Changes in kidney function among Nicaraguan sugarcane workers

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Background: There is an epidemic of chronic kidney disease (CKD) of unknown etiology in Central American workers.
Objectives: To investigate changes and job-specific differences in kidney function over a 6-month sugarcane harvest season, explore the potential role of hydration, and measure proteinuria.
Methods: We recruited 284 Nicaraguan sugarcane workers performing seven distinct tasks. We measured urine albumin and serum creatinine and estimated glomerular filtration rate (eGFR).
Results: eGFR varied by job and decreased during the harvest in seed cutters (−8.6 ml/min/1.73 m^2), irrigators (−7.4 ml/min/1.73 m^2), and cane cutters (−5.0 ml/min/1.73 m^2), as compared to factory workers. The number of years employed at the company was negatively associated with eGFR. Fewer than 5% of workers had albumin-to-creatinine ratio (ACR) >30 mg/g.
Conclusions: The decline in kidney function during the harvest and the differences by job category and employment duration provide evidence that one or more risk factors of CKD are occupational.

Keywords: Chronic kidney disease, Mesoamerican Nephropathy, Chronic kidney disease of nontraditional etiology, Estimated glomerular filtration rate, Serum creatinine, Tubulointerstitial

Introduction

In Central America, there is an epidemic of chronic kidney disease (CKD) and the prevalence has been increasing over the past two decades.[1,2] The etiology of this disease, also referred to as Mesoamerican Nephropathy (MeN) and CKD of nontraditional etiology (CKD_{nt}), is unknown and traditional CKD risk factors are not implicated.[3–10] In Nicaragua, Honduras, and El Salvador, the age-adjusted mortality rates due to CKD are among the 10 highest in the world, with El Salvador having the highest at 54 deaths per 100 000 people.[11] In the provinces of Chinandega and León in western Nicaragua, 73 and 77 deaths per 100,000 people are attributed to CKD, respectively.[12] Cross-sectional studies in affected communities in Nicaragua and El Salvador have found the prevalence of reduced kidney function (as defined by glomerular filtration rate <60 ml/min/1.73 m^2) to range between 12 and 18% in the general population and between 14 and 26% in men.[13–17]

In more industrialized countries, CKD occurs almost equally in older men and women (>60 years old) with antecedent diabetes and/or hypertension, and with proteinuria as a frequent clinical manifestation.[18–20] Studies in Nicaragua indicate that men are three to five times more likely to be affected than women, and that the disease develops at younger ages, often before the age of 40.[2,13,17] In one study, the prevalence of reduced kidney function among men aged 31–40 was nearly 15 times higher in Quezalguaque, Nicaragua than in the United States.[13] Little evidence of diabetes or hypertension, and the lack of proteinuria, suggest that the kidney disease is primarily tubulointerstitial in nature.[2,9,13–17,21]

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Agricultural workers are a high risk population, two to three times more likely to develop CKD compared to individuals who have never worked in agriculture.[8,9,15,16] A history of working in the sugarcane, cotton, corn, rice, and banana industries is associated with increased CKD risk, though increased risk has also been observed in communities of miners, subsistence farmers, and fishermen.[15–17] These findings have primarily been based on descriptive surveys and cross-sectional studies, with most studies focusing on current occupation rather than lifetime occupational history. Many occupational and non-occupational risk factors have been hypothesized, including heat stress, agrichemicals, heavy metals, aristolochic acid, nephrotoxic medications, systemic infections (e.g. leptospirosis), and genetic factors.[2,6,7,22] Several papers suggest that heat stress, described as repeated exposure to heat and strenuous labor with associated repeated volume depletion and dehydration, may play an important role.[7,15,17,23,24] Most researchers believe that the causal mechanism of MeN is multifactorial, though the specific risk factors remain unknown.[4,7,23]

Given the high prevalence of CKD in Central America and limited availability of treatment, this epidemic has been significantly understudied. Analyses to date have focused on between-industry comparisons, but not within-industry (i.e. job-level) comparisons. There is a need for repeated measures studies and an improved approach to exposure assessment.[3] Accordingly, the primary aims of this study were to (1) investigate changes in kidney function among sugarcane workers in northwestern Nicaragua during the 6-month harvest season; (2) evaluate differences in kidney function by job category; (3) explore associations between self-reported hydration and kidney function, and (4) measure presence of proteinuria as an indicator of location of injury within the kidney.

Methods

Study design

The study population included sugarcane workers employed by one company in northwestern Nicaragua. All participants were at least 18 years of age and provided informed consent. The Institutional Review Boards at the Boston University Medical Center and the Nicaraguan Ministry of Health approved this study.

Prior to each harvest season, the company conducts a medical exam, which includes screening for elevated serum creatinine, to determine whether workers are healthy for employment. During the study period, workers with serum creatinine levels $\geq$1.4 mg/dl were not hired. Study participants (n=1249) were enrolled and provided pre-harvest samples between October and December 2010. The baseline assessment included collection of blood and urine samples and information about personal characteristics and occupational history.

Approximately 4–6 months later, toward the end of the harvest season (March–May 2011), we obtained a second sample from 506 workers at their respective work locations (Fig. 1). All workers were sampled during the morning hours, which occurred prior to starting the work shift for all field workers, prior to or during the work shift for factory workers, and at the end of the night work shift for drivers. The follow-up assessment collected blood and urine samples and information about work practices, hydration, and alcohol consumption. To assess hydration, workers were asked about the quantity of water and electrolyte solution packets (100 ml, distributed by the company) consumed during a typical workday.

A subset of the 506 workers sampled at pre- and late-harvest was included in the final study population. First, participants who performed more than one job during the harvest (n=34) were excluded from analysis. Next, we included all cane cutters, seed cutters, seeders, and agrichemical applicators. A random sample of irrigators, drivers, and factory workers was selected, as there were a larger number of workers in these jobs. Workers who reported performing jobs other than these seven were excluded due to small sample sizes. The final study population included 284 workers representing seven jobs with biological samples from pre- and late-harvest (Fig. 1). Our goal was to select job categories that were emblematic of certain exposures hypothesized to cause MeN and to include enough workers in each job to have adequate power. For each job, assessment of the likelihood of exposure to putative causal agents, relative to other jobs, was determined based on a prior industrial hygiene assessment (Table 1).[25]

Biomarkers of kidney function

Immediately following collection, blood and urine samples were processed at the local health center and stored at −20°C. Within 1 week, samples were transported to the ISO certified *Centro Nacional de Diagnóstico y Referencia* (CNDR) in Managua, a division of the Ministry of Health (MINSA), and stored at −80°C.

Serum creatinine was measured at CNDR using a kinetic-rate Jaffe method; 0.2 mg/dl was subtracted from creatinine results to calibrate to an isotopic dilution mass spectrometry (IDMS) standard. Urine samples were shipped to the Division of Nephrology and Hypertension at the Cincinnati Children's Hospital Medical Center (Cincinnati, OH, USA) for analysis of urine creatinine and albumin (to assess proteinuria).

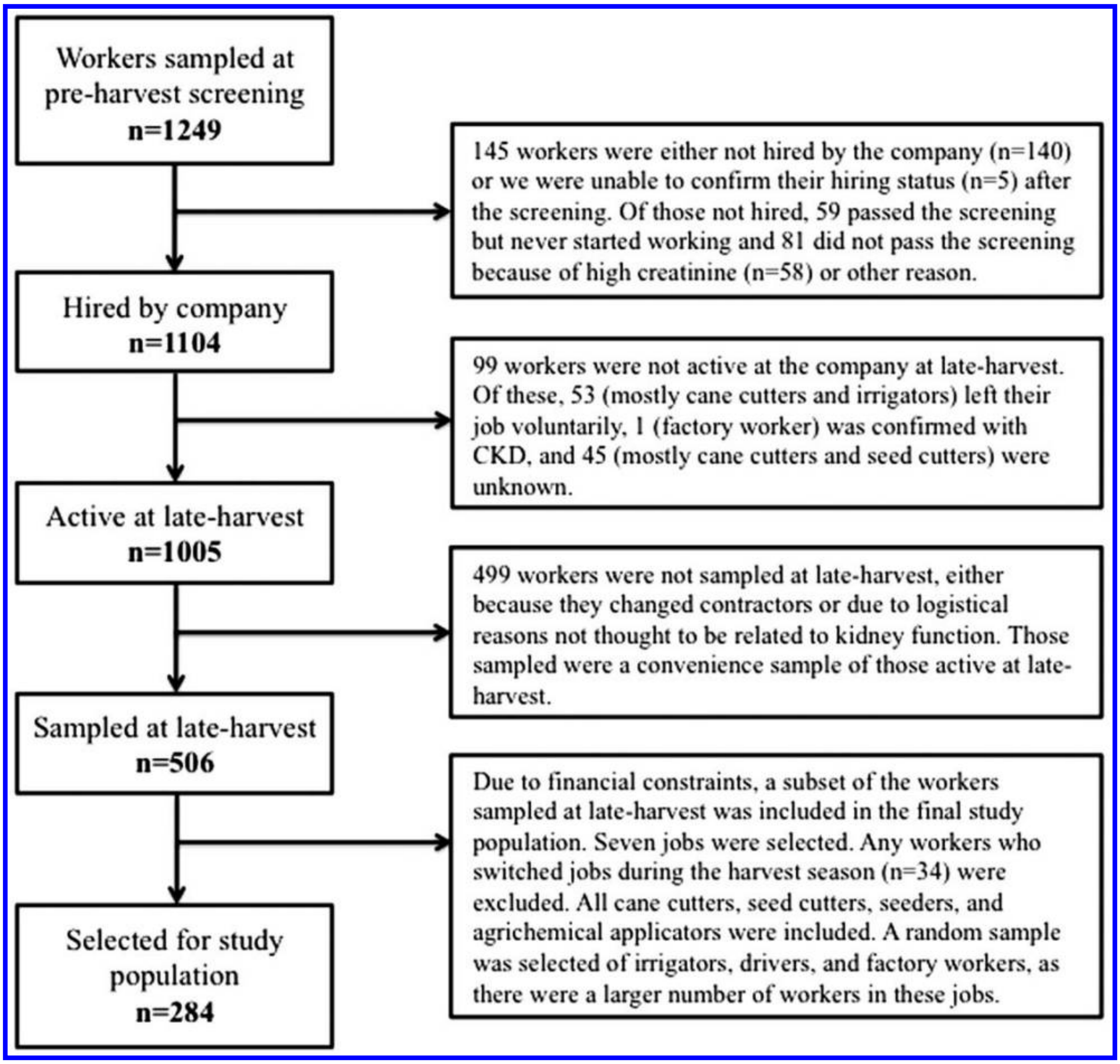

Figure 1 Derivation of study population (n=284).

Urine albumin and creatinine were measured by immunoturbidimetry and a colorimetric modification of the Jaffe reaction, respectively. The limit of detection (LOD) for urine albumin was 1.3 mg/l.

Data analysis

Data were analyzed using Statistical Analysis Software (SAS version 9.3, Cary, NC, USA). The distribution of each biomarker was examined using histograms, graphical displays, and summary statistics, to determine if eGFR and serum creatinine were normally distributed. For albumin values below the LOD, the LOD/$\sqrt{2}$ was substituted. To account for urine concentration, albumin was normalized to urine creatinine concentration and expressed as albumin-to-creatinine ratio (ACR) (mg/g). Serum creatinine (mg/dl) was used to estimate glomerular filtration rate (eGFR) (ml/min/1.73 m^2) using the Chronic Kidney Disease epidemiology collaboration (CKD-EPI) equation.[26] Lower eGFR and higher serum creatinine levels are indicative of worse kidney function. Race was considered ''non-black'' for purposes of calculating the CKD-EPI equation.

Paired t-tests were performed on unadjusted data to determine if eGFR and serum creatinine changed from pre-harvest to late-harvest by job category. Using multiple linear regression models, the association between job category and eGFR at pre-harvest, late-harvest, and change-during-harvest (calculated by subtracting each pre-harvest measurement from the corresponding late-harvest measurement) was evaluated. In the first set of models, ''field worker'' (yes/no) was the primary predictor of interest (reference: non-field worker). In the second set of models, the ''job category'' variable was the independent variable (reference: factory workers) (Table 1). Sensitivity analyses, restricted to men and to field workers, were performed to test for residual confounding by exposures associated

Table 1 Job category description and likelihood of exposure to putative causal agents[a]

Job category	Description	Likelihood of exposure			
		Heat stress	Agrichemicals	Metals (dust)	Leptospirosis
Field workers					
Cane cutter	Manually harvests the cane using machetes	H	M	H	H
Seeder	Transports seed billets and plants seeds	H	L	H	H
Seed cutter	Cuts sugarcane stalks to make seed billets	M	L	M	M
Agrichemical applicator	Mixes and applies agrichemicals (primarily herbicides) using backpack sprayers	H	H	M	M
Irrigator	Diverts water using a gravity-fed approach to irrigate cane fields	M	M	M	H
Non-field workers					
Driver	Operates vehicles during all steps of the sugarcane process	L	L	L	L
Factory worker	Processes the cane to create sugar, ethanol, and biofuels	L	L	L	L

[a]Likelihood of exposure to putative causal agents was determined relative to the other jobs.
H: high likelihood of exposure; M: medium likelihood of exposure; L: low likelihood of exposure.

with sex or field worker status. Because the CKD-EPI equation is not as accurate at higher levels of eGFR, sensitivity analyses were performed both truncating any eGFR values >120 ml/min/1.73 m^2 to 120 ml/min/1.73 m^2 and restricting analyses to workers with eGFR ≤120 ml/min/1.73 m^2.

Additional predictors of interest included years worked at the company, self-reported daily water/electrolyte solution intake, and weekly alcohol consumption. We explored the effects of these variables on kidney function overall and within categories of job and field worker status. Sex and age were included in all adjusted models. For the two workers missing information on the number of years worked, data were imputed using age, sex, and job. Though age was correlated with years worked at the company (r=0.67; P<0.001), the two variables were not collinear (i.e. tolerance greater than 0.1 and variance inflation factor less than 10). To evaluate previous employment, we analyzed pre-harvest eGFR by job according to whether participants had worked at the company during the previous year. In separate models, we assessed predictors of hydration to explore how water and electrolyte solution consumption differs by job category and sex.

Results

Study population and biomarkers of kidney function

Figure 1 summarizes the derivation of the final study population. The majority of workers were men, with women only employed as seed cutters, seeders, and factory workers. The mean age of workers was 33.6 years (Table 2), and on average, drivers and factory workers were older than field workers (Figure S1 in Supplementary material). The number of years worked at the company ranged from less than 1 to 40, with a mean of 9.4 years (Table 2).

Unadjusted values of serum creatinine and eGFR, stratified by job, are presented in Table 3. Both variables were normally distributed. Mean eGFR was lowest for drivers at pre-harvest and for cane cutters at late-harvest. Comparisons of pre-harvest to late-harvest measurements indicated that, on average, serum creatinine increased and eGFR decreased during the harvest for seed cutters, irrigators, cane cutters, and to a lesser degree, agrichemical applicators.

Thirteen workers (4.6%) had serum creatinine concentrations that increased during the harvest by at least 0.3 mg/dl, a magnitude unlikely to be consistent with random variability. The number of workers with eGFR<60 ml/min/1.73 m^2 (the cutoff for Stage 3 CKD) increased from one worker at pre-harvest (0.4%) to eight workers at late-harvest (2.8%). All eight were field workers and included three cane cutters, three seeders, one seed cutter, and one irrigator.

Table 2 Characteristics of study population (*n*=284)

	N (%)
Job	
Cane cutter	51 (18%)
Seeder	36 (13%)
Seed cutter	19 (7%)
Agrichemical applicator	29 (10%)
Irrigator	49 (17%)
Driver	41 (14%)
Factory worker	59 (21%)
Age	
18–24	58 (20%)
25–34	112 (39%)
35–44	65 (23%)
45–54	34 (12%)
55–63	15 (5%)
Sex	
Female	33 (12%)
Male	251 (88%)
Years worked at company	
<1–4	117 (41%)
5–9	62 (22%)
10–19	57 (20%)
20–29	38 (13%)
30–40	10 (4%)
Water per day (l)	
≤1	1 (0.4%)
2–3	66 (23%)
4–5	121 (43%)
6–7	41 (14%)
8–9	34 (12%)
≥10	14 (5%)
Missing	7 (2%)
Electrolyte solution packets per day	
0	65 (23%)
1–2	82 (29%)
3–4	87 (31%)
≥5	43 (15%)
Missing	7 (2%)
Alcoholic drinks per week	
0	189 (67%)
1–2	43 (15%)
3–5	17 (6%)
>5	27 (10%)
Missing	8 (3%)

Urine ACR was generally low in all workers at pre-harvest (median=2.4 mg/g) and late-harvest (median=2.1 mg/g). At both time points, fewer than 5% of workers had ACR>30 mg/g, the clinical threshold for abnormal urine ACR. Of the eight workers who had eGFR <60 ml/min/1.73 m^2 at late-harvest, only one had ACR>30 mg/g. Two of 29 workers (6.9%) with eGFR<90 ml/min/1.73 m^2 at late-harvest had ACR>30 mg/g.

Job category and eGFR

At pre-harvest, there was no difference in eGFR between field workers and non-field workers (Table 4, Model 1). When analyzed by job category, cane cutters had the lowest mean eGFR at pre-harvest, an average of 9.2 ml/min/1.73 m^2 (95% CI: −14.9, −3.4) less than factory workers (Table 4, Model 2). In a sub-analysis of cane cutters, those who had worked in the previous year had a mean pre-harvest eGFR that was 13.1 ml/min/1.73 m^2 (95% CI: −24.6, −1.7) lower than those who had not worked (age adjusted). Similar sub-analyses were not possible for additional job categories due to an insufficient number of new employees. In Model 2, years worked at the company was a predictor of eGFR, such that eGFR decreased by 0.3 ml/min/1.73 m^2 (95% CI: −0.6, −0.04) for each additional year of employment. The effect of employment duration did not differ by job.

At late-harvest, field workers had a mean eGFR that was 7.8 ml/min/1.73 m^2 (95% CI: −12.3, −3.2) lower than non-field workers (Table 4, Model 1). eGFR among cane cutters, seed cutters, seeders, and irrigators was lower than factory workers (Table 4, Model 2). The association between years worked at

Table 3 Summary statistics for biomarkers of kidney function and paired *t*-tests by job

	Pre-harvest		Late-harvest		Change during harvest[a]		
	Mean	Range	Mean	Range	Mean	Range	(95% CI)[b]
Serum creatinine (mg/dl)							
Cane cutter	0.89	0.6–1.5	0.96	0.5–2.7	0.07	−0.5–2.0	(−0.04, 0.2)
Seeder	0.72	0.4–1.5	0.73	0.4–1.5	0.005	−0.2–0.4	(−0.03, 0.04)
Seed cutter	0.73	0.5–1.1	0.79	0.5–1.5	0.06	−0.04–0.4	(0.004, 0.1)
Agrichemical applicator	0.80	0.6–1.1	0.82	0.6–1.2	0.03	−0.09–0.3	(−0.005, 0.06)
Irrigator	0.78	0.5–1.2	0.85	0.6–1.8	0.07	−0.09–1.0	(0.02, 0.1)
Driver	0.86	0.5–1.2	0.81	0.5–1.1	−0.06	−0.4–0.1	(−0.09, −0.02)
Factory worker	0.82	0.4–1.4	0.79	0.3–1.1	−0.02	−0.7–0.4	(−0.06, 0.02)
eGFR (ml/min/1.73 m^2)							
Cane cutter	111	61–137	108	29–150	−3.0	−90–34	(−9.1, 3.0)
Seeder	118	50–148	117	50–148	−0.2	−34–23	(−3.7, 3.4)
Seed cutter	116	72–135	112	52–135	−4.5	−31–2.7	(−8.7, −0.2)
Agrichemical applicator	116	74–143	114	82–139	−1.8	−23–7.7	(−4.1, 0.5)
Irrigator	120	84–144	116	46–143	−4.9	−63–9.1	(−8.3, −1.5)
Driver	106	67–147	110	78–139	4.6	−12–30	(1.7, 7.4)
Factory worker	113	69–161	115	76–181	2.1	−41–60	(−1.6, 5.8)

[a] Calculated by subtracting each pre-harvest measurement from the corresponding late-harvest measurement.
[b] 95% Confidence interval for paired *t*-test comparing pre-harvest measurement to late-harvest measurement.
eGFR: estimated glomerular filtration rate.

Table 4 Multivariable analysis of eGFR (ml/min/1.73 m²) by job

	Pre-harvest	Late-harvest	Change during harvest
Variables	Mean difference (95% CI)	Mean difference (95% CI)	Mean difference (95% CI)
Model 1[a]			
Job			
Field worker	−0.8 (−4.7, 3.1)	−7.8 (−12.3, −3.2)	−6.9 (−10.6, −3.2)
Non-field worker	Ref	Ref	Ref
Sex			
Female	1.7 (−3.8, 7.1)	5.5 (−0.9, 11.8)	3.7 (−1.4, 8.9)
Male	Ref	Ref	Ref
Age			
Years (continuous)	−0.9 (−1.1, −0.6)	−1.0 (−1.2, −0.7)	−0.1 (−0.3, 0.1)
Years worked			
Years (continuous)	−0.1 (−0.4, 0.2)	−0.05 (−0.4, 0.3)	0.06 (−0.2, 0.3)
Model 2[b]			
Job			
Cane cutter	−9.2 (−14.9, −3.4)	−14.1 (−20.9, −7.3)	−5.0 (−10.5, 0.6)
Seeder	−3.7 (−10.9, 3.5)	−8.9 (−17.5, −0.2)	−4.7 (−11.9, 2.4)
Seed cutter	−5.2 (−13.5, 3.1)	−14.0 (−23.8, −4.1)	−8.6 (−16.7, −0.5)
Agrichemical applicator	1.6 (−4.7, 7.9)	−2.2 (−9.6, 5.2)	−3.8 (−9.9, 2.3)
Irrigator	1.8 (−3.6, 7.3)	−5.5 (−12.0, 0.9)	−7.4 (−12.6, −2.1)
Driver	−3.4 (−9.1, 2.3)	−0.2 (−7.0, 6.5)	3.2 (−2.3, 8.7)
Factory worker	Ref	Ref	Ref
Sex			
Female	2.2 (−4.8, 9.3)	6.7 (−1.7, 15.2)	4.2 (−2.8, 11.1)
Male	Ref	Ref	Ref
Age			
Years (continuous)	−0.8 (−1.0, −0.5)	−0.9 (−1.2, −0.6)	−0.1 (−0.4, 0.1)
Years worked			
Years (continuous)	−0.3 (−0.6, −0.04)	−0.2 (−0.6, 0.1)	0.08 (−0.2, 0.3)

[a] Model 1: individual job categories grouped into field workers (cane cutters, seeders, seed cutters, agrichemical applicators, and irrigators), and non-field workers (drivers and factory workers).
[b] Model 2: all job categories analyzed individually.
eGFR: estimated glomerular filtration rate.

the company and late-harvest eGFR, though of lesser magnitude, followed a similar trend as at pre-harvest and did not vary by job.

Finally, we evaluated the change in eGFR during the 6-month harvest (late-harvest eGFR minus pre-harvest eGFR). Field workers experienced a 6.9 ml/min/1.73 m^2 (95% CI: −10.6, −3.2) decrease in eGFR during the harvest compared to non-field workers (Table 4, Model 1). Seed cutters (−8.6 ml/min/1.73 m^2; 95% CI: −16.7, −0.5), irrigators (−7.4 ml/min/1.73 m^2; 95% CI: −12.6, −2.1), and cane cutters (−5.0 ml/min/1.73 m^2; 95% CI: −10.5, 0.6) experienced the largest mean decreases in eGFR during the harvest, compared to factory workers. The number of years worked at the company was not a predictor of change in eGFR during the harvest. As an alternative to evaluating change-during-harvest, similar analyses were conducted using general regression models accounting for repeated measures with an unstructured covariance matrix, and results were similar to those presented (not shown).

For the pre- and late-harvest models, differences by job category were still observed when the sample was restricted to males or field workers (not shown). In the models evaluating change in eGFR, differences were still observed when restricted to males. When the sample was restricted to field workers, the trends and differences by job category were in a similar

Table 5 Multivariable analysis of eGFR (mL/min/1.73 m²) by hydration

	Late-harvest		Change during harvest	
Variables	β[a]	(95% CI)	β[a]	(95% CI)
Model 1[b]				
Water consumption (l/day)	−0.8	(−1.7, 0.2)	−0.5	(−1.3, 0.3)
Electrolyte solution consumption (n/day)	−0.4	(−1.5, 0.7)	0.02	(−0.9, 0.9)
Model 2[c]				
Water consumption (l/day)	−0.07	(−1.1, 0.9)	0.03	(−0.8, 0.9)
Electrolyte solution consumption (n/day)	0.3	(−1.0, 1.6)	0.7	(−0.3, 1.7)

[a] Estimate (β) shown is per unit increase of consumption.
[b] Adjusted for sex, age, and years worked
[c] Adjusted for sex, age, years worked, and job category
eGFR=estimated Glomerular Filtration Rate

direction but of lesser magnitude (not shown). Trends and differences by job category were similar when eGFR values >120 ml/min/1.73 m^2 were truncated to 120 ml/min/1.73 m^2 and when restricting to workers with eGFR ≤120 ml/min/1.73 m^2 (not shown).

Hydration and eGFR

Overall, workers reported consuming a mean of 5.1 liters of water and 2.4 electrolyte solution packets (100 ml each) while at work each day (Table 2). Seed cutters, cane cutters, and irrigators reported drinking the most water each workday, on average consuming 2.9, 2.3, and 2.3 liters more than factory workers each day, respectively, after adjusting for sex. On average, women reported drinking less water (difference=−2.2 l) than men, while controlling for job (Table S2 in Supplementary material). Cane cutters and agrichemical applicators reported consuming the most electrolyte solution packets each day, at an average of nearly four per day. There was no difference in electrolyte solution consumption by sex, after controlling for job (Table S2 in Supplementary material).

Each additional liter of water consumed during the workday was associated with an average 0.8 ml/min/1.73 m^2 lower eGFR at late-harvest (95% CI: −1.7, 0.2) and a 0.5 ml/min/1.73 m^2 decrease in eGFR during the harvest (95% CI: −1.3, 0.3), after controlling for sex, age, years worked, and electrolyte solution consumption (Table 5, Model 1). These associations with water consumption disappeared after also adjusting for job category (Table 5, Model 2).

We did not find an association between eGFR and electrolyte solution consumption, after controlling for sex, age, years worked, and water consumption (Table 5). When stratified by job, electrolyte solution consumption appeared protective only for cane cutters. For every additional electrolyte solution packet consumed by cane cutters during the workday, mean late-harvest eGFR was 6.1 ml/min/1.73 m^2 higher (95% CI: −0.06, 12.2), and eGFR increased during the harvest by a mean of 7.0 ml/min/1.73 m^2 (95% CI: 1.9, 12.1).

Self-reported alcohol consumption was low, with 67% of workers reporting that they did not drink at all and 10% reporting >5 drinks per week (Table 2). Alcohol is generally not an independent predictor of CKD and was not associated with eGFR, so was excluded from final models.

Discussion

This is the first longitudinal study, to our knowledge, to assess kidney function in a population at risk for MeN. We found that the kidney function of Nicaraguan sugarcane workers decreased during the harvest season, varied by job category, and was associated with the number of years worked at the company. These findings suggest that one or more of the underlying risk factors is related to occupational exposure. We also found that self-reported hydration with water and electrolyte solution varied by job category and sex and was not associated with kidney function, with the exception of electrolyte solution consumption among cane cutters. Additionally, given that albuminuria was rare, our results suggest that the disease is likely tubulointerstitial rather than glomerular in nature.

Field workers were at greater risk of kidney function decline over one harvest season compared to non-field workers (factory workers and drivers). This finding suggests that fieldwork is associated with reduced kidney function, though not all field workers were equally affected. Additionally, we cannot rule out non-occupational differences between field workers and non-field workers that may also play a role in the development of MeN in combination with occupational exposures. For example, it is possible that a community may experience low-level exposure to a nephrotoxic agent (via food or drinking water) that by itself is insufficient to cause disease, but in combination with occupational factors such as heat stress and dehydration causes CKD. These potential etiologic occupational exposures may be explored more closely by examining differences in kidney function by individual job categories, each of which require different tasks with varying exposures to putative causal agents (Table 1). Though future investigations will benefit from direct monitoring of such agents, this preliminary study uses job category as a proxy for exposure, improving on past exposure assessments conducted at the industry level.

Over the 6-month harvest season, the decline in kidney function was greatest among seed cutters, irrigators, and cane cutters. These jobs require strenuous labor in a hot and humid environment, and cane cutters and seed cutters (as well as seeders) are paid piecemeal, which is often more physically demanding due to the financial incentives. During a previous industrial hygiene assessment, we measured wet-bulb globe temperatures (WBGT) ranging from 26.9 to 33.2°C.[25] Other assessments in sugarcane fields in Nicaragua and Costa Rica have documented similar ambient conditions and found that sugarcane workers, particularly cane cutters, are at risk of heat stress during their work shift.[27–30] The differences we found in eGFR by job category are consistent with the hypothesis that heat stress may be a contributing factor, and future studies should quantify personal exposure to heat and dehydration. However, other unmeasured factors that vary by job could also contribute to the observed results. For example, cane cutters and seeders work in dusty conditions, likely with exposure to metals, and irrigators would have the greatest exposure to agents in water (e.g. leptospirosis). Among the field workers, agrichemical applicators experienced the least decline in kidney function. Because these workers have the most direct contact with agrichemicals, this finding does not support the hypothesis that agrichemicals are a causal agent. However, agrichemicals are used extensively in the region and widely believed among community members as the cause of the epidemic; additionally, several studies have found an association between self-reported agrichemical use and CKD.[1,8,9,14] Furthermore, in September 2013, El Salvador's National Assembly voted to ban 53 agrichemicals, including 2,4-D, paraquat, DDT, and glyphosate, among others, due to the perceived link to CKD.[31] Agrichemicals, therefore, remain an important hypothesis and warrant further study.

Many researchers hypothesize that inadequate hydration, coupled with repeated volume depletion from exposure to heat and strenuous labor, is partially responsible for MeN.[7,15,17,23,24] There is also a strong perception among community physicians and pharmacists that poor hydration and strenuous labor are the major occupational factors responsible for the disease, and that there may be a reluctance to hydrate during the workday due to fear of contaminated water.[4] We found a modest association between water consumption and eGFR, with water consumption appearing harmful for kidney function, similar to a finding by Sanoff *et al.*[16] Although this finding could be interpreted as consistent with the presence of a nephrotoxin in water, increased water consumption may also indicate a heavy workload, suggesting that the increased risk might be associated with working in strenuous jobs. Consistent with this latter interpretation, the association between increased water consumption and lower eGFR disappeared after controlling for job. We did observe a protective effect of electrolyte solution consumption among cane cutters. This suggests that for the most high-risk jobs, rehydrating with electrolyte solutions during the workday may be protective against renal damage; however, additional research should confirm this observation. Our protective finding contrasts with a recent study that found electrolyte solution consumption during the workday to be harmful for renal function.[9] It is possible, however, that the harmful effect observed in that study is an artifact and would have disappeared with an adjustment for job category (as did our similar finding for water consumption).

Prior to the harvest, cane cutters had the lowest eGFRs of any job. There are several possible explanations for this finding: (1) cane cutters have held the same job for multiple years and their job exposures reduce kidney function more than other positions, (2) cane cutters were more likely to work in occupations with similar conditions/exposures during the non-harvest season, (3) cane cutters have greater muscle mass leading to higher creatinine generation (rather than decreased clearance), or (4) there are other shared characteristics of individuals who apply for cane cutting jobs that account for the decreased eGFR. Sub-analyses restricted to cane cutters indicated that those who worked at the company the previous year had a mean eGFR that was 13 ml/min/1.73 m^2 lower than those who did not. This result is consistent with the explanation that exposures related to working as a cane cutter reduce kidney function more than other jobs. However, we did not have data to address the other possible explanations.

We also found that the number of years employed by the company was associated with reduced eGFR, after controlling for age. Pre-harvest eGFR decreased on average by 0.3 ml/min/1.73 m^2 for each additional year employed by the company. Therefore, 10 years of employment would, on average, be associated with a reduction in eGFR of 3.0 ml/min/1.73 m^2. At late-harvest, the association followed a similar trend. This is consistent with the findings of Peraza *et al.*, in which years of coastal sugarcane or cotton work were associated with elevated serum creatinine levels.[15] Specifically, they found that for every 10 years of employment in these industries, men (OR = 3.1) and women (OR = 2.3) had an increased risk of elevated serum creatinine levels, after controlling for age and smoking history.

Several studies conducted in the region have found that proteinuria is uncommon in individuals with CKD, and, when present, is generally low-grade.[8,9,13–15,17] Consistent with these findings, few workers in the current study ($<5\%$) had albuminuria. Of those workers with reduced kidney function, only 13% with eGFR <60 ml/min/1.73 m^2 and 7% with eGFR <90 ml/min/1.73 m^2 had low levels of albuminuria, suggesting that the disease process does not appear to be primarily affecting the glomerulus. Kidney biopsy specimens from CKD patients in El Salvador showed signs of damage to both glomerular and tubulointerstitial compartments; biopsies showed varying amounts of glomerular sclerotic lesions with extensive tubular atrophy and interstitial fibrosis.[32,33] Given the paucity of albuminuria, these biopsies support a primary tubulointerstitial disease with secondary glomerular manifestations.

A limitation of this study is the use of job category as a surrogate exposure metric. Though an improvement on previous exposure surrogates such as "community of residence" or "industry category," job category is a surrogate for one or more unknown risk factors. These factors could include occupational or non-occupational exposures. Of most concern is that field workers may be different from non-field workers with respect to non-occupational risk factors. However, we found that kidney function was different by job category even when restricted to field workers, suggesting that there is an occupational component to the disease.

An additional limitation is the fact that the assessment of hydration and alcohol was based on self-reported consumption. We found no association between water consumption and eGFR after controlling for job. While it is possible that there is no association, it is also possible that the null finding is attributable to exposure misclassification.

There were two types of loss-to-follow-up (LTF) during the harvest: (1) 99 workers who were no longer working at the time of the late-harvest sampling and (2) 499 workers who were not sampled even though they were considered active employees at the time of the late-harvest sampling (Fig. 1). Regarding the first type of LTF, 53 of the 99 workers left their jobs voluntarily, one factory worker left due to CKD, and the reasons for the remaining 45 were unknown. The majority of these 45 were cane cutters (n=18) and seed cutters (n=10), and it is possible that the reason for leaving was related to kidney function. We found that cane cutters and seed cutters experienced decreases in kidney function; therefore, if LTF was related to kidney function, the effects we observed would more likely be an underestimate than an overestimate. Regarding the second type of LTF, financial and logistical constraints forced us to select a sample of those active in the same job at the late-harvest investigation. Each workday, field workers were dispersed across an area of 35 000 hectares, and it was not possible to visit each area to obtain samples from all workers. We were, however, able to confirm that these 499 workers LTF were actively working at late-harvest, and it is unlikely that the placement of workers in particular fields would be related to their kidney function. We would be more concerned about bias if these 499 workers were no longer employed by the company at late-harvest.

Importantly, it was not a goal of this investigation to characterize the prevalence of CKD. The sugarcane company has a health surveillance program that is designed to identify workers with elevated serum creatinine. When workers are screened prior to the harvest, those with elevated creatinine are not hired. Accordingly, these screening procedures reduce the number workers with CKD, creating a healthy worker effect. However, the repeated measures study design addressed this issue by evaluating change in kidney function in the same workers over a 6-month harvest period. Because this investigation was only during one harvest, we had limited ability to assess incidence or progression of CKD; however, 2.8% of workers did develop new occurrence of eGFR <60 ml/min/1.73 m^2, all of whom were field workers. This number could be an underestimate due to the first type of LTF described above.

Conclusion

Our results suggest that the kidney disease is primarily tubulointerstitial, and not glomerular, in nature. This is important because the primary causes of CKD globally are diabetes and hypertension, which more often result in glomerular disease manifestations. The observed decline in kidney function during the harvest as well as the differences in kidney function by job category and employment duration provide evidence that one or more risk factors are occupational. These results, as well as the protective effect of consuming electrolyte solution, are consistent with the hypothesis that heat stress and dehydration may play a role in MeN. Future studies that directly measure exposure to heat and dehydration are needed. Our results are not consistent with the hypothesis of agrichemicals as causal agents. However, given the extent of use in the region and widely held belief in the community that agrichemicals play a role, future studies should characterize personal exposure to agrichemicals.

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