Prospective Biopsy-Based Study of CKD of Unknown Etiology in Sri Lanka

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Abstract

Background and objectives A kidney disease of unknown cause is common in Sri Lanka's lowland (dry) region. Detailed clinical characterizations of patients with biopsy-proven disease are limited, and there is no current consensus on criteria for a noninvasive diagnosis.

Design, setting, participants, & measurements We designed a prospective study in a major Sri Lankan hospital servicing endemic areas to ascertain pathologic and clinical characteristics of and assess risk factors for primary tubulointerstitial kidney disease. We used logistic regression to determine whether common clinical characteristics could be used to predict the presence of primary tubulointerstitial kidney disease on kidney biopsy.

Results From 600 new patients presenting to a tertiary nephrology clinic over the course of 1 year, 87 underwent kidney biopsy, and 43 (49%) had a biopsy diagnosis of primary tubulointerstitial kidney disease. On detailed biopsy review, 13 (30%) had evidence of moderate to severe active kidney disease, and six (15%) had evidence of moderate to severe chronic tubulointerstitial kidney disease. Patients with tubulointerstitial kidney disease were exclusively born in endemic provinces; 91% spent a majority of their lifespan there. They were more likely men and farmers (risk ratio, 2.0; 95% confidence interval, 1.2 to 2.9), and they were more likely to have used tobacco (risk ratio, 1.7; 95% confidence interval, 1.0 to 2.3) and well water (risk ratio, 1.5; 95% confidence interval, 1.1 to 2.0). Three clinical characteristics—age, urine dipstick for protein, and serum albumin—could predict likelihood of tubulointerstitial kidney disease on biopsy (model sensitivity of 79% and specificity of 84%). Patients referred for kidney biopsy despite comorbid diabetes or hypertension did not experience lower odds of tubulointerstitial kidney disease.

Conclusions A primary tubulointerstitial kidney disease occurs commonly in specific regions of Sri Lanka with characteristic environmental and lifestyle exposures.

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Introduction

Residents of Sri Lanka's dry zone face a high risk for kidney disease characterized predominantly by tubulointersititial kidney disease of as yet unknown etiology (1,2). Termed CKD of unknown etiology (CKDu), this condition has emerged as a leading cause of hospitalization and death, with significant political and economic upheaval in the region (3). Care for this single disease now consumes 5% of the total health care budget in Sri Lanka (4). The demographic features (2,5) and the timeline of CKDu's emergence in Sri Lanka align with descriptions of Mesoamerican nephropathy (6–8) and Uddanam nephropathy in Andhra Pradesh, India (9).

Despite considerable efforts to improve the understanding of CKDu epidemiology and investigate its etiology, several questions remain unanswered, including whether a set of clinical features can inform reliable, noninvasive diagnosis for persons living in recognized "hot spots." At the current time, the "gold standard" for a diagnosis of CKDu is biopsy-proven primary tubulointerstitial kidney disease with no evident predisposing condition and residence in an area with high prevalence of similar disease. However, kidney biopsies are impractical for large-scale surveillance, geographic mapping, and entry into a patient-control study. Thus, varying approaches have been applied for categorization and study inclusion. For example, the CKDu National Project Team surveyed nearly 5000 persons in a population-based study in three endemic regions and one nonendemic region of Sri Lanka (10). Persons who had albuminuria without a reduction in eGFR were classified as "CKDu cases," despite numerous studies of CKDu pointing to tubulointerstitial kidney disease (2,6,7,11,12) as unlikely to present as albuminuria without reduced eGFR. Any assessment of exposures was biased (13). Other studies have accepted clinician- or self-reported

diagnosis and restricted to variable thresholds of serum creatinine (5,14–16).

Working in a major tertiary care hospital servicing central Sri Lanka, we investigated whether a set of routinely measured clinical features was associated with biopsy-proven tubulointerstitial kidney disease and could inform standardized approaches to select patients for patient-control studies investigating CKDu etiology in endemic regions. To identify potential risk factors for further study, we also compare the demographic, occupational, environmental, and selected lifestyle exposures of patients with tubulointerstitial versus other kidney disease.

Materials and Methods

We systematically approached persons referred to Kandy Teaching Hospital nephrologists as new patients over a 1-year time period (October 1, 2016 to September 30, 2017). Persons serviced by Kandy Teaching Hospital reside primarily in the Central Province of Sri Lanka, which includes both CKDu endemic and non endemic areas. Participants provided informed consent for the study, which was approved by the ethics committees at Stanford University and Kandy Teaching Hospital.

Questionnaire Administration

A trained research coordinator administered a study questionnaire, which captured detailed data on residential, family, occupational, drinking water, and tobacco and alcohol use history. Patients were asked to report history of diabetes, hypertension, cardiovascular disease, or autoimmune disease and whether they were using any nonsteroidal anti-inflammatory agents (NSAIDs) or ayurvedic supplements routinely. At the completion of clinical visit, nephrologists reported preliminary/presumed cause(s) of kidney disease. The research coordinator abstracted BP, body weight, and laboratory measures (serum creatinine, basic metabolic panel, urine studies [including urine protein-to-creatinine ratio], and kidney ultrasound). In patients returning for kidney biopsy, the research coordinator abstracted repeat measurements. We entered data into Redcap, an open source, privacy-protected platform.

Biopsy Indications

The participants' attending nephrologists determined the need for kidney biopsy. Standard clinical practice in this region is to refer patients with unexplained abnormal urine sediment or elevated serum creatinine for kidney biopsy, with the most common contraindication for biopsy being small kidneys (<9 cm in length on ultrasound measurement).

Biopsy Review

A single nephropathologist working with Kandy Teaching Hospital reviewed all biopsies. We triaged biopsy samples for light microscopy and immunofluorescence microscopy. The light microscopy sample was fixed, processed, and paraffin embedded. We obtained multiple hematoxylin and eosin– and silver methanamine–stained sections for examination, with additional sections stained with periodic acid–Schiff, Congo red, and Perl stain as needed. The immunofluorescence microscopy tissue was fresh frozen and stained with antisera to IgG, IgA, IgM, and C3. Electron microscopy examination was unavailable.

The pathologist made a diagnosis of primary tubulointerstitial kidney disease if the immunofluorescence study was predominantly negative and if active inflammation and/or chronic tubulointerstitial damage was the only or predominant pathology rather than glomerular and vascular abnormalities. Periglomerular fibrosis was considered a sequela of adjacent interstitial inflammation or disease. For the initial 6 months of the study period, a second nephropathologist coreviewed electronically transmitted images (n=45; 52% of biopsies) and traveled to Kandy Teaching Hospital for review of select patients. The two pathologists concurred on initial diagnoses. In patients with a diagnosis of tubulointerstitial kidney disease (n=43), the onsite pathologist reassessed histologic compartments and scored for severity (grades 1–3) of interstitial fibrosis, tubular atrophy, and tubulointerstitial inflammation using a standardized form (Supplemental Appendix) (n=41 [95%] with slides available for scoring).

Statistical Analyses

We present comparisons between patients recommended and not recommended for kidney biopsy and between patients with a diagnosis of tubulointerstitial versus other kidney disease on biopsy using t tests for continuous variables and chi-squared or Fisher exact tests for categorical variables. To evaluate the feasibility of a noninvasive approach to identification of CKDu, we preselected nine routinely available clinical and laboratory features hypothesized to be associated with presence or absence of tubulointerstitial kidney disease on kidney biopsy: age, sex, self-reported or physician-diagnosed diabetes mellitus, physician-diagnosed or measured hypertension, serum potassium and albumin, and urine microscopy characteristics (dipstick proteinuria, hematuria, and pyuria). We ran a logistic regression model; we computed the c statistic as a measure of discrimination and the calibration slope to evaluate model fit. In addition to a full model including nine preselected features, we tested five parsimonious versions to determine if a simpler model would achieve comparable predictive ability (17). In evaluating the models, we defined a patient as having probable CKDu if the predicted probability exceeded 0.5 (Supplemental Appendix 2).

To assess correlates of tubulointerstitial kidney disease, we used modified Poisson models to compute the risk ratio (and 95% confidence interval) for tubulointerstitial kidney disease after accounting for patients' age (18). We used StataMP, version 13.1 (Stata Corporation, College Station, TX) and R (19) (version 3.4.3) package *rms* (20) to conduct the analyses.

Results

A total of 606 newly referred patients presented to Kandy Hospital nephrologists over the course of 1 year; 600 (99%) consented to participate in the study, and 87 (15%) underwent successful kidney biopsies (Figure 1). In contrast to patients presenting to nephrologists who were not recommended for biopsy, patients recommended for biopsy were younger, were more likely to have hematuria or

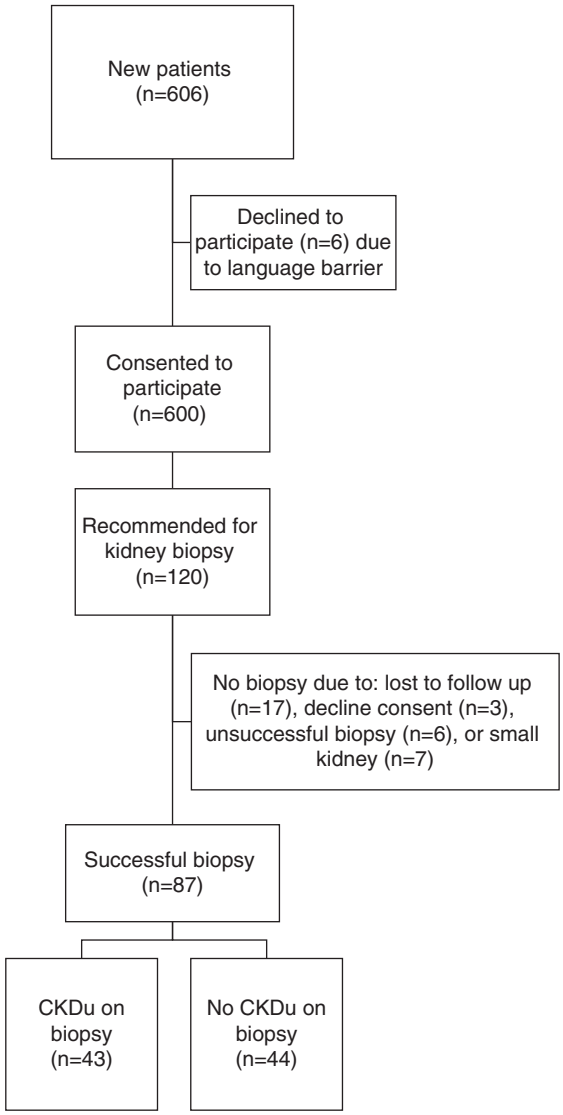

Figure 1. | **Flowchart of study participants.** Of the 606 patients approached during the 1-year study period, 600 agreed to further participate; nephrologists recommended biopsy in 120, and 87 underwent successful biopsy. CKDu, CKD of unknown etiology.

pyuria, were more likely to have lower serum albumin concentrations, and were more likely to have kidney size <9 cm (Table 1). Patients recommended for biopsy were also less likely to carry diagnosis of diabetes mellitus and/or hypertension.

Kidney Biopsy Findings

Of the 87 patients undergoing biopsy, 43 (49%) had a diagnosis of tubulointerstitial kidney disease. The tubulointerstitial inflammation was predominantly lymphocytic, with less frequent plasma cells and occasional neutrophils (Figure 2). Thirteen (30%) patients had moderate to severe (grades 2–3) tubulitis or interstitial inflammation in the nonatrophic cortex, which qualified as active tubulointerstitial disease. Six (15%) patients had moderate to severe chronic tubulointerstitial damage, tubular atrophy, or interstitial fibrosis. The remaining patients had mild (grade 1 interstitial fibrosis or tubular atrophy) chronic tubulointerstitial kidney disease. A high degree of global glomerulosclerosis (grades 2–3) was identified in six (15%) patients; arteriosclerosis was insignificant, with only mild changes observed. Tubular neutrophilic casts were observed in five (12%) patients; coexistent glomerular disease was evident in four (10%) patients, but on the basis of prominent tubulointerstitial inflammation with a mild degree of glomerular involvement, these patients were given the diagnosis of tubulointerstitial kidney disease. The most common diagnoses in patients without tubulointerstitial kidney disease were IgA nephropathy (n=12; 27%) and membranous nephropathy (n=7; 16%) (Supplemental Figure 1).

Characteristics of Patients with Tubulointerstitial Kidney Disease on Kidney Biopsy

Patients with tubulointerstitial kidney disease were geographically clustered in the eastern regions of Central Province (Figure 3). They were more likely to have spent most of their lifespan in the endemic region (Table 2). Family history of any kidney disease (but not necessarily kidney disease associated with death or dialysis) was more

Table 1. Characteristics of patients recommended or not recommended for kidney biopsy at Kandy Teaching Hospital in 2016–2017

Selected Characteristics	Biopsy Recommended, *n*=120	Biopsy Not Recommended, *n*=480
Age	45 (12)[a]	57 (12)
Women	31 (26)	122 (25)
Systolic BP, mm Hg	128 (21)	131 (20)
Diastolic BP, mm Hg	80 (9)	81 (10.4)
Hypertension[b]	54 (45)[c]	300 (63)
Physician diagnosed	27 (50)	149 (50)
Diabetes[b]	20 (17)[c]	157 (33)
Physician diagnosed	17 (85)	114 (73)
Serum creatinine, mg/dl	1.9 (1.4)	2.1 (1.5)
eGFR, ml/min per 1.73 m^2	54 (29)[c]	44 (25)
Missing		15 (3)
Serum albumin, g/dl	3.9 (0.9)[a]	4.4 (0.6)
Low serum albumin[d]	27 (23)	15 (3)
Missing	19 (16)	209 (44)
Hematuria[e]	21 (18)[c]	41 (9)
Missing	2 (2)	54 (11)
Pyuria[e]	50 (42)[c]	116 (24)
Missing	2 (2)	54 (11)
Urine dipstick protein negative	66 (55)	281 (59)
Missing	2 (2)	53 (11)
Small kidney[f]	22 (18)[a]	247 (52)
Missing	20 (17)	153 (32)

Numbers are mean (SD) or *N* (%).
[a]$P<0.001$.
[b]Patients without physician-diagnosed hypertension had either a clinic measurement of systolic BP ≥140 mm Hg or diastolic BP ≥90 mm Hg or self-reported hypertension. Participants without physician-diagnosed diabetes self-reported diabetes.
[c]$P<0.05$.
[d]Serum albumin <3.5 g/dl.
[e]Hematuria was defined as more than three urine red blood cells per high power field. Pyuria was defined as more than three urine white blood cells per high power field. Urine dipstick protein trace or nil was categorized as negative.
[f]A participant was categorized as having a small kidney if one kidney was <9 cm.

common in patients with tubulointerstitial kidney disease. More than 80% of patients with tubulointerstitial kidney disease were farmers at one point in their lives. Rice was the primary crop, and most reported doing all tasks associated with farming. More than 90% drank well water as a primary water source at one point. Self-reported tobacco use was high in patients with tubulointerstitial kidney disease.

Symptoms of recent fever, back pain or joint pain, and/or dysuria in the past 6 months were more common in patients with tubulointerstitial kidney disease, but there was a high background rate in patients with other kidney diseases as well (81% versus 66%). Serum urate concentrations were not higher in the patients with tubulointerstitial kidney disease (median of 6.4; interquartile range, 5.4–7.7 versus median of 7.1; interquartile range, 5.8–7.7).

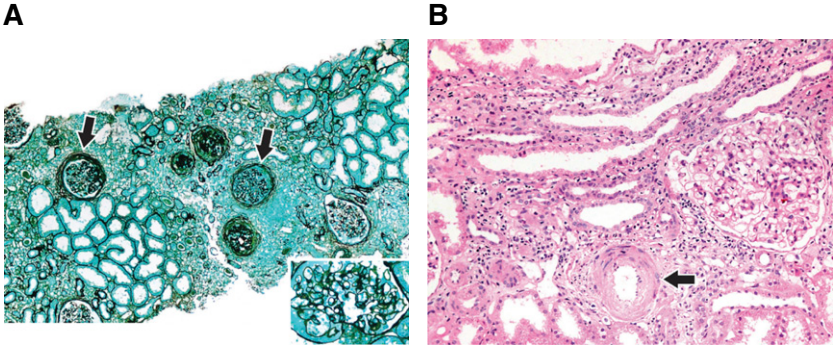

Figure 2. | Biopsy features of tubulointerstitial kidney disease in Sri Lanka demonstrating marked fibrosis and active interstitial inflammation. (A) Broad bands of tubular atrophy and interstitial fibrosis with relative sparing of glomeruli. In addition to global glomerulosclerosis, this atrophic cortex shows glomeruli with periglomerular fibrosis (arrows). The preserved glomeruli are unremarkable (inset), and the immunofluorescence microscopy is negative (methanamine silver stain, ×100). (B) Active interstitial inflammation in nonatrophic cortex. The infiltrate is composed of lymphocytes admixed with a few plasma cells and rare eosinophils. The glomerulus is normal, and the interlobular artery (arrow) shows mild arteriosclerosis (hematoxylin and eosin, ×200).

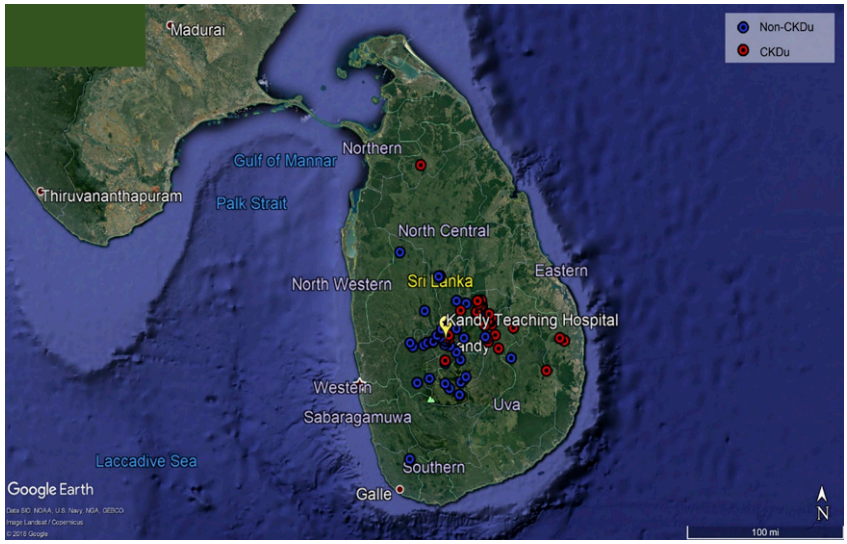

Figure 3. | Patients with tubulointerstitial kidney disease on biopsy (red dots) are primarily from the Eastern part of Central Province in Sri Lanka. Kandy Teaching Hospital serves as a major tertiary referral center for the Central Province of Sri Lanka. Although patients with other kidney diseases on biopsy resided throughout the province, a vast majority of patients with CKD of unknown etiology (CKDu) on biopsy are residing in the eastern portion of the Central Province, the Uva Province, or the Eastern Province.

Despite questioning in detail using local drug names, no patients reported using NSAIDs routinely (*i.e.*, more than ten times a month). No patients with tubulointerstitial kidney disease self-reported a diagnosis of an autoimmune disease.

Prediction of Tubulointerstitial Kidney Disease in CKDu Endemic Regions

Forty-two (98%) patients with and 29 (66%) patients without CKDu on biopsy had eGFR<90 ml/min per 1.73 m^2; 41 (95%) patients with and 26 (59%) patients without CKDu on biopsy had serum creatinine ≥1.2 mg/dl. Of the 43 patients diagnosed with tubulointerstitial kidney disease on biopsy, nephrologists had *a priori* diagnosed 25 (58%) as having CKDu (Figure 1).

Table 3 lists selected characteristics of patients with tubulointerstitial versus other diagnoses on biopsy as entered into the prediction model. The full model validation *c* statistic was 0.82; validation calibration slope was 0.59 (Supplemental Table 1). Of the parsimonious models, model E (including age, urine dipstick for protein, and serum albumin as key predictors, with an interaction term for the laboratory variables) had a validation *c* statistic similar to the one for the full model (0.84) and a validation calibration slope of 0.84, with sensitivity of 79%, specificity of 84%, positive predictive value of 83%, and negative predictive value of 80% (Supplemental Figure 2 has the relative ranking of strength of predictors, Supplemental Figure 3 shows calibration plots, and Supplemental Appendix 2 has model equations. Patients referred for kidney disease evaluation ages 35–60 years old with urine dipstick negative and normal serum albumin had high likelihood of tubulointerstitial kidney disease (predicted probability ≥0.6). For example, a 50-year-old patient with serum albumin of 4.0 g/dl and urine dipstick trace or negative for protein had a predicted probability of primary tubulointerstitial kidney disease of 0.85 (95% confidence interval, 0.74 to 0.99).

Presence or absence of comorbid diabetes mellitus or hypertension was not associated with the likelihood of tubulointerstitial kidney disease. However, there was clear physician selection in referral to biopsy: patients with diabetes or hypertension who were recommended for biopsy were younger and more likely to have low serum albumin, hematuria, or pyuria (Supplemental Table 2) than patients with these conditions not referred for kidney biopsy.

Discussion

In our study to characterize CKDu as seen in Sri Lanka's endemic areas, we found that active tubulitis and interstitial nephritis and moderate to severe chronic tubulointerstitial damage were common findings. Compared with patients with other identified kidney diseases (*e.g.*, autoimmune or primary glomerular disease), patients with tubulointerstitial kidney disease on biopsy had several characteristic exposures, including residence, farm work, well water use, and tobacco use. Three clinical parameters —age, urine dipstick for protein, and serum albumin— predicted presence of tubulointerstitial kidney disease on biopsy, with positive and negative predictive values exceeding 80%. If nephrologists referred patients with comorbid diabetes mellitus and/or hypertension, they did not experience significantly lower odds of tubulointerstitial kidney disease on biopsy.

Currently available biopsy studies from endemic regions have generally been small in size (n=8–64) (2,11,21) and/or have focused solely on patients with possible CKDu or Mesoamerican nephropathy (2,22). Their goal has been to describe pathology features in detail rather than to validate approaches for a clinical diagnosis. Clinical patient definitions developed by expert committees in Sri Lanka (23) and Mesoamerica (24) were not informed by data on sensitivity

Table 2. Demographic, behavioral, and occupational characteristics of persons with biopsy-proven tubulointerstitial kidney disease

Selected Characteristic	Tubulointerstitial Kidney Disease, *n*=43	Other Kidney Disease, *n*=44	Age-Adjusted Risk Ratio[a] (95% CI)
Demographics			
Education ≥10 yr	17 (40)	31 (71)	0.7 (0.4 to 1.1)
Endemic providence[b]			
Birth	43 (100)	33 (75)	1.4 (1.1 to 1.7)
Current residence	31 (72)	31 (73)	0.8 (0.6 to 1.1)
≥80% of lifespan	39 (91)	29 (66)	
Family history			
Any kidney disease	16 (37)	7 (16)	2.6 (0.4 to 4.7)
Death or dialysis requirement	5 (31)	5 (71)	—
Occupation			
Current occupation			
Professional	3 (7)	6 (14)	—
Service	2 (5)	3 (7)	—
Factory worker or day laborer	1 (2)	8 (18)	—
Farmer	28 (65)	8 (18)	—
Never worked	2 (5)	6 (14)	—
Not currently working	7 (16)	12 (270)	—
Ever farmer	36 (84)	17 (39)	2.0 (1.2 to 2.9)
Primary crop, rice	29 (81)	12 (71)	1.2 (0.8 to 1.6)
Primary crop, fruits and vegetables	6 (17)	2 (12)	—
Primary job, everything on farm	33 (92)	12 (71)	1.3 (0.9 to 1.7)
Primary job, manual harvesting	3 (8)	4 (24)	—
Water			
Current water source			
Public tap	11 (26)	16 (36)	0.5 (0.2 to 0.9)
Wells	21 (49)	9 (21)	—
Spring or surface	0 (0.0)	9 (21)	—
Reverse osmosis, tanker, or bottle	11 (26)	10 (23)	1.1 (0.2 to 2.0)
Ever used well water	39 (91)	25 (57)	1.5 (1.1 to 2.0)
Lifestyle			
Tobacco use, ever	35 (81)	19 (43)	1.7 (1.0 to 2.3)
Chewable tobacco use	25 (71)	10 (53)	1.3 (0.7 to 2.0)
Alcohol use, ever	27 (63)	20 (46)	1.3 (0.8 to 1.9)
Local alcohol use	23 (85)	13 (65)	1.3 (0.8 to 1.8)
Ever used traditional medications	2 (5)	3 (7)	—
Clinical symptoms or history			
Acute symptoms of fever, back pain, or dysuria over the past 6 mo	35 (81)	29 (66)	1.2 (0.9 to 1.5)
Dysuria, ever	18 (42)	13 (30)	1.5 (0.5 to 2.6)
Kidney stones	3 (7)	3 (7)	—
Snake bites	7 (16)	8 (18)	—

Numbers are *N* (%). 95% CI, 95% confidence interval.
[a]Risk ratios are performed for variables with sufficient sample (denominator and/or numerator with at least ten observations).
[b]Endemic province defined as Central Province, Northern Province, Uva Province, or Eastern Province.

and specificity relative to a criterion standard, and they have large differences between them. Compared with clinical descriptions of CKDu, the current patient definitions cast a wider net for some parameters (*e.g.*, including patients with renal tubular acidosis alone or albuminuria alone without reduction in eGFR) while leaving others out (*e.g.*, excluding patients with diabetes or hypertension).

Accurately identifying persons with abnormal kidney function as potentially having CKDu using simple clinical tools can strengthen surveillance, geographic mapping, and entry into patient-control or cohort studies. Notably, knowledge of overall CKD (*i.e.*, data on kidney disease parameters eGFR and albuminuria as defined by Kidney Disease Improving Global Outcomes criteria) is lacking in many of the regions presumed to have an epidemic of CKDu (25). Any population-based surveys of chronic diseases should integrate markers of kidney disease to gauge its overall and likely growing burden. An expert committee report on Mesoamerican nephropathy organized by the Pan American Health Organization echoes this recommendation (26). At the same time, to inform investigations into potential causes of the tubulointerstitial kidney disease afflicting select populations in select regions of Sri Lanka and Mesoamerica, it is also important to attempt to distinguish this specific disease entity from other forms of kidney disease. A standard approach to do so could allow for comparison of data across studies, over time, and across regions (27). Given the resource and research capacity constraints in the afflicted regions, a uniform patient definition could also facilitate pooling of smaller studies and yield better capacity to assess multiple risk factors.

Our work indicates that a clinical, noninvasive approach to identifying patients as having probable CKDu is feasible.

Table 3. Selected characteristics of persons with tubulointerstitial kidney disease (versus other kidney disease) on kidney biopsy

Selected Characteristic	Tubulointerstitial Kidney Disease, *n*=43	Other Kidney Disease, *n*=44
Age	48 (11)[a]	38 (12)
Women	6 (14)[b]	13 (30)
Hypertension[c]	14 (33)	19 (43)
Diabetes[c]	7 (16)	6 (14)
Serum albumin, g/dl	4.2 (0.9)[b]	3.5 (0.9)
Low serum albumin[d]	6 (14)	19 (43)
Missing	4 (9)	—
Serum potassium, mEq/L	4.0 (0.7)[b]	4.4 (0.8)
Low serum potassium[e]	6 (14)	3 (7)
Missing	3 (7)	—
Hematuria[f]	5 (12)	14 (32)
Pyuria[f]	20 (47)	21 (48)
Urine dipstick protein negative[f]	35 (81)[a]	13 (30)

Numbers are mean (SD) or *N* (%). Diabetes was defined as self-reported or physician-diagnosed diabetes.
[a]$P<0.001$.
[b]$P<0.05$.
[c]Hypertension was defined as self-reported or physician-diagnosed hypertension, systolic BP ≥140 mm Hg, or diastolic BP ≥90 mm Hg.
[d]Serum albumin <3.5 g/dl.
[e]Serum potassium <3.5 mEq/L.
[f]Hematuria was defined as more than three urine red blood cells per high power field. Pyuria was defined as more than three urine white blood cells per high power field. Urine dipstick protein trace or nil was categorized as negative.

The parameters seen commonly in patients with tubulointerstitial kidney disease in our study—that is, young to middle age, urine dipstick negative for proteinuria, and normal serum albumin—align with clinical descriptions of CKDu (2,22,28) and a published protocol aimed at studying CKD in disadvantaged populations (29). A negative result on urine dipstick for protein (rather than more sensitive measures of proteinuria) provided acceptable sensitivity and specificity in Sri Lanka's endemic region. We also find that, in regions with known high prevalence of CKDu, persons with a diagnosis of diabetes or hypertension may still require consideration for possible CKDu. If a nephrologist recommended a patient with diabetes for biopsy (because for example, he or she was young and did not have accompanying hypertension), this patient was nearly equally as likely to have tubulointerstitial kidney disease as other diagnoses on kidney biopsy. In areas where the background prevalence of diabetic nephropathy and renovascular disease is low but that of CKDu is high, patients with diabetes or hypertension, CKD, and no proteinuria may require additional investigation with either ancillary testing to determine the extent of other end organ damage or kidney biopsy to determine a definitive diagnosis. Additional biopsy studies in endemic regions could better define the prevalence of tubulointerstitial kidney disease in patients with diabetes and/or hypertension.

Evaluation of the demographic, occupational, environmental, and selected lifestyle correlates of patients with tubulointerstitial kidney disease on biopsy shows that they have experienced certain exposures at a high frequency. These include birth and residence in endemic regions, farm work, well water use, and tobacco use. All four of these exposures were nearly ubiquitous (present in >80% of patients with tubulointerstitial kidney disease), and they point us in the direction of environment and lifestyle rather than genetics alone (because although a positive family history was more prevalent in patients with tubulointerstitial kidney disease, it was reported in only about one third of patients). The same correlates, with the exception of well water use, were observed in a recent study evaluating risk factors for postharvest eGFR decline in Guatemala (30). In contrast to patients with Mesoamerican nephropathy who seem to have clearly defined tasks on the sugarcane farm and may experience differential task-dependent risks for kidney disease (31,32), patients with tubulointerstitial kidney disease in Sri Lanka were essentially running the whole farm. Thus, task-based studies are not likely to be as relevant in this area. We also did not find high prevalence of traditional medication or NSAID use, and we did not observe higher serum urate as was described in one study from Mesoamerica (33).

Our study has several limitations. First, our results require additional external validation and apply only to persons living in regions with a high prevalence of CKDu in Sri Lanka. Furthermore, persons presenting to a nephrology clinic for additional evaluation may differ from persons who may not have consented to follow through on the large-scale screening efforts put forth by the Sri Lankan Ministry of Health. Second, we did not dictate kidney biopsy using a study protocol, but rather, we allowed physicians to evaluate for need for biopsy, thus leaving room for inconsistencies in selection for biopsy. Younger patients with fewer comorbidities underwent biopsies. Lack of data on the feasibility, risks, and benefits of kidney biopsy in this region meant that following standard of care rather than prescribing an external protocol adhered to best practices principles of research in low-resources settings (34). Because we observed that a substantial burden of tubulointerstitial kidney disease coexists with a high burden of diabetes and hypertension among those with kidney disease in this region, our work could provide rationale for studies with *a priori* biopsy protocols. Third, we assume that persons with tubulointerstitial kidney disease on biopsy have CKDu rather than other possible

causes; none reported prior diagnosis of an autoimmune disease or regular NSAID use. Until specific molecular signatures of CKDu on biopsy are discovered, we were limited to this assumption. Furthermore, although urine protein by dipstick was among the variables identified as distinguishing CKDu from other causes of kidney disease, quantitative assessment of proteinuria could provide additional discrimination, but it was not routinely available. The high positive and negative predictive values obtained by our model result from the high prevalence of CKDu in the population studied, and they may not translate to other regions with lower prevalence of CKDu.

Within the constraints of a medical system servicing patients with a variety of clinical presentations, most traveling from significant distance, we performed standardized questionnaire administration and data abstraction with pathology review at two institutions in patients with and without tubulointerstitial kidney disease on biopsy. In addition to finding that specific environmental and lifestyle exposures are ubiquitous, we identified common clinical parameters that could serve as a first step toward a standardized noninvasive approach to identifying CKDu, which in turn, is essential for rigorous investigation into its cause.

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Disclosures

None.

Supplemental Material

This article contains the following supplemental material online at http://cjasn.asnjournals.org/lookup/suppl/doi:10.2215/CJN.07430618/-/DCSupplemental.

Supplemental Table 1. Discrimination and calibration of all preselected characteristics: (full) model and parsimonious models

Supplemental Table 2. Comparisons of patients with diabetes and/or hypertension by physician recommendation for biopsy

Supplemental Figure 1. Distribution of diagnoses in patients without CKDu on biopsy.

Supplemental Figure 2. Ranking of the relative strength of the nine preselected characteristics used in the full model.

Supplemental Figure 3. Calibration plots of the full model and five parsimonious models.

Supplemental Appendix 1. Definition of CKDu histologic scoring.

Supplemental Appendix 2. Methods for prediction modeling.

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