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Original Investigation

Clinical and Pathological Characterization of Mesoamerican Nephropathy: A New Kidney Disease in Central America

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Background: An endemic of chronic kidney disease (CKD) of unknown cause among rural inhabitants in Central America has been identified. Young and otherwise healthy men working in plantations are frequently affected. The name Mesoamerican nephropathy (MeN) has been suggested. Clinically, MeN presents with low-grade proteinuria and progressive kidney failure. The renal pathology of this disease has not yet been described.

Study Design: Case series.

Setting & Participants: 8 male patients with CKD of unknown cause and clinically suspected MeN were recruited from a nephrology unit in El Salvador. All recruited patients had been working on plantations. Kidney biopsies, blood, and urine samples were collected.

Outcomes & Measurements: Renal morphology examined with light microscopy, immunofluorescence, and electron microscopy; clinical and biochemical characteristics.

Results: A similar pattern was seen in all 8 biopsy specimens, with extensive glomerulosclerosis (29%-78%) and signs of chronic glomerular ischemia in combination with tubular atrophy and interstitial fibrosis, but only mild vascular lesions. Electron microscopy indicates podocytic injury. Biochemical workup showed reduced estimated glomerular filtration rate (27-79 mL/min/1.73 m^2 with the CKD Epidemiology Collaboration [CKD-EPI] creatinine equation), low-grade albuminuria, and increased levels of tubular injury biomarkers. Hypokalemia was found in 6 of 8 patients.

Limitations: Small number of patients from one country.

Conclusions: This study is the first report of the biochemical and morphologic findings in patients with MeN. Our findings indicate that MeN constitutes a previously unrecognized kidney disease with damage to both glomerular and tubulointerstitial compartments.

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INDEX WORDS: Chronic kidney disease; Central America; sugarcane; renal pathology; occupational and environmental health; El Salvador; Mesoamerican nephropathy.

Rural inhabitants in Central America have an endemic form of chronic kidney disease (CKD) of unknown cause and with as yet insufficiently described clinical and morphologic characteristics.[1,2] The nephropathy was reported to affect primarily young male workers engaged in sugarcane cultivation, but later the disease also was reported in other types of farming. According to local physicians, the affected individuals present with various degrees of CKD but usually are normotensive and have no hematuria, and proteinuria is absent or of non–nephrotic range. At a workshop in Costa Rica in 2012, the disease was named Mesoamerican nephropathy (MeN). Among local health professionals and inhabitants, awareness of the problems involving CKD and subsequent morbidity and mortality in certain regions of Nicaragua, Costa Rica, and El Salvador has been emerging for several years.[3] Health statistics from the World Health Organization show that El Salvador has the highest mortality rate from kidney disease in the world, especially among male inhabitants.[4]

Four extensive cross-sectional examinations of populations in Central America have used measurements of serum creatinine to confirm the existence of an increased prevalence of CKD in certain rural populations.[5-8] Three of these studies were conducted in Nicaragua, and one, in El Salvador. These studies show a pattern of more males than females being affected,[5-8]

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villages in which agricultural work (particularly plantation work at low altitude) is the predominant source of employment having a higher prevalence of CKD than service-oriented villages,[5,7,8] and proteinuria among affected individuals usually being low.[5-7] In the most recent study performed in El Salvador by Peraza et al,[7] 664 inhabitants aged 20-60 years in 5 communities were studied; 2 were coastal communities with sugarcane production and 3 communities were located at higher altitude, with sugarcane, coffee, and service-oriented economies. The prevalence of decreased estimated glomerular filtration rate (eGFR) <60 mL/min/1.73 m^2 in men was 18% in the coastal communities with sugarcane production compared to 1% in the communities at higher altitude. Nicaragua has similar areas with low prevalence of CKD.[9]

Many of the affected slowly develop terminal kidney failure. There is a lack of renal replacement therapy facilities with dialysis or kidney transplantation in Nicaragua and El Salvador, and thus many of those affected eventually will die of uremia. According to a recent overview, the death toll from CKD is likely to have reached at least 20,000 in this region.[2]

Considerable efforts have been made to elucidate the cause, or causes, of this endemically occurring CKD. At an early stage, suspicion was raised that the CKD was brought about by occupational exposure to pesticides that frequently are used during sugarcane and cotton production. A research group from Boston (the Boston University investigation of CKD in Western Nicaragua) has carried out a series of studies in Nicaragua in collaboration with a local team of researchers. To date, little evidence has come forth supporting the contention that exposure to agrochemicals, pesticides, heavy metals, or locally occurring infections are causative.[10] Instead, it has been hypothesized that repeated heat stress with excessive sweating and volume depletion during heavy manual work may be a causative factor, perhaps in combination with the use of nonsteroidal anti-inflammatory drugs (NSAIDs), which are easily available over the counter and frequently used.[11] The significance of heat stress during long days of hard manual work and possible repeated episodes of fluid depletion is supported by male predominance, a high prevalence in farming villages at a low altitude close to the Pacific coast (where the climate is hottest and most humid), and the occurrence of MeN irrespective of the type of crop produced. In further support of an association between long-term and repeated heat stress, a strong statistical association has been found between the relative risk for elevated creatinine level and years of work on coastal sugarcane or cotton plantations.[7]

With the low degree of proteinuria involved, MeN being caused by a tubulointerstitial type of nephritis rather than a glomerular disease has been suggested.[5-7] However, only relatively few kidney biopsies have been done in patients from the area, and to our knowledge, no report of the morphology of MeN has been published to date. Thus, the real nature of MeN is unknown.

In this report, we present clinical and renal morphology features from 8 men who have undergone kidney biopsy and, on a clinical basis, are considered as having MeN. To our knowledge, this report constitutes the first detailed clinical and morphologic description of this kidney disease.

METHODS

Ethical Statement

Approval was obtained from the Ethical Committee in Stockholm, Sweden, and Hospital Nacional Rosales, San Salvador, El Salvador. All participating patients gave their informed consent before the study.

Patients

Eight male plantation workers with CKD of unknown cause and clinical suspicion of MeN were recruited at the Department of Renal Medicine, Hospital Nacional Rosales, San Salvador, El Salvador. Kidney biopsies, blood and urine samples were collected. The inclusion criteria were CKD of unknown cause, age 20-60 years, and plasma creatinine levels of 1.36-2.49 mg/dL (120-220 μmol/L) or eGFR of 30-60 mL/min/1.73 m^2 by the CKD-EPI (CKD Epidemiology Collaboration) creatinine equation. Exclusion criteria were diabetes mellitus (fasting blood glucose >126 mg/dL [>7.0 mmol/L]), uncontrolled hypertension (blood pressure $>140/90$ mm Hg or treatment with >1 hypertension medicine), or nephrotic-range proteinuria (24-hour protein excretion >3.5 g).

Procedure

Overview

Participants presented fasting on the day of the biopsy. Blood and urine samples were collected before the biopsy procedure was conducted, and blood pressure was tested. No intravenous fluid was given before the collection of blood samples. All patients answered a questionnaire about how long they had been working at plantations; their current use of hypertension medicines, painkillers (NSAIDs and paracetamol), and herbal medicines; daily fluid intake; and smoking habits. The patients stayed at the hospital for observation for 24 hours after the biopsy.

Kidney Biopsies

The kidney biopsy procedure was performed at Hospital Nacional Rosales with an ultrasound-guided percutaneous technique using a spring-loaded biopsy needle (DANA/Biocore II MG, 14 gauge; Histo). Preparation of the kidney biopsy specimens was done according to standard procedure and the biopsy specimens were examined with light microscopy, immunofluorescence, and electron microscopy (EM). See Item S1, available as online supplementary material, for more detailed description of the preparation of the biopsies.

Histologic evaluation of masked samples was performed by 2 experienced renal pathologists (A.W. and M.S.). Tubular atrophy,

Table 1. Patient Characteristics

Patient No.	Age (y)	BMI (kg/m^2)	Chronic Disease	Elevation of Residence Area (masl)	Fluid intake (L/d)	Duration of Plantation Work (y)	Smoking (y)
1	47	26[a]	No	42	2.5	30	0
2	47	29[a]	Hypertension	360	5	39	6
3	28	29[a]	No	378	5	20	6
4[b]	44	20	No	522	5	35	0
5	22	20	No	522	2.5	8	0
6	57	27[a]	Hyperuricemia	41	4	44	25
7	53	35[a]	No	476	3	40	0
8	56	21	No	164	4	41	40

Note: Data from medical records and questionnaire.
Abbreviations: BMI, body mass index; masl, meters above sea level.
[a]Results outside of normal range.
[b]Father of patient 5.

interstitial fibrosis, and interstitial inflammation were semiquantified as follows: mild, affecting 6%-25% of the cortical area; moderate, 26%-50% of the cortical area; and severe, >50%.

Thickness of the glomerular basement membrane (GBM) was calculated by measuring the thickness in 5 areas in 5 randomly selected capillaries. The degree of foot-process effacement was semiquantified in the same capillaries by calculating the numbers of slits per length of the GBM.

Blood Samples

Two weeks prior to the kidney biopsy, blood samples were collected from the patients for measurements of red and white blood cells and screening for hepatitis B and C virus and HIV (human immunodeficiency virus). These measurements were performed at the Hospital Nacional Rosales laboratory.

Blood samples obtained on the morning of the kidney biopsy were analyzed for the following constituents at Karolinska University Hospital laboratory according to standard protocols: creatinine (assay standardized to isotope-dilution mass spectrometry), serum urea nitrogen, sodium, potassium, calcium, albumin, uric acid, alanine aminotransferase, phosphate, antineutrophil cytoplasmic antibody and antinuclear antibody screening, complement levels, and anti-GBM antibodies. Specific biochemical markers for renal damage, such as cystatin C (assay standardized to IFCC [International Federation of Clinical Chemistry and Laboratory Medicine] standard) and serum β_2-microglobulin, also were measured. eGFR was calculated using the CKD-EPI creatinine equation[12] and the CKD-EPI cystatin C equation.[13]

Urine Samples

Urine samples were shipped to Karolinska University Hospital laboratory to be analyzed. Urinary albumin, creatinine, and uric acid, as well as biomarkers for proximal tubular injury, urinary *N*-acetyl-β-D-glucosaminidase (NAG), and urinary α_1-microglobulin (also known as protein HC),[14,15] were measured (see Item S1 for more detailed description). Additional urine samples were collected 2-3 months after the biopsy for dipstick (Combur-Test; Roche) and manual microscopic examination of sediment at Hospital Nacional Rosales.

RESULTS

Study Participants

Eight male patients aged 22-57 years with CKD of unknown cause were included in the study. All participants had been or were currently working at plantations in rural areas of El Salvador. All patients had normal blood pressure, 100-130/60-80 (mean, 112/71) mm Hg, at the time of the biopsy.

Questionnaire

Data from the questionnaire are listed in Tables 1 and 2. All patients had been doing agricultural plantation work, mainly sugarcane or corn, bean, and sorghum production. The number of years working at plantations varied from 8-44 (mean, 32) years. All participants reported that they have a physically strenuous occupation. None of the patients had a history of urinary stones, and only one patient (patient 2) had treatment for hypertension. The patients' own estimated daily liquid intake was 2.5-5 (mean, 3.9) L, of which 50%-90% (mean, 80%) was water.

Two of the patients were maintained on aspirin, 100 mg daily. One of the patients was treated with allopurinol due to hyperuricemia. Two of the patients (patients 1 and 7) had been treated with ciprofloxacin for urinary tract infection (UTI) for 2 weeks during the past 6 months. Participants' use of hypertension medicines, herbal medicines, and NSAIDs are presented in Table 2.

Biochemical Workup

Blood

Results of relevant plasma and serum measurements are listed in Table 3. Additional analysis demonstrated that all patients had normal glucose, calcium, albumin, antinuclear antibody, antineutrophil cytoplasmic antibody, anti-GBM, and complement levels. Serum β_2-microglobulin levels were elevated at 2.5-6.8 (reference value, < 2.0) mg/L, as was expected due to reduced GFR. Alanine aminotransferase levels were normal in all cases, with the exception of patient 7, who had slightly increased levels: 82.6 U/L (1.38 μkat/L).

Table 2. Regular Use of Hypertension Medications, Herbal Medicines, and NSAIDs at the Time of Biopsy

Patient No.	Hypertension Medicine	Herbal Medicines	NSAID Use	
			Every Day	Every Week
1	0	0	No	No
2	Nifedipine 30 mg 1×/d	Tea from "Jiote" bark[a]	No	Yes
3	Enalapril 5 mg 2×/d	0	Yes	Yes
4	0	Magnus[b]	No	Yes
5	0	Magnus[b]	No	No
6	Enalapril 5 mg 2×/d	Water from Coquillo root[c]	No	No
7	0	0	Yes	Yes
8	0	Cat's claw[d]	No	Yes

Abbreviation: NSAID, nonsteroidal anti-inflammatory drug.
[a]*Bursera simbaruba.*
[b]Water-soluble powder with fructose, vitamins and minerals (manufacturer: Omnilife).
[c]*Astrocaryum alatum.*
[d]*Uncaria tomentosa.*

Urine

Results from urine measurements are listed in Table 4. Urinary NAG-creatinine ratio and urinary α_1-microglobulin–creatinine ratio were elevated in the majority of patients, indicating tubular injury. Urine dipstick and sediment were analyzed 2-3 months after the biopsy and showed trace protein in patient 8, 1+ protein in patient 7, and 2+ protein in patient 2. None of the urine samples showed microscopic hematuria, glucosuria, or casts.

Kidney Biopsies

Overview

All biopsy specimens were evaluated by light microscopy, immunofluorescence, and EM. The light microscopic picture is described in Table 5. Biopsy specimens included 10-27 glomeruli.

Glomerular Changes

All biopsy specimens showed varying degrees of global glomerulosclerosis, affecting 29%-78% of included glomeruli (Fig 1A-C). Wrinkling of glomerular capillary basement membranes and/or thickening of Bowman capsules were seen in all but one biopsy specimen (Fig 1D and Fig S1A). In patient 8, these changes were mild. All biopsy specimens showed glomerular enlargement (Fig 2A), and in 2 patients, segmental glomerular sclerotic lesions were observed. In patient 2, segmental sclerosis was of the cellular type (Fig 2B). In patient 6, sclerotic lesions were perihilar (Fig S1B). There was no

Table 3. Results of Blood Analyses

Patient No.	Sodium[a] (mEq/L)	Potassium (mEq/L)	Cr[a] (mg/dL)	SUN (mg/dL)	Uric Acid[a] (mg/dL)	Hb[b] (g/dL)	$eGFR_{cr}$ (mL/min/1.73 m^2)	$eGFR_{cys}$ (mL/min/1.73 m^2)
1	138	3.4[c]	1.64[c]	27[c]	9.6[c]	12.8[c]	49	61
2	129[c]	3.3[c]	2.73[c]	56[c]	7.8	11.1[c]	27	23
3	138	4.1	2.22[c]	19	7.1	11.2[c]	39	34
4	141	2.8[c]	1.97[c]	13	7.5	12.4[c]	40	34
5	140	2.4[c]	1.28[c]	8[c]	7.1	14.4	79	60
6	140	4.7	2.53[c]	30[c]	6.3	12.1[c]	27	25
7	140	2.9[c]	1.13[c]	11	9.3[c]	15.0	74	50
8	122[c]	3.0[c]	2.02[c]	24[c]	6.7	12.2[c]	36	34

Note: Reference values (Laboratory at Karolinska University Hospital): sodium, 1.55-1.64 mg/dL; potassium, 3.6-4.6 mEq/L; Cr, <1.13 mg/dL; SUN, 9-23 mg/dL; uric acid, 3.9-8.1 mg/dL; Hb, 13.4-17.0 g/dL. Conversion factors for units: Cr in mg/dL to μmol/L, ×88.4; SUN in mg/dL to mmol/L, ×0.357; uric acid in mg/dL to μmol/L, ×59.48.

Abbreviations: Cr, creatinine; $eGFR_{cr}$, glomerular filtration rate estimated from creatinine; $eGFR_{cys}$, glomerular filtration rate estimated from cystatin C; Hb, hemoglobin; SUN, serum urea nitrogen.

[a]Plasma.
[b]Serum.
[c]Results outside of normal range.

Table 4. Results of Urine Analyses

Patient No.	ACR (mg/g)	NAG:Cr Ratio (nkat/g)	A1M:Cr Ratio (mg/g)
1	<2	33	4
2	788[a]	169[a]	92[a]
3	6	110[a]	119[a]
4	8	149[a]	73[a]
5	<7	82[a]	51[a]
6	12	53	16[a]
7	17	163[a]	50[a]
8	192[a]	140[a]	39[a]

Note: Reference values (Laboratory at Karolinska University Hospital): ACR, <30 mg/g; NAG:Cr ratio, <71 nkat/g; A1M:Cr ratio, <6 mg/g. Conversion factors for units: ACR in mg/g to mg/mmol, ×0.113; NAG:Cr ratio in nkat/g to nkat/mmol, ×0.113; A1M:Cr ratio in mg/g to mg/mmol, ×0.113.

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

[a]Results outside of normal range.

endocapillary cell proliferation. Discrete mesangial matrix increase was seen in a few patients, but there was no increase in number of mesangial cells. No crystals were seen in polarized light. In all patients, immunoglobulins, complement, fibrinogen, and light chains were undetectable by immunofluorescence.

The findings of the electron microscopic evaluation are presented in Table 6. The specimens included one glomerulus, except the specimen from patient 7, in which the material contained 5 glomeruli. In general, the material for EM showed fixation artifacts, most prominently in tubuli. Segmental foot-process effacement was observed in 3 patients. In 6 of 8 patients, the podocyte cytoplasm contained a variable number of vacuoles, most prominently in patient 1 (Fig 3A and Fig S2C). The membranes of most vacuoles were smooth and only a few had ribosomes (Fig 3A). In 3 of 8 patients, fat droplets were found (Fig 3B and Fig S2C). Mesangial areas did not show significant pathology, except in patient 7, in which small amounts of electron-dense deposits were found, indicating immune complexes (Fig S2D). Immunofluorescence also was performed on pronase-treated paraffin-embedded material in this patient, and small amounts of mesangial immunoglobulin G (IgG) deposits could thereby be identified. However, IgA and IgM results were negative. Based on results from these findings, we conclude that the immune complexes probably represent a remnant of a previous episode of glomerulonephritis, such as postinfectious glomerulonephritis, which in itself was not enough to explain the overall morphologic picture.

Tubulointerstitial Changes

In general, tubuli showed extensive fixation artifacts. Long-term changes were seen in all patients, with varying degrees of tubular atrophy and interstitial fibrosis (Figs 1A and 2C and Figs S1C, S1D and S2A). In 4 of 8 patients, the changes were mild, and in the other 4, they were moderate. Varying degrees of chronic inflammation were seen (Fig 2C and Fig S2B). None of the patients showed severe changes. Patient 2 showed mild tubulitis. There were no granulocytes found in the tubular lumina and thus no signs of acute pyelonephritis. No eosinophils were found in 7 patients, but in patient 8, a few eosinophils were found in peritubular capillaries.

EM showed no lead inclusions in tubular nuclei, but further examination of tubular cells could not be done due to fixation artifacts.

Table 5. Light Microscopy Findings

	Pt 1	Pt 2	Pt 3	Pt 4	Pt 5	Pt 6	Pt 7	Pt 8
Total no. of glomeruli	18	20	16	10	18	27	17	30
Globally sclerosed glomeruli	50%	65%	38%	40%	44%	78%	29%	70%
Segmentally sclerosed glomeruli	0%	5%	0%	0%	0%	7%	0%	0%
Interstitial fibrosis[a]	1-2 F	2-3 F	1 F	2 F	1 F	2 F	1 F	2 F
Tubular atrophy[b]	1	2	1	2	0-1	2	0-1	2
Tubulitis[b]	0	1	0	0	0	0	0	0
Interstitial inflammation[b]	1	2	1	2	1	1	0	2
Glomerular size[b]	2	3	1	2	1	3	1	2
Mesangial matrix expansion[b]	0	1	0	0	0-1	0	0-1	0
Wrinkled GBM/thickened Bowman capsule[c]	1	1	1	1	1	1	1	0-1
Intimal thickening[b]	0	1	0	0	0	0	0	1
Smooth muscle hyperplasia[b]	0	1-2	1	1-2	1	1	1	1
Arteriolar hyalinosis[b]	0	1	0	0	0	1	0	1

Abbreviations: D, diffuse; F, focal; GBM, glomerular basement membrane; pt, patient.

[a]Scale, 0-3 D/F.

[b]Scale, 0-3.

[c]Scale, 0-1.

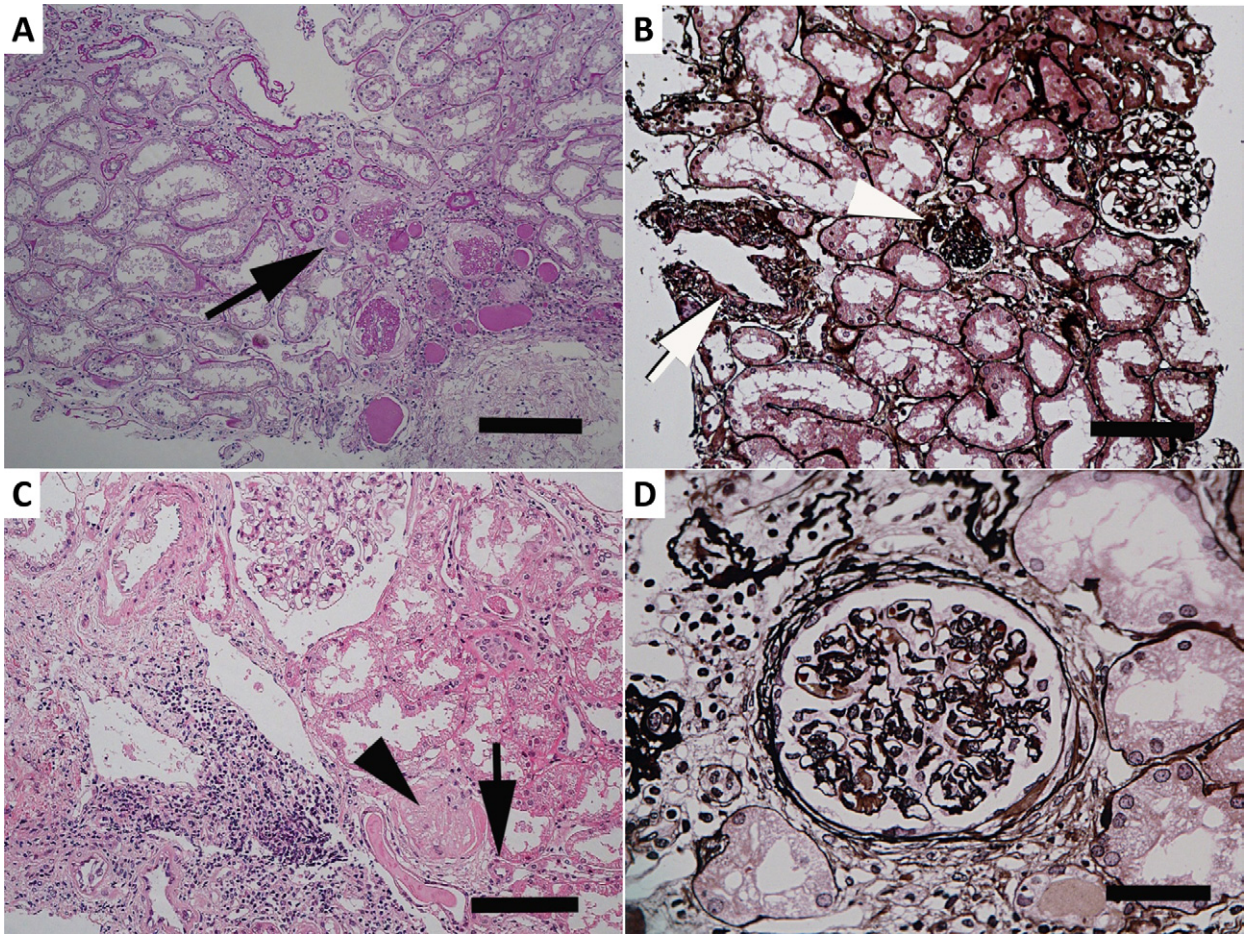

Figure 1. Light microscopic changes in kidney biopsy specimens from patients with Mesoamerican nephropathy. All biopsy specimens showed varying extent and distribution of global glomerulosclerosis, both in (A) small scars (arrow; periodic acid–Schiff) and (B, C) more dispersed (B: arrowhead; C: arrowhead; periodic acid–Schiff–methenamine silver and hematoxylin and eosin, respectively). (D) Most biopsy specimens showed signs of chronic ischemia with wrinkling of capillary basement membranes and/or thickening of Bowman capsules (periodic acid–Schiff–methenamine silver). (A) Varying degrees of tubular atrophy were seen in all biopsy specimens (arrow). (C) Chronic mononuclear inflammation was seen in atrophic areas. (B) Arterial smooth muscle hyperplasia was found in some patients (arrow). (C) Arteriolae were mostly normal (arrow), but some showed mild hyalinosis. Bars = 50 μm (D), 200 μm (B, C), 500 μm (A).

Vascular Changes

Arterial changes were mild (Fig 2D). In only 2 biopsy specimens was mild intimal fibrosis observed. In most patients, mild hyperplasia of smooth muscle cells was identified (Fig 1B and Fig S1A). Only 3 biopsy specimens showed mild arteriolar hyalinosis (Fig 2A), whereas no arteriolar changes were found in 5 biopsy specimens (Fig 1C).

DISCUSSION

To our knowledge, the present study is the first report correlating clinical data with biochemical and morphologic findings in patients with MeN.

The 8 patients in this study all have risk factors associated with MeN, such as plantation work and male sex. They display a homogenous clinical appearance with normal blood pressure, no diabetes, and low-grade or absent albuminuria. Most of them showed increased urinary excretion of NAG and/or α_1-microglobulin (Table 4). The morphologic evaluations of the kidney biopsy specimens show a similar pattern that does not resemble any other common specific kidney disease. This indicates that MeN, clinically and morphologically, constitutes a new and unique diagnostic entity.

The morphologic picture was similar in biopsy specimens of all included patients. Chronic tubulointerstitial damage with tubular atrophy and interstitial fibrosis was found in combination with chronic glomerular changes. The most striking and surprising finding was the presence of relatively extensive globular sclerosis, involving 29%-78% of glomeruli analyzed. This morphologic pattern contrasts with the chronic interstitial nephritis described in 2 other types of endemic nephropathies, Chinese herb nephropathy and Balkan nephropathy, characterized by chronic

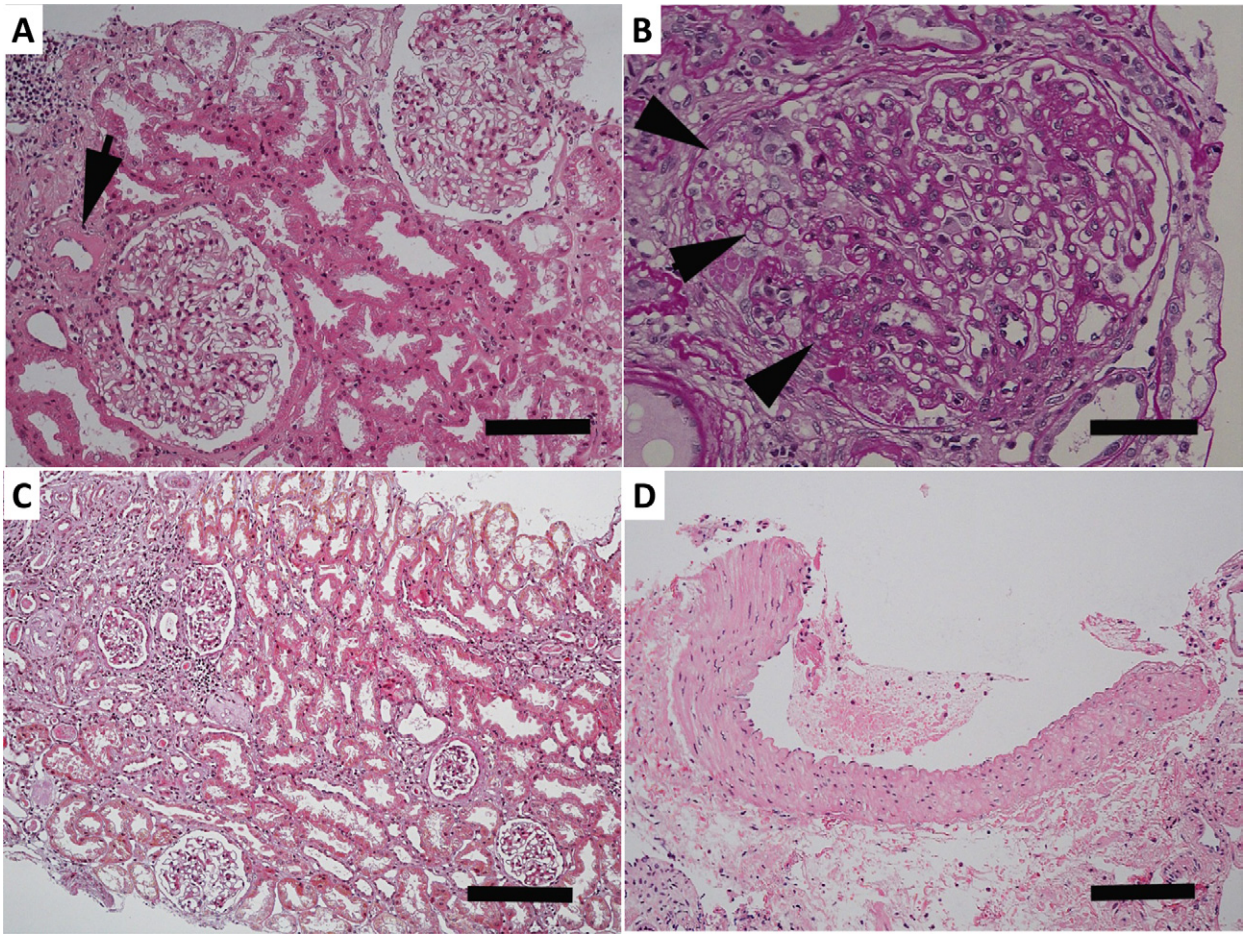

Figure 2. Light microscopy changes in kidney biopsy specimens from patients with Mesoamerican nephropathy. (A) Glomerular enlargement was seen in all biopsy specimens (hematoxylin and eosin). In 2 patients, focal segmental lesions were found in glomeruli; (B) in patient 2, the lesion was of cellular type (periodic acid–Schiff). (C) Mild to moderate tubular atrophy and chronic interstitial inflammation was seen in most patients (Ladewig). (D) Most arteries showed no intimal fibrosis (hematoxylin and eosin). (A) Three of 8 patients displayed mild arteriolar hyalinosis.

tubulointerstitial changes and preserved glomeruli.[16-18] The pathogenesis of the glomerular sclerosis in our patient cohort is unclear. In all but one biopsy specimen, glomeruli showed changes typical of chronic ischemia, with wrinkling of glomerular capillaries and/or thickening of Bowman capsule. Chronic glomerular ischemia is a common finding in diseases or conditions with vascular damage, such as hypertensive kidney disease[19] or aging.[20] However, all our patients were normotensive and showed only mild changes in the arteries and arterioli, which makes the finding of widespread global sclerosis even more noteworthy. The absence of proteinuria and negative immunofluorescence findings in all but one patient do not support that chronic glomerulonephritis is an underlying cause.

The glomerular enlargement found in all biopsy specimens could be secondary to the widespread glomerulosclerosis with nephron loss, but other factors such as ischemia or other hemodynamic derangements in the kidney might contribute. Glomerular hypertrophy often is seen in adaptive focal segmental glomerulosclerosis, and the increase in glomerular volume may be one of mechanisms in the development of glomerulosclerosis.[21,22] Focal segmental sclerotic lesions were observed in 2 patients, albeit without significant proteinuria, which usually is the typical clinical manifestation of focal segmental glomerulosclerosis. Both the biochemical data and morphologic picture indicate a component of chronic tubulointerstitial damage that was both focal and diffuse. Chronic inflammation also was present, although not more than could be expected in atrophic areas. We did not see acute inflammation, but chronic pyelonephritis is more difficult to exclude because the morphologic picture in chronic pyelonephritis is unspecific, with tubular atrophy and interstitial fibrosis. Two patients in our study had been treated for UTIs within the previous 6 months, but had no history of repeated UTIs. There have been reports of a high incidence of

Table 6. Electron Microscopy Findings

Patient No.	GBM Thickness (nm)	Podocyte Foot Processes (slits/μm GBM)	Endothelial Cells	Mesangium	Podocyte Cytoplasm
1	430[a]	1.7 (1.4-2.0)	Normal	Normal	Great no. of vacuoles; lipid droplets
2	625[b]	0.9 (0.3-1.4)[c]	Normal	Normal	Great no. of vacuoles
3	470[d]	1.6 (1.3-1.9)	Normal	Normal	Slightly increased no. of vacuoles; lipid droplets
4	380[a]	1.0 (0.4-1.7)[c]	Normal	Normal	Moderate increased no. of vacuoles
5	360[a]	1.3 (1.0-1.9)	Normal	Normal	Moderate increased no. of vacuoles
6	390[a]	1.3 (1.0-1.9)	Normal	Normal	Normal
7	360[a]	1.3 (1.0-1.6)	Normal	Electron-dense deposits	Moderate increased no. of vacuoles; lipid droplets
8	340[a]	1.2 (0.6-1.4)[c]	Normal	Normal	Normal

Note: Values for podocyte foot processes are given as mean number of slits per micrometer GBM (range of the 5 different areas of measurements).

Abbreviation: GBM, glomerular basement membrane.

[a]Normal.

[b]Thickened.

[c]Segmental effacement.

[d]Mildly thickened.

UTI symptoms among sugarcane workers in Nicaragua, but a recent investigation with urine cultures from 50 of these patients showed no bacteriuria.[10]

The clinical presentation of our 8 patients show CKD stages 2-4 (eGFR, 27-79 mL/min/1.73 m^2) with urinary findings of no or non−nephrotic-range albuminuria, no hematuria, and increased levels of biochemical urinary markers for tubular damage (NAG and α_1-microglobulin). These results mainly indicate a tubulointerstitial disease, and the morphologic finding of extensive glomerular sclerosis therefore was surprising. However, this observation provides important information for future studies regarding the pathogenic mechanisms of this disease.

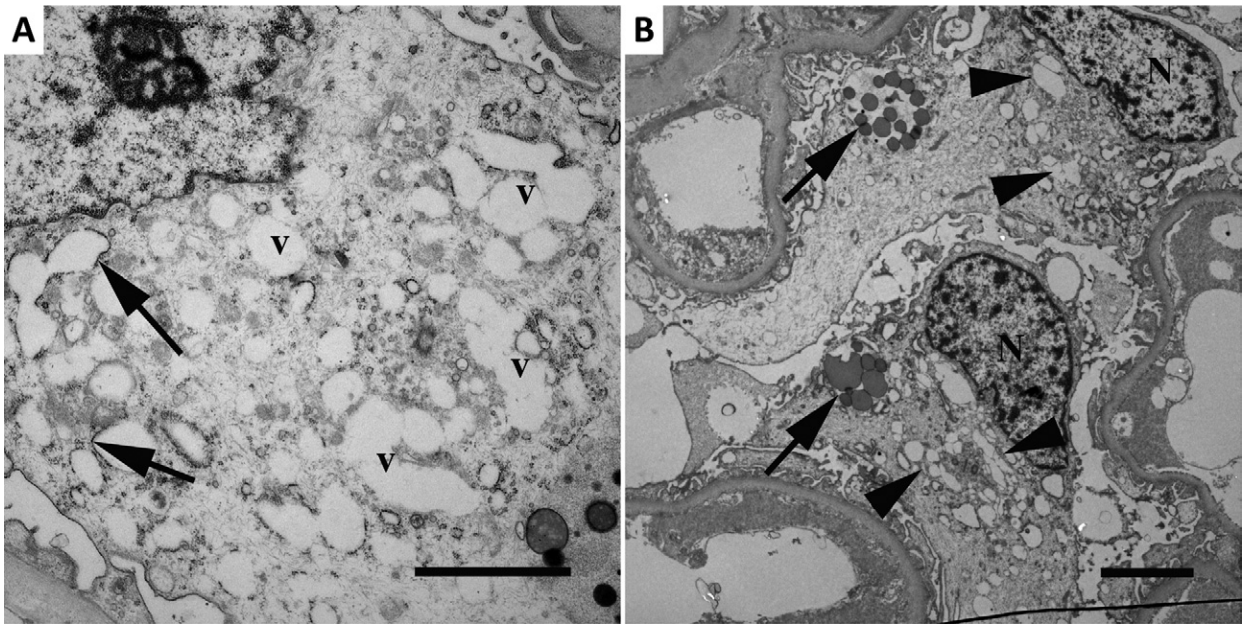

Figure 3. Transmission electron image from biopsy specimen from patient 1 shows (A) numerous empty vacuoles (v) with smooth membranes in the podocyte cytoplasm. In some areas, similar vacuoles covered with ribosomes are found (arrows). (B) In podocytes from patient 7, the cytoplasm contains some vacuoles (arrowheads) and lipid droplets in clusters (arrows). Bars = (A) 500 nm, (B) 5 μm.

Interestingly, a morphologic picture similar to that found in our patients has been described in endemic CKD of unknown cause in farmers in Sri Lanka.[23-25] In the most recent study by Nanayakkara et al,[24] 64 patients with CKD of unknown cause underwent kidney biopsy and it was concluded that tubulointerstitial damage was the main pathologic lesion. However, they also reported global glomerular sclerosis and ischemic glomerular changes, and the authors concluded that neither of these glomerular changes correlated with clinical parameters such as hypertension. Most likely, hypertension contributed to the glomerulosclerosis found in that study because most of the patients had hypertension (55%) and chronic vascular lesions.

Environmental toxins have been discussed as a potential cause of the endemic nephropathy in Sri Lanka,[23,24] as well as in MeN. However, there is no evidence of, for example, cadmium or lead toxicity in the patients with MeN.[6,10] Moreover, the morphologic picture of lead toxicity differs from those seen in our patients. In 1974, Cramer et al[26] studied kidney biopsy specimens from lead-exposed workers. They found ultrastructural tubular changes and interstitial fibrosis, while the glomeruli and blood vessels were spared. In another study of chronic lead nephropathy, chronic tubulointerstitial changes were described in combination with some sclerotic glomeruli, but also marked chronic vascular lesions.[27] Glomerular sclerosis has not been described in cadmium toxicity.[28,29]

Repeated dehydration due to heavy physical labor in a hot climate has been suggested as one of the causes of MeN.[1-3,6,7] Severe dehydration is a well-known cause of acute kidney injury, and during the past decade, a number of studies have shown that patients who have had transient acute kidney injury have an increased risk of developing CKD/end-stage renal disease.[30-33] The renal morphology in patients developing CKD after acute kidney injury has not been described, thus making a comparison with the morphology found in this study impossible. However, a recent experimental study by Grgic et al[34] demonstrated that repeated injury to proximal tubules could result in global glomerular sclerosis. One therefore can speculate whether the glomerular sclerosis seen in our patients is only a phenomenon secondary to tubular damage. Although we cannot exclude that the tubular injury may contribute to the glomerular damage, the relation between the chronic tubulointerstitial and glomerular changes suggests a primary injury to the glomeruli.

A finding that indicates glomerular injury is the observation of vacuoles and lipid droplets in the podocyte cytoplasm. This finding was not expected considering the low range of proteinuria found in our cohort. The podocytic ultrastructural changes in our material do not resemble those previously observed in lead toxicity.[26]

A majority of our patients had a frequent intake of analgesic drugs, including NSAIDs. Intake of NSAIDs may cause kidney failure due to allergic tubulointerstitial nephritis, characterized by tubulitis and interstitial inflammatory infiltrate containing eosinophils and sometimes granuloma. These changes were not found in the biopsy specimens from our patients. There is no epidemiologic evidence that nonphenacetin analgesics or NSAIDs cause CKD.[35-37] However, NSAIDs inhibit the vasodilative effect of prostaglandins and thereby change renal hemodynamics. Constriction of the afferent arteriole decreases kidney perfusion pressure, which hypothetically may contribute to the chronic glomerular ischemia found in our patients. One can speculate whether these changes might be aggravated further by dehydration and subsequent activation of the renin-angiotensin-aldosterone system because the plantation workers operate in extremely hot areas. The hypokalemia found in 6 of our patients is noteworthy as a possible result of renin-angiotensin-aldosterone system activation and calls for further investigation.

Taken as a whole, our data indicate both tubulointerstitial and glomerular damage in the kidney. However, this study presents only a small number of patients with clinical MeN from El Salvador and may not be representative for other cases of clinically suspected MeN in Central America. Additional studies of biopsies from other endemic areas are warranted.

The pathogenic mechanisms causing the changes described in this study are unclear, and further studies are needed. However, our data indicate that steroids probably will not be beneficial for these patients because the inflammatory component is limited. A brief overview of the findings in this study was presented at the First International Research Workshop on MeN held in 2012 in Costa Rica,[38] and we believe that our findings have and will generate new and important hypotheses for future studies about the cause of this major health problem in Central America.

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A summary of the findings in this study has been presented at the First International Research Workshop on Mesoamerican Nephropathy in San José, Costa Rica, November 28-30, 2012.

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SUPPLEMENTARY MATERIAL

Figure S1: Light microscopy images.

Figure S2: Light microscopy and transmission electron microscopy images.

Item S1: Supplementary methods.

Note: The supplementary material accompanying this article (http://dx.doi.org/10.1053/j.ajkd.2013.05.019) is available at www.ajkd.org.

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