BIOLOGICAL SAMPLING REPORT:
Investigating biomarkers of kidney injury and chronic kidney disease among workers in Western Nicaragua

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I. INTRODUCTION

From October 2010 to June 2011, the Boston University team conducted an investigation of biological markers of kidney injury and chronic kidney disease (CKD) among workers in Western Nicaragua. Workers in the sugarcane industry were recruited from the Ingenio San Antonio (ISA) and tested at the beginning and during the latter part of the 2010-2011 zafra (sugar cane harvest). Workers in three other industries in Western Nicaragua (miners, construction workers, and port workers) were also tested once between March and June of 2011. The primary objectives of the investigation were to:

- Evaluate characteristics of the disease to determine whether kidney damage is tubulointerstitial or glomerular.
- Evaluate biomarkers of kidney injury and CKD among ISA workers by investigating changes during the zafra and differences by ISA job.
- Determine whether there is evidence of kidney injury or CKD among workers in other industries who have never worked in the sugarcane industry.
- Analyze heavy metals in biological samples collected at both pre-zafra and late-zafra to characterize metals exposure in the region and explore relationships with biomarkers of kidney injury and CKD.
- Culture urine samples collected from ISA workers at late-zafra to investigate the frequent clinical diagnosis of UTIs among young men in this region.
- Determine whether hydration practices or alcohol consumption are associated with biomarkers of kidney injury or CKD.

It should be noted that the objective of this investigation was NOT to characterize the prevalence of CKD in the region or to compare the prevalence of CKD at ISA to the prevalence in other industries in the region. Addressing these objectives was not possible because ISA has a health surveillance program that is designed to identify workers with elevated serum creatinine. The largest component of the ISA surveillance program is conducted prior to the start of each zafra (November) and is required of all temporary employees and subcontracted workers. Workers with elevated creatinine at the pre-zafra screening are not hired. An optional "mid-zafra" screening, primarily for subcontracted workers, is conducted in January or February, and typically 50-70% of subcontracted workers elect to participate. In addition, employees who come to the ISA Hospital for medical attention are also tested for creatinine as part of their visit. At late-zafra, some workers were not available for testing. For some of these workers, the reason may have been related to elevated creatinine identified during the zafra. Other workers may have stopped working for reasons that were unrelated to health, either due to a decreased need for labor in the final month of the zafra or simply due to a voluntary decision to leave.

However, similar screening procedures are not used in the other industries in the region. Accordingly, the prevalence of CKD among actively engaged ISA workers is biased toward a lower percentage because these screening procedures reduce the number of ISA workers with CKD. The reduction occurs because workers with CKD are not hired at the beginning of the zafra and may not be actively working at the end of the zafra as a result of screening. This concern was addressed in the study design, which evaluates a population of ISA workers at pre-zafra and then again during the latter part of the zafra, so that the focus is on *change* in biomarkers of kidney injury and CKD, with each worker serving as his or her own control.

It should also be noted that it was not possible to include an assessment of heat stress (i.e., volume depletion and muscle damage) or agrichemical exposure as part of this investigation. These are clearly important hypotheses that require investigation, but there is no single study that can adequately address all hypotheses. We designed the current investigation to evaluate changes in biomarkers of kidney injury and CKD during the zafra, a time period of approximately five months. However, an investigation of heat stress and agrichemicals would require a different study design in which biological samples would be collected at the beginning and at the end of a workday, in addition to monitoring ambient conditions and core body temperature throughout the workday. Each type of study would allow us to address some questions but not others, and we are currently designing a study that specifically will investigate the potential role of volume depletion, muscle damage, and agrichemical exposure.

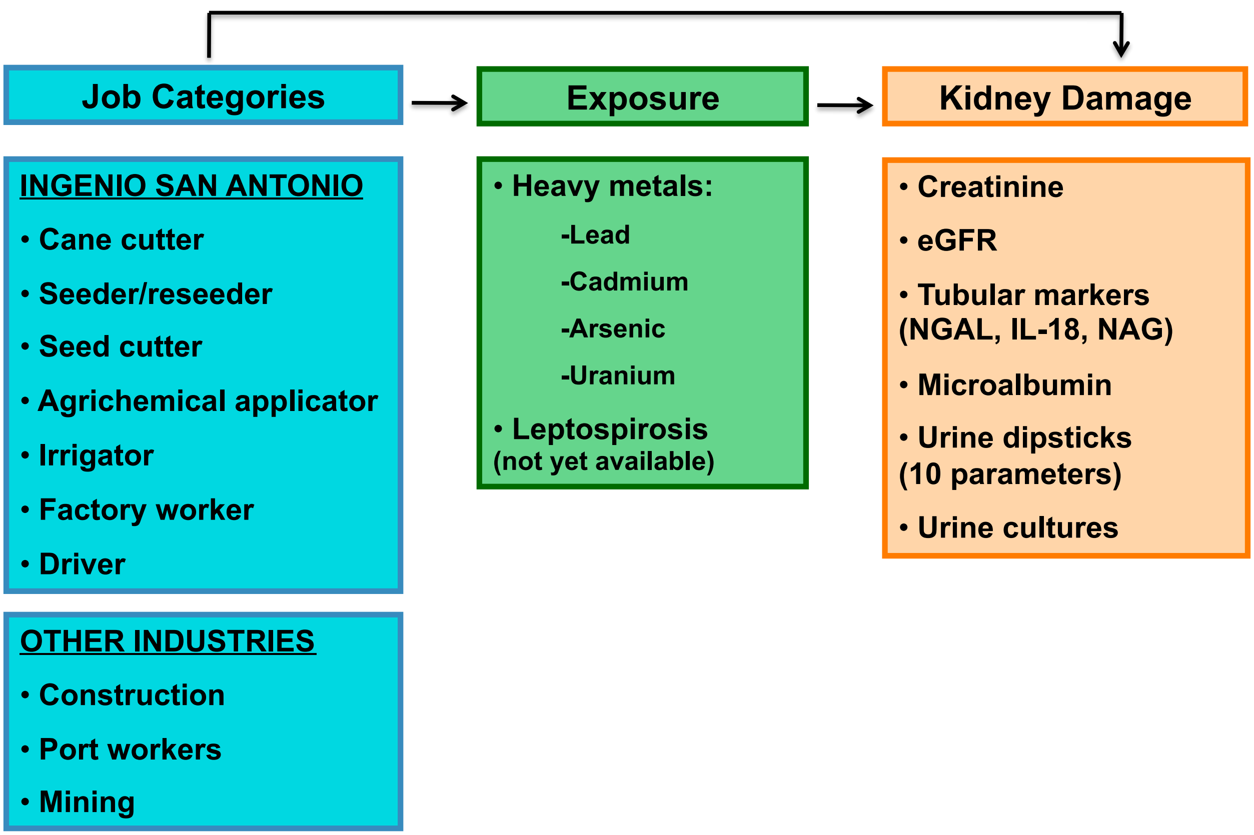
II. METHODS

Study protocols were reviewed and approved by the Institutional Review Boards at the Boston University Medical Center and at the Nicaraguan Ministry of Health. All study subjects provided informed consent prior to participation in research activities.

2.1 Study design

Figure 1 presents an overview of the study design. The study population included workers in four industries: sugarcane, mining, construction, and port workers. Sugarcane workers were selected to represent seven different job categories: cane cutters, seed cutters, seed planters, agrichemical applicators, irrigators, drivers, and factory workers. The miners, construction workers, and port workers were selected to represent other jobs in the same region that also require heavy manual labor.

Figure 1. Overview of study design



Within the sugarcane industry, "job category" is used as a surrogate exposure variable since the specific causal agent(s) are unknown. Job characteristics likely vary with respect to the potential for many different exposures of interest, such as heat stress, agrichemicals, metals, silica, and leptospirosis. If we were to observe no differences in biomarkers of kidney injury and CKD by job category, this would suggest that the primary CKD risk factors are not occupational. However, if there are significant differences in biomarkers of kidney injury and CKD by job category, then identifying which jobs appear to have the highest risk will represent an important step toward identifying the causal agent(s).

Two possible causal agents, specifically heavy metals (lead, cadmium, arsenic, and uranium) and leptospirosis, were evaluated as part of this investigation. For these agents, we first evaluated whether exposures vary by job category and then evaluated whether they are associated with biomarkers of kidney injury and CKD. The results for heavy metals are included in this report; however, leptospirosis data are not yet available and will be summarized in a separate report at a later date.

2.2 Study population

The study population included workers in four industries: sugarcane, mining, construction, and port workers. To be eligible for participation, workers were required to be at least 18 years of age and to be employed in one of the jobs of interest. The recruitment process and study population is described in the sections below.

2.2.1 Sugarcane Workers

At the beginning of the zafra (October to December 2010), we tested a total of 1,249 sugarcane workers representing 10 different jobs at ISA. Workers were primarily recruited and tested at the same time and location of the pre-zafra screening sessions conducted by ISA. The pre-zafra investigation included the collection of blood and urine samples, as well as basic demographic information and a short work history using a brief questionnaire. Of the 1,249 applicants who were tested at pre-zafra, at least 140 never started working, either because they had elevated creatinine or for other reasons. As a result, only 1,109 of the 1,249 applicants were hired and worked at some point during the zafra.

At the late-zafra sampling investigation (March to April 2011), 1,005 of the 1,109 hired sugarcane workers (91%) were still working at ISA. The other 104 workers (9%) were lost-to-follow-up for various reasons. It is likely that some stopped working due to personal choice or a decreased need for labor in the final month of the zafra, while others may no longer have been part of the active workforce due to elevated serum creatinine. Accordingly, the 1,005 workers who were available to be tested at the late-zafra investigation do not represent a random sample of the 1,109 hired workers.

Of the 1,005 workers who were still employed at ISA, 506 workers (50%) were tested at their respective work locations at the late-zafra investigation. In this case, the 499 workers who were not tested (48%) were primarily missed due to the logistics of the late-zafra investigation. Each workday, field workers were dispersed across an area of 35,000 hectares and the administrative practices at ISA do not allow the daily work locations to be tracked for individual workers. Accordingly, the logistics of the late-zafra investigation were much more complicated and challenging than for the pre-zafra investigation. The late-zafra investigation included the collection of blood and urine specimens as well as information about work practices, urinary symptoms, and hydration practices using a questionnaire. The potential impact of the combined losses from the 1,109 hired workers is addressed in Section IV (Limitations).

The final study population of 284 ISA workers was selected from the 506 workers who had been tested at both the beginning of the zafra and at the end of the zafra. First, we excluded workers who changed jobs during the zafra. Next, we included all remaining cane cutters, seed cutters, seed planters, and agrichemical applicators. Finally, we selected a random sample of the remaining irrigators, drivers, and factory workers to bring the total study population to 284 workers. This last step was conducted because of the large number of workers in these three jobs. Machinery operators, maintenance workers, and puchos (workers who collect the cut cane) were excluded from the final study population, which represented 7 of the 10 jobs. The cane cutters, seed cutters, seed planters, and agrichemical applicators are subcontracted workers. The irrigators, drivers, and factory workers are temporary workers, subcontracted workers, and/or permanent workers. Throughout the report, we will generally refer to this portion of the study population (n=284) as 'ISA workers.'

We also analyzed samples collected from a second population of study participants at ISA, which included 47 job applicants who were randomly selected from among the 59 applicants determined to have elevated serum creatinine (≥1.4 mg/dL) at the pre-zafra screening, based on serum creatinine data provided by ISA. These workers were not tested again at the late-zafra because most were not hired. This second population of 47 workers was included for the purpose of evaluating biomarkers of kidney injury in a subgroup of workers who arrived at the pre-zafra screening with elevated creatinine levels. Throughout the report, we will generally refer to this portion of the study population (n=47) as 'ISA applicants.'

Table 1 summarizes the characteristics of the 284 ISA workers who were tested at the beginning of the zafra and at the end of the zafra, as well as the 47 ISA applicants with elevated creatinine who were only tested at the beginning of the zafra.

2.2.2 Miners, Construction Workers, and Port Workers

At around the same time as the late-zafra investigation of ISA workers, we recruited 164 workers from three other industries in the Western region of Nicaragua who had never worked in the sugarcane industry. The 51 miners, 60 construction workers, and 53 port workers were recruited and tested at their respective work locations from May to June 2011. It was not possible to recruit and test these workers in the first round of sampling (pre-zafra).

The workers in the construction industry primarily included laborers involved in various aspects of constructing traditional houses and commercial buildings. The workers in the mining industry primarily included laborers and operators who work in and around the mine. The port workers were selected from among those who are affiliated with unions and are brought in to perform heavy manual labor when ships require assistance. The port workers in our population did not work for the governmental institution, *The Portuaria*, where the use of large machines is more common and the work is therefore less labor intensive.

Table 1. Characteristics of study population

	ISA Workers	ISA Applicants	Miners	Construction	Port Workers
	N (%)	N (%)	N (%)	N (%)	N (%)
Total	284 (100%)	47 (100%)	51 (100%)	60 (100%)	53 (100%)
Age					
18-25	57 (20%)	5 (11%)	1 (2%)	15 (25%)	6 (11%)
25-34	113 (40%)	27 (57%)	21 (41%)	20 (33%)	13 (25%)
35-44	64 (23%)	10 (21%)	18 (35%)	9 (15%)	15 (28%)
45-54	35 (12%)	3 (6%)	10 (20%)	12 (20%)	11 (21%)
55-63	15 (5%)	2 (4%)	1 (2%)	4 (7%)	8 (15%)
Sex					
Male	254 (89%)	47 (100%)	48 (94%)	60 (100%)	53 (100%)
Female	30 (11%)	0 (0%)	3 (6%)	0 (0%)	0 (0%)
Department of residence					
Chinandega	264 (93%)	NA	1 (2%)	60 (100%)	53 (100%)
Leon	18 (6%)	NA	50 (98%)	0 (0%)	0 (0%)
Missing	2 (1%)	NA	0 (0%)	0 (0%)	0 (0%)
ISA Job					
Cane cutter	51 (18%)	18 (38%)			
Seeder/reseeder	28 (10%)	3 (6%)			
Seed cutter	26 (9%)	13 (28%)			
Factory worker	59 (21%)	0 (0%)			
Agrichemical applicator	29 (10%)	0 (0%)			
Irrigator	50 (18%)	2 (4%)			
Driver	41 (14%)	0 (0%)			
Machinery operator	0 (0%)	0 (0%)			
Maintenance worker	0 (0%)	0 (0%)			
Pucho	0 (0%)	11 (23%)			

NA = Not available

The late-zafra protocols for the investigation of miners, construction workers, and port workers were the same as those used for the investigation of sugarcane workers. Accordingly, the investigation included the collection of blood and urine samples, as well as information about work practices, urinary symptoms, and hydration practices using a questionnaire. Table 1 summarizes the characteristics of the 51 miners, 60 construction workers, and 53 port workers who were tested at the late-zafra.

2.3 Collection and analysis of blood and urine samples

2.3.1 Collection of blood and urine samples

At the pre-zafra sampling, blood and urine samples were collected from ISA workers and ISA applicants by our study personnel inside the same building and at the same time as the pre-zafra screening conducted by ISA personnel. At the late-zafra sampling, blood and urine samples were collected from ISA workers, miners, construction workers, and port workers at their respective work locations (e.g., in the field, at the factory, etc.). All ISA field workers were sampled pre-shift prior to entering the field for work that day. Most factory workers and drivers were also sampled pre-shift, although some factory workers were sampled mid-morning after they had already been working a few hours and some drivers were sampled in the early morning as they concluded a night-shift. Port workers and construction workers were always sampled pre-shift, but some miners were sampled mid-morning after they had already been working a few hours.

Prior to providing a urine sample, subjects washed their hands with soap and then cleaned the perineal/genital area with an alcohol pad. Each subject was asked to void into a sterile 100mL container. At the late-zafra sampling of ISA workers, each subject was asked to first void into a sterile 100mL plastic container and then to void into a second 100mL plastic container, which was included for the purpose of culturing urine and for analysis of metals. The urine containers included no metal inserts, glued caps, or preservatives. The urine samples were immediately placed in a cooler for transport to the Chichigalpa Health Center. At the Chichigalpa Health Center, urine samples were aliquoted and stored at -20 °C until transported to CNDR in Managua for storage at -80 °C.

Blood samples were collected from each subject by experienced phlebotomists using one 4mL Vacuette® K_3EDTA tube, followed by two 8 mL red top Vacuette® tubes. All collection tubes were lead free. Following collection, all three tubes were gently inverted 10 times to ensure mixing of blood with the anticoagulant or serum clot activator. The blood samples were immediately placed in a cooler for transport to the Chichigalpa Health Center. At the Chichigalpa Health Center, the red top tubes were centrifuged for 5 minutes at a speed of 3500 rpm, after which the serum was transferred to multiple 1.5 mL cyrovials (Greiner Bio-One). The serum samples and whole blood samples (in original K_3EDTA tubes) were stored at -20 °C until transported to CNDR in Managua where the whole blood was stored at -20 °C and serum was stored at -80 °C.

2.3.2 Urine dipstick analyses

A urine dipstick (Combur 10UX®, Roche Diagnostics) was immersed in the urine container and placed into the urine strip reader (Urisys 1100, Roche Diagnostics). The results of the dipstick measures (specific gravity, pH, leukocyte esterase, nitrite, protein, glucose, ketones, urobilinogen, bilirubin, blood) were recorded on the data collection sheet.

2.3.3 Analysis of serum creatinine

Serum samples were analyzed for creatinine at the Centro Nacional de Diagnóstico y Referencia (CNDR), a division within the Nicaraguan Ministry of Health (MINSA). Serum creatinine levels were measured using an alkaline picric acid kinetic assay (Creatinine Jaffé Gen. 2. Cobas Integra 400. Roche Diagnostics). To correct the non-specific reaction with serum pseudo-creatinine chromogens, including proteins and ketones, the results were automatically corrected by -18 µmol/L (-0.2 mg/dL) as part of the internal quality control designed by the manufacturer. Final results were expressed in milligrams of creatinine per deciliter of serum (mg/dL).

2.3.4 Analysis of kidney injury biomarkers and creatinine in urine

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 creatinine, albumin, neutrophil gelatinase-associated lipocalin (NGAL), N-acetyl--D-glucosaminidase (NAG), and interleukin-18 (IL-18). Urinary creatinine was analyzed for the purpose of normalizing other urinary biomarkers to account for urine dilution. The urine analytes, NGAL, NAG, and IL-18, are considered markers of tubular injury; their interpretations are described later in the report.

Urine creatinine and albumin were measured by immunoturbidimetry and a colorimetric modification of the Jaffe reaction, respectively, on a Siemens Dimension Xpand plus HM chemistry analyzer (Siemens Healthcare Diagnostics, Deerfield, IL). Urine creatinine was reported in grams per liter of urine (g/L) and all reported values were above the limit of detection. Urine albumin was reported in milligrams per liter of urine (mg/L) with a limit of detection of 1.3 mg/L. Urine albumin to creatinine ratio (ACR) was calculated as mg of albumin per gram of creatinine (mg/g).

NAG activity was measured using a colorimetric assay (Roche Diagnostics, USA). Briefly, 5 µl of sample was incubated with 100 µl of substrate solution (3-cresolsulfonphthaleinyl-N-acetyl-b-D-glucosaminide) for 20 min at 37 °C. The reaction was stopped with a stop solution containing sodium carbonate and the optical density measured at 580 nm. The optical density values were subtracted from blank and NAG activity was calculated and the activity was reported in units per liter of urine (U/L) but for analysis was converted to units per gram of creatinine (U/g). All reported values were above the limit of detection.

NGAL (Bioporto, Gentofte, Denmark) and IL-18 (MBL, Intl., Woburn, MA) were measured by ELISA according to the manufacturer's instructions. NGAL was reported in nanograms per milliliter of urine (ng/ml) but for analysis was converted to micrograms per gram of creatinine (µg/g). All reported NGAL values were above the limit of detection. IL-18 was reported in picograms per milliliter of urine (pg/ml) but for analysis was converted to nanograms per gram of creatinine (ng/g). The detection limit for IL18 was 4 pg/ml.

2.3.5 Analysis of heavy metals

Urine and blood samples were shipped to the Centre de toxicologie of the Institut national de santé publique du Québec (INSPQ) in Quebec, Canada for analysis of heavy metals. The laboratory is accredited under ISO 17025 and uses numerous external quality control programs, including the German External Quality Assurance Scheme and Center for Disease Control's Lead and Multi-element Proficiency Testing.

Cadmium, uranium, and arsenic (total) were analyzed in urine. A 500 µL aliquot was diluted twenty-fold in dilute nitric acid and analyzed by inductively coupled plasma mass spectrometry (ICP-MS). An internal standard was added for improved precision and calibration was performed in a urine-based matrix. The detection limits were 0.09 µg/L for cadmium, 0.01 µg/L for uranium, and 0.22 µg/L for arsenic. To facilitate comparison with other reference values, metals in urine were reported in units of micrograms per liter of urine (µg/L) and were not normalized to creatinine (µg/g creatinine). However, in statistical models, urinary creatinine was included as a covariate to adjust for urine dilution.

Lead was analyzed in whole blood. A 500 µL aliquot was diluted twenty-fold in a dilute ammonium hydroxide / Triton-X solution and analyzed by ICP-MS. An internal standard is added for improved precision. Calibration is performed in a blood-based matrix. The detection limit for blood lead was 0.22 µg/L. To facilitate comparison with other reference values, lead in blood was reported as micrograms per deciliter (µg/dL).

2.3.6 Urine cultures

During the late-zafra round of sampling, urine samples collected from a subset of 114 ISA workers were transported in a cooler (2-8°C) to UNAN-Leon, where urine samples were examined under a microscope and cultured within 3 hours of collection. Workers were selected for screening if they reported experiencing urinary symptoms within the past 24 hours or if dipstick analyses indicated the presence of leukocyte esterase. The subset of selected workers also included workers who did not exhibit either condition. Table 2 summarizes the characteristics of the 103 men and 11 women who were selected to have urine cultures.

Table 2. Characteristics of workers selected for urine cultures

<table>
<tr><th colspan="2" rowspan="3"></th><th colspan="4">Males</th><th colspan="4">Females</th></tr>
<tr><th colspan="3">Symptoms</th><th>Total</th><th colspan="3">Symptoms</th><th>Total</th></tr>
<tr><th>Pos</th><th>Neg</th><th>Missing</th><th>(Men)</th><th>Pos</th><th>Neg</th><th>Missing</th><th>(Female</th></tr>
<tr><td rowspan="2">Leukocyte Esterase</td><td>Pos</td><td>9</td><td>18</td><td>3</td><td>30</td><td>0</td><td>8</td><td>0</td><td>8</td></tr>
<tr><td>Neg</td><td>20</td><td>51</td><td>2</td><td>73</td><td>3</td><td>0</td><td>0</td><td>3</td></tr>
<tr><td colspan="2">Total</td><td>29</td><td>69</td><td>5</td><td>103</td><td>3</td><td>8</td><td>0</td><td>11</td></tr>
</table>

A microbiologist first examined the sample under a microscope and reported abnormalities. Then, the urine was cultured to evaluate the presence of bacteria. A culture was only determined to be positive if there was growth of at least 100,000 colonies, or if there was growth of at least 20,000 colonies with other factors such as nitrates or high quantities of leukocytes in urine.

2.4 Questionnaires

At the pre-zafra investigation, participants completed a short questionnaire to collect information about personal characteristics and a brief work history. At the late-zafra investigation, participants completed a longer questionnaire that included a more detailed work history, a survey of symptoms experienced during the previous 24 hours and previous 3 months, and questions about hydration practices and alcohol consumption. Questionnaires were administered by study staff.

2.5 Data analysis

Biomarker data were evaluated using SAS statistical software (version 9.1 - Cary, NC). The distribution of each biomarker was characterized using graphical displays and summary statistics. When the reported value was below the limit of detection, the limit of detection divided by the square root of 2 was substituted. Biomarkers exhibiting a lognormal distribution were natural log-transformed prior to analysis to satisfy normality assumptions.

Linear regression models were used to evaluate predictors of biomarkers of metals exposure, kidney injury, and CKD. For ISA workers, job category was used as the primary predictor of interest in separate models evaluating biomarkers at pre-zafra, late-zafra, and “change during zafra.” “Change during zafra” was a secondary variable that was calculated by subtracting the pre-zafra measurement from the corresponding late-zafra measurement for each ISA worker. Since samples were collected from each worker at two time points, similar analyses were conducted using linear mixed-effects models with a compound symmetry covariance matrix structure. For ISA workers, age and sex were included as covariates in all models evaluating biomarkers of kidney injury and CKD. For workers in other industries, age was included as a covariate in all models but sex could not be included since only 3 of the 164 workers were female.

III. RESULTS & DISCUSSION

This section has been organized into six subsections: (1) serum biomarkers of CKD, which includes serum creatinine and estimated glomerular filtration rate (eGFR); (2) urine biomarkers of kidney injury, which include urinary ACR, NGAL, IL-18, and NAG; (3) an analysis of self-reported symptoms; (4) an analysis of infection and inflammation, which includes the results of the urine dipsticks and cultures; (5) biomarkers of metals exposure, which includes lead, cadmium, uranium and arsenic; and (6) an analysis of hydration practices and alcohol consumption. (See previous Section 2.2.1 for a description of ISA workers and Section 2.2.2 for a description of workers in other industries.)

3.1 Serum Biomarkers of CKD

3.1.1 Serum creatinine

The assay of creatinine in serum is the most commonly used test to assess kidney function. Creatinine is a break-down product of creatine phosphate in muscle and is produced at a fairly constant rate by the body (depending on muscle mass). People with more muscle mass produce more creatinine each day and those with less muscle mass produce less creatinine. Accordingly, on average men have higher serum creatinine levels than women. Creatinine is freely filtered by the glomeruli and, under normal conditions, is not re-absorbed by the tubules to any appreciable extent. A small but significant amount is also actively secreted. Normal serum creatinine levels can range from approximately 0.6 mg/dL in women to 1.4 mg/dL in very muscular individuals (Antunes et al. 2004), with the range of normal values influenced by factors such as muscle mass, use of medications that can affect tubular secretion of creatinine, and diet (Rigalleau et al. 2011).

Figure 2 shows the distribution of serum creatinine at pre-zafra (Figure 2a) and late-zafra (Figure 2b) for the 284 ISA workers. The number of workers with serum creatinine ≥1.2 mg/dL increased from 6 workers at pre-zafra (2%) to 10 workers at the late-zafra (3.5%), which included 6 of the 51 cane cutters (12%), 3 of the 26 seed cutters (12%), and 1 of the 50 irrigators (2%). These are valid estimates of the prevalence of elevated serum creatinine in our study population of 284 ISA workers, but are not valid estimates of the prevalence of elevated creatinine among ISA workers in general for the reasons explained in Section I (Introduction), Section II (Methods), and Section IV (Limitations).

Figure 2. Distribution of serum creatinine (mg/dL) at (a) pre-zafra and (b) late-zafra among sugarcane workers

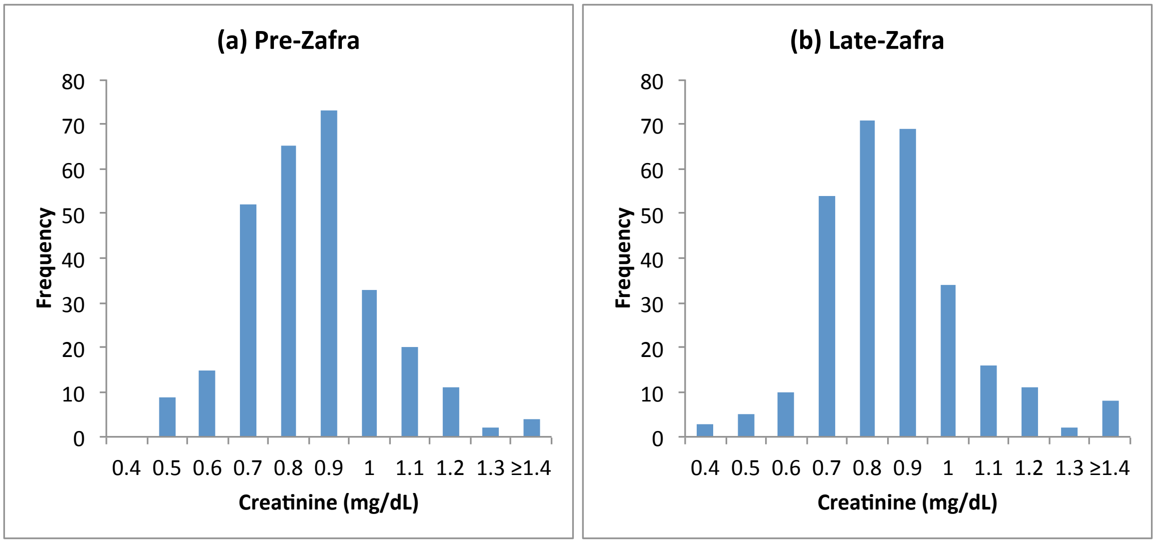


Table 3 presents the summary statistics for serum creatinine among the 284 ISA workers by job. The data are shown separately for pre-zafra, late-zafra, and change during zafra. A total of 13 workers (4.6%) had serum creatinine concentrations that increased during the zafra by at least 0.3 mg/dL, a magnitude of change that is unlikely to be attributable to method variability. The raw data summarized in this table do not account for differences in age or sex and may not match the results of the multivariate models in Table 4.

Table 3. Summary statistics for serum creatinine (mg/dL) among sugarcane workers

ISA Job	n	Pre-zafra		Late-zafra		Change during zafra	
		Mean	Range	Mean	Range	Mean	Range
Cane cutter	51	0.89	0.6 - 1.5	0.96	0.5 - 2.7	0.07	-0.5 - 2.0
Seed cutters	26	0.78	0.5 - 1.5	0.85	0.5 - 1.5	0.06	-0.1 - 0.4
Irrigators	50	0.78	0.5 - 1.2	0.85	0.6 - 1.8	0.06	-0.1 - 1.0
Drivers	41	0.87	0.5 - 1.2	0.80	0.5 - 1.1	-0.06	-0.4 - 0.1
Seeders	28	0.67	0.4 - 1.1	0.66	0.4 - 1.2	-0.01	-0.2 - 0.3
Agrichemical applicators	29	0.80	0.6 - 1.1	0.82	0.6 - 1.2	0.03	-0.1 - 0.3
Factory workers	59	0.82	0.4 - 1.4	0.79	0.3 - 1.1	-0.02	-0.7 - 0.4

Linear regression models were used to determine whether serum creatinine concentrations among ISA workers were significantly different by job, age, sex, or department of residence. As expected, serum creatinine concentrations were significantly higher in older individuals and among men. Accordingly, age and sex were retained as covariates in statistical models. As serum creatinine concentrations were not significantly different in Leon as compared to Chinandega while adjusting for age and sex, department of residence was not retained as a covariate.

Table 4 presents the results of three different linear regression models evaluating differences in serum creatinine by job, using factory workers as the reference group. The β's indicate the mean difference between workers in each job as compared to factory workers and p-values less than or equal to 0.05 indicate that the difference is statistically significant.

Table 4. Multivariate analysis of serum creatinine (mg/dL) by ISA job

ISA Job	Pre-zafra		Late-zafra		Change during zafra	
	β	p-value	β	p-value	β	p-value
Cane cutter	**0.08**	**0.01**	**0.18**	**<0.0001**	**0.10**	**0.01**
Seed cutters	0.06	0.2	**0.17**	**0.007**	**0.11**	**0.05**
Irrigators	-0.03	0.4	0.07	0.1	**0.10**	**0.02**
Drivers	0.03	0.3	-0.01	0.8	-0.05	0.3
Seeders	-0.03	0.6	0.03	0.7	0.05	0.4
Agrichemical applicators	-0.02	0.5	0.03	0.6	0.05	0.3
Factory workers	reference		reference		reference	

Analyses adjusted for age and sex.

At pre-zafra, ISA workers being hired as cane cutters and seed cutters had the highest mean serum creatinine levels as compared to other jobs, though only cane cutters had levels that were significantly higher than factory workers (p=0.01). When restricted to only field workers (excluding drivers and factory workers), pre-zafra serum creatinine was lowest among seeders and still significantly different by job (p=0.002). This is consistent with our observation that cane cutters (38%) and seed cutters (28%) represented the majority of the 47 ISA applicants who were determined to have serum creatinine ≥1.4 mg/dL, based on the ISA results from the pre-zafra screening.

This analysis of pre-zafra serum creatinine indicates that when workers are hired as cane cutters at the beginning of the zafra, their serum creatinine levels are significantly higher than workers who are being hired to perform other jobs. However, in this analysis of pre-zafra serum creatinine, ISA job is not temporally relevant since serum creatinine was measured prior to working in the reported job. Therefore, serum creatinine at pre-zafra could be highest among workers being hired as cane cutters because they have previously worked as cane cutters or because the type of workers who apply for those jobs have other characteristics that account for the increased levels, such as greater muscle mass.

To address this question, we analyzed the pre-zafra serum creatinine levels for the 51 cane cutters according to whether they had worked for ISA during the previous year. The 26 cane cutters who worked at ISA during the previous year had an average serum creatinine level (0.95 mg/dL) that was higher than those who did not work at ISA during the previous year (0.84 mg/dL), a difference that was borderline statistically significant (p=0.06). Of those 26 workers, 23 worked the previous year as cane cutters, 2 worked as seed cutters, and 1 worked as a weeder.

These results suggest that previous employment at ISA as a cane cutter is associated with higher serum creatinine at pre-zafra the following season.

At late-zafra, cane cutters and seed cutters still had the highest mean serum creatinine levels, and both were significantly higher than factory workers. When restricted to only field workers (excluding drivers and factory workers), late-zafra serum creatinine was lowest among agrichemical applicators and still significantly different by job (p=0.03). In contrast to the analysis of pre-zafra measurements, here the analysis of ISA job has temporal relevance since serum creatinine is being measured at the end of the zafra, after performing the reported job.

The increase in serum creatinine during the zafra was greatest for cane cutters, seed cutters, and irrigators. The mean increases in serum creatinine among workers in these jobs were approximately 0.1 mg/dL higher than those experienced by factory workers, differences that were statistically significant. When restricted to only field workers (excluding drivers and factory workers), the mean increases in serum creatinine by job were consistent with the differences observed by job at pre-zafra and late-zafra (*i.e.* highest among seed cutters and lowest among agrichemical applicators), but these differences were not statistically significant. In general, the jobs clustered into three groups with respect to the estimated change in serum creatinine during the harvest as follows:

drivers/factory workers < agrichemical applicators/seeders < irrigators/cane cutters/seed cutters

Table 5 presents the summary statistics for "late-zafra" serum creatinine among 51 miners, 60 construction workers, and 53 port workers. These workers were only sampled during one round and at approximately the same time as the collection of "late-zafra" samples among sugarcane workers, so we will continue with this terminology for consistency. The number of workers with serum creatinine ≥1.2 mg/dL included 6 miners (12%), 6 construction workers (10%), and 7 port workers (13%). As above, the data summarized here are raw data and do not account for differences in age or sex.

Table 5. Summary statistics for serum creatinine (mg/dL) among workers in other industries

Industry	n	Mean	Range
Port workers	53	0.95	0.6 - 2.9
Miners	51	0.91	0.6 - 2.0
Construction	60	0.89	0.5 - 4.1

Table 6 presents the results of the linear regression model evaluating differences in serum creatinine by industry, while adjusting for age. For the assessment of industry, construction workers were used as the reference group to which workers in other industries were compared. There were no significant differences

Table 6. Multivariate analysis of serum creatinine (mg/dL) by industry

Industry	β	p-value
Port workers	0.01	0.95
Miners	0.003	0.97
Construction	reference	

Analyses adjusted for age

in serum creatinine among port workers, miners, and construction workers. Sugarcane workers were not included in this comparison because of the bias that would be introduced due to the health surveillance program that is in place at ISA but not in place at these other companies.

3.1.2 Estimated Glomerular Filtration Rate (eGFR)

Glomerular filtration rate (GFR) is a measure of the volume of blood per minute that passes through the glomeruli of the kidney. Estimating GFR is the approach recommended by the National Kidney Foundation for diagnosing kidney disease. GFR is an indicator of the effectiveness of kidney filtration, such that a decrease in GFR may indicate progression to CKD. GFR and serum creatinine levels are related in that glomeruli remove creatinine from the blood during filtration, thus decreased function of the glomeruli often correspond with elevated levels of serum creatinine (Rigalleau et al. 2011). A GFR <60 ml/min per 1.73 m^2 indicates a moderate decrease in kidney function and is the cutoff value for CKD stage 3.

Estimated GFR (eGFR) was calculated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation (Levey et al. 2009). The CKD-EPI equation has been shown to be less biased and more accurate than the Modification of Diet in Renal Disease Study (MDRD) equation, especially when eGFR is >60 mL/min/1.73 m^2 (Stevens et al. 2010). The CKD-EPI equation is presented here:

$$\mathbf{eGFR = 141 \times \min(S_{cr}/\kappa, 1)^{\alpha} \times \max(S_{cr}/\kappa, 1)^{-1.209} \times 0.993^{Age} \times 1.018_{[if\ female]} \times 1.159_{[if\ black]}}$$

where S_{cr} is serum creatinine (mg/dL), κ is 0.7 for females and 0.9 for males, α is –0.329 for females and –0.411 for males, min indicates the minimum of S_{cr}/κ or 1, and max indicates the maximum of S_{cr}/κ or 1. In cases where GFR was estimated to be >120 mL/min/1.73 m^2, a maximum value of 120 mL/min/1.73 m^2 was assigned.

Figure 3 shows the distribution of eGFR at pre-zafra (Figure 3a) and late-zafra (Figure 3b) for the 284 ISA workers. The number of workers with eGFR <60 mL/min/1.73 m^2 increased from 1 worker at pre-zafra (0.4%) to 7 workers at the late-zafra (2.5%); this included 3 of the 51 cane cutters (6%), 3 of the 26 seed cutters (12%), and 1 of the 50 irrigators (2%). These are valid estimates of the prevalence of CKD (Stage 3 and higher) in our study population of 284 ISA workers, but are not valid estimates of the prevalence of CKD among ISA workers in general for the reasons explained in Section I (Introduction), Section II (Methods), and Section IV (Limitations).

Figure 3. Distribution of eGFR (mL/min/1.73 m^2) at (a) pre-zafra and (b) late-zafra among sugarcane workers

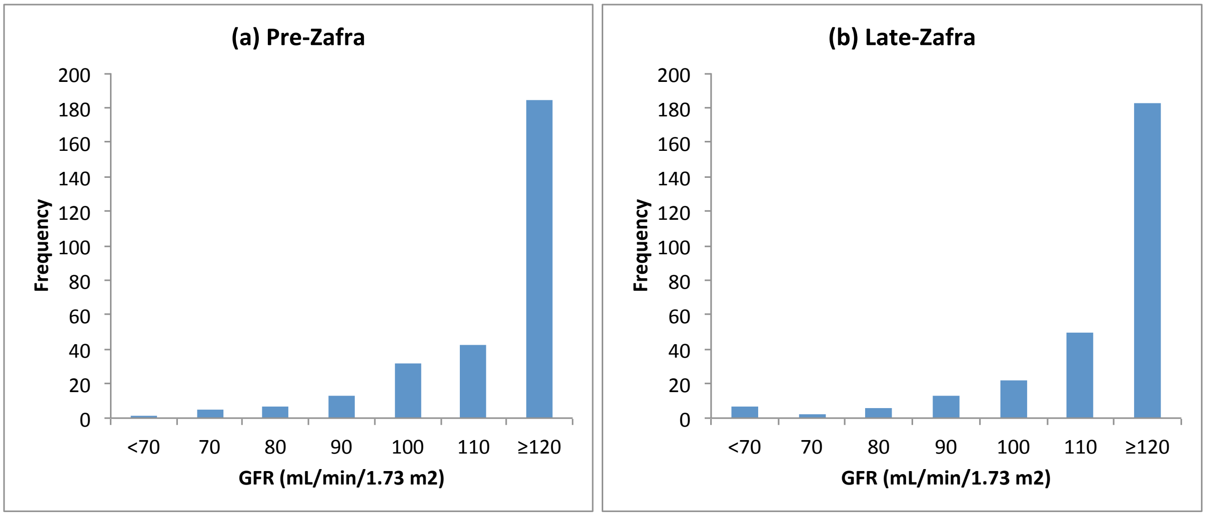


Table 7 presents the summary statistics for eGFR among the 284 ISA workers by job. The data are shown separately for pre-zafra, late-zafra, and change during zafra. The raw data summarized in this table do not account for differences in age or sex and may not match the results of the multivariate models in Table 8. As expected, the 47 ISA applicants that were selected based on elevated serum creatinine at pre-zafra had a mean eGFR (79 mL/min/1.73 m^2) and minimum eGFR (27 mL/min/1.73 m^2) that were lower than in ISA workers.

Table 7. Summary statistics for eGFR (mL/min/1.73 m^2) among sugarcane workers

ISA Job	n	Pre-zafra		Late-zafra		Change during zafra	
		Mean	Minimum	Mean	Minimum	Mean	Range
Cane cutter	51	108	61	104	29	-4.1	-90 - 34
Seed cutters	26	109	50	105	50	-4.0	-31 - 3
Irrigators	50	115	84	112	46	-2.9	-63 - 9
Drivers	41	104	67	108	78	3.9	-9 - 30
Seeders	28	116	90	115	80	-0.9	-34 - 23
Agrichemical applicators	29	113	74	112	82	-1.6	-23 - 8
Factory workers	59	110	69	112	76	2.0	-16 - 45

Linear regression models were used to determine whether eGFR was significantly different by job among ISA workers, while controlling for age and sex. Table 8 presents the results of three different linear regression models evaluating differences in eGFR by job, using factory workers as the reference group. The β's indicate the mean difference between workers in each job as compared to factory workers and p-values less than or equal to 0.05 indicate that the difference is statistically significant.

Table 8. Multivariate analysis of eGFR (mL/min/1.73 m^2) by ISA job

ISA Job	Pre-zafra		Late-zafra		Change during zafra	
	β	p-value	β	p-value	β	p-value
Cane cutter	**-5.0**	**0.03**	**-11.4**	**<0.0001**	**-6.4**	**0.006**
Seed cutters	-4.3	0.2	**-12.0**	**0.002**	**-7.0**	**0.03**
Irrigators	1.2	0.6	-4.1	0.1	**-5.3**	**0.03**
Drivers	-2.4	0.3	-0.1	0.9	2.3	0.4
Seeders	2.4	0.5	-2.4	0.6	-4.4	0.2
Agrichemical applicators	2.5	0.3	-1.1	0.7	-3.6	0.2
Factory workers	reference		reference		reference	

Analyses adjusted for age and sex.

At pre-zafra, workers being hired as cane cutters and seed cutters had the lowest mean eGFR as compared to other jobs, though only cane cutters had levels that were significantly lower than factory workers (difference of 5.0, p=0.03). When restricted to only field workers (excluding drivers and factory workers), pre-zafra eGFR was highest among seeders and agrichemical applicators and still significantly different by job (p=0.008). This finding is consistent with our observation that cane cutters (38%) and seed cutters (28%) represented the majority of the 47 ISA applicants who were determined to have serum creatinine ≥1.4 mg/dL, based on the ISA results from the pre-zafra screening.

This analysis of pre-zafra eGFR indicates that when workers are hired as cane cutters at the beginning of the zafra, their eGFR is significantly lower than workers who are being hired to perform other jobs. However, in this analysis of pre-zafra eGFR, ISA job is not temporally relevant since eGFR was derived from serum creatinine measured prior to working in the reported job. Therefore, eGFR at pre-zafra could be lowest among workers being hired as cane cutters because they have previously worked as cane cutters or because the type of workers who apply for those jobs have other characteristics that account for the decreased levels.

To address this question, we analyzed pre-zafra eGFR for the 51 cane cutters according to whether they had worked for ISA during the previous year. The 26 cane cutters who worked at ISA during the previous year had an average eGFR that was 8.4 mL/min/1.73 m^2 lower than those who did not work at ISA during the previous year (p=0.05). These results suggest that previous employment at ISA as a cane cutter may be associated with lower eGFR at pre-zafra the following season, although greater muscle mass leading to higher creatinine generation is another possible explanation.

At late-zafra, cane cutters and seed cutters still had the lowest mean eGFR and both were significantly lower than factory workers. The cane cutters were 11.4 mL/min/1.73 m^2 (p<0.0001) lower than factory workers and seed cutters were 12.0 mL/min/1.73 m^2 (p=0.002) lower than factory workers. When restricted to only field workers (excluding drivers and factory workers), late-zafra eGFR was highest among agrichemical applicators and still significantly different by job (p=0.01). In contrast to the analysis of pre-zafra eGFR, here the analysis of ISA job has temporal relevance since eGFR was derived from serum creatinine measured at the end of the zafra, after performing the reported job.

The decrease in eGFR during the zafra was highest for seed cutters, cane cutters, and irrigators. The mean decreases in eGFR among workers in these jobs were approximately 5 to 7 mL/min/1.73 m^2 higher than those experienced by factory workers, differences that were statistically significant. When restricted to only field workers (excluding drivers and factory workers), the mean decreases in eGFR by job were consistent with the differences by job observed at pre-zafra and late-zafra (*i.e.* mean decrease in eGFR highest among seed cutters and lowest among agrichemical applicators), but these differences were not statistically significant. In general, the jobs clustered into three groups with respect to the decrease in eGFR during the harvest as follows:

drivers/factory workers < seeders/agrichemical applicators < irrigators/cane cutters/seed cutters

Table 9 presents the summary statistics for "late-zafra" eGFR among 51 miners, 60 construction workers, and 53 port workers. The number of workers with eGFR <60 mL/min/1.73 m^2 included 3 miners (6%), 3 construction workers (5%), and 4 port workers (8%). As above, the data summarized here are raw data and do not account for differences in age and sex.

Table 9. Summary statistics for late-zafra eGFR (mL/min/1.73 m^2) among workers in other industries

Industry	n	Mean	Minimum
Miners	51	103	39
Construction	60	107	15
Port workers	53	101	26

Table 10 presents the results of the linear regression model evaluating differences in eGFR by industry, while adjusting for age. For the assessment of industry, construction workers were used as the reference group to which workers in other industries were compared. There were no significant differences in eGFR among port workers, miners, and construction workers. Sugarcane workers were not included in this comparison because of the bias that would be introduced due to the health surveillance program that is in place at ISA but not in place at these other companies.

Table 10. Multivariate analysis of late-zafra eGFR (mL/min/1.73 m^2) by industry

Industry	β	p-value
Port workers	-0.3	0.9
Miners	-1.1	0.8
Construction	reference	

Analyses adjusted for age

Typically, the prevalence of CKD in a population less than 60 years old would be expected to be quite low. For example, in the United States, the prevalence of CKD (Stages 3 and 4) in men between the ages of 20 and 59 is approximately 1% (Levey, 2009). If we restrict our study population of ISA workers to only include men between the ages of 20 and 59, we find that the prevalence of CKD (Stages 3 and 4) is 12% among seed cutters and 6% among cane cutters. Because of the health surveillance program at ISA, the prevalence observed in our study population is almost certainly lower than among all seed cutters and cane cutters at ISA. If we also restrict our study population of workers in other industries to only include men between the ages of 20 and 59, we find that the prevalence of CKD (Stages 3 and 4) is 6% among miners, 3% among construction workers, and 8% among port workers. In summary, the prevalence of CKD in all five of these jobs is much higher than expected in a population of relatively young men.

3.2 Urine Biomarkers of Kidney Injury

3.2.1 Urine ACR

Elevated urine albumin, normalized for creatinine (ACR), is a measure of kidney damage that most often indicates increased glomerular permeability to proteins. The presence of albumin in the urine can also indicate tubular dysfunction, reflecting an inability of the proximal tubules to fully reabsorb albumin; in this setting, the urine ACR typically is lower than in cases of glomerular proteinuria. Albumin is the most common circulating protein in blood and is not normally present in urine; in settings of glomerular disease (most commonly diabetes or primary glomerular kidney diseases), small amounts of albumin may leak into urine. When the amount of albumin leaking across the glomerular capillaries exceeds the ability of the tubules to reabsorb albumin, albuminuria results. Very low levels (ACR of 30-299 mg/g) are categorized as microalbuminuria while higher levels (ACR 300+ mg/g) are categorized as macroalbuminuria. Macroalbuminuria almost always indicates significant glomerular disease, and is commonly seen in hypertensive and diabetic nephropathies. ACR calculated from a single urine specimen collected at one point in time has similar utility to measuring albumin in a 24 hour sample.

Figure 4 shows the distribution of urine ACR at pre-zafra (Figure 4a) and late-zafra (Figure 4b) for the 284 ISA workers. ISA workers with ACR ≥30 mg/g creatinine, a cutpoint for defining elevated ACR (NKF, 2002), included 12 workers at pre-zafra (4.2%) and 10 workers at late-zafra (3.5%). Urinary ACR exhibits a lognormal distribution and was therefore natural log-transformed prior to analysis.

Figure 4. Distribution of urinary ACR (mg/g creatinine) at (a) pre-zafra and (b) late-zafra among sugarcane workers

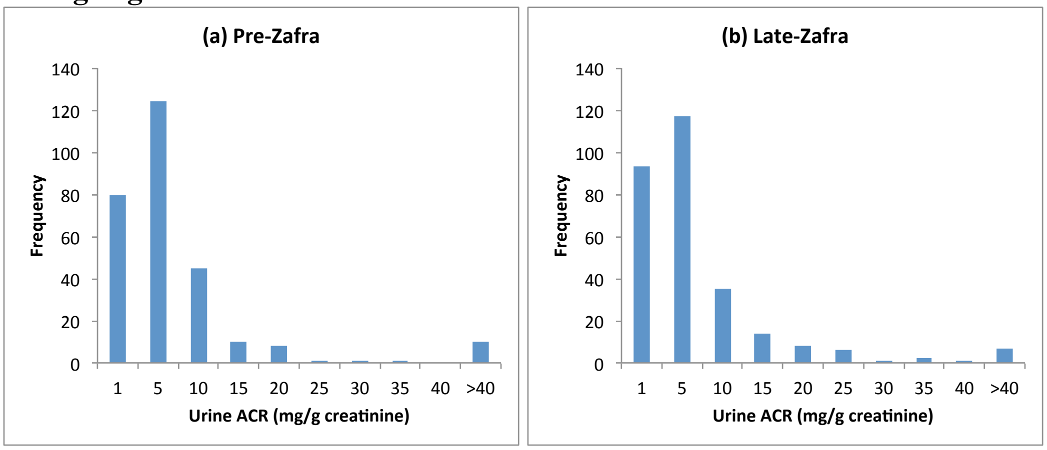


Table 11 presents the summary statistics for urine ACR among the 284 ISA workers by job. The data are shown separately for pre-zafra, late-zafra, and change during zafra. Due to the lognormal distribution, we present the geometric mean (GM) concentrations of ACR at pre-zafra and late-zafra. The raw data summarized in this table do not account for differences in age or sex and may not match the results of the multivariate models in Table 12. The 47 ISA applicants that were selected based on elevated serum creatinine at pre-zafra had geometric mean (6.0 mg/g creatinine) and maximum ACR concentrations (889 mg/g creatinine) that were higher than in ISA workers, with 7 workers (15%) who exceeded the cutpoint of 30 mg/g creatinine.

Table 11. Summary statistics for urinary ACR (mg/g creatinine) among sugarcane workers

ISA Job	n	Pre-zafra		Late-zafra		Change during the zafra	
		GM	Maximum	GM	Maximum	Mean	Range
Cane cutter	51	3.4	621	1.7	16	-20	-619 - 14
Seed cutters	26	3.0	136	3.1	115	4.8	-26 - 111
Irrigators	50	1.7	175	3.6	205	3.8	-8.5 - 31
Drivers	39	2.6	64	1.7	486	11	-12 - 422
Seeders	28	3.9	129	1.1	127	-2.0	-38 - 17
Agrichemical applicators	28	1.9	150	2.4	29	-5.8	-121 - 19
Factory workers	59	2.1	20	1.3	35	-0.6	-11 - 16

Linear regression models were used to determine whether ACR was significantly different by job, while controlling for age and sex. Table 12 presents the results of three different linear regression models evaluating differences in ACR by job, using factory workers as the reference group. Because the ACR concentrations were natural log-transformed for the purpose of analysis, the exponentiated β estimates (e^{β}) can be interpreted as the multiplicative difference between each job and the reference job.

Table 12. Multivariate analysis of ln(ACR) and change in ACR (mg/g creatinine) by ISA job

ISA Job	Pre-zafra			Late-zafra			Change during zafra	
	β	e^{β}	p-value	β	e^{β}	p-value	β	p-value
Cane cutter	0.5	1.6	0.09	0.3	1.3	0.2	**-18.7**	**0.05**
Seed cutters	0.4	1.6	0.2	**0.8**	2.2	**0.02**	3.3	0.8
Irrigators	-0.3	0.8	0.3	**1.1**	3.0	**<0.0001**	5.4	0.6
Drivers	0.3	1.3	0.4	0.2	1.2	0.5	10.9	0.3
Seeders	0.7	2.0	0.06	**1.0**	2.7	**0.02**	-4.9	0.7
Agrichemical applicators	-0.1	0.9	0.7	**0.6**	1.8	**0.04**	-4.9	0.7
Factory workers	reference			reference			reference	

Adjusted for age and sex.

At pre-zafra, workers being hired as seeders and cane cutters had the highest mean ACR as compared to other jobs, though none of the jobs were significantly different from factory workers. By exponentiating the β estimate of 0.7, we see that ACR among seeders is 2.0 times higher than ACR among factory workers. However, as indicated above, the overall concentrations of ACR at pre-zafra were quite low.

At late-zafra, ACR concentrations among irrigators, seeders, seed cutters, and agrichemical applicators workers were significantly higher than among factory workers, ranging from 1.8 to 3.0 times as high. However, as indicated above, the overall concentrations of ACR at late-zafra were quite low.

There was no evidence of an increase in ACR during the zafra and urinary ACR was not predictive of eGFR (p=0.9). Overall, ACR concentrations were quite low, did not increase during the zafra, and were not associated with eGFR, providing little evidence that glomerular injury is occurring during the zafra.

Table 13. Summary statistics for ACR (mg/g creatinine) among workers in other industries

Industry	n	GM	Maximum
Miners	51	4.0	502
Construction	60	2.7	124
Port workers	53	2.0	241

Table 13 presents the summary statistics for "late-zafra" ACR among port workers, miners, and construction workers. Workers with ACR ≥30 mg/g creatinine included 11 miners (22%), 4 port workers (7.5%), and 6 construction workers (10%). Urinary ACR among workers in these industries exhibited a lognormal distribution and was therefore natural log-transformed prior to analysis. The ACR concentrations were highest among miners but not significantly different by industry (p=0.2), while adjusting for age.

3.2.2 Urinary NGAL

Neutrophil gelatinase-associated lipocalin (NGAL) is an ion-transporting protein produced in the distal tubules. NGAL expression is substantially upregulated in kidney tubules that are acutely damaged (Devarajan 2008). NGAL is easily detected in both serum and urine within hours of initial insult to the kidney, providing earlier detection of kidney injury than can be achieved by measuring serum creatinine (Devarajan 2008). NGAL may also identify those patients who will develop CKD (Bolignano et al. 2009; Devarajan 2008).

Figure 5 shows the distribution of NGAL at pre-zafra (Figure 5a) and late-zafra (Figure 5b) for the 284 ISA workers. Since NGAL is a relatively new biomarker, there is no established cutpoint to define elevated NGAL. Urinary NGAL exhibited a lognormal distribution and was therefore natural log-transformed prior to analysis.

Figure 5. Distribution of urinary NGAL (μg/g creatinine) at (a) pre-zafra and (b) late-zafra among sugarcane workers

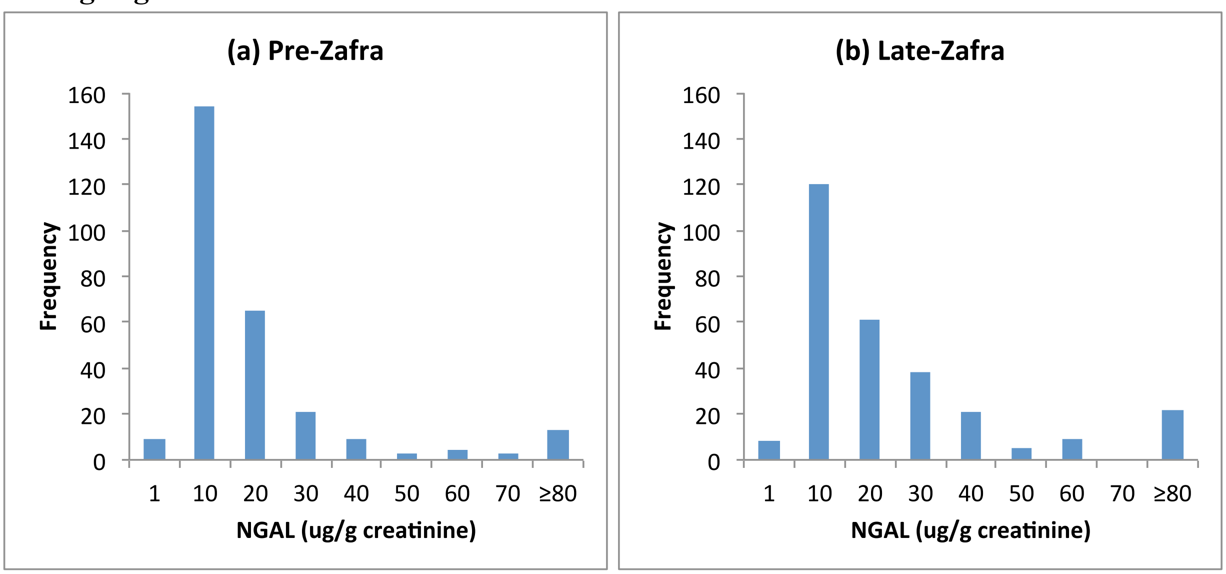


Table 14 presents the summary statistics for NGAL among the 284 ISA workers by job. The data are shown separately for pre-zafra, late-zafra, and change during zafra. Due to the lognormal distribution, we present the geometric mean concentrations of NGAL at pre-zafra and late-zafra. The raw data summarized in this table do not account for differences in age or sex and may not match the results of the multivariate models in Table 15. The 47 ISA applicants that were selected based on elevated serum creatinine at pre-zafra had geometric mean (13 μg/g creatinine) and maximum NGAL concentrations (91 mg/g creatinine) that were consistent with those observed in ISA workers.

Table 14. Summary statistics for NGAL (µg/g creatinine) among sugarcane workers

ISA Job	n	Pre-zafra		Late-zafra		Change during the zafra	
		GM	Maximum	GM	Maximum	Mean	Range
Cane cutter	51	7.6	80	19	406	24	-58 - 400
Seed cutters	26	16	118	15	156	-3.2	-65 - 130
Irrigators	50	7.2	660	14	335	8.9	-325 - 230
Drivers	39	6.8	34	7.7	36	0.7	-25 - 26
Seeders	28	23	245	20	187	-3.2	-170 - 149
Agrichemical applicators	28	7.0	43	6.9	76	3.0	-27 - 70
Factory workers	59	7.2	174	7.2	165	3.5	-163 - 133

Linear regression models were used to determine whether NGAL was significantly different by job, while controlling for age and sex. Table 15 presents the results of three different linear regression models evaluating differences in NGAL by job, using factory workers as the reference group. Because the NGAL concentrations were natural log-transformed for the purpose of analysis, the exponentiated β estimates (e^{β}) can be interpreted as the multiplicative difference between each job and the reference job.

Table 15. Multivariate analysis of ln(NGAL) and change in NGAL (µg/g creatinine) by ISA job

ISA Job	Pre-zafra			Late-zafra			Change during zafra	
	β	e^{β}	p-value	β	e^{β}	p-value	β	p-value
Cane cutter	0.1	1.1	0.6	**1.1**	**3.0**	**<0.0001**	**19.2**	**0.04**
Seed cutters	**0.6**	**1.8**	**0.04**	0.5	1.6	0.1	-2.8	0.8
Irrigators	0.05	1.1	0.8	**0.8**	**2.2**	**0.0007**	4.4	0.6
Drivers	-0.08	0.9	0.7	0.01	1.0	0.9	-2.3	0.8
Seeders	**0.8**	**2.3**	**0.005**	0.6	1.8	0.08	-0.6	0.9
Agrichemical applicators	0.02	1.0	0.9	-0.01	1.0	0.9	-0.8	0.9
Factory workers	reference			reference			reference	

Adjusted for age and sex.

At pre-zafra, workers being hired as seeders and seed cutters had the highest NGAL concentrations as compared to other jobs. Compared to factory workers, NGAL among seeders was 2.3 times higher (p=0.005) and among seed cutters was 1.8 times higher (p=0.04). However, when restricted to only field workers (excluding drivers and factory workers), pre-zafra NGAL was lowest among agrichemical applicators but not significantly different by job (p=0.1).

At late-zafra, NGAL concentrations among cane cutters and irrigators were significantly higher than among factory workers, 3.0 and 2.2 times as high, respectively. When restricted to only field workers (excluding drivers and factory workers), late-zafra NGAL was lowest among agrichemical applicators and still significantly different by job (p=0.004).

An increase in urinary NGAL during the zafra was primarily evident in the cane cutters, with an increase that was 19.2 µg/g creatinine higher than among factory workers (p=0.04). Similarly, when restricted to only field workers (excluding drivers and factory workers), the mean increase in urinary NGAL among cane cutters was 18.7 µg/g creatinine higher than among other field jobs ($p=0.01$).

When evaluated as a continuous variable, increased urinary NGAL was significantly associated with decreased eGFR at late-zafra ($p<0.0001$) but not at pre-zafra ($p=0.6$). When late-zafra NGAL was categorized using tertiles as cutpoints, the highest tertile of NGAL (>18.3 µg/g creatinine) was associated with an eGFR that was 7.3 mL/min/1.73 m^2 lower than the lowest tertile ($p=0.0005$). There was no difference in eGFR between the middle and lowest tertile of NGAL. Overall, NGAL increased during the zafra among cane cutters but not among other ISA workers and was associated with decreased eGFR at late-zafra but not pre-zafra, suggesting that cane cutters experience tubulointerstitial kidney damage during the zafra that may be increasing their risk of CKD.

Table 16 presents the summary statistics for "late-zafra" NGAL among port workers, miners, and construction workers. Urinary NGAL among workers in these industries exhibited a lognormal distribution and was therefore natural log-transformed prior to analysis. The NGAL concentrations were not significantly different by industry ($p=0.5$), while adjusting for age.

Table 16. Summary statistics for NGAL (µg/g creatinine) among workers in other industries

Industry	n	GM	Maximum
Miners	51	9.9	130
Construction	60	11.7	224
Port workers	53	12.4	255

Increased NGAL among workers in other industries was significantly associated with decreased eGFR ($p<0.0001$). When NGAL was categorized using the same cutpoints as above, the highest tertile of NGAL (>18.3 µg/g creatinine) was associated with an eGFR that was 13.4 mL/min/1.73 m^2 lower than the lowest tertile ($p=0.0008$). There was no difference in eGFR between the middle and lowest tertile of NGAL. Overall, as observed for ISA workers, NGAL was associated with decreased eGFR, possibly suggesting that workers in these other industries experience tubulointerstitial kidney damage that may be increasing their risk of CKD or that there is ongoing tubular damage in people with lower eGFR.

3.2.3 Urinary NAG

N-acetyl-beta-D-glucosaminidase (NAG) is a sensitive biomarker for kidney injury. NAG is an enzyme located in the lysosome, where it plays a role in the breakdown of glycoprotein. A small amount of NAG is normally present in urine, but urinary NAG activity is increased when renal proximal tubular epithelial cells are damaged (Lin et al. 2007).

Figure 6 shows the distribution of NAG at pre-zafra (Figure 6a) and late-zafra (Figure 6b) for the 284 ISA workers. Since NAG is a relatively new biomarker, there is no established cutpoint to define elevated NAG. Urinary NAG exhibited a lognormal distribution and was therefore natural log-transformed prior to analysis.

Figure 6. Distribution of urinary NAG (U/g creatinine) at (a) pre-zafra and (b) late-zafra among sugarcane workers

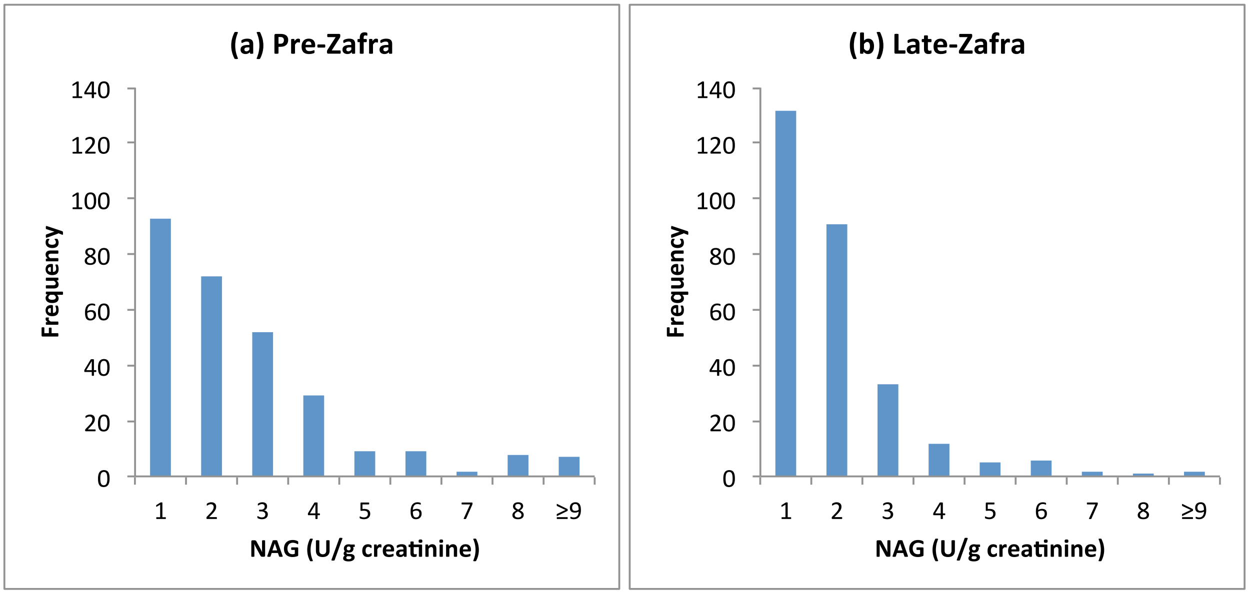


Table 17 presents the summary statistics for NAG among the 284 ISA workers by job. The data are shown separately for pre-zafra, late-zafra, and change during zafra. Due to the lognormal distribution, we present the GM concentrations of NAG at pre-zafra and late-zafra. The raw data summarized in this table do not account for differences in age or sex and may not match the results of the multivariate models in Table 18. The 47 ISA applicants that were selected based on elevated serum creatinine at pre-zafra had geometric mean (2.2 U/g creatinine) and maximum NAG concentrations (17 U/g creatinine) that were higher than most ISA workers, with the exception of factory workers.

Table 17. Summary statistics for NAG (U/g creatinine) among sugarcane workers

ISA Job	n	Pre-zafra		Late-zafra		Change during the zafra	
		GM	Maximum	GM	Maximum	Mean	Range
Cane cutter	51	1.3	11	1.5	9.6	0.3	-9.2 - 8.3
Seed cutters	26	1.5	7.2	1.3	9.2	0.4	-1.6 - 3.6
Irrigators	50	1.1	7.8	1.0	7.6	-0.2	-6.9 - 4.0
Drivers	39	1.9	14	0.7	2.2	-1.7	-12 - 1.0
Seeders	28	1.3	7.4	1.4	5.8	0.1	-4.7 - 2.3
Agrichemical applicators	28	1.7	8.3	0.9	4.2	-1.4	-7.5 - 0.6
Factory workers	59	2.3	21	0.6	6.5	-2.3	-20 - 4.5

Linear regression models were used to determine whether NAG was significantly different by job, while controlling for age and sex. Table 18 presents the results of three different linear regression models evaluating differences in NAG by job, using factory workers as the reference group. Because the NAG concentrations were natural log-transformed for the purpose of analysis, the exponentiated β estimates (e^{β}) can be interpreted as the multiplicative difference between each job and the reference job.

Table 18. Multivariate analysis of ln(NAG) and change in NAG (U/g creatinine) by ISA job

ISA Job	Pre-zafra			Late-zafra			Change during zafra	
	β	e^{β}	p-value	β	e^{β}	p-value	β	p-value
Cane cutter	**-0.6**	**0.6**	**0.001**	**1.1**	**2.9**	**<0.0001**	**2.9**	**<0.0001**
Seed cutters	-0.4	0.7	0.1	**0.8**	**2.1**	**0.001**	**3.0**	**<0.0001**
Irrigators	**-0.7**	**0.5**	**0.0001**	**0.7**	**1.9**	**0.0002**	**2.4**	**<0.0001**
Drivers	-0.2	0.8	0.4	0.01	1.0	0.9	0.4	0.4
Seeders	**-0.6**	**0.5**	**0.03**	**0.8**	**2.1**	**0.004**	**2.8**	**<0.0001**
Agrichemical applicators	-0.3	0.7	0.2	**0.5**	**1.6**	**0.02**	1.0	0.06
Factory workers	reference			reference			reference	

Adjusted for age and sex.

At pre-zafra, factory workers had the highest NAG concentrations and workers being hired as irrigators, cane cutters, and seeders had NAG concentrations that were significantly lower. At the late-zafra, factory workers had the lowest NAG concentrations and workers in almost all other jobs had NAG concentrations that were significantly higher than factory workers (with the exception of drivers). Cane cutters had the highest NAG concentrations at late-zafra, which were 2.9 times as high as those among factory workers ($p<0.0001$). When restricted to only field workers (excluding drivers and factory workers), late-zafra NAG concentrations were lowest among agrichemical applicators and still significantly different by job ($p=0.05$).

As shown in Table 17, the increases in urinary NAG during the zafra were highest in seed cutters and cane cutters, but these increases were not significantly different from zero. Table 18 shows that the increases among seed cutters and cane cutters were 3.0 and 2.9 U/g creatinine higher than among factory workers, respectively. However, this result is primarily driven by the fact that factory workers experienced a decrease of 2.3 U/g creatinine during the zafra. In summary, none of the jobs were associated with significant increases in NAG during the zafra.

Table 19. Summary statistics for NAG (U/g creatinine) among workers in other industries

Industry	n	GM	Maximum
Miners	51	0.7	2.4
Construction	60	1.0	5.2
Port workers	53	0.8	4.1

When evaluated as a continuous variable, increased urinary NAG was significantly associated with decreased eGFR at late-zafra ($p<0.0001$) but not at pre-zafra ($p=0.4$). When late-zafra NAG was categorized using tertiles as cutpoints, the highest tertile of NAG (>1.4 U/g creatinine) was associated with an eGFR that was 6.8 mL/min/1.73 m^2 lower than the lowest tertile ($p=0.001$). There was no difference in eGFR between the middle and lowest tertile of NAG. Overall, NAG was associated with

decreased eGFR at late-zafra but not pre-zafra, suggesting that ISA workers experience tubulointerstitial injury that may be increasing their risk of CKD.
Table 19 presents the summary statistics for "late-zafra" NAG among port workers, miners, and construction workers. Urinary NAG among workers in these industries exhibited a lognormal distribution and was therefore natural log-transformed prior to analysis. The NAG concentrations were not significantly different by industry (p=0.2), while adjusting for age.

Higher NAG among workers in other industries was significantly associated with lower eGFR (p<0.0001). When NAG was categorized using the same cutpoints as above, the highest tertile of NAG (>1.4 U/g creatinine) was associated with an eGFR that was 15.2 mL/min/1.73 m^2 lower than the lowest tertile (p=0.0002). There was no difference in eGFR between the middle and lowest tertile of NAG. Overall, as observed for ISA workers, NAG was associated with lower eGFR, suggesting that workers in these other industries may experience tubulointerstitial kidney damage that may be increasing their risk of CKD.

3.2.4 Urinary IL-18

Interleukin-18 (IL-18), a pro-inflammatory cytokine produced during active immune response by macrophages and dendritic cells, is another urinary biomarker that may be elevated early in the course of kidney injury. IL-18 functions by binding to the IL-18 receptor, and together with IL-12 it induces cell-mediated immunity. IL-18 in urine is indicative of renal tubule inflammation, and high concentrations of urinary IL-18 are associated with acute tubule necrosis (Parikh et al. 2005).

Figure 7 shows the distribution of IL-18 at pre-zafra (Figure 7a) and late-zafra (Figure 7b) for the 284 ISA workers. Since IL-18 is a relatively new biomarker, there is no established cutpoint to define elevated IL-18. Urinary IL-18 exhibited a lognormal distribution and was therefore natural log-transformed prior to analysis.

Figure 7. Distribution of urinary IL-18 (ng/g creatinine) at (a) pre-zafra and (b) late-zafra among sugarcane workers

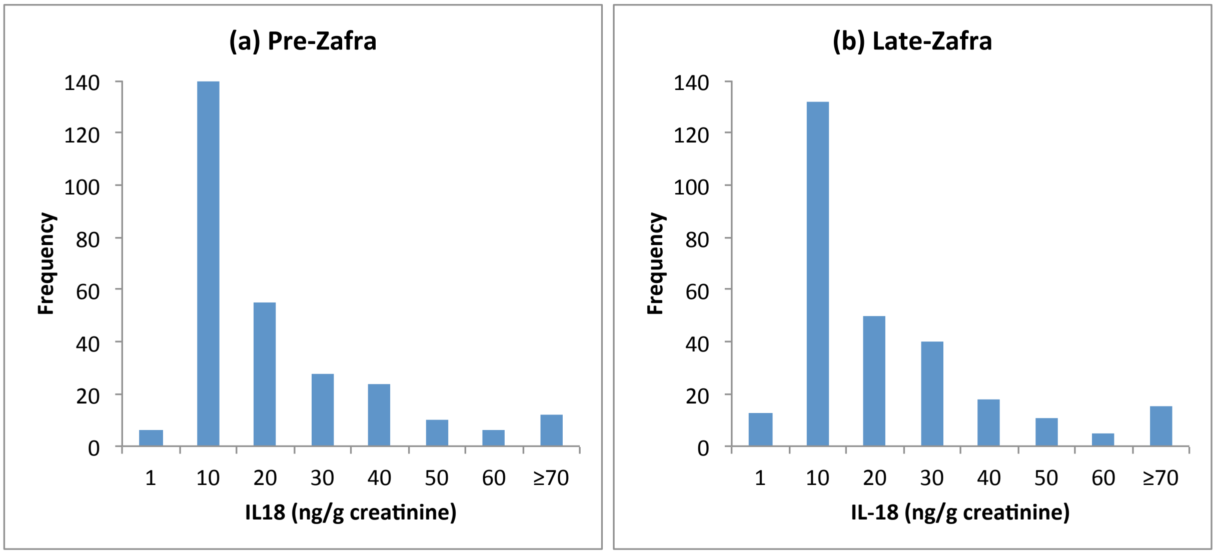


Table 20 presents the summary statistics for IL-18 among the 284 ISA workers by job. The data are shown separately for pre-zafra, late-zafra, and change during zafra. Due to the lognormal distribution, we present the GM concentrations of IL-18 at pre-zafra and late-zafra. The raw data summarized in this table do not account for differences in age or sex and may not match the results of the multivariate models in Table 21. The 47 ISA applicants that were selected based on elevated serum creatinine at pre-zafra had geometric mean (10 ng/g creatinine) and maximum IL-18 concentrations (167 ng/g creatinine) that were consistent with ISA workers.

Table 20. Summary statistics for IL-18 (ng/g creatinine) among sugarcane workers

ISA Job	n	Pre-zafra		Late-zafra		Change during the zafra	
		GM	Maximum	GM	Maximum	Mean	Range
Cane cutter	51	7.9	85	11	367	11	-71 - 365
Seed cutters	26	7.0	212	11	146	-5.9	-194 - 81
Irrigators	50	5.4	38	6.2	130	3.5	-29 - 127
Drivers	39	13.0	69	9.1	52	-5.9	-67 - 49
Seeders	28	20.0	281	20	137	-3.2	-212 - 101
Agrichemical applicators	28	8.4	66	7.8	67	-2.7	-54 - 39
Factory workers	59	7.8	104	5.9	85	-1.3	-95 - 44

Linear regression models were used to determine whether IL-18 was significantly different by job, while controlling for age and sex. Table 21 presents the results of three different linear regression models evaluating differences in IL-18 by job, using factory workers as the reference group. Because the IL-18 concentrations were natural log-transformed for the purpose of analysis, the exponentiated β estimates (e^{β}) can be interpreted as the multiplicative difference between each job and the reference job.

Table 21. Multivariate analysis of ln(IL-18) and change in IL-18 (ng/g creatinine) by ISA job

ISA Job	Pre-zafra			Late-zafra			Change during zafra	
	β	e^{β}	p-value	β	e^{β}	p-value	β	p-value
Cane cutter	-0.1	0.9	0.7	**0.7**	**2.0**	**0.005**	**14.5**	**0.05**
Seed cutters	**-0.7**	**0.5**	**0.03**	0.3	1.4	0.3	10.5	0.7
Irrigators	**-0.5**	**0.6**	**0.03**	0.1	1.1	0.8	6.8	0.4
Drivers	**0.6**	**1.8**	**0.02**	0.44	1.6	0.09	-6.7	0.4
Seeders	0.2	1.2	0.5	0.7	2.0	0.06	10.5	0.3
Agrichemical applicators	0.1	1.1	0.8	0.3	1.3	0.3	-1.1	0.9
Factory workers	reference			reference			reference	

Adjusted for age and sex.

At pre-zafra, drivers had the highest IL-18 concentrations (significantly higher than factory workers) and workers being hired as seed cutters and irrigators had the lowest IL-18 concentrations (significantly lower than factory workers). When restricted to only field workers (excluding drivers and factory workers), pre-zafra IL-18 concentrations were still significantly different by job (p=0.03).

At the late-zafra, factory workers had the lowest IL-18 concentrations and cane cutters had the highest, approximately twice as high as factory workers (p=0.005). When restricted to only field workers (excluding drivers and factory workers), irrigators had the lowest IL-18 concentrations such that late-zafra IL-18 concentrations among cane cutters were approximately twice as high as irrigators (p=0.01).

Cane cutters experienced the largest increase in IL-18 during the zafra, with an average increase that was 14.5 ng/g creatinine higher than the change experienced by factory workers (p=0.05). Similarly, when restricted to only field workers (excluding drivers and factory workers), the mean increase in IL-18 among cane cutters was 12.8 ng/g creatinine higher than among other field jobs (p=0.04).

When evaluated as a continuous variable, increased urinary IL-18 was not associated with eGFR at late-zafra (p=0.6) or at pre-zafra (p=0.1). When IL-18 was categorized using tertiles as cutpoints, IL-18 was still not associated with eGFR. Overall, IL-18 was highest among cane cutters, which was also the group that experienced the largest increase during the zafra; however, IL-18 was not associated with eGFR. These results may suggest that cane cutters experience tubulointerstitial kidney damage during the zafra.

Table 22 presents the summary statistics for "late-zafra" IL-18 among port workers, miners, and construction workers. Urinary IL-18 among workers in these industries exhibited a lognormal distribution and was therefore natural log-transformed prior to analysis. IL-18 concentrations were highest among miners, significantly higher than among construction workers (p=0.03).

It initially appeared that higher IL-18 among workers in other industries was significantly associated with lower eGFR (p=0.002), but when IL-18 was categorized using tertiles as cutpoints, there was no association with eGFR (p=0.4). Investigation of the scatterplot of IL-18 and eGFR showed the linear association was driven by two extreme values (data not shown), and the categorical analysis confirms that there does not appear to be an association between IL-18 and eGFR among workers in these other industries.

Table 22. Summary statistics for IL-18 (ng/g creatinine) among workers in other industries

Industry	n	GM	Maximum
Miners	51	8.3	95
Construction	60	5.2	99
Port workers	53	5.8	134

3.2.5 Correlation among eGFR and biomarkers of kidney injury

Table 23 presents a correlation matrix for eGFR and the four biomarkers of kidney injury (ACR, NGAL, NAG, and IL-18) for ISA workers at pre-zafra and late-zafra. These analyses describe the relationship between each pair of markers but do not control for other variables such as age and sex, and therefore may not be consistent with the analyses above.

Table 23. Spearman correlations of eGFR and biomarkers of kidney injury in ISA workers

	Pre-zafra				Late-zafra			
	ACR	NGAL	NAG	IL-18	ACR	NGAL	NAG	IL-18
GFR	r = 0.03 [p=0.6]	r = 0.02 [p=0.7]	**r = -0.12 [p=0.04]**	**r = 0.12 [p=0.05]**	r = -0.03 [p=0.6]	**r = -0.12 [p=0.05]**	**r = -0.23 [p<0.0001]**	r = 0.03 [p=0.6]
ACR		**r = 0.29 [p<0.0001]**	**r = 0.21 [p=0.0003]**	**r = 0.21 [p=0.0004]**		**r = 0.38 [p<0.0001]**	**r = 0.45 [p<0.0001]**	**r = 0.43 [p<0.0001]**
NGAL			**r = 0.16 [p=0.006]**	r = 0.12 [p=0.7]			**r = 0.40 [p<0.0001]**	**r = 0.39 [p<0.0001]**
NAG				**r = 0.25 [p<0.0001]**				**r = 0.38 [p<0.0001]**

We see that the relationship between NGAL and NAG were negatively associated with eGFR at late-zafra. At pre-zafra, the relationship between NAG and eGFR was not as strong but was still significant. In general, biomarkers of kidney injury were positively associated with each other, relationships that were much stronger at late-zafra than at pre-zafra.

Table 24 presents a correlation matrix for eGFR and the four biomarkers of kidney injury (ACR, NGAL, NAG, and IL-18) for the 47 ISA applicants at pre-zafra. We see that NGAL was negatively associated with eGFR among ISA applicants at pre-zafra, even more so than among ISA workers at late-zafra.

Table 24. Spearman correlations of eGFR and biomarkers of kidney injury in ISA Applicants

	ISA Applicants (Pre-zafra)			
	ACR	NGAL	NAG	IL-18
eGFR	r = -0.10 [p=0.5]	**r = -0.29 [p=0.05]**	r = -0.14 [p=0.4]	r = 0.23 [p=0.1]
ACR		r = 0.26 [p=0.07]	**r = 0.35 [p=0.02]**	**r = 0.34 [p=0.02]**
NGAL			**r = 0.52 [p=0.0002]**	r = -0.06 [p=0.7]
NAG				r = 0.16 [p=0.3]

The correlations among biomarkers of kidney injury were positively associated with each other and generally consistent with those observed among ISA workers.

Table 25 presents a correlation matrix for eGFR and the four biomarkers of kidney injury (ACR, NGAL, NAG, and IL-18) for workers in other industries at late-zafra. We see that the relationship between NGAL and NAG were negatively associated with eGFR, very similar to the relationships observed among ISA workers at late-zafra. The correlations among biomarkers of kidney injury were positively associated with each other and generally consistent with those observed among ISA workers at late-zafra.

Table 25. Spearman correlations of eGFR and biomarkers of kidney injury among workers in other industries

	Workers in other industries (Late-zafra)			
	ACR	**NGAL**	**NAG**	**IL-18**
eGFR	r = -0.13 [p=0.09]	**r = -0.19 [p=0.01]**	**r = -0.24 [p=0.002]**	r = 0.04 [p=0.6]
ACR		**r = 0.32 [p<0.0001]**	**r = 0.57 [p<0.0001]**	**r = 0.29 [p=0.0001]**
NGAL			**r = 0.41 [p<0.0001]**	**r = 0.18 [p=0.02]**
NAG				**r = 0.19 [p=0.02]**

3.3 Analyses of self-reported symptoms

At the late-zafra round of sampling, participants completed a questionnaire that included a survey of symptoms experienced during the previous 24 hours and previous 3 months, as well as some additional information about medical treatment during the previous 3 months. The questionnaires were administered by study staff. Table 26 presents a summary of the results for workers at ISA and in other industries.

One of the most striking findings is the high percentage of all study participants who sought medical treatment for urinary symptoms during the preceding 3 months. Even more surprising is the high percentage that was diagnosed with UTI during the preceding 3 months, particularly since the diagnosed workers included 47 men. UTIs are relatively common among women, but are quite rare among men (Shaeffer, 1994).

The symptom "chistata" is a colloquial term used to characterize a constellation of symptoms including "pain," "burning" and the "urgent" need to urinate, with chistata approximating the clinical term dysuria. Chistata was very commonly reported among study participants, as were the individual symptoms to which chistata refers (i.e., flank pain, burning while urinating, etc.). Dysuria may be a symptom of UTIs, sexually transmitted diseases, and kidney stones. Among physicians and pharmacists in Nicaragua, UTIs and dehydration are believed to be the two main causes of chistata (BU, 2011). Evidence of UTIs is explored in Section 3.4, which describes the urine dipstick data and the results of the urine cultures.

Table 26. Summary of self-reported symptoms for workers at ISA and in other industries

	ISA Workers		Construction		Port Workers		Miners	
	Past 3 Months	Past 24 Hours	Past 3 Months	Past 24 Hours	Past 3 Months	Past 24 Hours	Past 3 Months	Past 24 Hours
	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
Abdominal, Back, Flank Pain								
No	201 (73%)	238 (86%)	21 (35%)	33 (55%)	20 (38%)	38 (72%)	12 (24%)	32 (63%)
Yes	76 (27%)	39 (14%)	39 (65%)	27 (45%)	33 (62%)	15 (28%)	39 (76%)	19 (37%)
Burning or Pain during Urination								
No	246 (89%)	266 (96%)	32 (53%)	50 (83%)	32 (60%)	45 (85%)	33 (65%)	43 (84%)
Yes	31 (11%)	11 (4.0%)	28 (47%)	10 (17%)	21 (40%)	8 (15%)	18 (35%)	8 (16%)
Periods of Frequent Urination								
No	251 (91%)	269 (97%)	41 (68%)	52 (87%)	39 (39%)	48 (91%)	39 (76%)	45 (88%)
Yes	26 (9.4%)	8 (2.9%)	19 (32%)	8 (13%)	14 (53%)	5 (9.4%)	12 (24%)	6 (12%)
Any Fever or Chills								
No	240 (87%)	270 (97%)	41 (68%)	53 (88%)	41 (41%)	50 (94%)	41 (80%)	51 (100%)
Yes	37 (13%)	7 (2.5%)	19 (32%)	7 (12%)	12 (53%)	3 (5.7%)	10 (20%)	0 (0.0%)
Chistata								
No	186 (67%)	257 (93%)	21 (35%)	45 (75%)	24 (45%)	43 (81%)	19 (37%)	37 (73%)
Yes	91 (33%)	20 (7.2%)	39 (65%)	15 (25%)	29 (55%)	10 (19%)	32 (63%)	14 (27%)
Taken Antibiotics								
No	234 (84%)	266 (96%)	39 (65%)	57 (95%)	37 (70%)	50 (94%)	29 (57%)	46 (90%)
Yes	43 (16%)	11 (4.0%)	21 (35%)	3 (5.0%)	16 (30%)	3 (5.7%)	22 (43%)	5 (9.8%)
Sought Medical Treatment for Urinary Symptoms								
No	229 (83%)	NA	45 (75%)	NA	25 (47%)	NA	30 (59%)	NA
Yes	48 (17%)	NA	15 (25%)	NA	28 (53%)	NA	21 (41%)	NA
Taken Pain Medications for >3 days								
No	234 (84%)	NA	42 (70%)	NA	33 (62%)	NA	28 (55%)	NA
Yes	43 (16%)	NA	18 (30%)	NA	20 (38%)	NA	23 (45%)	NA
Diagnosed with a Urinary Tract Infection								
No	262 (95%)	NA	45 (75%)	NA	44 (83%)	NA	37 (73%)	NA
Yes	14 (5.1%)	NA	15 (25%)	NA	9 (17%)	NA	14 (27%)	NA

We evaluated whether the self-reported symptoms were associated with eGFR, while controlling for age and sex. Estimated GFR was lower among ISA workers who reported fever or chills (14.4 mL/min/1.73 m^2, p=0.01), burning or pain while urinating (7.4 mL/min/1.73 m^2, p=0.1), and chistata (6.4 mL/min/1.73 m^2, p=0.06) during the preceding 24 hours. Among workers in other industries, eGFR was lower among workers who reported fever or chills (24.6 mL/min/1.73 m^2, p=0.0002), abdominal/back/flank pain (6.3 mL/min/1.73 m^2, p=0.06), and periods of frequent urination (7.2 mL/min/1.73 m^2, p=0.1) during the preceding 24 hours.

We did a similar analysis to determine whether self-reported symptoms were associated with biomarkers of kidney injury, while controlling for age and sex. Compared to those who were negative for the same symptoms, NGAL was higher among ISA workers who reported fever or chills (2.5 times higher, p=0.05), burning or pain while urinating (2.3 times higher, p=0.03), and chistata (1.6 times higher, p=0.1) during the preceding 24 hours. Similar associations between NGAL and symptoms were not observed among workers in other industries. Additionally, symptoms during the preceding 24 hours were not associated with other biomarkers of kidney injury (ACR, IL-18, NAG) among workers at ISA or other industries.

3.4 Analyses of infection and inflammation

3.4.1 Dipstick Data

Urine dipsticks were used to semi-quantitatively assess urine specific gravity, pH, leukocyte esterase, nitrite, protein, glucose, ketones, urobilinogen, bilirubin, and blood. Table 27 presents a summary of the dipstick data for all study participants. Dipstick data were available for 280 of the 284 ISA workers, 45 of the 47 ISA applicants, and all workers in other industries. The only exception was for the test of blood in urine, which was missing for 15 ISA workers at pre-zafra, 6 ISA workers at late-zafra, and 3 ISA applicants. We will focus our analysis and discussion on protein, glucose, nitrite, leukocyte esterase, and blood.

One notable result from dipstick data is that protein or glucose is only rarely present in the urine of study participants, suggesting little evidence of glomerular disease, which was consistent with the finding described above for urinary ACR. The one exception appeared to be miners, who had a higher frequency of protein detected in urine, which was also consistent with the urinary ACR results.

Also of note is that the percentage of ISA workers who were positive for leukocyte esterase was higher at late-zafra (~16%) than at pre-zafra (~8%). As observed for protein, the percentage of miners with a positive test for leukocyte esterase was especially high (69%). Leukocyte esterase is an enzyme produced by white blood cells, such that a positive test typically indicates the presence of white blood cells in the urine. The presence of white cells in urine can be an indication of infection (i.e., bacteria, UTI) but can also be an indication of inflammation (i.e., crystals/stones, tubular injury). Accordingly, leukocyte esterase tests are often interpreted in combination with other measurements, such as tests for nitrite and blood in urine, as well as the actual presence of white blood cells in the urine on urine microscopy.

The presence of nitrites in urine is a specific but not sensitive test that is most consistent with the presence of gram-negative bacteria, the most common urinary pathogen. Typically, testing positive for leukocyte esterase and/or nitrite is suggestive of a UTI and is usually followed by a confirmatory urine culture. Nitrite was only very rarely detected in the study population, which suggests that the positive leukocyte esterase results are indicative of inflammation and not a bacterial infection.

Table 27. Summary of dipstick data for all study participants.

	ISA Workers		ISA applicants	Construction	Port Workers	Miners
	Pre-zafra	Late-zafra				
	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
Specific gravity						
1.005	29 (10%)	15 (5.3%)	9 (19%)	8 (13%)	7 (13%)	2 (3.9%)
1.010	104 (37%)	57 (20%)	16 (34%)	22 (37%)	15 (28%)	8 (16%)
1.015	84 (30%)	81 (29%)	15 (32%)	13 (22%)	15 (28%)	9 (18%)
1.020	50 (18%)	59 (21%)	4 (8.5%)	12 (20%)	14 (26%)	13 (25%)
1.025	9 (3.2%)	31 (11%)	1 (2.1%)	3 (5.0%)	2 (3.8%)	7 (14%)
1.030	4 (1.4%)	37 (13%)	0 (0%)	2 (3.3%)	0 (0%)	12 (24%)
pH						
5	60 (21%)	86 (30%)	19 (40%)	16 (27%)	13 (25%)	12 (24%)
6	79 (28%)	90 (32%)	13 (28%)	19 (32%)	18 (34%)	24 (47%)
6.5	43 (15%)	19 (6.7%)	2 (4.3%)	3 (5.0%)	5 (9.4%)	1 (2.0%)
7	70 (25%)	62 (22%)	10 (21%)	16 (27%)	14 (26%)	13 (25%)
8	26 (9.2%)	23 (8.1%)	1 (2.1%)	6 (10%)	3 (5.7%)	1 (2.0%)
9	2 (0.7%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Leucocyte esterase						
1+	8 (2.8%)	21 (7.4%)	2 (4.3%)	5 (8.3%)	4 (7.5%)	7 (14%)
2+	6 (2.1%)	15 (5.3%)	0 (0%)	3 (5.0%)	0 (0%)	20 (39%)
3+	8 (2.8%)	10 (3.5%)	0 (0%)	2 (3.3%)	8 (15%)	8 (16%)
Neg	258 (91%)	234 (82%)	43 (91%)	50 (83%)	41 (77%)	16 (31%)
Nitrite						
Pos	4 (1.4%)	2 (0.7%)	0 (0%)	0 (0%)	2 (3.8%)	3 (5.9%)
Neg	276 (97%)	278 (98%)	45 (96%)	60 (100%)	51 (96%)	48 (94%)
Protein						
1+	6 (2.1%)	6 (2.1%)	2 (4.3%)	5 (8.3%)	4 (7.5%)	1 (2.0%)
2+	0 (0%)	2 (0.7%)	2 (4.3%)	1 (1.7%)	1 (1.9%)	4 (7.8%)
3+	0 (0%)	1 (0%)	1 (2.1%)	0 (0%)	0 (0%)	4 (7.8%)
Neg	274 (96%)	271 (95%)	40 (85%)	54 (90%)	48 (91%)	42 (82%)
Glucose						
1+	1 (0.4%)	1 (0.4%)	0 (0%)	0 (0%)	0 (0%)	1 (2%)
2+	0 (0%)	1 (0.4%)	0 (0%)	1 (1.7%)	0 (0%)	0 (0%)
3+	1 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
4+	2 (0.7%)	2 (0.7%)	1 (2.1%)	1 (1.7%)	0 (0%)	1 (2.0%)
Normal	276 (97%)	276 (97%)	44 (94%)	58 (97%)	53 (100%)	49 (96%)
Ketones						
1+	3 (1.1%)	2 (0.7%)	2 (4%)	4 (6.7%)	0 (0%)	1 (2.0%)
Neg	277 (98%)	278 (98%)	43 (91%)	56 (93%)	53 (100%)	50 (98%)
Urobilinogen						
1+	2 (0.7%)	13 (4.6%)	1 (2.1%)	4 (6.7%)	1 (1.9%)	6 (12%)
2+	0 (0%)	0 (0%)	0 (0%)	1 (1.7%)	1 (1.9%)	0 (0%)
3+	0 (0%)	1 (0%)	0 (0%)	1 (1.7%)	0 (0%)	2 (3.9%)
Normal	278 (98%)	266 (94%)	44 (94%)	54 (90%)	51 (96%)	43 (84%)
Bilirubin						
1+	1 (0.4%)	1 (0.4%)	1 (2.1%)	0 (0%)	1 (1.9%)	1 (2.0%)
2+	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (2.0%)
Neg	279 (98%)	279 (98%)	44 (94%)	60 (100%)	52 (98%)	49 (96%)
Blood						
1+	0 (0%)	10 (3.5%)	0 (0%)	2 (3.3%)	0 (0%)	1 (2.0%)
2+	4 (1.4%)	6 (2.1%)	1 (2.1%)	2 (3.3%)	0 (0%)	3 (5.9%)
3+	0 (0%)	7 (2.5%)	0 (0%)	1 (1.7%)	1 (1.9%)	0 (0%)
4+	0 (0%)	2 (0.7%)	0 (0%)	1 (1.7%)	0 (0%)	2 (3.9%)
Neg	265 (93%)	253 (89%)	43 (91%)	52 (87%)	52 (98%)	45 (88%)

Hemoglobin in urine is detected using the dipstick, with the most common cause of a dipstick being positive for hemoglobin the presence of red blood cells. Other causes include the presence of myoglobin in the urine (from rhabdomyolysis) or hemoglobin itself in the urine (from intravascular hemolysis). There are numerous causes of blood in the urine, with injury anywhere along the genitourinary tract potentially causing hematuria. Causes include bladder lesions, ureteral lesions (e.g., trauma from kidney stones), inflammation in the setting of conditions like urinary tract infections and kidney stones and crystals, and kidney lesions, ranging from benign causes like thin basement membrane disease to severe glomerulonephritis. As was observed for leukocyte esterase, the percentage of ISA workers who were positive for hematuria was higher at late-zafra (~9%) than at pre-zafra (~1%). The combined results for leukocyte esterase, nitrite, and blood in urine could be consistent with either inflammation or infection, with the possibility of infection explored below in Section 3.4.2 (urine cultures).

We next evaluated whether leukocyte esterase positivity was associated with eGFR, while controlling for age and sex. At late-zafra, ISA workers who were leukocyte esterase positive had an eGFR that was an average of 14.0 mL/min/1.73 m^2 lower than workers who were negative ($p<0.0001$). The result was comparable for workers in other industries, with a difference of 10.1 mL/min/1.73 m^2 ($p=0.0025$).

We did a similar analysis to determine whether leukocyte esterase positivity was associated with biomarkers of kidney injury, while controlling for age and sex. At late-zafra, ISA workers who were leukocyte esterase positive had average NGAL concentrations that were 3.1 times higher ($p<0.0001$) and average NAG concentrations that were 1.4 times higher ($p=0.04$) than workers who were negative. For workers in other industries, leukocyte esterase positivity was associated with NGAL concentrations that were 1.6 times higher than workers who were negative ($p=0.009$), but was not associated with NAG concentrations. Leukocyte esterase was not associated with ACR or IL-18 for ISA workers or for workers in other industries.

Since NGAL and NAG are biomarkers of tubulointerstitial injury, the above results provide further evidence that leukocyte esterase positivity in this study population is predominantly a marker of inflammation. The association with eGFR suggests that leukocyte esterase positivity, whatever the reason for positivity may be, is indeed providing information about the process by which kidney function is being impaired in this population.

3.4.2 Urine Cultures

As shown earlier in Table 2, urine samples collected from 114 ISA workers (103 men, 11 women) were cultured at the late-zafra investigation. The results indicated that only 3 workers – all women – were positive for the presence of bacteria. The characteristics of these three women are summarized in Table 28.

Table 28. Characteristics of the 3 workers with positive urine cultures

	Worker #1	Worker #2	Worker #3
Sex	Female	Female	Female
Age	37	20	38
Job	Seeder	Seed cutter	Seeder
Leukocyte esterase	1+	2+	3+
Symptoms during past 24 hours	No	No	No
Symptoms during past 3 months	No	Yes	Yes

However, the most striking finding from the urine culture results is the fact that all of the men were negative. The 103 men included 30 who were positive for leukocyte esterase and 29 who reported having symptoms during the past 24 hours. There were 9 men who were positive for leukocyte esterase and reported have symptoms during the past 24 hours. Therefore, although a total of 50 men were positive for leukocyte esterase and/or reported have symptoms during the past 24 hours, none had a positive urine culture.

This is an important finding because physicians, pharmacists, and workers in Nicaragua have reported a high occurrence of clinically, but not microbiologically, diagnosed urinary tract infections (UTIs) among men (BU, 2011). In fact, most of the interviewed physicians and pharmacists believe that UTIs are a main cause of chistata. This belief seemed unlikely to be true since, while UTIs are relatively common among women, they are quite rare among men and typically the result of a urinary tract malformation or obstruction (Shaeffer, 1994).

Based on interviews with physicians and pharmacists, it is likely that many of the 50 men described above would be diagnosed with a UTI and/or treated for a UTI. However, based on the results of the urine cultures, we do not see evidence that these men have UTIs. Testing positive for leukocyte esterase can be an indication of infection, but can also be an indication of inflammation. There are many potential factors that could cause inflammation, including kidney injury. Overall, the urine culture results suggest that these men are testing positive for leukocyte esterase and experiencing symptoms due to factors that likely are associated with a process other than infection.

3.5 Analyses of heavy metals

A subpopulation of 100 workers was selected for the analysis of heavy metals in urine and blood, including 20 cane cutters, 20 irrigators, 20 seeders, 20 factory workers, and 20 miners. These workers were randomly selected from all workers with these job titles. One factory worker was later determined to be ineligible to participate (<18 years old) and was withdrawn from the study. Blood and urine samples collected from the 79 ISA workers at both pre-zafra and late-zafra were analyzed for heavy metals, but samples from miners were only available at late-zafra (total of

178 blood samples and 178 urine samples). Lead was analyzed in whole blood samples while cadmium, arsenic (total), and uranium were analyzed in urine samples.

3.5.1 Characterizing exposure to heavy metals

Lead, cadmium, arsenic, and uranium may enter the environment through natural sources or human activity. These metals are naturally occurring in soil, but also may be deposited as a result of volcanic activity, which is common in this particular region. It is also possible that agrichemicals containing arsenic were used historically in the region. There is the potential for ISA workers or miners to be exposed to these metals through contact with soil (inhalation of dust, incidental ingestion, dermal contact) or water (incidental ingestion, dermal contact) while performing their jobs. We included miners, cane cutters, and seeders due to their high potential for exposure to soil/dust, and we included irrigators due to their high potential for exposure to water. Factory workers were included as a group of workers in the sugarcane industry who do not work in the field.

Tables 29a and 29b present the summary statistics for heavy metals among the 79 ISA workers (both pre-zafra and late-zafra) and 20 miners (late-zafra only). Lead and cadmium are shown in Table 29a and arsenic and uranium and shown in Table 29b. For each metal, we have also shown an "Exposure Guideline Level" and the "GM in the US" as reference values. The exposure guideline for lead in whole blood is 30 μg/dL (ACGIH, 2004), for urinary arsenic (total) is 100 μg/L (WHO), for urinary uranium is 15 μg/L (U.S. Nuclear Regulatory Commission), and for cadmium was unavailable. The geometric mean (GM) values in the US are based on the National Health and Nutrition Examination Survey conducted by the Centers for Disease Control and Prevention to characterizes the distribution of chemical exposures in a random sample of the US general population (CDC, 2005). The values shown are GMs for both males and females in the age group 20 years and older. For metals measured in urine (cadmium, arsenic, uranium), the regression models examine differences by time and job while controlling for urinary creatinine to account for differences in urine dilution.

Table 29a. Summary statistics for exposure to heavy metals: lead and cadmium

	n	Lead (μg/dL)				Cadmium (μg/L)			
		Pre-zafra		Late-zafra		Pre-zafra		Late-zafra	
		GM	Range	GM	Range	GM	Range	GM	Range
ISA Job									
Cane cutter	20	2.0	0.8 - 24	1.8	0.8 - 8.7	0.4	0.1 - 0.82	0.2	0.1 - 0.7
Irrigator	20	1.7	1.0 - 4.1	1.4	0.8 - 3.4	0.4	0.1 - 0.80	0.3	0.1 - 1.0
Seeder	20	1.3	0.3 - 14	1.1	0.3 - 9.8	0.3	0.1 - 0.68	0.3	0.1 - 1.2
Factory workers	19	2.9	1.2 - 15	2.6	1.1 - 15	0.3	0.1 - 0.85	0.3	0.1 - 0.6
Miners	20	NA		1.8	0.7 - 13	NA		0.5	0.1 - 3.3
Exposure Guideline		30		30		NA		NA	
GM in US		1.5		1.5		0.3		0.3	

NA=Not available

Concentrations of lead in whole blood were below the ACGIH biological exposure index (BEI) of 30 μg/dL for all jobs at both pre-zafra and late-zafra. Among ISA workers, concentrations did not increase during the zafra and in fact were significantly lower at the late-zafra compared to pre-zafra ($p<0.0001$). There were also significant differences by job ($p=0.002$), with the highest levels among factory workers, miners, and cane cutters. Concentrations among factory workers (GM=2.6 μg/dL) were higher than in the US general population (GM=1.5 μg/dL) and would be between the 75th and 90th percentile values in the US. However, lead concentrations among all other workers were generally consistent with concentrations in the US general population (job-specific GMs between 25th and 75th percentile values).

Among ISA workers, concentrations of cadmium in urine did not increase during the zafra and in fact were significantly lower at the late-zafra compared to pre-zafra ($p=0.04$). Cadmium concentrations were not significantly different by job ($p=0.2$) and were generally consistent with concentrations in the US general population (job-specific GMs between 25th and 75th percentile values).

Table 29b. Summary statistics for exposure to heavy metals: arsenic and uranium

	n	Arsenic (μg/L)				Uranium (μg/L)			
		Pre-zafra		Late-zafra		Pre-zafra		Late-zafra	
		GM	Range	GM	Range	GM	Range	GM	Range
ISA Job									
Cane cutter	20	17	2.0 - 110	15	4.3 - 74	0.01	0.01 - 0.09	0.01	0.01 - 0.1
Irrigator	20	13	1.3 - 71	11	2.2 - 60	0.01	0.01 - 0.02	0.01	0.01 - 0.3
Seeder	20	12	2.7 - 52	13	2.2 - 48	0.01	0.01 - 0.03	0.01	0.01 - 0.04
Factory workers	19	8	2.5 - 21	13	1.6 - 45	0.01	0.01 - 0.08	0.01	0.01 - 0.3
Miners	20	NA		26	6.3 - 180	NA		0.01	0.01 - 0.02
Exposure Guideline		100		100		15		15	
GM in US		8.4		8.4		0.01		0.01	

NA=Not available

Concentrations of arsenic in urine exceeded the World Health Organization's guideline of 100 μg/L for 3 workers (2 miners and 1 cane cutter). While controlling for urinary creatinine, there were no significant differences in arsenic concentrations between pre-zafra and late-zafra ($p=0.3$) or by job ($p=0.4$). Concentrations among miners (GM=26 μg/dL) were higher than in the US general population (GM=8.4 μg/dL) and would be between the 75th and 90th percentile values in the US. However, arsenic concentrations among all other workers were generally consistent with concentrations in the US general population (job-specific GMs between 25th and 75th percentile values).

Concentrations of uranium in urine were below the U.S. Nuclear Regulatory Commission's action level of 15 μg/L for all jobs at both pre-zafra and late-zafra. While controlling for urinary creatinine, there were no differences in uranium concentrations between pre-zafra and late-zafra ($p=0.2$) or by job ($p=0.2$). Uranium concentrations among all workers were generally consistent with concentrations in the US general population.

3.5.2 Evaluating heavy metals as predictors of kidney injury and CKD

Chronic exposure to heavy metals has been shown to be associated with tubulointerstitial nephritis. Heavy metals may accumulate in proximal tubule cells, causing both functional and structural damage that results in reabsorptive and secretory defects. The mechanisms remain unknown but may involve local oxidative stress with associated lipid peroxidation, apoptosis, and necrosis as common phenomena in the course of nephrotoxicity of these metals (Sabolic, 2006).

For the 99 workers with metals exposure data, we evaluated each metal as a predictor of kidney injury (ACR, NGAL, IL-18, NAG) and kidney function (serum creatinine, eGFR) while controlling for age and sex. When evaluated as continuous variables, concentrations of lead, cadmium, arsenic, and uranium were not associated with any biomarkers of kidney injury or kidney function.

By evaluating metals exposure as continuous variables, we investigated a linear relationship between metal exposure and kidney biomarker. However, to explore whether a nonlinear relationship may exist, we also created categories of exposure to each metal. The only evidence of a non-linear relationship was for arsenic as a predictor of serum creatinine and eGFR. When dichotomized at the 90^{th} percentile value (48 µg/L), workers with the highest arsenic exposures were found to have significantly higher serum creatinine ($p=0.04$) and significantly lower eGFR ($p=0.01$) while controlling for age and sex. On average, the eGFR among workers with high arsenic was 9.0 mL/min/1.73 m^2 lower than workers with low arsenic.

Overall, we see no evidence that lead, cadmium, or uranium is associated with biomarkers of kidney injury or kidney function, and some evidence that high exposure to arsenic (total) is associated with biomarkers of kidney function. Total arsenic in urine includes all species of organic and inorganic arsenic. The inorganic fraction (and associated metabolites) is of primary concern with respect to kidney damage, such that it would be useful to further speciate those fractions, which could potentially show a stronger association with kidney function. Arsenic speciation was not possible as part of this investigation but should be considered in future studies.

3.6 Analysis of hydration practices and alcohol consumption

At the late-zafra round of sampling, participants completed a questionnaire that included questions about hydration practices and opinions, as well as alcohol consumption. The questions about hydration practices focused on the amount of water and *bolis* (an electrolyte solution provided by the company to ISA workers) consumed each workday, reported using categories in Table 30. Due to the large number of categories, a continuous estimate of water and bolis consumption was calculated and reported in Table 31.

Table 30. Hydration practices by industry (categorical)

	ISA Workers	Construction	Port Workers	Miners
	n (%)	n (%)	n (%)	n (%)
Water per day (liters)				
≤1	1 (0.4%)	4 (6.7%)	7 (13%)	2 (3.9%)
2-3	66 (23%)	23 (38%)	25 (47%)	12 (24%)
4-5	121 (43%)	19 (32%)	14 (26%)	32 (63%)
6-7	41 (14%)	9 (15%)	6 (11%)	4 (7.8%)
8-9	34 (12%)	3 (5.0%)	0 (0%)	1 (2.0%)
≥10	14 (4.9%)	2 (3.3%)	1 (1.9%)	0 (0%)
Missing	7 (2.5%)	0 (0%)	0 (0%)	0 (0%)
Bolis per day				
0	65 (23%)	NA	NA	NA
1-2	82 (29%)	NA	NA	NA
3-4	87 (31%)	NA	NA	NA
≥5	43 (15%)	NA	NA	NA
Missing	7 (2.5%)	NA	NA	NA

NA=Not available

Table 31. Hydration practices and alcohol consumption by ISA job (continuous)

ISA Job	n	Water per day (liters)		Bolis per day		Alcoholic drinks per week	
		Mean	Maximum	Mean	Maximum	Mean	Maximum
Cane cutter	44	6.3	11	3.6	5.5	2.3	24
Seed cutters	27	5.6	11	2.1	5.5	0.7	11
Irrigators	50	6.3	11	2.3	5.5	1.1	25
Drivers	40	4.6	8.5	0.4	5.5	0.6	6.0
Seeders	28	4.2	8.5	2.2	5.5	1.0	18
Agrichemical applicators	28	4.6	11	3.5	5.5	1.8	11
Factory workers	59	4.0	8.5	2.7	5.5	2.5	24

Water consumption varied significantly by ISA job ($p<0.0001$), highest among cane cutters and irrigators and lowest among factory workers. Bolis consumption also varied significantly by ISA job ($p<0.0001$), highest among cane cutters and agrichemical applicators and lowest among drivers. This is not surprising since field workers in the most physically demanding jobs would be expected to consume the most water and bolis. However, since the risk of kidney damage also appears to be highest among cane cutters and lowest among factory workers/drivers, the analysis evaluating the association between water consumption and markers of kidney function must be conducted carefully, otherwise it could appear that increased fluid consumption increases the risk of kidney damage.

Consumption of water and bolis was evaluated as a predictor of kidney injury (urinary biomarkers) and kidney function (eGFR), separately for workers in each job and while controlling age and sex. Self-reported water consumption was not associated with markers of kidney damage among workers at ISA or in other industries. Similarly, self-reported bolis consumption was not associated with kidney damage among ISA workers.

The questions about alcohol consumption asked about the number of days each week with at least one alcoholic drink and the typical number of drinks consumed on those days, reported using categories in Table 32. These two alcohol variables were used to create continuous estimates of 'total drinks of alcohol per week,' which was then summarized using four categories. The categories of 'total drinks of alcohol per week' are shown in Table 32 and the continuous estimates are shown in Table 31.

Table 32. Alcohol consumption by industry (categorical)

	ISA Workers	Construction	Port Workers	Miners
	n (%)	n (%)	n (%)	n (%)
Days per week with at least one drink of alcohol				
0	189 (67%)	30 (50%)	34 (64%)	24 (47%)
<1	65 (23%)	16 (27%)	13 (25%)	21 (41%)
1-2	17 (6%)	9 (15%)	2 (3.8%)	5 (10%)
2-3	4 (1.4%)	1 (1.7%)	1 (1.9%)	0 (0%)
4-5	1 (0%)	2 (3.3%)	1 (1.9%)	1 (2.0%)
6-7	0 (0%)	2 (3.3%)	2 (3.8%)	0 (0%)
Missing	8 (2.8%)	0 (0%)	0 (0%)	0 (0%)
Typical number of drinks on days with at least one drink				
0	188 (66%)	30 (50%)	34 (64%)	24 (47%)
1-2	16 (5.6%)	4 (6.7%)	3 (6%)	1 (2.0%)
3-5	40 (14%)	6 (10%)	6 (11%)	4 (7.8%)
6-8	18 (6.3%)	10 (17%)	6 (11%)	7 (14%)
9-11	5 (1.8%)	1 (1.7%)	2 (3.8%)	5 (9.8%)
12-14	1 (0.4%)	4 (6.7%)	0 (0%)	7 (14%)
≥15	8 (2.8%)	5 (8.3%)	2 (3.8%)	3 (5.9%)
Missing	7 (2.5%)	0 (0%)	0 (0%)	0 (0%)
Total drinks of alcohol per week				
0	189 (67%)	30 (50%)	34 (64%)	24 (47%)
1-2	43 (15%)	5 (8.3%)	6 (11%)	5 (9.8%)
3-5	17 (6.0%)	7 (12%)	8 (15%)	10 (20%)
≥5	27 (9.5%)	18 (30%)	5 (9.4%)	12 (24%)
Missing	8 (2.8%)	0 (0%)	0 (0%)	0 (0%)

Alcohol consumption was highest among factory workers and cane cutters and lowest among drivers and seed cutters, but overall did not vary significantly by job (p=0.1). Consumption of alcohol was evaluated as a predictor of kidney injury and kidney function, separately for workers in each job and while controlling age and sex. Self-reported alcohol consumption was not associated with markers of kidney damage and workers at ISA or in other industries.

To assess opinions about hydration, workers were asked about the extent to which they agreed or disagreed with the following two statements: "Drinking fluids during a workday is a sign of physical weakness", and "Drinking cold water while you are hot could result in negative health outcomes." These results are reported in Table 33.

Table 33. Opinions about hydration by industry

	ISA Workers	Construction	Port Workers	Miners
	n (%)	n (%)	n (%)	n (%)
"Drinking fluids during a workday is a sign of physical weakness"				
Strongly Agree	13 (4.6%)	0 (0%)	4 (7.5%)	4 (7.8%)
Agree	32 (11%)	8 (13%)	8 (15%)	13 (25%)
Disagree	216 (76%)	47 (78%)	41 (77%)	34 (67%)
Strongly Disagree	15 (5.3%)	5 (8.3%)	0 (0%)	0 (0%)
Missing	8 (2.8%)	0 (0%)	0 (0%)	0 (0%)
"Drinking cold water while you are hot could result in negative health outcomes"				
Agree	180 (63%)	45 (75%)	39 (74%)	41 (80%)
Disagree	97 (34%)	15 (25%)	14 (26%)	10 (20%)
Missing	7 (2.5%)	0 (0%)	0 (0%)	0 (0%)

It was surprising that such a high percentage of workers believe that drinking cold water while they are hot can result in negative health outcomes, ranging from 63% of ISA workers to 80% of miners. The results were consistent with what has repeatedly been mentioned as a common belief in Nicaragua. It was less common for workers to believe that drinking fluids during a workday is a sign of weakness, ranging from 13% of construction workers to 33% of miners (including both the 'Agree' and 'Strongly Agree' categories). Interestingly, self-reported water consumption was no different among workers who agreed or disagreed with these statements.

IV. LIMITATIONS

There were a number of limitations to our study that should be considered in interpreting the data.

- **Impact of health surveillance program at ISA**

The prevalence of elevated creatinine among the 1,249 ISA workers who were tested at pre-zafra was lower than would be expected in the absence of the health surveillance program at ISA, because workers who had been found to have elevated creatinine in previous zafras were removed (to some extent) from the pool of applicants. Because similar surveillance programs were not in place in the other industries, it was not appropriate to compare the prevalence of elevated creatinine at ISA to other industries. However, we still were able to (1) make internal comparisons across job and time among ISA workers and (2) assess whether creatinine levels were elevated among workers in other industries that shared strenuous outdoor manual labor but were not agricultural in nature.

- **Impact of Loss-To-Follow-up**

An important limitation of the study was the loss-to-follow-up (LTF) during the zafra. Of the 1,249 applicants who were tested at pre-zafra, at least 140 never started working, either because they had elevated creatinine or for other reasons. As a result, only 1,109 of the original 1,249 applicants worked at some point during the zafra. There were two types of LTF: (1) 104 (9%) of these 1,109 workers were no longer working at ISA at the time of the late-zafra testing, and (2) 499 (50%) of the remaining 1,005 workers were not reached for testing even though they were still working at ISA at the time of the late-zafra testing.

Regarding the first type of LTF, these 104 workers were no longer working at ISA for various reasons that have not yet been determined. Some may have stopped working because of their health, either because they had to stop (due to elevated creatinine) or because they chose to leave (due to symptoms). Some may have stopped working for reasons that were unrelated to health, either due to a decreased need for labor in the final month of the zafra or simply due to a voluntary decision to leave. Accordingly, the 1,005 workers who were available to be tested at the late-zafra investigation do not represent a random sample of the original 1,109 hired workers. If the workers who were no longer working at ISA had higher creatinine levels than the tested population, then the biomarker measures at late-zafra would have been higher than we observed. The LTF of workers with high creatinine likely decreased the observable differences between ISA jobs. Since we detected significant differences between ISA jobs, the actual differences may have been even greater than observed in this study.

Regarding the second type of LTF, the main reason for not being able to reach these 499 workers at the late-zafra investigation were logistical in nature and less likely to be related to the level of their biomarkers. Each workday, field workers were dispersed across an area of 35,000 hectares and the administrative practices at ISA do not allow the daily work locations to be tracked for individual workers. Accordingly, the logistics of the late-zafra investigation were much more complicated and challenging than for the pre-zafra investigation.

- **Analyzing biomarkers of kidney injury and CKD by ISA job category**

Within the sugarcane industry, job category was used as a surrogate exposure variable since the specific causal agent(s) are unknown. Workers in these different job categories likely vary with respect to the potential for many different exposures of interest, such as heat stress, agrichemicals, and leptospirosis. Workers in these different job categories may also be different in other ways, such as the extent to which they use nephrotoxic medications or the types of food they eat. If we had observed no differences in biomarkers of kidney injury and CKD by job category, this would suggest that the primary CKD risk factors are not occupational. However, we found that biomarkers of kidney injury and CKD were significantly different by job category, even when restricted to field workers. This finding is consistent with the hypothesis that there is an occupational component to the CKD epidemic, but does not rule out the possibility that confounding by unmeasured non-occupational factors may account for the observed differences by job. Additionally, the findings among workers in other industries suggest that the CKD epidemic is not restricted to sugarcane workers.

- **Cross-sectional assessment of CKD**

We were only able to compare measures over time for ISA workers and not for workers in other industries because we only added the latter group for the second round of testing. Therefore, we were not able to assess whether changes occurred over time among workers in these industries. Also, it is difficult to make statements about chronic disease based on cross-sectional associations and single measures. In particular, diagnosis of CKD requires two low eGFRs over a three-month period. As with most other field studies, we used a single measurement, which likely somewhat overestimated the prevalence of CKD in the study population.

- **Timing of sample collection**

It is also important to measure these biomarkers before work if possible as they may transiently rise during the day. Almost all workers were measured before work; however, some of the factory workers at ISA and the miners had to be tested in the middle of the workday. If the biomarkers of the factory workers were transiently elevated, this would have caused the difference between other ISA workers and factory workers to be underestimated. Because factory workers were found to have the lowest values on most measures, this would mean that the actual difference may have been even greater.

- **Self-reported hydration and alcohol consumption**

An additional limitation was the fact that the assessment of hydration practices and alcohol consumption was based on self-reported data about these behaviors. We found no association between these factors and biomarkers of CKD or kidney injury. One explanation for this finding is that there is in fact no association. An alternative explanation is that the null finding is attributable to a high degree of exposure misclassification.

V. CONCLUSIONS

From October 2010 to June 2011, the Boston University team conducted an investigation of biological markers of kidney injury and CKD among workers in Western Nicaragua. Workers in the sugarcane industry were recruited from ISA and monitored at the beginning and during the latter stages of the 2010-2011 zafra (see description of this population in Section 2.2.1). Workers in three other industries in Western Nicaragua (miners, construction workers, and port workers) were also monitored from March to June of 2011 (see description of this population in Section 2.2.2).

Here, we summarize our conclusions according to the objectives described in the Introduction. Our conclusions regarding CKD focus on eGFR rather than serum creatinine since eGFR is estimated using a combination of serum creatinine, age, and sex and therefore provides a better estimate of kidney function. Additionally, well established cutpoints for eGFR serve as the basis for defining the more advanced stages of CKD.

- **Evaluate characteristics of the disease to determine whether kidney damage is tubulointerstitial or glomerular.**

  Our results provide evidence that the type of kidney damage occurring in ISA workers, as well as among workers in other industries, is primarily tubulointerstitial in nature. At late-zafra, cane cutters had the highest concentration of urinary NGAL and NAG, both of which are biomarkers of tubulointerstitial kidney injury. This evidence of tubulointerstitial disease is important because the primary causes of CKD globally are diabetes and hypertension, which more often result in glomerular disease manifestations. However, protein or glucose was only rarely detected in urine, providing little evidence of diabetes or glomerular disease. The one possible exception was that miners had protein detected in urine with higher frequency (based on dipstick data) and at higher concentrations (based on urinary ACR) than ISA workers, port workers, or construction workers.

- **Evaluate biomarkers of kidney injury and CKD among ISA workers by investigating changes during the zafra and differences by ISA job.**

  Our results provide evidence that biomarkers of kidney injury and CKD were generally highest among cane cutters and seed cutters and lowest among factory workers. Estimated GFR was lowest among cane cutters and seed cutters at both pre-zafra and late-zafra (as compared to ISA workers in other jobs), though the difference was highest at late-zafra. This was consistent with the finding that cane cutters and seed cutters experienced the largest decreases in eGFR during the zafra, as compared to ISA workers in other jobs. As described above, we would expect the prevalence of CKD (Stages 3 and 4) to be quite low in a population between the ages of 10 and 59 years old (1% in US). If we restrict our study population of ISA workers to only include men between the ages of 20 and 59, we find that the prevalence of CKD (Stages 3 and 4) was 12% among seed cutters and 6% among cane cutters. Because of the health surveillance program at ISA, the prevalence observed in our study population is almost certainly lower than among all seed cutters and cane cutters at ISA. But even with the health surveillance program, the prevalence of CKD stage 3 or higher among cane cutters and seed cutters was much higher than expected in a population of relatively young men.

For biomarkers of kidney injury, late-zafra concentrations of NGAL and NAG were highest among cane cutters, approximately 3 times as high as among factory workers. The increases during the zafra were highest among cane cutters for NGAL and among both seed cutters and cane cutters for NAG. Interestingly, NGAL and NAG among ISA workers were significantly associated with decreased GFR at late-zafra but not at pre-zafra. The highest tertile of late-zafra NGAL (>18.3 µg/g creatinine) was associated with an eGFR that was 7.3 mL/min/1.73 m^2 lower than the lowest tertile (p=0.0005), while the highest tertile of NAG (>1.4 U/g creatinine) was associated with an eGFR that was 6.8 mL/min/1.73 m^2 lower than the lowest tertile (p=0.001). Overall, these results suggest that cane cutters and seed cutters experience tubulointerstitial injury during the zafra that may be increasing their risk of CKD.

- **Determine whether there is evidence of kidney injury or CKD among workers in other industries who have never worked in the sugarcane industry.**

Our results provide evidence of CKD among workers in other industries who have never worked in the sugarcane industry. Typically, the prevalence of CKD in a population less than 60 years old would be expected to be quite low. For example, in the United States, the prevalence of CKD (Stages 3 and 4) in men between the ages of 20 and 59 is approximately 1%. If we also restrict our population of miners, port workers, and construction workers to only include men between the ages of 20 and 59, we find that the prevalence of CKD (Stages 3 and 4) was 6% among miners, 3% among construction workers, and 8% among port workers. The prevalence of CKD in these industries was much higher than expected in a population of relatively young men.

For biomarkers of kidney injury, NGAL and NAG among workers in other industries were significantly associated with decreased eGFR. The highest tertile of NGAL (>18.3 µg/g creatinine) was associated with an eGFR that was 13.4 mL/min/1.73 m^2 lower than the lowest tertile, while the highest tertile of NAG (>1.4 U/g creatinine) was associated with an GFR that was 15.2 mL/min/1.73 m^2 lower than the lowest tertile (p=0.0002). These results suggest that workers in these other industries experience tubulointerstitial kidney injury that may be increasing their risk of CKD.

- **Analyze heavy metals in biological samples collected at both pre-zafra and late-zafra to characterize metals exposure in the region and explore relationships with biomarkers of kidney injury and CKD**

  Biomarkers of metals exposure among ISA workers did not increase during the zafra. Our results provide no evidence that lead, cadmium, or uranium is associated with biomarkers of kidney injury or CKD, and some evidence that high exposure to arsenic (total) is associated with biomarkers of CKD (*i.e.* lower eGFR). Total arsenic in urine includes all species of organic and inorganic arsenic. The inorganic fraction (and associated metabolites) is of primary concern with respect to kidney damage, such that it would be useful to further speciate those fractions, which could potentially show a stronger association with kidney function. Arsenic speciation was not possible as part of this investigation but should be considered in future studies.

- **Culture urine samples collected from ISA workers at late-zafra to investigate the frequent clinical diagnosis of urinary tract infections (UTIs) among young men in this region**

  Our results provide little evidence of UTIs in this population. We cultured urine samples from 50 men who were positive for leukocyte esterase and/or reported have symptoms during the past 24 hours, yet none of these men were found to have a positive urine culture. Additionally, nitrites were very rarely detected in urine, further supporting the absence of UTIs. Physicians and pharmacists report a high occurrence of diagnosed UTIs based on testing positive for leukocyte esterase and/or reporting symptoms, a strange phenomenon given that UTIs among men in the global population are quite rare. Accordingly, it is likely that many of the 50 men described above would be diagnosed with a UTI and/or treated for a UTI, even though they do not appear to have UTIs. We cannot rule out the possible role of sexually transmitted diseases, since we did not specifically culture for these pathogens.

  Testing positive for leukocyte esterase can be an indication of infection, but can also be an indication of inflammation. The percentage of ISA workers who were positive for leukocyte esterase was higher at late-zafra (~16%) than at pre-zafra (~8%). Blood in urine indicates the presence of red blood cells and is also suggestive of inflammation. As was observed for leukocyte esterase, the percentage of ISA workers who were positive for blood in urine was higher at late-zafra (~9%) than at pre-zafra (~1%). The combined results for the urine cultures, leukocyte esterase, nitrite, and blood in urine are suggestive of inflammation and not infection.

  There are many potential factors that could cause inflammation, including kidney injury, so we evaluated whether leukocyte esterase positivity was associated with biomarkers of tubulointerstitial injury. At late-zafra, ISA workers who were leukocyte esterase positive had average NGAL concentrations that were 3.1 times higher ($p<0.0001$), and average NAG concentrations that were 1.4 times higher ($p=0.04$), than workers who were negative. For workers in other industries, leukocyte esterase positivity was associated with NGAL concentrations that were 1.6 times higher than workers who were negative ($p=0.009$), but was not associated with NAG concentrations. Since NGAL and NAG are biomarkers of tubulointerstitial injury, the above results provide further evidence that leukocyte esterase positivity in this study population is predominantly a marker of inflammation.

- **Determine whether hydration practices or alcohol consumption are associated with biomarkers of kidney injury or CKD.**

  Workers self-reported the amount of water and bolis that they consume during the workday, as well as the amount of alcohol they consume in a typical week. Water consumption, bolis consumption, and alcohol consumption were not associated with biomarkers of kidney injury or kidney function.

Overall, our results show evidence of tubulointerstitial kidney injury among workers at ISA and in other industries, which also appears to be associated with decreased eGFR. These findings suggest that there is an occupational component to the CKD epidemic, but that the epidemic is not limited to sugarcane workers. Given the nature of the work performed by workers with the highest risk, volume depletion and muscle damage are important hypotheses that require further attention. This does not rule out the possibility that there could also be an important non-occupational component, which could include environmental exposures, personal behaviors, and/or other factors. For example, arsenic exposure did not vary by ISA job or increase during the zafra, but high exposure to arsenic was associated with lower eGFR and requires further attention. Exposures to lead, cadmium, and uranium did not increase by job and do not appear to be associated with kidney damage in this population. Finally, our results suggest that UTIs are likely being over-diagnosed in the region, especially in men who report symptoms and/or have white blood cells detected in urine, and that leukocyte esterase positivity in this population is more likely due to inflammation (possibly due to tubulointerstitial kidney injury).

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