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CLINICAL RESEARCH

Describing Natural History and Exploring Risk Factors for Kidney Function Decline in Persons With CKD of Uncertain Etiology in Sri Lanka

Pasan Hewavitharana[1,2,11], Stephen Schensul[3], Edison Lee[4], Maria Montez-Rath[4], Sachintha Senarathne[5], Sai Liu[4], Kaitlin Harold[4], Santhushya Hewapathiranage[1], Naduni Erandika[1], Hemalika T.K. Abeysundara[6], Xue Yu[4], Vivek Bhalla[4], Andrew Fire[7], Adeera Levin[8], Shuchi Anand[4], Penny Vlahos[9,11], Rohana Chandrajith[5,11] and Nishantha Nanayakkara[1,10,11]

[1]Center for Research, National Hospital Kandy, Sri Lanka; [2]Department of Nursing, Faculty of Health Sciences, The Open University of Sri Lanka, Kandy, Sri Lanka; [3]School of Medicine, University of Connecticut, Farmington, Connecticut, USA; [4]Division of Nephrology, School of Medicine, Stanford University, Palo Alto, California, USA; [5]Department of Geology, Faculty of Science, University of Peradeniya, Kandy, Sri Lanka; [6]Department of Statistics and Computer Science, Faculty of Science, University of Peradeniya, Kandy, Sri Lanka; [7]Department of Pathology, School of Medicine, Stanford University, Palo Alto, California, USA; [8]Division of Nephrology, University of British Columbia, Vancouver, Canada; [9]Department of Marine Sciences, University of Connecticut, Groton, Connecticut, USA; and [10]Nephrology and Kidney Transplant Unit, National Hospital Kandy, Sri Lanka

Introduction: Chronic kidney disease of uncertain etiology (CKDu) is a leading cause of death of adults in Sri Lanka's dry region.

Methods: We initiated the Kidney Progression Project (KiPP) to prospectively follow 292 persons with Chronic Kidney Disease Epidemiology Collaboration estimated glomerular filtration rate (eGFR) 20 to 60 ml/min per 1.73 m^2 living in a CKDu endemic area. Using data from 3-year follow-up, we assessed kidney function decline (>30% from baseline eGFR), and the composite outcome of >30% eGFR decline, eGFR <15 ml/min or death, and explored the association of the 2 outcomes with baseline demographic, residential, and clinical parameters accounting for baseline eGFR.

Results: Median eGFR at enrollment was 28 ml/min among 71 women; 30 ml/min among 221 men; 91% to 99% had trace or no proteinuria during follow-up. At enrollment, median serum sodium, uric acid, and potassium were 143 mmol/l, 6.3 mg/dl, 4.5 meq/l, respectively among women; and 143 mmol/l, 6.9 mg/dl, 4.3 meq/l among men. Mean slope of eGFR decline was −0.5 (SD 4.9) ml/min/yr. In exploratory analyses, men with greater years of education and those living in northern region of the study area experienced lower likelihood of disease progression (hazard ratios [HR] 0.87 [0.77–0.98] per additional year and 0.33 [0.12–0.89] for northern versus other subregions, respectively). There was a suggestion that men drinking well water had higher likelihood and men living further away from reservoirs had lower likelihood of >30% decline in eGFR (HR 2.07 [0.95–4.49] for drinking well water versus not, and HR 0.58 [0.32–1.05] per kilometer distance, respectively).

Conclusions: The overall rate of kidney function decline was slow in this CKDu cohort, similar to other nonalbuminuric CKD, and event rates were similar among men and women. Further etiologic investigations could focus on specific residence locale and water use.

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Correspondence: Shuchi Anand, Stanford University, 3180 Porter Drive, Palo Alto, California 94304, USA. E-mail: sanand2@stanford.edu

[11]PH, PV, RC, and NN contributed equally.

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Sri Lanka is one of the epicenters for the global epidemic chronic kidney disease of uncertain etiology (CKDu). This form of CKD, not triggered by hypertension or diabetes, was first described in Sri Lanka in the 1990s . In one of the earliest descriptions of the disease, Athuraliya *et al.*[1] reported a high

prevalence among male farmers residing in the country's dry zone regions, particularly in the North Central Province and its adjacent areas. Since the 1990s, diagnostic efforts have indicated hotspots exist in Sri Lanka and in many countries in the global tropical belt.[2,3] Despite the extensive search for etiology, results have been inconclusive. Further, there is insufficient understanding of the clinical profile of affected persons; rates of disease progression; incidence of end-stage kidney disease; and association of demographic, residential, or clinical parameters with disease progression.

Although it is established that CKDu has a primary tubulointerstitial pathology,[4,5] data are unclear about other purported hallmarks, including hypokalemia and hyperuricemia.[6] In addition, some studies suggest a disproportionately poor outcome among men. However, longitudinal prospective data from clinical cohorts are sparse.[1,3] Describing the laboratory characteristics of persons with CKDu over time could identify early or pathognomonic markers. Understanding the natural history of the disease among persons with CKDu could inform treatments and planning for dialysis or other supportive services in affected regions. Finally, assessing risk factors for faster decline in kidney function within a clinical CKDu cohort could identify potential candidates for early disease modifying interventions; as, for example, was the case when albuminuria was identified as a risk factor for faster progression in diabetic and glomerular kidney diseases.[7]

We conducted a prospective study of persons with CKDu in an endemic region in the Central Province of Sri Lanka (Kidney Progression Project or KiPP). We identified and have followed 292 participants with Chronic Kidney Disease Epidemiology Collaboration (CKD EPI 2009) eGFR 20 to 60 ml/min over 3 years.[8] As a result, KiPP provides the data to describe clinical and laboratory characteristics, and event rates for kidney-related outcomes (>30% sustained eGFR decline, eGFR < 15 ml/min per 1.73 m^2, or death), stratified by sex and baseline eGFR. We also explored a range of residential location, clinical, and laboratory data as risk factors for >30% sustained eGFR decline within this cohort.

METHODS

Kidney Progression Project (KiPP)

We initiated the KiPP in 2017 in the Wilgamuwa Divisional Secretariat, a CKDu endemic area of 40,000 people in the lowland dry zone area of the Central Province of Sri Lanka.[8] The study was reviewed and approved by the University of Connecticut, and National Teaching Hospital in Kandy, Sri Lanka. Stanford University approved follow-up after the second year of the study. The detailed methodological approach, including a description of clinical and expanded environmental variables, is described in Vlahos *et al*.[8] Briefly, in 2016, the Ministry of Health of Sri Lanka conducted a screening with serum creatinine and urinalysis of residents in Wilgamuwa to identify persons with CKDu. For KiPP, we approached persons with Stage 3 and 4 CKD (Chronic Kidney Disease Epidemiology Collaboration[9] eGFR 20–60 ml/min per 1.73 m^2). We excluded persons who had proteinuria above 1+ on dipstick, hematuria, self-reported or clinic-record history of diabetes, or a known kidney ultrasound with cysts. Our definition for CKDu was based on prior work demonstrating that among persons living in the endemic region, abnormal serum creatinine and a "bland" urine (negative for blood or protein) had 84% specificity for interstitial nephritis on biopsy.[10] Of the 330 persons meeting eligibility criteria, 304 agreed to participate, and 292 provided at least 2 serum creatinine measurements and were included in this analysis (Figure 1). We did not conduct a formal sample size estimation, rather our plan was to approach our study area's known eligible participants.

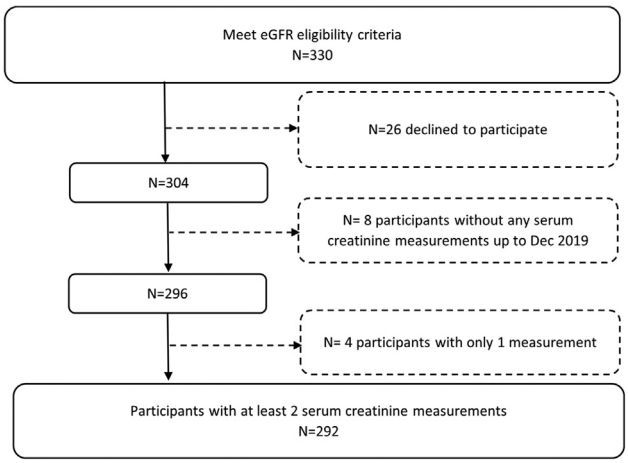

Figure 1. Study cohort. eGFR, estimated glomerular filtration rate.

Baseline Survey Components

We administered a baseline survey that focused on demographics, family history, residential, and medical history as described in the KiPP protocol.[8] In addition, we collected the GPS coordinates of the participant's well water source and that of the 10 largest reservoirs in the location. We calculated the distance relative to each of the reservoirs and evaluated distance from reservoir as an exposure because proximity to reservoirs may indicate participant exposure to concentrated environmental contaminants (e.g., agrochemicals or cyanobacteria). We used ArcGIS to map the

coordinates of participants' household wells relative to the reservoir locations. Based on these GPS coordinates, we also categorized a participant's relative location within the study region of Wilgamuwa (north, east, west, or south).

Clinical Parameters and Follow-Up

From December 2017 to December 2019, the study protocol involved 6 quarterly participant follow-up visits with an interview schedule and laboratory draw. Visit 1 encompassed a range of 6 months; this represented the time to recruit participants. At this visit, we assessed vitals (height, weight, systolic blood pressure, and diastolic blood pressure), serum biochemistry (sodium, potassium, calcium, phosphorus, albumin, uric acid, and creatinine), and hemoglobin. We measured serum creatinine using the Jaffe method via Indiko Plus Clinical Chemistry Analyzer (Thermofisher, Dreieich, Germany) at 1 of 2 study-affiliated laboratories. Serum creatinine was converted to eGFR using the Chronic Kidney Disease Epidemiology Collaboration 2009 equation, accounting for the body surface area of each participant. Methods for additional laboratory testing are in the Supplementary Methods.

Thereafter, we contacted patients within a 3-month window over 5 quarters, to track serum chemistries, hemoglobin, and urine dipstick. The last full laboratory visit was prolonged to a range of 6 months to capture a follow-up with as many participants as possible. In 2021, we conducted an abbreviated follow-up assessment of serum creatinine and participant status (alive, dead, or received a kidney transplant), and plan to continue annual follow-ups.

Outcome

We evaluated kidney function decline using time to >30% sustained decline in eGFR from baseline using observed eGFR data. To describe kidney-related event rates in the cohort, we additionally used a composite outcome of >30% sustained decline in eGFR from baseline, eGFR reaching below 15 ml/min, or death. The outcome of sustained decline required 2 consecutive measures meeting >30% decline from baseline. To ascertain date of death, we undertook a telephonic outreach to the next-of-kin after 3 attempts to contact the participant had failed. Patients were censored at end of study for both outcomes. Patients were further censored for death or last measurement when calculating time to the outcome of >30% sustained decline in eGFR.

Statistical Analysis

Because there were putative differences in exposures among men and women (e.g., in farming), we stratified all analyses by sex. We reported data on clinical characteristics and laboratory values of patients in the cohort stratified by sex and eGFR categories, following STROBE guidelines.[11] We presented characteristics with categorical variables as proportions and continuous variables as mean (SD) or median (Q1–Q3) depending on the distribution. We depicted the geolocation of the patients' well water source, by their eGFR decline status (>30% decline vs. not). To describe clinically significant event rates, we calculated the incidence of the composite outcome by looking at number of participant-experienced outcomes and number of events divided by total person-time at risk, and plotted Kaplan-Meier curves for the composite and >30% sustained eGFR decline outcomes. We investigated completeness of eGFR measurements over time for each patient (Supplementary Figure S1). We found that on average both men and women had at least 6 (out of 7 possible) measurements and that 37% of women and 46% of men had measurements in all collection periods. To evaluate patterns of missingness were correlated, we performed a logistic regression using missing eGFR as our outcome and using a delta eGFR (change in eGFR over antecedent 2 time periods) as our independent variable, and did not find a relationship with subsequent missing eGFR.

We performed cox proportional hazard models to compute baseline eGFR-adjusted HRs and 95% confidence intervals for the association of potential risk factors and the outcomes. Because death is a potential competing risk for the outcome of >30% sustained eGFR decline from baseline, we studied its effect in the analysis of the composite outcome inclusive of death. These were exploratory analyses, accounting for baseline eGFR and evaluating nearly all questionnaire variables with >5 values for each response level (among men, $n = 35$ questionnaire variables or geolocation-based variables). For anthropometric and laboratory data, we selected systolic blood pressure, baseline sodium, potassium, and uric acid measurements. The *P*-value for the test of proportional hazards assumption were all greater than 0.05 showing no evidence that the log-HR changed with follow-up time for any of the risk factors that were included in the model. However, the effect of baseline eGFR was found to vary over time in the composite outcome model. We included an interaction of baseline eGFR with log time as a time-dependent effect in the respective model. We required at least 5 persons with the outcome and 5 persons without the outcome per level of each categorical risk factor to fit the model. Therefore, results are not shown if this requirement was not met. Considering that a smaller subset of correlates was able

to be explored among women because of sample size, all regression analyses for women are presented in the Supplementary Material.

RESULTS

Clinical Characteristics of Cohort at Baseline and Over Time

Among the 292 participants comprising the cohort (Figure 1), 71 (24%) were women. Among both men and women, participants with eGFR >45 ml/min were younger and had higher body mass index (Tables 1 and 2). Participants with eGFR <30 ml/min had higher serum phosphorous and uric acid, and lower hemoglobin. Blood pressure was similar across eGFR strata among both men and women, and median serum sodium was >140 mmol/l for all eGFR strata among both men and women. During the 2-year follow-up period with intensive laboratory measures, a vast majority (>90%) of participants had no to 1+ proteinuria on urine dipstick (Supplementary Table S1). Mean blood pressure and median laboratory values, including uric acid were similar throughout 2 years of intensive laboratory follow-up.

Decline in Kidney Function

Overall, in the entire cohort, the mean slope of eGFR decline indicated small changes in kidney function (mean decline −0.5 [SD 4.9] ml/min/yr). There was no substantial difference in mean slope of decline among women compared with men (−0.6 [SD 5.3] ml/min/yr vs. −0.5 [SD 4.8] ml/min/yr) respectively.

Incidence of the composite outcome of >30% sustained reduction in eGFR from baseline, eGFR <15 ml/min, or death occurred at similar rates among men and women (Table 3, Figure 2). Among both men and women, participants with eGFR <30 ml/min had higher incidence rates of the composite outcome than participants with eGFR >45 ml/min. For the individual outcome of >30% sustained eGFR decline, 26 men and 14 women experienced this outcome (incidence rate 39 and 68 per 1000 person-years for men and women, respectively). Among men, the incidence rate was 92 (95% confidence interval 54–168) per 1000 person-years at baseline eGFR >45 ml/min, compared with 23 (95% confidence interval 11–46) per 1000 person-years at baseline eGFR <30 ml/min. Supplementary Table S2 lists the incidence rates of individual outcomes for both men and women. Figure 3 depicts location of participants within the endemic study area, stratified by sex and >30% eGFR decline versus not.

Table 1. Clinical characteristics of men in study cohort, by baseline eGFR[a]

Characersitic	eGFR < 30 *n* = 112	30 ≤ eGFR < 45 *n* = 62	eGFR ≥ 45 *n* = 47
Demographics			
Age at enrollment, yr	54 (8)	52 (9)	51 (9)
Vitals			
Systolic blood pressure	118 (13)	118 (12)	118 (11)
Diastolic blood pressure	77 (9)	77 (8)	78 (9)
BMI (kg/m^2)	19.6 (2.8)	21.0 (3.1)	21.2 (3.6)
Laboratory values[b]			
Sodium (mmol/l)	143 (139, 144)	143 (140, 145)	143 (141, 145)
Potassium (meq/l)	4.4 (4.0, 4.8)	4.2 (3.8, 4.6)	4.3 (3.9, 4.6)
Calcium (mg/dl)	9.1 (8.6, 9.7)	9.0 (8.6, 9.5)	9.1 (8.6, 9.5)
Phosphorus (mg/dl)	3.8 (3.1, 4.8)	3.8 (3.1, 4.5)	3.3 (2.9, 4.2)
Albumin (g/dl)	4.4 (4.2, 4.9)	4.6 (4.2, 5.0)	4.4 (4.1, 4.9)
Uric acid (mg/dl)	7.2 (6.3, 8.4)	6.7 (5.7, 7.5)	6.0 (5.4, 7.2)
Hemoglobin[c] (g/dl)	11.7 (10.6, 12.6)	12.7 (11.8, 13.3)	13.1 (12.5, 14.4)

BMI, body mass index; eGFR, estimated glomerular filtration rate.
Units in parenthesis.
[a]Data are presented as *n* (%) or mean (SD). Data for baseline were obtained during a 6 month period from December 2017 to May 2018 men.
[b]Laboratory values are presented as median (25th, 75th percentile).
[c]Median (25th, 75th percentile).

Table 2. Clinical characteristics of women in study cohort, by baseline eGFR[a]

Characteristics	eGFR < 30 *n* = 40	30 ≤ eGFR < 45 *n* = 18	eGFR ≥ 45 *n* = 13
Demographics			
Age at enrollment, yr	58 (6)	56 (6)	47 (7)
Vitals			
Systolic blood pressure	121 (17)	122 (11)	119 (14)
Diastolic blood pressure	77 (10)	81 (10)	79 (12)
BMI (kg/m^2)	21.8 (3.1)	21.8 (4.9)	23.1 (4.3)
Laboratory values[b]			
Sodium (mmol/l)	144 (142, 147)	142 (140, 144)	143 (140, 144)
Potassium (meq/l)	4.5 (4.1, 4.7)	4.6 (4.3, 4.7)	4.3 (3.9, 4.7)
Calcium (mg/dl)	8.9 (8.7, 9.3)	9.1 (8.7, 9.5)	9.1 (8.7, 9.3)
Phosphorus (mg/dl)	4.4 (3.9, 5.6)	3.8 (3.6, 5.0)	3.6 (3.0, 4.7)
Albumin (g/dl)	4.3 (4.0, 4.7)	4.4 (4.2, 4.8)	4.4 (4.2, 4.7)
Uric Acid (mg/dl)	6.5 (5.8, 7.5)	6.3 (6.0, 7.5)	5.4 (4.4, 6.3)
Hemoglobin (g/dl)	10.6 (9.8, 11.5)	11.3 (10.8, 11.6)	11.2 (10.9, 12.1)

BMI, body mass index; eGFR, estimated glomerular filtration rate.
Units in parenthesis.
[a]Data are presented as *n* (%) or mean (SD) unless otherwise noted;
[b]Laboratory values are presented as median (25th, 75th percentile).

Table 3. Incidence rates for the composite outcome of >30% sustained decline in eGFR, eGFR<15 ml/min, or death

Baseline eGFR categories (ml/min)	No. of patients	No. with composite outcome	Person-time at risk (yr)	Incidence rate (events/ 1000 person-yr)
Women				
Overall	71	27	166	163 (112–238)
eGFR < 30	40	21	77	273 (178–419)
30 ≤ eGFR < 45	18	3	53	56 (18–174)
eGFR ≥ 45	13	3	35	85 (27–262)
Men				
Overall	221	64	562	114 (89–145)
eGFR< 30	112	44	249	177 (132–238)
30 ≤ eGFR < 45	62	8	187	43 (21–85)
eGFR ≥ 45	47	12	126	95 (54–168)

eGFR, estimated glomerular filtration rate.
Incidence rates were stratified according to baseline eGFR

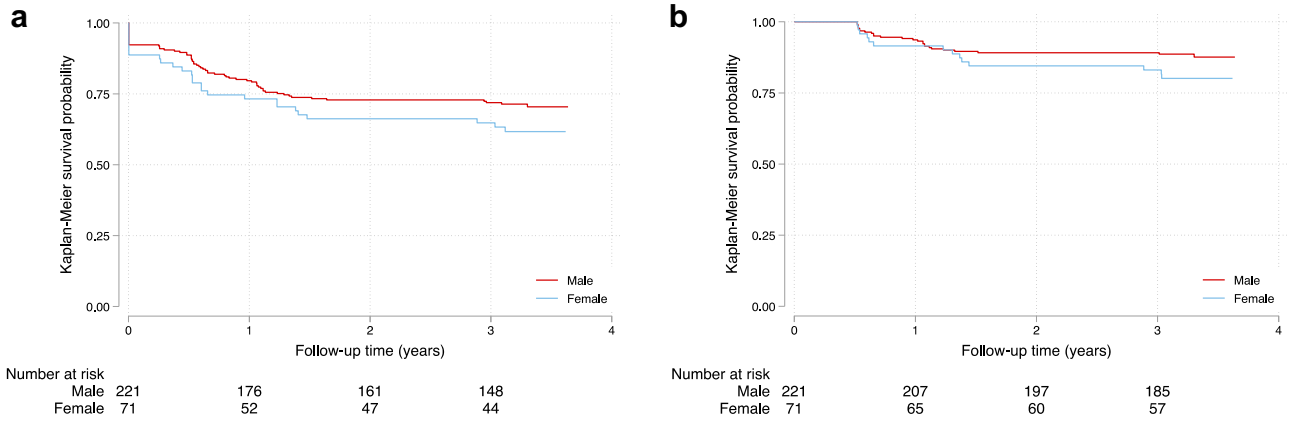

Figure 2. Survival curves for kidney-related events among men and women. (a) Kaplan-Meier curve for the probability of the composite outcome of >30% sustained decline in eGFR, eGFR<15 ml/min, or death. (b) Kaplan-Meier curve for the probability of >30% sustained decline in eGFR. eGFR, estimated glomerular filtration rate.

Exploring Risk Factors for Kidney Function Decline

Among men, for the outcome of >30% sustained eGFR decline, number of years of education and residence in the northern subregion within the study locale were associated with lower hazards of kidney function decline (Table 4). In addition, living farther away from the major regional reservoirs and drinking well water (vs. not drinking any well water) at enrollment were associated with kidney function decline, although confidence intervals crossed 1. No consistent association was evident for any putative occupational risk factors. Older age was associated with higher likelihood of experiencing the composite outcome; farming or any other occupation at enrollment and higher serum sodium were associated with lower likelihood of the composite outcome. Eating lunch in the field, a potential additional route of occupational exposures, was also associated with lower likelihood of the composite outcome.

Among women, in contrast to men, paradoxically, older age, and higher serum sodium were associated

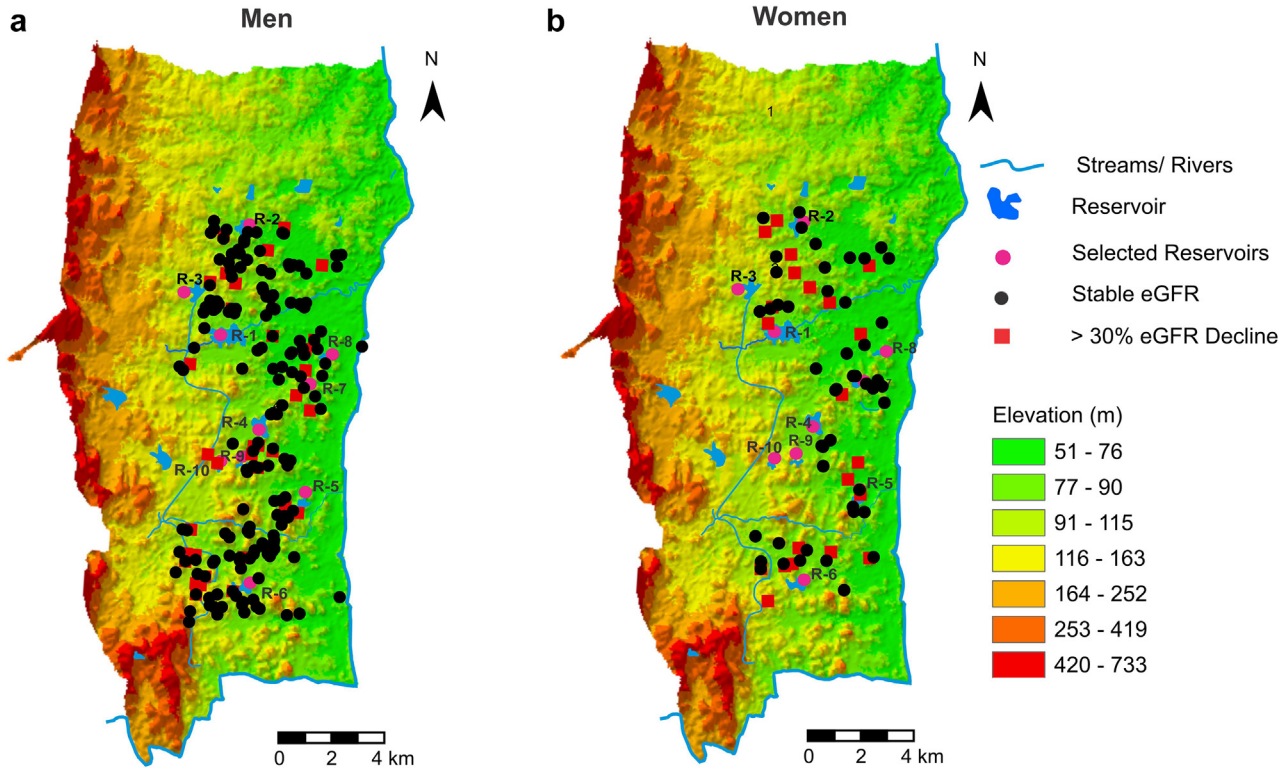

Figure 3. Spatial distribution of wells according to participant's CKDu progression as stratified by >30% sustained decline in eGFR. The plot of the participant well water is generated via ArcGIS. The base map is generated using USGS Earth Explorer and is available at https://earthexplorer.usgs.gov/. Also illustrated on maps are major reservoirs (R-1 to R-10) as well as topography.

Table 4. Demographics, residence, and clinical correlates of rapid kidney function loss among men participating in the Kidney Progression Project cohort, accounting for baseline eGFR

Variable	Summary statistic[a]	>30% sustained decline in eGFR HR (95% CI)	>30% sustained decline in eGFR, eGFR<15 ml/min, or death HR (95% CI)
Demographics			
Age, per 5 yr increment	54 (48, 60)	1.21 (0.94–1.56)	1.26 (1.06–1.49)[d]
Years of education, per yr increment	5 (3, 8)	0.87 (0.77–0.98)[c]	0.95 (0.88–1.02)
Extended (vs. nuclear family)	24%	1.20 (0.47, 3.06)	1.47 (0.87–2.50)
Household size	3 (2, 4)	1.04 (0.78–1.39)	0.94 (0.78–1.13)
Any household member had kidney disease	10%	-	-
Received supplemental income	41%	1.33 (0.61–2.89)	0.93 (0.56–1.54)
Currently drinking well water	39%	2.07 (0.95–4.49)	1.00 (0.61–1.66)
Ever drank well water[b]	90%	-	-
Residence history			
Residence distance from reservoir (per kilometer)	1.3 (0.9–1.9)	0.58 (0.32–1.05)	0.83 (0.60–1.16)
Residence in North Wilgamuwa (vs. other subregions)	38%	0.33 (0.12–0.89)[c]	0.84 (0.50–1.41)
Lived entire life in Wilgamuwa	57%	1.25 (0.57–2.75)	0.80 (0.49–1.31)
Birthplace located in wet zone	48%	1.32 (0.60–2.91)	1.24 (0.76–2.03)
Ever lived in wet zone	49%	1.26 (0.57–2.79)	1.19 (0.73–1.94)
Number of years lived in wet zone, per year increment	29 (13, 51)	1.01 (0.98–1.03)	1.00 (0.98–1.02)
Own land	72%	1.68 (0.63–4.47)	1.38 (0.76–2.49)
Lifestyle/habits			
Ever consumed alcohol	48%	0.58 (0.26–1.29)	0.71 (0.43–1.17)
Ever used tobacco	97%	-	-
Currently use tobacco	87%	-	-
Chew betel while working in the field	67%	1.06 (0.46–2.43)	0.65 (0.40–1.07)
First generation family history			
Any kidney disease	52%	0.94 (0.43–2.06)	1.25 (0.76–2.06)
Sibling	39%	0.89 (0.37–2.12)	1.07 (0.65–1.76)
Brother	35%	0.94 (0.38–2.31)	1.08 (0.64–1.80)
Any hemodialysis	25%	1.50 (0.61–3.70)	1.47 (0.86–2.49)
Occupational variables			
Ever farmed	96%	-	-
Currently farming	76%	0.76 (0.33–1.78)	0.54 (0.32–0.90)[c]
Worked an occupation other than farming	37%	0.61 (0.26–1.40)	0.46 (0.25–0.82)[d]
Hours worked per week during harvest season[e]	8 (7, 9)	0.98 (0.80–1.21)	1.01 (0.88–1.16)
Reduced number of hours and days for farming	54%	1.08 (0.50–2.35)	0.96 (0.58–1.56)
Agrochemical use: fertilizers	62%	0.82 (0.38–1.80)	0.63 (0.38–1.03)
Total hours spent applying fertilizer	6 (4, 9)	0.93 (0.81–1.06)	0.97 (0.91–1.04)
Agrochemical use: herbicides	56%	0.78 (0.36–1.68)	0.63 (0.38–1.03)
Total hours spent applying herbicide	2 (1, 4)	0.80 (0.62–1.03)	0.98 (0.90–1.08)
Agrochemical use: pesticides	51%	0.79 (0.37–1.72)	0.78 (0.47–1.27)
Total hours spent applying pesticide	2 (1, 4)	0.90 (0.74–1.08)	0.99 (0.92–1.05)
Eats lunch in the field while working	67%	0.68 (0.31–1.48)	0.54 (0.33–0.89)[c]
Severely thirsty while working	73%	0.80 (0.35–1.85)	0.72 (0.43–1.22)
Clinical variables			
Hospitalized in the past yr	26%	0.80 (0.35–1.85)	1.14 (0.66–1.96)
Self-reported pain while urinating	32%	1.37 (0.62–3.03)	1.02 (0.60–1.71)
Self-reported burning while urinating	43%	0.44 (0.19–1.05)	0.71 (0.43–1.19)
Self-reported hypertension	21%	1.51 (0.59–3.89)	1.70 (0.99–2.92)
Systolic blood pressure, per mm Hg	120 (110, 130)	1.01 (0.98–1.05)	1.01 (0.99–1.03)
Baseline sodium, per mmol/l	143 (140–145)	0.92 (0.82–1.03)	0.93 (0.87–1.00)[c]
Baseline potassium, per meq/l	4.3 (3.9, 4.6)	1.09 (0.55–2.17)	1.44 (0.96–2.15)
Baseline uric acid, per mg/dl	7.0 (5.9, 8.0)	0.95 (0.73–1.23)	1.09 (0.93–1.27)

CI, confidence interval; eGFR, estimated glomerular filtration rate; HR, hazard ratio.
[a]Data are presented as N (%) or median (IQR); HR reported for categorical variables with a minimum of 5 events or nonevents.
[b]Includes tube well, paddy well, protected dug well, unprotected dug well, paddy water, or from a surface water source (tank, stream, channel, lake)
[c]<0.05.
[d]<0.01.
[e]Among those with any working hours.

with lower hazards of >30% sustained eGFR decline (Supplementary Table S3).

DISCUSSION

In this cohort of persons with moderate and advanced nonproteinuric kidney disease living in a CKDu endemic region, kidney function decline was slow, implying that a prolonged duration of follow-up or initiation of follow-up at higher level of kidney function may be necessary to increase likelihood of identifying risk factors for significant kidney function decline. There was no evidence of universal hypokalemia or hyperuricemia as potential pathognomonic findings for CKDu. We did find that median serum sodium levels were consistently above 140 mmol/l, with 25% of both men and women having frank hypernatremia (≥145 mmol/l) at baseline. There was a small subset of participants experiencing >30% sustained eGFR decline; however, unlike in prior studies suggesting disproportionately poor outcomes among men, the proportion experiencing kidney function decline and the incidence rate of kidney-related events was similar among women compared with men. In an exploratory evaluation of demographic and clinical risk factors for >30% sustained eGFR decline, our analyses suggested that the 'micro-geographic' variables of residence within a specific locale, residence near a reservoir, and use of well water at enrollment were potential risk factors for kidney function decline among men, but not among women.[12] Further prospective work is required to confirm these preliminary findings, and could focus on participants with higher levels of kidney function so as to avoid potential reverse causation and survivor bias.

KiPP participants had entry criteria similar to participants in the Chronic Renal Insufficiency Cohort in the USA. Koye *et al.*[13] reported annual eGFR change of −0.17 ml/min per 1.73 m^2 and −1.35 ml/min per 1.73 m^2 among Chronic Renal Insufficiency Cohort (CRIC) participants with albuminuria <30 mg/g and albuminuria between 30 and 299 mg/g, respectively. Presuming our participants similarly had little to no albuminuria, as evidenced by the trace or negative protein urine dipsticks in the vast majority throughout out follow-up, the annual eGFR change we observed was similar (mean decline −0.5 [SD 4.9] ml/min/year). Our observed events rates for eGFR <15 ml/min were higher than those reported for end-stage kidney disease in the CRIC cohort and in the Kidney Early Evaluation Program for persons with eGFR <60 ml/min per 1.73 m^2 and systolic blood pressures <130 mm Hg; however, these differences may be due to differences in proportions with advanced kidney disease.[14]

Despite decades of research, without a confirmatory biopsy showing tubulointerstitial disease in a region with high reported prevalence, CKDu remains a diagnosis of exclusion without any pathognomonic clinical or laboratory findings (e.g., hyperuricemia and gout for persons with lead toxicity or urothelial cancer for persons with aristolochic acid nephropathy). One study from Nicaragua reported mean uric acid levels as high 9.6 mg/dl among affected men, whereas others from Nicaragua and Sri Lanka reported mean uric acid levels within or only slight above assay range among affected persons.[5,15,16] We do not find supportive evidence for significant hyperuricemia in this Sri Lankan cohort, although data on population-normative ranges among Sri Lankans are sparse. Hypokalemia has also been suggested as an early indicator of developing tubulopathy with renal potassium wasting.[17] In our study, although prevalence of hypokalemia was low, patients had established and moderately advanced kidney disease, perhaps masking earlier hypokalemia.

Consistent with findings of higher serum osmolality and mildly elevated serum sodium as reported by Fernando *et al.*[15] in a cohort of persons with CKDu, we did find median serum sodium consistently above 140 mmol/l in our study. These findings are in contrast to the US National Health and Nutritional Examination Survey data reporting population distributions of serum generally at or below <140 mmol/l, even when restricted to persons with CKD (median 139 [25th, 75th percentile 137–140]). Our data could imply either a tendency to ignore thirst, a urinary concentrating defect, or osmotic diuresis.[18] Further investigations to confirm this finding of hypernatremia, and to differentiate the potential etiologies could yield important insights into the pathophysiology of CKDu.

In these exploratory analyses, among men, there was a modest association between well water use and likelihood of >30% sustained eGFR decline. These findings are consistent with our earlier follow-up of this cohort,[12] and a case-control study from the northern province of Sri Lanka.[19] We have previously reported that household wells in Sri Lanka's dry region, most of them shallow and constructed during the settlements of these dry regions in the 1970s, are likely contaminated with the agrochemicals applied to rice paddy.[20] Others have reported the presence of cyanobacteria in these household wells.[21,22] Many strains of cyanobacteria produce the neurotoxin B-N-methylamino-L-alanine, and it is conceivable that given their vast genetic diversity, specific strains produce a kidney trophic toxin.[23] Other geographic correlates identified in exploratory analyses in our study were also linked to the hydrology, including residence in the northern region of the study area and closer to a reservoir. In the

northern region of our study area, the water flow is more likely downstream because there is higher relative elevation compared to the rest of our study area. The Sri Lankan reservoir system uniquely conserves water using a "tank cascade," where upstream earthen dams built in axis with the watershed capture largely rainfall and other fresh water, whereas downstream reservoirs have their own catchment area but may also receive irrigation drainage water returns and over-spill from tanks higher in the catchment.[24] In this way the reservoirs may concentrate hydrogeological exposures.

Observed associations with the composite outcome, specifically the protective association with working at enrollment and eating lunch in the field, likely reflect the health of the participant engaging in these behaviors, compared with those who have had to stop working. In addition, findings among men were not replicated among women in our study. We were limited by small sample sizes with only 14 women experiencing >30% sustained decline in eGFR. In general, our case cohort is vulnerable to survivor bias, such that men and women with protective factors for survival were more likely enrolled in our cohort.

The strengths of our study include a detailed questionnaire, geographic, and clinical characterization of persons with CKDu in Sri Lanka, and prospective, community-based follow-up illustrating the natural history of the disease, both of which can inform future investigations. We gathered data on multiple potential candidate risk factors at baseline and a comprehensive set of laboratories over time. There were several limitations. The overall slow decline observed in the cohort, and 3 years of follow-up may not be adequate to observe significant changes in kidney function. Many exposures were pervasive, limiting our ability to differentiate risk factors for faster decline, and our analyses only accounted for baseline eGFR and thus were exploratory. Furthermore, because an extant case cohort is subject to survivor bias, it implies that we are not capturing the exposures or rate of decline among patients with severe disease. We did not measure bioburden of agrochemicals, nor did we question practices about slash and burn cultivation, another putative risk factor for CKDu through silica exposure. Finally, given the limited number of men and even more limited number of women experiencing kidney function decline, our analyses of any potential risk factors are exploratory, adjusted only for baseline eGFR, and require further studies to confidently determine associations while accounting for a greater range of confounders.

In summary, in a cohort of persons with CKDu living in Sri Lanka's central province, the overall decline of kidney function was slow. A modest and similar proportion of both men and women experienced significant kidney function decline. We observed mild hypernatremia in this cohort, and associations with specific residence locale and source of water among men with kidney function decline. These need further study. Future research could build on our observed disease progression variation within endemic areas to interrogate environmental differences within these subregions, and therefore, narrow potential candidate causes of CKDu.

DISCLOSURE

SA reports consulting contracts with Vera Therapeutics and HealthPals Inc. All the other authors declared no competing interests.

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Data Sharing Statement

Data are not publicly available; however, anonymized data used to generate this analysis can be made available for researchers on review of request by study investigators.

SUPPLEMENTARY MATERIAL

Supplementary File (PDF)

Supplementary Methods for Laboratory Measures.

Figure S1. Missing data patterns during the study divided by 8 major data collection periods.

Table S1. Vitals and laboratory values of participants in study cohort over time[#].

Table S2. Incidence rates for death, eGFR < 15 ml/min, and sustained eGFR decline greater than 30% among men and women.

Table S3. Demographics, residence, and clinical correlates of rapid kidney function loss among women participating in the KiPP cohort, accounting for baseline eGFR.

STROBE Statement—Checklist of items that should be included in reports of cohort studies.

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