; potassium, 3.5 to 5.0 mEq/L; sodium, 137 to 145 mEq/L; magnesium, 1.4 to 1.9 mEq/L; uric acid, 3.0 to 7.0 mg/dL; osmolality, 280 to 300 mOsm/kg; renin, 2.8 to 40 mIU/L; aldosterone, <650 pmol/L; albumin, 3.6 to 4.5 g/dL; alanine aminotransferase, <72 U/L; and aldosterone to renin ratio, <60 pmol/mIU. Conversion factors for units: creatinine in mg/dL to μmol/L, ×88.4; magnesium in mEq/L to mmol/L, ×0.5; uric acid in mg/dL to μmol/L, ×59.48; alanine aminotransferase in U/L to μkat/L, ×0.0167.

Abbreviations: $eGFR_{cr}$, estimated glomerular filtration rate based on creatinine; $eGFR_{cr-cys}$, estimated glomerular filtration rate based on creatinine and cystatin C; $eGFR_{cys}$, estimated glomerular filtration rate based on cystatin C; SD, standard deviation.

[a]Four (21%) patients had plasma potassium <3.5 mEq/L.
[b]Nine (47%) patients had plasma sodium <137 mEq/L.
[c]Seven (37%) patients had plasma magnesium <1.4 mEq/L.
[d]Eleven (58%) patients had plasma uric acid >7.0 mg/dL.

Table 3. Nicaragua Biopsy Study: Urine Test Results at Time of Biopsy

Urine Variable	Mean ± SD (Range)
Culture	All negative
Potassium, mEq/L	33 ± 17 (4-61)
Potassium-creatinine ratio, mEq/mmol	4.2 ± 1.3 (2.7-8.9)
Sodium, mEq/L	119 ± 59 (21-254)
pH	6.2 ± 0.4 (6-7)
24-h urine volume, L	3.1 ± 1.4 (1.3-6.7)[a]
ACR, mg/g	64 ± 194 (0.8-809)[b]
NAG-creatinine ratio, nkat/g	68 ± 53 (5-186)[c]
A1M-creatinine ratio, mg/g	27 ± 33 (3-141)[d]
Arsenic, μg/L	10.3 ± 6.6 (1.31-20.1)
Cadmium-creatinine ratio, μg/g	0.10 ± 0.09 (0.02-0.336)[e]
Mercury-creatinine ratio, μg/g	0.32 ± 0.25 (0.05-0.88)[f]
Lead-creatinine ratio, μg/g	0.98 ± 0.88 (0.15-2.93)[g]

Note: n = 19. Reference values: 24-hour urine volume, 0.5 to 3 L; ACR, <30 mg/g; NAG-creatinine ratio, <71 nkat/g; A1M-creatinine ratio, <6.2 mg/g; arsenic, <100 μg/L; cadmium-creatinine ratio, <2 μg/g; mercury-creatinine ratio, <5 μg/g; and lead-creatinine ratio, <50 μg/g. Conversion factors for units: ACR in mg/g to mg/mmol, ×0.113; NAG-creatinine ratio in nkat/g to nkat/mmol, ×0.113; A1M-creatinine ratio in mg/g to mg/mmol, ×0.113.

Abbreviations: A1M, α_1-microglobulin; ACR, albumin-creatinine ratio; NAG, *N*-acetyl-β-D-glucosaminidase; SD, standard deviation.

[a]Nine (47%) patients had 24-hour urine volume >3 L.
[b]Two (11%) patients had ACRs >30 mg/g.
[c]Eight (42%) patients had NAG-creatinine ratio > 71 nkat/g.
[d]Thirteen (68%) patients had A1M-creatinine ratios > 6.2 mg/g.
[e]Eight (42%) patients had no detectable urinary cadmium (ie, <0.05 μg/L).
[f]Ten (53%) patients had no detectable urinary mercury (ie, <0.2 μg/L).
[g]Ten (53%) patients had no detectable urinary lead (ie, <0.5 μg/L).

glucosuria; dipstick protein was increased in 2 patients (trace and 3+, respectively). Sediment showed no casts. Patients 7 and 15 had increased urinary albumin-creatinine ratios at 316 and 809 mg/g, respectively.

Kidney Biopsies

All 19 participants underwent kidney biopsy. One participant developed transient macroscopic hematuria; the other participants had no adverse events.

Light Microscopy and Immunofluorescence

Overview

There were 13 biopsy specimens that were representative; 3, marginally representative; and 3, not representative. Polarized light on paraffin-embedded tissue detected no crystals. No uric acid crystals were found using de Galantha staining. In 9 patients, frozen tissue was available for evaluation with polarized light; in 1 patient (patient 12), 1 round-shaped crystal was seen. Evaluation of the representative and marginally representative biopsy specimens (n = 16) are presented in the following text and Table 4. Information about the not representative biopsies (n = 3) is presented in Table S1.

Glomerular Changes

Specimens showed global glomerulosclerosis of varying proportions, 7% to 70%, and hypertrophy of the remaining glomeruli (Fig 1A). In patients 7 and 15, glomerular segmental sclerosis were found. Discrete mesangial matrix expansion was seen in most patients, but no mesangial cell proliferation. Focal wrinkling of the GBM and/or periglomerular fibrosis was seen in all but 1 patient (Fig 1A, C, and D). In 4 patients, no representative material for immunofluorescence was collected due to limited biopsy material. In the other patients, immunofluorescence showed no signs of immune complex disease.

Table 4. Nicaragua Biopsy Study: Light and Electron Microscopy Findings of Representative and Marginally Representative Biopsies

Microscopy Findings	Value
Light microscopy findings (n = 16)	
Glomerular changes (n = 16)	
Mean globally sclerosed glomeruli	38% ± 21% (7%-70%)
Percentage globally sclerosed glomeruli	
<25%	4 (25%)
25%-50%	7 (44%)
>50%	5 (31%)
Segmental scleroses	2 (12.5%)
Glomerular hypertrophy	
0	0 (0%)
1	0 (0%)
2	11 (69%)
3	5 (31%)
Wrinkled GBM/periglomerular fibrosis	
Yes	15 (94%)
No	1 (6%)
Tubulointerstitial changes (n = 16)	
Tubular atrophy[a]	
0	1 (6%)
1	13 (81%)
2	2 (13%)
3	0 (0%)
Interstitial fibrosis[a]	
0	1 (6%)
1F	8 (50%)
2F	7 (44%)
3F	0 (0%)
Interstitial inflammation[a]	
0	2 (13%)
1	12 (75%)
2	2 (13%)
3	0 (0%)
Vascular changes (n = 15)	
Intimal thickening	
0	11 (73%)
1	3 (20%)
2	1 (7%)
3	0 (0%)
Smooth muscle hyperplasia	
0	5 (33%)
1	6 (40%)
2	4 (27%)
3	0 (0%)
Electron microscopy findings (n = 16)	
GBM thickness, nm[b]	441 ± 63 (333-566)[c]
Podocyte foot processes, slits/μm GBM	1.0 ± 0.5 (0.1-1.7)
Podocyte foot process effacement[d]	
No (normal)	7 (44%)
Segmental effacement	4 (25%)
Widespread effacement	5 (31%)
Endothelial cells	
Normal	10 (62%)
Swollen	6 (38%)
Podocyte cytoplasm inclusions	
Yes	12 (75%)
No	4 (25%)
Immune complex deposits	
Yes	0 (0%)
No	16 (100%)

Note: Values are given as cases (percentage) or mean ± standard deviation (range). Mean number of glomeruli (n = 16): 17 ± 7 (7-28). Grading: 0, normal; 1, mild; 2, moderate; and 3, severe.

Abbreviations: F, focal; GBM, glomerular basement membrane.

[a]Grading of tubular atrophy, interstitial inflammation, and interstitial fibrosis is defined as 1 = mild, affecting <25% of area; 2 = moderate, affecting 26% to 50%; and 3 = severe, affecting >50%.

[b]GBM thickness normal range, 250 to 457 nm.

[c]Five (31%) patients had thickened GBM.

[d]Podocyte foot-process effacement defined as widespread, ≥80% of evaluated areas with <1.0 slit/μm GBM; segmental, 21%-79% of evaluated areas with <1.0 slit/μm GBM.

Tubulointerstitial Changes

Interstitial fibrosis was mild to moderate (Fig 1B) in most patients. Interstitial inflammation was generally mild, located in fibrotic areas, and consisting of lymphocytes. A few neutrophil granulocytes were found in tubuli of 2 patients. In 2 patients, a few eosinophils were found in the interstitium. Mild tubular atrophy was common (Fig 1C and D). Mild tubulitis was seen in 1 patient.

Vascular Changes

Arteries were found in all but 1 patient. Arterial changes were generally mild (Fig 1C and D; Table 4). Five specimens showed arteriolar hyalinosis (mild in 3 patients, moderate in 2).

Electron Microscopy

Results are presented in Tables 4 and S2. No immune deposits were found. Mild thickening of the GBM due to homogenous thickening of the lamina densa was seen in 5 patients. The podocytic cytoplasm often contained vacuoles (Fig 2A) or lipofuscin-like bodies (Fig 2B). Cell debris, probably derived from podocytes, was seen between capillary loops in Bowman's space in 3 patients (Fig 2C). The parietal epithelial cells of Bowman's capsule displayed no apparent pathology. Podocytic foot-process effacement was seen in 9 patients (Fig 2D). The endothelium was mostly normal, but in 6 patients, it was swollen at varying degrees (Fig 2D). In 4 patients, the swollen endothelium also showed reduced fenestration. Tubular structures showed varying

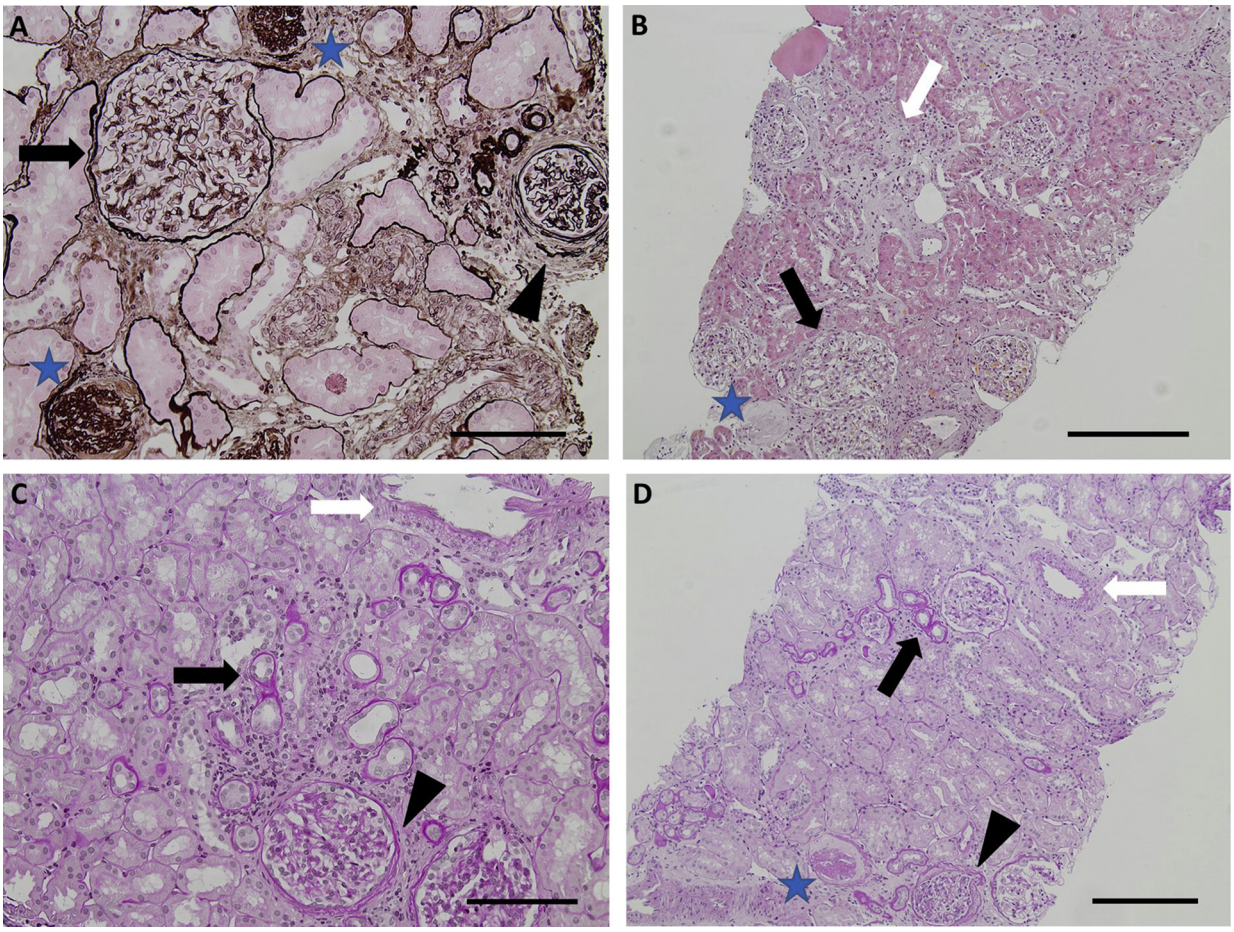

Figure 1. Light microscopy findings in kidney tissue from the Nicaragua biopsy study. Global glomerulosclerosis of varying degree (stars in A [periodic acid–Schiff (PAS)-methenamine staining from patient 1], B [Ladewig staining from patient 9], and D [PAS staining from patient 9]) and (A, B) moderate to severe glomerular hypertrophy (black arrow) were found in all patients. Signs of glomerular ischemia with thickening of Bowman's capsule or wrinkling of capillary walls (arrowheads in A, C [PAS staining from patient 6], and D) were found in all but 1 patient. (B) Mild to moderate interstitial fibrosis (white arrow) and (C, D) tubular atrophy of varying degree (black arrows) were seen in most patients. (C, D) Arteries were in most cases normal (white arrows) or only mildly changed. Scale bars = (A, C) 100 μm, (B, D) 200 μm.

degrees of tubular atrophy, but otherwise no apparent pathology.

Follow-up of El Salvador and Nicaragua Biopsy Studies

In total, 25 of 27 participants in the 2 biopsy studies were included in the follow-up, and they had a mean change in $eGFR_{cr}$ per year of -4.4 ± 8.4 (range, -27.7 to 10.2) mL/min/1.73 m^2.

El Salvador Follow-up

Seven of 8 patients were followed up 19 to 26 months after kidney biopsy (Table 5). One patient had initiated hemodialysis therapy after 19 months (with $eGFR_{cys}$ of 9 mL/min/1.73 m^2 3 days before dialysis therapy initiation). Urine samples were available for 5 patients; 2 had increased urine albumin-creatinine ratios (2,458 and 45 mg/g). Two patients were working with sugarcane, 4 with other agricultural work, and 1 was unemployed.

Nicaragua Follow-up

Eighteen of 19 patients were followed up 13 to 17 months after kidney biopsy (Table 5). Urine albumin-creatinine ratios were increased in patients 7 and 19 (462 and 2,270 mg/g). Ten patients were unemployed or on pension; 2, construction workers; 1, an agricultural worker (not sugarcane); 1, a baker; 1, a mechanic; and 3, in non–physically strenuous professions.

Possible Predictors of Decrease in eGFR

Simple linear regression was performed to predict change in $eGFR_{cr}$ based on various biochemical data at the time of the biopsy (t_0); results are presented in Table S3. A significant correlation was seen between plasma sodium level and change in $eGFR_{cr}$ per year when combining both cohorts (El Salvador and Nicaragua, n = 25). Introducing baseline eGFR as a covariate in the equation did not significantly ($P = 0.7$) influence the decline in eGFR related to plasma sodium

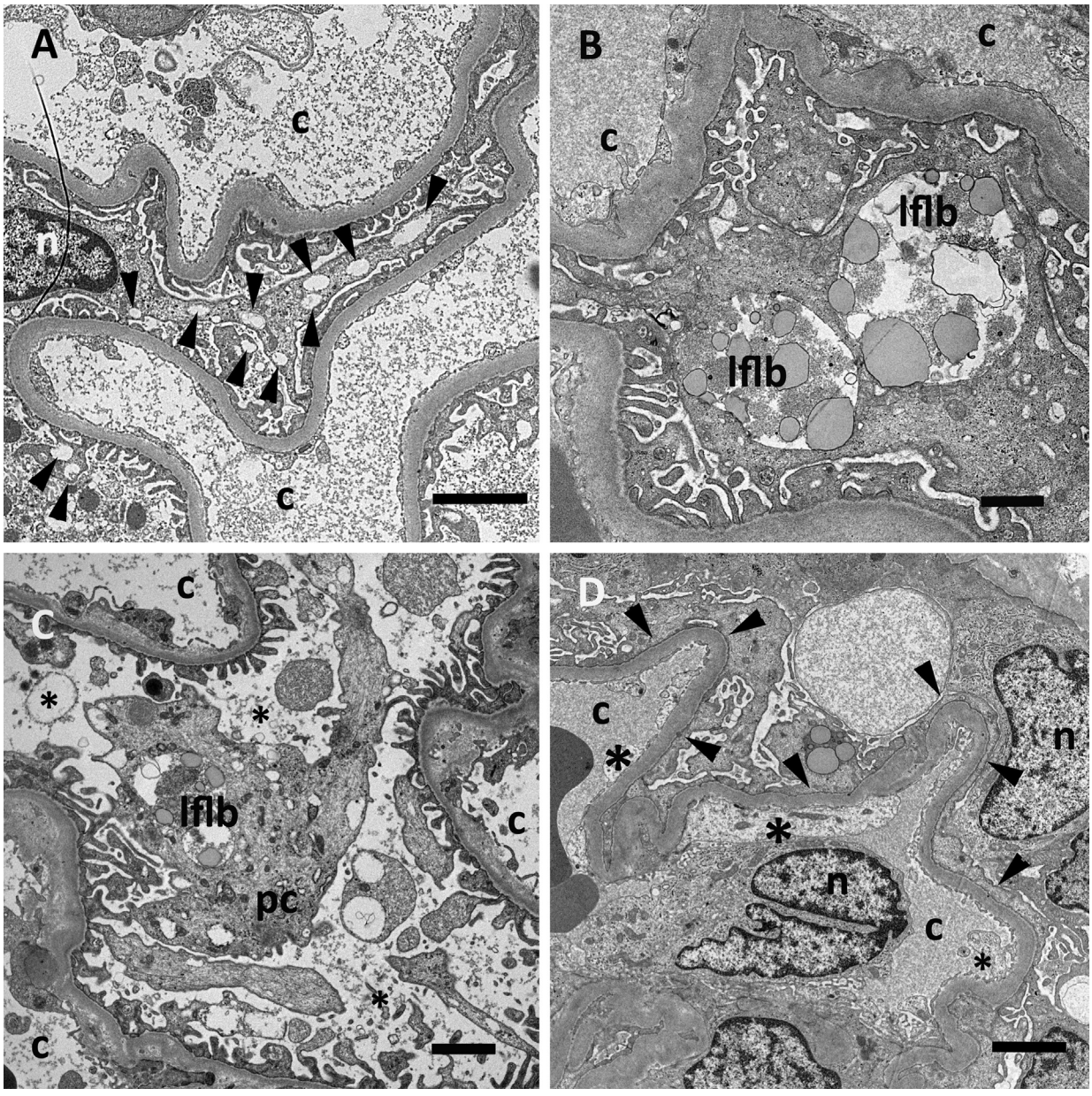

Figure 2. (A) Transmission electron microscopy image from patient 10 shows cytoplasm of a podocyte (pc) containing vacuoles (arrowhead). (B) Image from patient 17 shows lipofuscin-like bodies (lflb) in cytoplasm of a podocyte. (C) Image from patient 6 shows cell debris in Bowman's space (⋆), probably derived from podocytes because it was mostly found within the glomerular tuft between capillary loops and Bowman's capsular epithelium showed no apparent pathology. (D) Image from patient 17 shows widespread foot-process effacement (arrowhead) and a focally swollen endothelium (*). Abbreviations: c, capillary space; n, nucleus. Scale bars = (A) 5 μm, (B-D) 1 μm.

level ($P = 0.02$). Therefore, baseline eGFR was not used as a covariate in any of the calculations in Table S3.

Patients with severe glomerular enlargement had a significantly larger decrease in $eGFR_{cr}$ compared with patients with mild to moderate enlargement (Table S4).

DISCUSSION

This unique study describes the renal morphology and biochemical characteristics of 19 patients with MeN in Nicaragua and also presents 1- to 2-year follow-up data from this cohort, as well as from our previous biopsy study of 8 patients with MeN in El Salvador.

The renal morphology shows glomerulosclerosis of varying degrees, glomerular hypertrophy, and signs of chronic glomerular ischemia, together with mild to moderate chronic tubulointerstitial damage. The morphology is very similar to findings in our previous study of MeN cases.[8]

Table 5. Follow-up Plasma and Urine Data From El Salvador and Nicaragua Kidney Biopsy Studies

	El Salvador Biopsy (n = 7)	Nicaragua Biopsy (n = 18)
Postbiopsy follow-up time, mo	25 ± 3 (19-27)	14 ± 2 (13-17)
$eGFR_{cr}$, mL/min/1.73 m^2	45 ± 25 (9 to 76)	52 ± 20 (20 to 87)
$\Delta eGFR_{cr}$, mL/min/1.73 m^2 per y	−3.6 ± 5.2 (−13.3 to 2.8)	−5.3 ± 9.2 (−27.7 to 10.2)
$eGFR_{cr\text{-}cys}$, mL/min/1.73 m^2		48 ± 20 (18 to 85)
$\Delta eGFR_{cr\text{-}cys}$, mL/min/1.73 m^2 per y		−7.0 ± 8.2 (−24.9 to 6.5)
Plasma		
Potassium, mEq/L	3.3 ± 0.7 (1.9 to 4)[a]	4.3 ± 0.6 (2.8 to 4.8)[b]
Sodium, mEq/L	132 ± 4 (126 to 136)	138 ± 1 (135 to 140)[b]
Uric acid, mg/dL	—	7.30 ± 2.52 (4.12 to 12.19)[b]
Urine		
ACR, mg/g	508 ± 1,091 (4 to 2,458)[a]	155 ± 539 (1 to 2,270)[b]
NAG:Cr ratio, nkat/g	108 ± 46 (53 to 168)[a]	
A1M:Cr ratio, mg/g	-	24 ± 34 (4.5 to 144)[b]

Note: Values are given as mean ± standard deviation (range). Reference values: potassium, 3.5 to 5.0 mEq/L; sodium, 137 to 145 mEq/L; uric acid, 3.0 to 7.0 mg/dL; ACR, <30 mg/g; NAG:Cr ratio, <71 nkat/g; A1M/Cr ratio, <6.2 mg/g. To convert uric acid in mg/dL to μmol/L, ×59.48.

Abbreviations: A1M, α_1-microglobulin; ACR, albumin-creatinine ratio; Cr, creatinine; $eGFR_{cr}$, estimated glomerular filtration rate based on creatinine; $eGFR_{cr\text{-}cys}$, estimated glomerular filtration rate based on creatinine and cystatin C; NAG, *N*-acetyl-β-D-glucosaminidase.

[a]Potassium was <3.5 mEq/L in 4 patients; ACR, >30 mg/g in 2 patients; and NAG:Cr ratio, >71 nkat/g in 4 patients.

[b]Potassium was <3.5 mEq/L in 2 patients; sodium, <137 mEq/L in 4 patients; uric acid, >7.0 mg/dL in 7 patients; ACR, >30 mg/g in 2 patients; and A1M:Cr ratio, >6.2 mg/g in 11 patients.

We used similar inclusion and exclusion criteria as in our previous biopsy study, but this study included twice as many patients, enabling a more detailed description of the disease. Furthermore, $eGFR_{cr\text{-}cys}$ in this study is higher (mean, 56 compared to 42 mL/min/1.73 m^2) and participants are younger (mean, 33 compared to 44 years), thus reducing possible age-related morphologic changes and describing earlier lesions in MeN.

Chronic glomerular changes were seen in all patients. Glomerular hypertrophy was present in all patients and could be the result of compensatory mechanisms due to nephron loss/low nephron count or chronic ischemia.[17,18] In all but one patient, wrinkling of the GBM and/or periglomerular fibrosis was seen. These changes, indicating glomerular ischemia, are often found in nephrosclerosis,[19] but neither hypertension nor vascular changes consistent with hypertension were found. Immune complex glomerulonephritis could be excluded in all representative biopsies by immunofluorescence or electron microscopy.

The chronic tubulointerstitial damage was in most cases mild and in some cases was moderate. Signs of tubular damage were also evident in urine, in which NAG or α_1-microglobulin levels often were elevated. A few granulocytes were found in tubuli of 2 patients, but because urine culture results were negative, acute pyelonephritis is unlikely. Chronic pyelonephritis is more difficult to exclude. However, in a previous report, urine cultures from 103 sugarcane workers all gave negative results, indicating that infections are uncommon.[20]

Podocyte foot-process effacement was found in 9 of 16 patients, but was not correlated to albuminuria because only 1 of these patients displayed albuminuria. Other signs of podocytic injury, including podocyte debris and lipofuscin-like bodies, were also found. Whether there is primary damage to the podocytes or secondary damage due to glomerular hypertrophy is unclear.

In 2014, López-Marín et al[21] published a study of kidney biopsies from 46 Salvadoran patients with eGFRs of 30 to 89 mL/min/1.73 m^2. They concluded that their main finding was "chronic tubulointerstitial nephropathy" with subsequent glomerular and vascular damage. However, they also reported that 67% of the men in the study had >25% global glomerulosclerosis and 83% of the men had <25% tubular atrophy (ie, results similar to our findings).

Based on our previous study, others have described the morphology of MeN as "chronic tubulointerstitial nephropathy with secondary glomerular damage."[22] We do not agree with this phrase because we would describe the morphology as a combination of chronic glomerular and tubulointerstitial changes for which the mechanism has to be elucidated. That tubulointerstitial damage can cause glomerular damage or vice versa is well known.[23] In our experience, the ratio between the glomerular and cortical tubulointerstitial damages found in our studies of MeN suggest that the tubulointerstitial damage alone might not be enough to explain the glomerular changes.

The finding of low plasma sodium, potassium, and magnesium levels in many patients (Tables 2 and 5) is striking. Spot urine samples suggest that the hypokalemic patients in our Nicaraguan cohort have renal potassium losses, a finding that also has been reported from another MeN study.[24] Interestingly, when combining data from both biopsy cohorts, low sodium at baseline was correlated with a higher progression rate (Table S3). To establish if this is a correlation or causation, further studied in larger cohorts are needed. Hyponatremia correlated to sugarcane work,[25] as well as extreme exercise,[26,27] has been described, but our study was performed outside of harvest season, indicating a chronic state independent from the extreme conditions during sugarcane harvest. The participants' large urine volumes (mean, 3.1 L/24 h) are noteworthy. Excessive water intake may contribute to the hyponatremia in some patients. We have previously argued for possible renin-angiotensin-aldosterone system activation as one of the mechanisms behind the glomerular ischemia and hypokalemia seen in MeN.[8] Our findings further support the hypothesis that chronic sodium depletion may stimulate the renin-angiotensin-aldosterone system, whereby angiotensin II would cause glomerular ischemia by constriction of glomerular capillaries and aldosterone would cause hypokalemia. Plasma renin level was increased in 8 patients, who also were low or in the lower reference range for plasma sodium. However, aldosterone levels were in most cases within the reference ranges, making the data difficult to interpret. Analysis of renin, angiotensin II, and aldosterone during harvest would be of great interest.

To our knowledge, this is the first report presenting follow-up data from patients with kidney biopsy−verified MeN. Our data show a mean decline in $eGFR_{cr}$ of 4.4 mL/min/1.73 m^2 per year. However, individual variation in change in eGFR over time was great (Table 5).

When comparing kidney morphology with progression rate, we found that patients with severe glomerular hypertrophy had a worse prognosis (Table S4). This finding needs to be confirmed in larger studies.

The cause of MeN is not fully understood, but the leading hypothesis is occupational heat exposure with repeated episodes of volume and salt depletion.[6,28,29] That volume depletion can lead to acute kidney injury (AKI) is known, and in recent years, AKI has been found to be a risk factor for CKD development.[30,31] Three studies of sugarcane workers report increasing serum creatinine levels of about 10% to 20% after a workday,[25,29,32] supporting the hypothesis that repeated AKI caused by extreme working conditions may be the major cause of MeN.

Interestingly, a morphologic picture similar to that of MeN has been described in rats developing CKD after AKI.[33-35] Kidney morphology 9 months after ischemic AKI is described as glomerular hypertrophy, glomerulosclerosis, podocyte foot-process effacement, and tubulointerstitial damage. Furthermore, rats developing CKD after AKI were normotensive, another similarity with patients with MeN. Spironolactone and losartan treatment prevented the development of CKD, suggesting that these agents, affecting the renin-angiotensin-aldosterone system, are of importance in the pathophysiology.

A possible mechanism for developing CKD due to repeated dehydration involving fructose metabolism in tubuli has been presented in a study of mice.[36] The authors suggest the main pathologic mechanism to be tubular damage caused by end products in the fructose metabolism (inflammatory mediators, oxidants, and uric acid). Hyperuricemia and hyperuricosuria have also been hypothesized as possible mechanisms.[37] In our study, we did not find support for uric acid nephropathy; no uric acid crystals or lesions related to hyperuricemia were seen. However, we cannot exclude the possibility because biopsies were not performed during dehydration.

The role of NSAIDs in the development of MeN has been discussed.[1,38] In the present study, most Nicaraguan participants did not use NSAIDs. However, the pharmacodynamic properties of NSAIDs, reducing renal blood flow, are probably harmful in MeN, in which glomerular ischemia is already present.

Heavy metals have been raised as possible cofactors in MeN development.[39] In this study, we could not find evidence for this because all values were below toxic levels.[12,13,40]

In recent years, the resemblance between MeN and CKD of unknown origin affecting rural inhabitants in Sri Lanka has been discussed.[38,41] Tubulointerstitial damage has been described as the main lesion in kidney biopsy specimens, but glomerulosclerosis, glomerular enlargement, and thickening of Bowman's capsule have also been reported,[42,43] indicating a resemblance with MeN. Most biopsy studies in Sri Lanka have included participants by screening for proteinuria, and this makes comparison difficult because patients with MeN often lack proteinuria.

In summary, we propose that the findings in our study are compatible with the hypothesis that MeN is caused by recurrent kidney injury due to occupational heat stress with subsequent volume and salt depletion. Kidney function often deteriorates in patients with MeN, and risk factors for this should be further studied to enable preventive strategies. Comparative studies with CKD of unknown cause in other countries are needed to elucidate whether MeN might be a global disease, accelerated by global warming.

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SUPPLEMENTARY MATERIAL

Table S1: Nicaragua biopsy study, light microscopy findings of not-representative biopsies.

Table S2: Nicaragua biopsy study, electron microscopy findings.

Table S3: Biochemical predictors of change in eGFR.

Table S4: Morphology predictors of change in eGFR per year.

Item S1: Tissue preparation of kidney biopsies.

Note: The supplementary material accompanying this article (http://dx.doi.org/10.1053/j.ajkd.2016.10.036) is available at www.ajkd.org

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