Kidney function changes in sugarcane workers in the south coast, KwaZulu Natal, South Africa.

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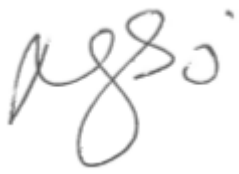
A research report submitted to the Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, in partial fulfilment of the requirements for the degree of Master of Medicine (Community Health) in the field of Public Health Medicine

October 2019

Declaration

I, Mollen Magombo, declare that this research report is my own, unaided work. It is being submitted as per the requirements for the degree of Master of Medicine in the Specialty of Community Health (Public Health Medicine). It has not been submitted for any other degree or examination at this or any other university.



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18th day of October 2019

Dedication

This work is dedicated to my loving family, my husband Mike and my children Jayden and Michaella, my mom, my dad and my lovely brothers.

Disclosure

The authors in this paper do not have any conflicts of interest or financial benefits to disclose.

Abstract

Introduction: Epidemic chronic kidney disease of unknown cause (CKDu) occurs among sugarcane workers, notably cane cutters doing heavy manual work in hot tropical environments in Mesoamerica. Repetitive dehydration consequent on strenuous work in heat is postulated to be the cause. A Nicaraguan cohort study showed remarkable kidney function decreases across six weeks of the cane-cutting season consistent with the dehydration hypothesis. This Nicaraguan study was replicated on a sugar plantation in South Africa about 3 400 kms from the equator and cooler than previous study locations to examine whether less extreme ambient conditions resulted in reduced kidney stress.

Methods: 37 cane cutters and 36 referents of similar socio-economic status but doing less strenuous work on the same plantation had their blood and urine samples assessed for biomarkers of kidney function with the aim of assessing changes in kidney function and hydration across ten weeks of cane cutting. The cutters were examined on Day 1 (start of cutting season), Day 10 and Week 10 of the cutting season, pre- and post-shift. The reference group was examined only on Day 1 and Week 10. CKD-EPI equation was used to calculate the estimated glomerular filtration rate (eGFR) base on creatinine.

Results: Among cane cutters, mean pre-shift serum creatinine increased by 7% and 14% on Day 1 and week 10 respectively; and eGFR decreased across shift by 3% and 5% respectively. These changes were consistent with kidney stress over the shift. Whereas no change was observed across the season in eGFR. Pre-shift Cystatin C increased by 15%, NGAL decreased by 3.6%, serum creatinine decreased by 1% and eGFR increased by 1.4% across the 10 weeks of cutting.

Conclusion: Overall, our study found evidence of effects on kidney function after 10 weeks of cane cutting, but milder than those reported in hotter and lower altitude settings. Our findings are of concern as it is postulated that minor effects when repeated frequently over years may result in significant renal injury.

Key words:

Chronic kidney disease of unknown origin, Heat stress, Dehydration, eGFR, Cystatin C

Acknowledgements

Many thanks and appreciation to my supervisors Professor Rees and Dr Kgalamono for trusting me and giving me an opportunity to carry out the research. I am most grateful to you for being patient with me throughout the research, for your assistance, and for liberally giving your time and expertise to improve my work.

I need to express my gratitude and deep appreciation to Professor Barregard, Professor George and Professor Naicker for persevering with me, for your advice and expertise throughout the research. Many thanks also to Felix Made for assisting me with data analysis, your hospitality, knowledge, and wisdom will not go unnoticed. I am most grateful to Tracy Snyman for great help in the chemical analyses. The study would not have been if not for your assistance in this regard. Your dedication and enthusiasm in making sure we got the blood results inspired me to keep working on the research. Thank you for keeping your door open to us always as we repeatedly asked for results.

My thanks must go also to Lucky Mkhize, Dr Dorkin and the Farm management and workers for giving us access to conduct the research at the farm. Thank you for your hospitality and for persistently supporting us throughout data collection.

I must acknowledge as well Dr Ndaba, Sister Buffel. Thank you for your selfless willingness to travel and take time away from your families to assist with the data collection. Not forgetting Cynthia Dintwe and Jacob Senamela, thank you for coordinating and taking care of the administrative matters that made it possible for the completion of the study.

I owe everything to my loving family, to my husband Mike and my children Jayden and Michaella. I could not have done this work without my husband being the most amazing father and friend; taking over when l had to work and needed space to write. To my lovely parents, Petros and Flora thank you for always believing in me. Finally, I am most grateful to God Almighty, for being the wind beneath my wings “Lord l am grateful, this is a testimony”.

MMed submission format

This research report is in the format of a manuscript for submission to the Environmental Research Journal. I confirm that this article has conformed to the author guidelines of Environmental Research Journal.

Contribution of the candidate to the paper

Contribution of the candidate to the paper

We, the co-authors certify that Mollen Magombo is the first author of this paper. She is responsible for the content of the paper and worked on the following areas regarding the research execution and writing of the article:

- Conception and design of the work outlined in the paper
- Data collection
- Data analysis and interpretation of the results
- Writing of the drafts of the article
- Writing of the final article

Dr Spo Kgalamono

Role: conceptualisation, supervising, review of article drafts

Signature

Date: 27 March 2019

Prof David Rees

Role: conceptualisation, supervising, review of article drafts

Signature.....

Date .....27 March 2019.....

Felix Made

Role: Data analysis, statistical support, review of article drafts

Signature.....

Date ...27 March 2019.....

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Abbreviations: BMI = Body mass index. CKD = Chronic kidney disease. eGFR = estimated glomerular filtration rate. CKD-EPI= Chronic kidney disease epidemiology collaboration. HIV= Human Immunodeficiency Virus. MeN = Mesoamerican nephropathy. NGAL = neutrophil gelatinase-associated lipocalin. NHLS= National Health Laboratory Services. NSAIDS = Non-steroid anti-inflammatory drugs. OHSA= Occupational Health and Safety Act.

Ethics review: The study protocol was reviewed and approved by the Research Ethics Committee of the University of Witwatersrand Medical School All participants gave written informed consent prior to participating in the research.

1. Introduction

The prevalence of chronic kidney disease (CKD) defined as kidney damage that is evidenced by structural damage or functional abnormalities of the kidney for more than three months with health implications, or estimated glomerular filtration rate (eGFR) <60 ml/min/1.73m (García-Trabanino et al., 2015; Paula Santos U., 2015); varies widely from country to country, ranging from approximately 1 to 18 percent (Laws RL et al., 2016; Lozier et al., 2016; Orantes et al., 2014; Raines et al., 2014). As early as the 1990s studies have documented an excess of CKD in Central America and South America, an occurrence that is termed "Mesoamerican nephropathy" (MeN) because of its geographic origin (Lozier M et al., 2016; Peraza et al., 2012; Torres C., 2010; Trabanino et al., 2002; Wegman et al., 2018; Wesseling et al., 2014, 2016). Since the cause is not known, the disease is often known as CKD of unknown cause (CKDu). According to the World Health Organization (WHO), kidney disease is the third most common cause of death in Nicaragua and the fifth most common health related cause of death in El Salvador (Wesseling et al., 2014; El Salvador: WHO Statistical Profile 2012; Nicaragua: WHO Statistical Profile, 2012). Many studies hypothesize that repetitive dehydration consequent on strenuous labour in heat is responsible for CKDu (Bodin et al., 2016; Crowe et al., 2013; García-Trabanino et al., 2005; Glaser et al., 2016; Roncal-Jimenez et al., 2016). The epidemiology of CKDu is consistent with this hypothesis because: mortality is highest in lowland areas with high temperatures (Ekiti et al., 2018; García-Trabanino et al., 2015; Moyce et al., 2017; Santos et al., 2015; Wesseling et al., 2013); occupational groups most affected perform heavy manual labour in hot environments (García-Trabanino et al., 2005, 2015, 2016; Jayatilake et al., 2013; Nathan et al., 2014; Peraza et al., 2012; Wanigasuriya , 2014a, 2014b; Weaver et al., 2015); and

communities in cooler, high altitude areas have lower CKD prevalence than similar communities in hotter, low altitude areas (Ekiti et al., 2018; Peraza et al., 2012; Wegman et al., 2018; Wijkström et al., 2013). Additionally, CKDu occurs predominantly in healthy young males (Laws et al., 2016; Peraza et al., 2012; Raines eta al., 2014).Sugarcane cutters probably have the highest rates of the farming groups as they are commonly exposed to extreme ambient temperatures during hard strenuous work (Jayatilake et al., 2013; Nathan et al., 2014; Wanigasuriya , 2014a, 2014b; Weaver et al., 2015; Wegman et al., 2018). The work is strenuous as in many parts of the world the cane is cut manually because the topography of the farms hinders mechanization. Studies have reported varying working hours and environmental temperatures , Sugarcane harvesting typically involving four to 12 hours of mostly uninterrupted intense physical exertion, and temperatures that range on average between 31 to 42^{o}C (García-Trabanino et al., 2015; KDIGO, 2012;Wegman et al., 2016; Weiner et al., 2013; Wesseling et al., 2013).

CKDu is characterised by the absence of known traditional causes of kidney disease such as diabetes mellitus and hypertension (Orantes et al., 2011, 2014; Raines et al., 2014; Ramirez-Rubio et al., 2013; Van-Biesen et al., 2006; Wesseling et al., 2016); and absent or low-grade proteinuria (Laws et al., 2016; Machiraju et al., 2009; Orantes et al., 2009; Peraza et al., 2012; Raines et al., 2014; Weiner et al., 2013). The disease is believed to occur because of recurrent episodes of acute kidney injury (AKI) (Ekiti et al., 2018; Sanoff et al., 2010); induced by heat stress in conjunction with dehydration and manifests initially as AKI (García-Trabanino et al., 2015; Roncal-Jimenez et al., 2016). Most workers with CKDu are asymptomatic and are diagnosed based on a decreased eGFR based on serum creatinine (Machiraju et al., 2009;

Peraza et al., 2012; Wesseling et al., 2016). More severe cases have markedly elevated serum-creatinine, low plasma sodium, hypokalemia, and features of glomerular ischemia with no established causes of kidney disease (Bedford M et al., 2014; Machiraju RY et al., 2009; Sanoff SL et al., 2010; Yang HY et al., 2015; Wesseling et al., 2016). Renal biopsies have shown a component of tubular interstitial damage (Wesseling et al., 2016; Wijkström et al., 2018).

Besides the heat and dehydration hypothesis, other possible etiologies have been discussed, such as the use of non-steroidal anti-inflammatory drugs (NSAIDS); other nephrotoxic medications; fructose consumption; pesticides/agrochemicals, heavy metals, fluoride in the drinking water and leptospirosis (Brooks DR et al.,2012; Orantes et al., 2014; Wesseling et al., 2013, 2014). Heat and repetitive dehydration as the cause of CKDu is, however, supported notably by a prospective study of sugarcane cutters in Nicaragua. This study monitored markers of kidney function and hydration in cane cutters and referents across shifts and across the first nine weeks of the sugarcane harvest. There was a remarkable decrease in the eGFR over the ten weeks and a cross shift increase in the serum creatinine that could have been caused by dehydration. The authors concluded that repeated dehydration could result in CKD.

Generally, the heat and strenuous work is similar in other sugar growing parts of the world to those of Mesoamerica. An exception to these conditions is the southern coastal area of KwaZuluNatal Province: sugarcane farms here are at the southernmost growing latitude in the world with cooler temperatures during the harvest (Wikipedia, The Free Encyclopedia, 2019). This reduction in temperature but with similar farming methods as elsewhere, gave us the opportunity to investigate whether the same disruptions in renal function and hydration

markers as occurred in the Nicaraguan study occurred in this less heat-stressed locale. Hence, the aim of the study was to determine whether changes in kidney function and hydration across shift and across the early part of the harvest season in cooler ambient conditions were the same as those shown in the hotter climate of Nicaragua. For this purpose, we analysed the following specific objectives:

- To determine cross shift changes in biomarkers of acute kidney injury across shift by comparing pre and post shift measurements.
- To determine the long-term changes in biomarkers of kidney function across the harvest by comparing the baseline and week 10 measurements.
- to determine whether job category is an important determinant of renal function by comparing the kidney function changes between the cutters and the referents doing less strenuous work in less hot environments.

2. Methods

2.1 Study design and setting

We performed a longitudinal study to assess the change in the kidney function and hydration markers across shifts and across the early part of the sugarcane harvest season among sugarcane cutters and less heat-stressed referent sugar plantation workers. The study largely replicated that of the Nicaraguan study mentioned above (Wesseling et al., 2016). For cutters, serum and urine biomarkers of kidney function were measured at Day 1 of the season prior to acclimatization, Day 10 of the season when cutters were operational and at Week 10, after nine weeks of strenuous work and potential heat stress. Referents had their serum and urine parameters measured at Day 1 and at Week 10.

The study was conducted at a sugarcane plantation in the South Coast region of KwaZulu Natal Province, South Africa from March to May 2017. The plantation is roughly 80 kilometers south of Durban, the major business Centre of the KwaZulu Natal Province. It is about 3 400 kms from the equator making it cooler than previously studied locations which reported temperatures ranging from 31 to 42 degrees Celsius (García-Trabanino et al., 2005, 2015, 2016; Jayatilake et al., 2013; Nathan et al., 2014; Peraza et al., 2012; Wanigasuriya , 2014a, 2014b; Weaver et al., 2015). The maximum temperatures during the first ten weeks of cutting season were typically 16 to 30 degrees Celsius.

The plantation employees included seasonal cane cutters – most of whom come from the Eastern Cape Province - and seeders, irrigators, workshop artisans, tractor drivers and milling factory workers. The harvest season lasted nine months from mid-April to early December.

During the harvest cutters lived in dormitories at the plantations provided by the employer. Cutters were given the first week to acclimatise to the manual work and heat. During this week of acclimatisation, workers were not expected to meet the daily cane tonnage target of four tonnes per day, a requirement later in the season for full pay and bonuses. Workers manually harvest previously burnt cane with a machete. Shifts, including transport to and from fields, vary from eight to ten hours per day, six days a week, depending on how quickly an individual reaches their daily targets. The company provided us with the data on the daily average tonnes of cane cut for each of the two operational days of the study (Day 10 and Week 10).

The occupational health clinic does pre-employment health checks consisting of blood pressure, heart rate, height, weight, and physical examination and urine dipstick. Workers with abnormal urine dipstick would not be hired; however, none of the employees has been rejected on those grounds.

2.2 Study Participants

A convenience sample of 37 male cane cutters and 36 male referents (73 in all) volunteered for the study. Thirty-seven of the 68 employed cane cutters from the main farm participated in the study. All participants were older than 18 years and had passed the pre-employment medical screening. During the pre-employment medical examinations Participants did not have proteinuria of more than +1 on dipstick, did not have uncontrolled blood pressure of > 160/90 or uncontrolled diabetes mellitus.

The referents were sugarcane farm workers of a similar work grade to the cutters who did less strenuous work. Their numbers by occupation were: weeders (7), fire team (4), truck drivers (12), cane burners (2), security guards (3), cane cutters' supervisors (3), maintenance team (3) and cane weighers (3). Working conditions for field workers and referents varied substantially. The referents worked half day on short-term contracts. Their working hours generally lasted from 5h00 or 6h00 to 12h00. Each workweek was six days long.

2.3 Measurement tools

2.3.1 Questionnaires

Two questionnaires were administered by trained interviewers to the participants in their preferred language. Questionnaire 1 was administered during the pre-employment screening prior to the start of the cutting season. The questionnaire included questions about: i) sociodemographic data, ii) work history (past and present) including exposure to heat and pesticides; iii) general health including history of hypertension and diabetes, medication used, symptoms of urinary tract infections and self-reported HIV status if known; iv) use of tobacco, alcohol and recreational drugs; and v) family history of renal disease. The questionnaire was based on the questionnaire used in the study done in Nicaragua (Laws RL et al,. 2015).

Questionnaire 2 was administered at the end of each shift to ascertain how much fluid each worker consumed during the work shift, the type of fluids consumed, and the number of rest breaks taken. The questionnaire was also developed based on the Nicaraguan study (Laws RL et al,. 2015).

2.3.2 Physical Examination

All participants were required to undergo a physical examination during which weight and height were measured on a combined digital weighing scale with a mechanical height rod. Blood pressure and heart rate were measured once after the participant had been seated for a minimum of five minutes using a digital automatic sphygmomanometer. The blood pressure was measured on the right arm. The data for the examination and investigations was entered into the questionnaire.

2.3.3 Collection of blood and urine samples and transport to the laboratory

Blood and urine samples were collected pre- and post-shift at the dormitories of the workers or at worker pick-up and drop-off points on the farms. Samples were placed in cooler bags with ice packs until all samples had been collected from the group (typically an hour or so). They were then immediately transported by the last author (DR) to a local National Health Laboratory Service (NHLS) pathology laboratory where they were spun down as appropriate and frozen. At the end of the collection period (usually a couple of days), the samples were transported frozen to the analytical laboratory (see below) by DR and placed by him in -80 ^{o}C freezers. All samples were still frozen on reaching the laboratory.

2.4 Biochemical analysis

Blood and urine samples were analysed at the laboratory of the Faculty of Health Sciences, Chemical Pathology Department at the University of the Witwatersrand, Johannesburg. Serum was analysed for creatinine, cystatin C, potassium, sodium, uric acid, calcium, phosphate, urea, neutrophil gelatinase-associated lipocalin NGAL), myoglobin and osmolality. Urinary sodium,

creatinine, osmolality and albumin were also measured. Hemoglobin A1c and blood glucose were measured only at the start of the cutting season. Urine dipstick analysis was performed using the Roche Combur-test reagent strips for semi-quantitative measurements of proteinuria, urinary specific gravity, ph., blood, nitrite, leucocytes, bilirubin, ketones, and urobilinogen. Reference values for all tests were those used by the laboratory, which did the analyses.
All serum creatinines were done by an IDMS traceable enzymatic method (Cobas 702, Roche Diagnostics Mannheim, Germany). Electrolytes were measured by ion specific electrodes (ISE) on the same instrument. Cystatin C was measured by immunoturbidimetry (Cobas 502, Roche Diagnostics, Mannheim, Germany). Urine creatinine was measured by an enzymatic method on a Cobas 702 (Roche Diagnostics, Manheim) and urine electrolytes by ISE. Urine osmolality was measured by freezing point depression and NGAL by a two-step immunoassay (Architect, Abbot, Longford, Ireland). The laboratory participates in external quality assurance programmes (RCPA).

Estimated glomerular filtration rate (eGFR) was calculated using the kidney disease epidemiology collaboration (CKD-EPI) equation based on serum creatinine (Levey AS et al., 2012).

2.5 Work and environment measures

Physical exertion were estimated by daily average tonnes of cane cut. Daily environmental temperatures were measured at the plantation and provided by the weather station in Durban, KwaZulu Natal (World Weather Information Service—Durban).

2.6 Data analyses

The de-identified data set was prepared in an excel spreadsheet. Errors in data entry were corrected after crosschecking both the laboratory records and clinical case recording forms. All data were analysed using Stata 14. The distribution of the data was examined using the Shapiro-wilk tests, to determine the normality of the parameters. The biomarkers were either normally / not normally distributed. Descriptive statistics were computed for demographic data. The categorical variables were expressed as frequencies, percentages, counts and proportions. Continuous variables were expressed with the mean if normally distributed and median within the 10^{th} and 90^{th} centile range if non-normally distributed.

Cross shift and cross harvest changes were assessed for both the referents and the cutters. To evaluate differences between biomarkers at pre- and post-shift, we used Wilcoxon's signed rank for non-normally distributed biomarkers and t-test for the normally distributed parameters. Cross harvest, differences in pre-shift and post-shift levels of selected kidney function markers were assessed at the beginning of the harvest season and ten weeks into the harvest season, in those individuals who took part in both examinations of each variable for both cutters and referents. Mixed effect models produced P-values for changes across harvest and for the differences between the groups.

3. Results

Seventy-three male sugarcane workers (37 cutters and 34 referents) were enrolled and assessed. The baseline characteristics of the study participants are summerised in Table 1. Overweight and obesity was uncommon, only 21% and 7% of the subjects had a BMI > 25 and 30 kg/m^2, respectively. Whereas over half of the participants used alcohol in each group. Self-reported diabetes mellitus among participants was very low at 5.5%. None of the participants was hyperglycemic at the beginning of the cutting season: none one of the cane cutters had random glucose levels of >11 mmol/l, whereas two referents exceeded 11 mmol/l. About 14% of the workers had HbA1c in the diabetic range (> 6.5 mmol/mol), all of whom were referents. A considerable number of the study population reported having been diagnosed with HIV: 28.8% - with more cane-cutters reportedly infected 14 (39%) compared to seven (19.4%) referents. About 29.7% and 36% of the cutters and non-field workers respectively reported ever using NSAIDS for a period of more than three months. The median years worked cutting cane for the cutters was 17 years (10-90 percentile range 8- 30).

No cane cutter started the season with an eGFR < 90 ml/min/1.73m^2, but two referents did; none was < 60 ml/min/1.73m^2. Of the five cutters participants who had +1 proteinuria at the start of the cutting season, two of them self-reported being HIV positive.

3.1. Work Characteristics

Table 2 presents some aspects of the work conditions on the plantation. The production data provided by the Mill shows an average production of 3.6 tonnes/individual on Day 10 and an average of 5.48 tonnes at Week 10. On average, the cane cutters took at least one break during

the cutting shift. Overall, workers had a poor water uptake. The mean self-reported water intake was approximately 400ml per shift, a volume that is too low to be plausible. About 18 % started the day dehydrated (USG ≥ 1.020). Environmental temperature readings for day 1, day 10 and week ten were 26.5, 28.2 and 26.6 ^{o}C respectively. Ambient temperatures were lower than those reported in Mesoamerica: The means of the maximum temperatures were 26.3 ^{o}C 24.8 ^{o}C 23.2 ^{o}C in March, April and May respectively. The dipstick data showed a low-grade detection of proteinuria during the ten weeks of the study. None of the participants had higher-grade proteinuria (+3) at the end of week ten.

3.2 Cross-shift changes

Several kidney function markers demonstrated significant post-shift changes relative to pre-shift at all measurement points for both cutters and referents as seen in Tables 3A and 3B. Comparisons of the mean difference in pre-shift and post-shift levels of the kidney function markers for cane cutters showed significant increases in creatinine, phosphate, calcium and Cystatin-C. On average, there was a decrease in the eGFR during the harvest season at all-time points. Overall, the average percent decline in cross-shift eGFR on Day 1 and Week 10 was 2 and 5% respectively. The cross-shift changes in the eGFR were smaller for the referents. Overall serum potassium decreased across shift. There was a small increase in uric acid whereas NGAL decreased across shift. NGAL, which increases if there is acute kidney injury, was unaffected by working a shift of cane cutting and serum uric acid only increased marginally during the Week 10 shift. Average serum sodium was normal, with a 10th to 90th centile range of 137 - 143 (normal = 135 - 145). Few of these workers had substantially decreased eGFR.

3.3. Cross harvest changes

Cutters

Cross harvest, changes for cane cutters and referents are shown in Table 4. For the evaluation of longitudinal changes in key kidney function biomarkers, we restricted the analysis to individuals who took part in examinations both at baseline and at end of follow-up for each parameter. In the cane cutting group, mainly cystatin c, urea, potassium, eGFR, uric acid, and myoglobulin. Levels of post shift potassium significantly decreased across the harvest season. Serum creatinine decreased significantly in the sugarcane cutters over the nine-week period, pre-shift means from 79.6 to 74.9 µm/L, and serum urea N even more. There was a non-significant tendency towards an increase in pre-shift serum urea (Table 4). Pre and post-shift serum phosphate and pre-shift decreased among cane cutters. Urinary NGAL also decreased substantially. Individual daily average production increased over the ten weeks of harvest from (Table1.)

Referents

In the reference group, most parameter showed no change across the first ten weeks of cutting. There was a significant tendency towards an increase in pre-shift Cystatin C. eGFR showed a non-significant decrease over the nine-week period from 114.5 to 115.7 mL/min/1.73m^2.

3.4 Differences between cane cutters and referents across harvest

The pre-harvest and week 10 measurements of the biomarkers of kidney function indicated an increase in some parameters in both groups. Comparison of the mean pre-shift values between

cane-cutters and referents shows a smaller change for referents compared to the cane cutters (Table 4). Most of the key parameters did not show any significant differences between the groups. At Day 1 and Week 10 cane cutters had significantly lower serum creatinine (pre- and post-shift) and higher eGFR, post shift potassium and Cystatin C than referents (P-values are in the second last and last columns). At the end of ten weeks, there was a significant increase in cystatin C in both groups with cane cutters showing a higher increase and a significant group difference in increase. Serum creatinine was significantly lower in the cutters than the referents on both day 1 and week 10. Pre-shift phosphate was higher in cane cutters than in referents after 10 weeks, while post-shift levels at follow-up were not significantly different.

The sugarcane cutters demonstrated an improvement in their eGFR across harvest whilst the referents did not show any change in theirs throughout the ten weeks. However, the cane cutters demonstrated a higher percentage change in GFR across harvest of 2% as compared to 1% in the referent group. On average, pre-shift eGFR improved by 2.1 mL/min/1.73m2 for cutters groups over the first ten weeks of harvest and that of referents by 0.46 mL/min/1.73m2. On average, none of the workers experienced a decline more than 20 % (severe eGFR decline). At pre- harvest no workers had an eGFR < 60 ml/min/1.73m^2 and none at the end of ten weeks of harvest. More so none of the participants had a persistent eGFR < 90 ml/min/1.73m^2. Although the mean eGFR for the referents was, lower than that of the cane cutters it remained constant with no substantial changes throughout the ten weeks of the harvest as compared to the significant changes that were experienced by the cane cutters. Both groups had a mean decrease in serum NGAL across harvest. Although there were, changes in both groups the changes were more pronounced in cane cutters. Sixteen percent of the participants had

persistent low-grade proteinuria (18% of the cane cutters). However, none had a persistent eGFR < 60 ml/min/1.73m2. We found that change in eGFR over the ten weeks appeared to be associated with an increase in the serum-creatinine across shift at cut1 (r_p= -0.22, P= 0.001). In addition, there was an association in the longitudinal change in eGFR over the ten weeks and the average cross shift changes in creatinine at cut1 and cut2(r_p= 0.122, P=0.012) respectively

4. Discussion

The aim of this study on a South African sugarcane plantation was to determine whether markers of kidney function across shift and across the early part of the cane-cutting season indicted renal injury in response to the strenuous work done by the cutters. Cane cutters were examined pre- and post-shift on Day 1 (start of the cutting season), Day 10 of the season and Week 10. The study replicated an investigation of Nicaraguan sugarcane workers but the South African plantation was much further from the equator and hence cooler than previously studied settings, including that in Nicaragua. The cooler environment is of interest given the hypothesis that repetitive dehydration consequent on strenuous work in heat is the cause of the CKDu identified in sugarcane workers. Our findings are not straightforward. Based on serum creatinine and eGFR derived from it, we did not find the "clear and significant decrease in pre-shift renal function" after 10 weeks of cutting as shown in Nicaragua after nine weeks (Wesseling et al., 2016). Nor did we find any cutter whose eGFR fell below 60mL min/1.73 m^2 after this period – Wesseling et al. 2016, reported three. Serum NGAL, which rises in acute kidney injury, did not increase significantly pre-shift in cutters during 10 weeks of the study. Contrary to these findings, cystatin C levels did rise across the 10 weeks both pre-shift and post-shift. Increased Cystatin C values without a concomitant elevation in serum creatinine levels have been reported in subjects with normal or close to normal renal function (Lima et al., 2014; Legand et al., 2017). The discordance between the two measures is thought to be due to the higher sensitivity of Cystatin C for early kidney injury. Overall, therefore, our study found evidence of effects on kidney function after 10 weeks of cane cutting, but milder than those reported in the hotter and lower altitude setting of Nicaragua and other sugar growing areas.

Whether the effects we describe are sufficient to result in CKDu has still to be determined, but they are of concern as it is postulated that relatively minor effects repeated over years may result in significant renal injury (Ekiti et al., 2018; García-Trabanino et al., 2015; Roncal-Jimenez et al., 2016; Sanoff et al., 2010).

In cutters, post-shift serum creatinine values were higher than the pre-shift ones at Day 1, Day 10 and Week 10, and eGFR lower, although modestly so on average: for example at Week 10 median serum creatinine levels increased by 11 µmm/L; and eGFR fell by -5.8 mL/min/1.73 m^2. This cross-shift decline in renal function is consistent with previous studies of workers exposed to heat stress and strenuous work (Ekiti et al., 2018; García-Trabanino et al., 2015; Moyce et al., 2017; Wegman et al., 2018; Wesseling., 2016); but some of the change may be variation both biologically and in measurement unrelated to effects on the kidney. A study by Wegman et al, described a change in eGFR of < 10 percent as normal variation (Wegman et al., 2018). As noted in previous studies in El Salvador and Nicaragua (Peraza et al., 2012; Wesseling et al., 2016), these post-shift changes likely reflect poor hydration with a loss of extracellular volume, i.e. not due to renal damage or dysfunction. This interpretation is somewhat at odds with the absence of increases across shift in urinary specific gravity in our study at Day 10 and Week 10: the median change was zero on both occasions. Pre-shift changes would be more indicative of renal injury as workers would have had the opportunity to re-hydrate after the shift and overnight, thus pre-shift values would remove the transitory effects of under-hydration. The mean pre-shift rise in cystatin C from 0.88 mg/L at Day 1 to 1.04 mg/L at Week 10 is, therefore, probably a true effect on kidney function. eGFR cystatin C (i.e. derived from this biomarker) changed from a median of 100.1 to 81.7 ($p = < 0.001$), a substantial reduction. Previous studies of sugarcane

workers have not derived eGRF from cystatin C; whether or not our findings are typical of cane-cutting induced injury is unknown.

Referents were similar to the cutters in that they did not show increases in serum creatinine and decreases in eGFR across the 10 weeks of the study: these biomarkers were essentially unchanged, for example pre-shift eGFR mean values were 114.5 ml/min/1.73 m^2 at Day 1 and 115.7 ml/min/1.73 m^2 at Week 10. NGAL also did not increase. As with cutters, though, cystatin C did rise over the course of the investigation: from a mean of 0.77 mg/L to 0.87 mg/L, an increase of 0.1 mg/L which is slightly less than the 0.13 mg/L found in the cutters. Post-shift changes in serum creatinine and eGFR were not evident in the non-cutters; the only significant change being a cross-shift increase in cystatin C at Day 1 and at Week 10. The referents' post-shift levels were lower than those of the cutters, though, with median values of 0.99 versus 1.11 mg/L respectively. An a explanation is that factors or agents unrelated to heat or which increased the response to moderate or even mild heat stress caused the increase in cystatin C in both groups or the referents alone. Human immunodeficiency virus (HIV) infection is one such consideration: possibly HIV infection is an effect modifier of the relation between heat stress and kidney injury. We were unable to find published investigations on this issue. Additionally, cystatin C was not significantly higher in the HIV positive workers pre-shift at Week 10 in all 68 plantation workers analysed together (0.93 HIV - vs 0.99 HIV +; $p = 0.19$) or in 34 cutters (0.99 HIV - vs 1. 04 HIV +; $p = 0.19$) or 34 referents (0.87 HIV - vs 0.90 HIV +; $p = 0.71$) analysed alone. It is notable, though, that the HIV positive workers on average had higher cystatin C levels than their negative counterparts; small numbers may explain the p values > 0.05.

Other longitudinal studies in the sugar industry are relatively scant. There have been two from Nicaragua (Laws et al., 2016; Wesseling et al., 2016), and one each from Brazil (Santos et al., 2014), El Salvador (Wegman et al., 2018) and Guatemala (Butler-Dawson et al., 2019). All except Santos et al. found increases in serum creatinine and decreases in eGFR derived from the biomarker over the study periods. The "negative" Brazilian investigation found substantial decreases in eGFR across work shifts (Santos et al., 2015). Table 5 presents key findings from the five publications. In the main, they show larger effects on markers of renal function than we found on the South African plantation.

Elevated urinary concentrations of uric acid have been implicated in the kidney damage of Mesoamerican nephropathy (Roncal-Jimenez et al., 2015, 2016). We did not measure this agent in urine but did so in serum; the levels did not increase significantly across shifts in cutters (Table 3A)

5. Limitations

Almost all of the cane cutters were migrant workers from the Eastern Cape, a province over 100 kms from the sugar plantation. The cutters were recruited annually through announcements in the labour-sending area that the harvest was soon to begin. It can be presumed that the strenuous nature of the work would inhibit sick individuals from returning to the plantation, resulting in a healthy worker effect. Additionally, possibly only workers more resistant to the kidney-damaging effects associated with cane cutting would return year after year producing a tolerant survivor population. These pressures could explain the absence of reduced eGFR-creatinine across the harvest in our study. These assumptions are countered by the fact that no

cutter had an eGFR- Creatinine < 90 ml/min/1.73m^2 pre-harvest; the lowest value was 95 ml/min/1.73m^2.Workers were not screened for renal dysfunction pre- or post-harvest and eGFRs modestly < 90 ml/min/1.73m^2 would typically be asymptomatic, thus not resulting in self-selection for non-presentation at the start of the harvest. The cutters generally had long service - a mean 17.4 years and 10th to 90th percentiles of 8-30 years – so if CKDu was occurring in this workforce, even with a healthy worker effect, one would expect at least some individuals to have mildly low eGFRs at the start of the season and reduced rates across the season.

Our study was done over a relatively short period of 10 weeks from the end of summer into early winter; most other longitudinal studies span most of the six or so months of harvest (Table 5). Had we continued until the end of harvest, which is in mid-summer and a hotter time of the year, we may have observed more damaging renal effects. Interestingly, though, the Wesseling et al., (2016) study found increased creatinine levels and reduced eGFRs already at six days and no further deterioration in average values at nine weeks. The intervention study in El Salvador (Wegmann et al., 2018) reported decreased average eGRFs about two months into the study, although with further decline in the non-intervention arm some four months later. Based on these data, it would be reasonable to expect changes in serum creatinine and eGFR-creatinine 10 weeks into the season, which we did not find.

The discordance between cystatin C and serum creatinine can be explained by the higher sensitivity of cystatin C for early renal injury, but also by unreliable measurement of either or both biomarkers. This latter explanation seems unlikely: quality assurance during cystatin C analyses shows good performance (See Supplementary Table 1 Cystatin C quality standards).Serum creatinines were rerun for six specimens there was no general tendency to

have underestimated the initial creatinine measurement based on the repeat measurements (see Supplementary Table 2).

An additional possible limitation is that analyses were done on frozen samples after long-term storage of some months. Theoretically, this could result in decreased concentrations of some biomarkers. If this occurred, it would not alter the main findings of our study; changes would be proportionate for cutters and referents and across shifts and harvest, meaning changes in renal function would still be evident.

HIV infection status was self-reported and not objectively determined. This was mainly due to our anticipation that a mandatory HIV test would discourage participation in the study. There is still stigma associated with being HIV positive in South Africa (Koodibetse KG., 2015; Maretha J et al., 2009) so misclassification of HIV status based on self-report is to be expected. Nevertheless, it may not have been substantial: the occupational health service at the plantation offers voluntary HIV testing and counselling; most workers participate in the programme, know their status and are aware that the occupational health service also knows it (Personal communication, Dorkin E, 2018). That 21 (28.8%) of subjects reported being HIV positive suggests that under-reporting was limited.

In conclusion, our study was done on a sugar plantation much further from the equator and cooler than previously studied settings. Based on serum creatinine and eGFR derived from it, we did not find a decrease in renal function after 10 weeks of cutting as shown in Nicaragua after nine weeks (Wesseling et al., 2016) and in other longitudinal studies. Nor did we find any cutter whose eGFR fell below 60mL min/1.73 m^2 after the 10 weeks of cane cutting. We did,

however, find substantial but reversible cross shift deleterious effects on renal biomarkers, indicating kidney stress over the shift. Additionally, Cystatin C levels rose across the 10 weeks both pre-shift and post-shift. Based on this biomarker, quite substantial reduction in glomerular filtration occurred in the study subjects. Attention to hydration, rest and shade, as recommended elsewhere (OHSA, 1999) and interventions to reduce workload (Wegman et al., 2018) are, therefore, indicated. Overall, therefore, our study found evidence of effects on kidney function after 10 weeks of cane cutting, but milder than those reported in hotter and lower altitude settings. This suggests that the cooler ambient conditions may have moderated the effects of heat stress on the kidney. Nevertheless, our findings are of concern as it is postulated that relatively minor effects repeated over years may result in significant renal injury. An investigation of CKDu in former cane cutters in the labour-sending area of the Eastern Cape Province would help in determining whether the condition does occur in these workers and its magnitude if it does. In addition, kidney function should be ascertained in the cane cutters of the study plantation at the end of the harvest in mid-summer when the effects would be expected to be most pronounced.

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Table 1. Characteristics of sugarcane cutters and referents at baseline. Median (10 – 90 percentile range) are shown for continuous variables, number and percentages N (%) for categorical variables and medical conditions.

Characteristics of study population- mean median (range)	**Cutters (N=37)**	**Referents (N=36)**	**Both groups (N=73)**
Age (years)	42 (28 - 53)	45.9 (24.3 - 61.6)	44.2 (29.3 - 53.1)
Body weight (kg)	61 (45.9 - 90.1)	64.9 (45.7 - 115.3)	63.9 (51.9 - 81.9)
Height (cm)	170 (159 - 178)	167 (155 - 177)	168 (160 - 170)
BMI (kg/m^2)	21.7 (18.9 - 27)	23.2 (19.3 - 31.4)	22.5 (19.2 - 27.3)
% BMI >: 25 (overweight) N (%) 30 (obese) N (%)	 5 (14) 0	 7 (19) 5 (14)	 12 (16) 5 (7)
Current smokers N (%)	10 (27)	18 (50)	28 (38.4)
Ex-smokers N (%)	9 (24.3)	2 (5.71)	11 (15.07)
Current use of alcohol N (%)	20 (54)	22 (61)	42 (58)
Education (formal) N (%)	34 (91.9)	24 (66)	58 (79)
Hypertension N (%)[a]	8 (21.6)	7 (19)	15 (20.5)
Diabetes N (%)[a]	1 (2.7)	3 (8.3)	4 (5.5%)
Kidney disease N (%)[a]	2 (5.4)	4 (11)	6 (8.2)
HIV N (%)[a]	14 (37.8)	7 (19.4)	21 (28.8)
NSAIDS N (%)[b]	11 (29.7)	13 (36)	24 (32.9)
Serum-glucose mmol/L	5 (4.5-6.2)	5.2 (4.5-8.8)	5.1 (4.5- 7)
Random glucose > 11 mmol/L (N) (%)	0	2 (6)	2 (3)
HbA1c mmol/mol	5.2 (4.9 - 5.8)	5.4 (5 - 8.7)	5.4 (5 - 6)
HbA1c mmol/mol >6.5 (%)	0	14 (39)	14 (19)
Pre-shift s-creatinine,mmol/L	81 (68 - 90)	88 (72 - 103)	
Years of cutting	17 (8 - 30)		
Took breaks during shift N (%)	35 (94.6)		
Previous canecutter N (%)		12 (33.3)	
Pesticides use N (%)	9 (24.3)	2 (5.5)	11 (15)
eGFR , ml/min/1.73m^2 (creatinine)	123.0 (114.9 - 135.8)	114.8 (103.5 - 123.7)	118.5 (106.8 - 134.0)
Reduced eGFR, 60-89 ml/min/1.73m^2 , N (%)	0	2 (6)	2 (3)
Specific gravity (USG) ≥1.020, N (%)	7 (19)	6 (17)	13

[a]Self-reported. [b]Used for > 3 months in the last 3 months prior to the study

Table 2. Working conditions across the first ten weeks of harvest.

Condition	**Cutters (N=37)**
Workload (tonnes cut/day) - Mean, median (10 – 90 percentile range)	
Day 1	Acclimatisation
Day 10	3.4 (2.4 - 5.3)
Week 10	5.5 (3.9 - 7)
Number of breaks / shift - Mean, median (10 – 90 percentile range)	
Day 1	1.1, 1 (1 - 1.5)
Day 10	1.4, 1 (0 - 4)
Week 10	1.5, 1 (0 - 3)
Water-intake (mL)[a] - Mean, median (10 – 90 percentile range)	
Day 1	450 (200 - 700)
Day 10	400 (200 - 800)
Week 10	600 (200 - 800)
Maximum temperatures ^{o}C - mean (SD) (min-max)	
March	26.36 (2.09) **(**19.3 - 30)
April	24.83 (2.01) (19.9- 28.9
May	23.28 (2.71) (16.1- 26.8)
Across ten weeks	24.7 (2.59) (16.1- 30)
Minimun temperatures ^{o}C - mean (SD) (min-max)	
March	18.9 (1.9) (15.7 - 22)
April	17.24 (2.46) (12 - 21.3)
May	15.2 (1.96) (11.7- 18.7)
Across ten weeks	17.1 (2.60) (11.7- 22)

[a] Self-reported.

Table 3A. Pre-shift and cross-shift changes in serum and urine biomarkers at the beginning of the harvest season (Day 1), Day10 and ten weeks into the harvest season for 37 cane-cutters. Median (10 – 90 percentile range) and mean (standard deviation) for non-normally distributed and normally distributed variables respectively.

Serum biomarkers (cane cutters)	**Day1 median** (10 – 90 percentile range)	**Day 1 mean (SD)**	**P-value[b]**	**Day 10 median** (10 – 90 percentile range)	**Day 10 mean (SD)**	**P-value[b]**	**Week 10 median** (10 – 90 percentile range)	**Week 10 mean (SD)**	**P-value[b]**
S-creatinine pre (µm/L) S-creatinine change,	81 (68 - 90) 6 (-3 - 20)		0.0002	79 (65 - 89) 6 (-3 - 13)		0.005	75 (62 - 84) 11 (-1 - 20)		<0.001
S-urea N pre (mmol/L) S-urea N change	3.8 (2.8 - 5.3) 0 (-2.1 - 0.7)		0.22	3.8 (2.6 - 5.6) 0.05 (-0.07 - 1.1)		0.29	3.8 (2.7 - 4.9) 0.9 (-0.3 - 1.4)		0.006
S-uric acid pre (mmol/L) S-uric acid change		0.24 (0.08) 0.01 (0.06)	0.28	0.29 (0.16 - 0.35) -0.02 (-0.02 - 0.04)		0.61	0.29 (0.17 - 0.37) 0.03 (-0.02 - 0.14)		0.007
S-Na pre (mmol/L) S-Na change	140 (137 - 143) 0 (-5 - -10)		0.66	141 (139 - 143) - 3 (-3 - -2)		0.04		137 (6.73) -0.80 (5.79)	0.38
S-K pre (mmol/L) S-K change		4.35 (0.513) -0.31 (0.598)	0.01		4.3 (3.75) 0.2 (0.46)	0.01		4.47 (0.433) -0.07 (0.614)	0.61
S-Ca pre (mmol/L) S-Ca-change		2.26 (0.1197) 0.13 (0.2201	0.0009		2.29 (0.111) 0.05 (0.121)	0.03	2.22 (2.03 - 2.33) 0.09 (-0.11 - 0.26)		0.09
S-phosphate pre (mol/L) S-phosphate change	1.08 (0.76 - 1.48) 0.05 (-0.22 - 0.27)		0.25		1.03 (0.244) 0.053 (0.242)	0.24		0.967 (0.186) 0.155 (0.22)	0.64
NGAL pre (ng/mL) NGAL-change		41.2 (101.1) -16.2 (110.3)	0.45		27.8 (7.4- 129.5) 4 (-28.8 - 63.5	0.4	28.8 (4 - 103) -4.9 (-58 - 91.9)		0.76
S-Cystatin C pre (mg/L) S-Cystatin C change	0.88 (0.69 - 0.98) 0.08 (0.02 - 0.42)		<0.001				1.015 (0 .88 - 1.11) 0.14 (0.01 - 0.26)		<0.001
S-Osmolality pre (osmo/L) S-Osmolality change		278 (18) -9.1 (39)	0.33	268 (267 - 269) -47.5 (-54 - -41)		0.18		280 (20.4) 8.2 (23)	0.06
eGFR pre (Creat)[a] (mL/min/1.73m^2) eGFR change eGFR %change	123.0 (114.89 - 135.8) -2.9 (-9.4 - 1.2) -2.3 (-6.9 - 1.1)		0.0002	121.9 (112.9- 138.4) -3.2 (-8.0 - 1.7) -2.6 (-5.6 - 1.4)		0.0004	123.3 (116.9 - 138.2) -5.8 (-9.5 - 0.9) -4,0 (-7.51 - 0 .57)		<0.001
Myoglobin post (nmols/L) Myoglobulin change	4.4 (2.1 - 10.3)			1.8 (1.0 - 2.5)			5.8 (2.3 - 10.8) 1.37 (-0.8 - 4.9)		0.0005
Urine biomarkers									
pH pre	5 (5 - 6)		0.72	6 (5 - 7)		0.21	6 (5 - 6)		0.51

pH change	0 (-1 - -1)			0 (-1 - 1)			0 (-1 - 1)		
Creatinine pre , g/L Creatinine change	10.4 (5.6 - 30.6) 12.9 (-16.3 - 39.3)		0.01	11.6 (4.6 - 38.2) 0.8 (-15.7 - 45.8)		0.09		13.6 (9.59) 3.6 (13.05)	0.34
U-Osmolality pre (mOsmo/kg) U-Osmolality change		942 (284) 322 (718)	0.01	585 (289 - 940) 71 (-534 - 1337)		0.09		571.5 (222.3) -55.3 (269.7)	0.46
Specific-gravity pre (g/ML) Specific-gravity change	1.015 (1.015 - 1.025) 0 .005 (-0.005 - 0.015)		0.05	1.025 (1.010 - 1.03) 0 (-0.02 - 0.01)		0.71	1.025 (1.015 - 1.03) 0 (-0.02 - 0 .01)		0.16

[a]eGFR calculated using CKD-EPI equation based on serum creatinine.

[b]p- values represent delta (changes) for each variable across shift

Table 3B. Serum and urine biomarkers at the beginning of the harvest season, and ten weeks into the harvest season. (N=36 Referents.). Median (10-90 percentiles range) and mean (standard deviation) for non-normally distributed and normally distributed variables respectively. P values are given for cross-shift changes (Wilcoxon signed rank and t-test).

Serum biomarkers (referents)	**Day1 Ref1 Median** (10 – 90 percentile range)	**Day1mean(SD)**	**P-value[b]**	**Week 10 median** (10 – 90 percentile range)	**Week 10 mean(SD)**	**P-value[b]**
S-creatinine pre (µm/L) S-creatinine change	88 (72 - 103) 3 (-14 - 19)		0.41	90 (70 - 98) 1 (-14 - 21)		0.24
S-urea pre (mmol/L) S-urea change	3.4 (2.7 - 6.6) -0.3 (-3 - 0.8)		0.30	4.25 (3.0 - 5.6) 0 (-2.4 - 0.9)		0.24
S-uric acid pre (mmol/L) S-uric acid change	0.28 (0.17 - 0.42) -0.01 (-.02 - 0.01)		0.07	0.28 (0.17 - 0.36) 0 (-0.24 - 0.04)		0.59
S-Na pre (mmol/L) S-Na change	139 (136.5 - 140.5) 0.5 (-1 - 6)		0.13	140 (134 - 144) 0 (-4 - 2)		0.35
S-K pre (mmol/L) S-K change	4.3(3.9 - 4.8) 0.2 (-0.5 - 0.4)		0.25	4.5 (4 - 5.5) 0 (-0.6 - 0.5)		0.90
S-Ca pre (mmol/L) S-Ca change	2.28 (2.2 - 2.4) 0.02 (-0.1 - 0.15)		0.63	2.26 (2.1 - 2.4) 0.01 (-0.07 - 0.220)		0.37
S-phosphate pre (mmol/L) S-phosphate change		1.05 (0.18) -0.063 (0.20)	0.63	1.045 (0.154) -0.029 (0.177)		0.63
NGAL pre (ng/ml) NGAL change	33.7 (10.5 - 118.7) 2.6 (-43.1 - 52.5)		0.73		43.5 (37) -5.6 (41.9)	0.51
S-Cystatin C pre (mg/L) S-Cystatin C change	0.8 (0.6 -0.9) 0.09 (-0.01 - 0.36)		<0.001	0 .9 (0.7 - 1.0) 0.06 (-0.03 - 0.25)		0.0002
S-Osmolality pre (Osmo/L) S-Osmolality change		279.6 2 (18.5) 7.5 7 (15.4)	0.24		279.6 (18.5) 0 (11.1)	1.00
GFR pre (Creat)[a] ml/min/1.73m^2 GFR change GFR %change	114.85 (103.56 - 123.69) -1.3 (-6.3 - 4.9) -1.19 (-5.87 - 4.92)		0.40	115.19 (101.77 - 128.91 -0.6 (-8.5 - 5.3) -0.412 (-7.68 - 5.03)		0.22
Urine biomarkers						
pH pre pH change	6 (5 - 8) 0 (-1.5 - 1)		0.43		6 (5 - 7) 0 (-1 - 0)	0.03
Creatinine pre (g/L)	9.4 (2.3 - 23.7)		0.63		10.5 (3.2 - 20.7)	0.78

Creatinine change	1.5 (-16.1 - 15.9)				-0.1 (-9.8 - 12.7)	
U-Osmolality pre (mOsmo/kg) U-Osmolality change	525 (196 - 846) 156 (-218 - 444)		0.02		598 (398 - 801) -100 (-549 - 459)	0.23
Specific-gravity pre (g/mL) Specific-gravity change	1.020 (1-1.03) 0 .010 (-0.01-0.012)		0.13	1.020 (1.010 - 1.030) 0 (-0.01 - 0.01)		0.07

[a]eGFR calculated using CKD-EPI equation based on serum creatinine.

[b]p- values represent delta (changes) for each variable across shift

Table 4. Cross harvest changes in pre-shift and post-shift levels (median (10 – 90 percentile range)) of selected kidney function markers at the beginning of the harvest and ten weeks into the harvest season for Cutters and Referents, in those individuals who took part in both examinations of each variable. P-values are given for changes across harvest and for the differences between the groups of Cutters and Referents at Day 1 and Week 10 (mixed effect model).

Kidney function parameters	Cutters N =12 - 35 [a]		P for change Week 10 vs Day 1	Referents N =12 - 35 [a]		P for change Week 10 vs. Day 1	P for group diff Cutters vs Referents Day 1	P for group diff Cutters vs Referents Week 10
	Day 1	Week 10		Day 1	Week 10			
S-creatinine pre (µm/L)	80 (68 - 90)	75 (62 - 84)	0.005 0.007[b]	88 (72 - 104)	92 (70 - 98)	0.28; 0.29[b]	0.003	<0.001
S-creatinine post	90 (74 - 105)	86 (70 - 103)	0.69; 0.61[b]	86 (74 - 111)	90 (75 - 101)	0.9; 0.66[b]	0.50	0.28
eGFR pre mL/min/1.73m^2	122.3 (115.3 - 135.8)	123.7 (117.4 - 138.2)	0.01; 0.01[b]	114.8 (101.7 - 123.8)	114.0 (101.8 - 126.7)	0.28; 0.28[b]	0.001	<0.001
eGFR post	118.9 (110.3- 128.7)	116.8 (108.0 - 134.7)	0.78; 0.87[b]	112.2 (101.3 - 130.9)	115.4 (103.2 - 126.7)	0.31; 0.45[b]	0.09	0.07
S-Cystatin C pre, mg/L	0.84 (0 .69 - 0 .98)	1.02 (0.88 - 1.11)	<0.001 <0.001[b]	0.8 (0.5 5 - 0.95)	0.94 (0.66 - 1.02	0.001; 0.12[b]	0.05	<0.001
S-Cystatin C post mg/L	0.95 (0.8 - 1.10)	1.15, 1.11 (0 .91- 1.4)	<0.001- <0.001[b]	0.88 (0 .74 - 1.03)	0.98 (0.88 - 1.09)	<0.001	0.08	<0.001
S_urea pre (mmol/L)	3.8 (2.7 - 5.2)	3.95 (2.65 - 5.05)	0.89	3.45 (2.7 - 5.2)	4.3 (3 - 5.6)	0.06	0.87	0.11
S_urea post	3.85 (2.6 - 4.8)	4.5 (3.3 - 5.5)	0.03	3.3 (2.6 - 5.7)	3.7 (2.6 - 6.1)	0.93	0.70	0.02
S-K pre (mmol/L)	4.4 (3.85 - 5.1)	4.5 (4 - 5.10)	0.29	4.2 (3.8 - 5.2)	4.5 (3.9-5.8)	0.10; 0.11[b]	0.89	0.50
S-K post	4 (3.9 - 5.5)	4.4 (3.9 - 5.5)	<0.001	4.5 (2.5 - 5.1)	4.5 (3.8-5.1)	0.97	0.04	0.88
S-phosphate pre (mmol/L)	1.23 (0.24 - 1.48)	0.99 (0 .65 - 1.36)	0.11	1.01 (0.78 - 1.33)	1.01 (0.86 - 1.3)	0.92	0.29	0.37
S-phosphate post	1.17 (0.74 - 1.45)	1.06 (0 .82 - 1.42)	0.26	1.03 (0.79 - 1.5)	0.95 (0 .72 - 1.15)	0.18	0.27	0.16
S-NGAL pre (ng/mL)	16.2 (3.5- 86.3)	29.05 (1.3- 156.10)	0.95; 0.17	38.05 (10.5 - 118.7)	37.5 (14.9 , 117)	0.93	0.655	0.56
S-NGAL post	11.8 (1.6- 66)	3.1 (1. 9- 149.30)	0.38; 0.14[b]	23 (4 - 191.7)	24.5 (6.6-196.5)	0.60; 0.64[b]	0.277	0.57
S-uric acid pre mmol/L	0.22 (0.02 - 0.34)	0.24 (0.13 - 0 .99)	0.28; 0.26[b]	0.28 (0.15 - 0.44)	0.28 (0.15-0.36)	0.51	0.084	0.98
S-uric acid post	0.27 (0.17 - 0.42)	0.29 (0.15 - 0.38)	0.20	0.25 (0.17 - 0.39)	0.33 (0.027-0.38)	0.32	0.388	0.98

S- osmolality pre (Osmo/L)	283 (254 - 294)	283 (256 - 301)	0.80; 0.85[b]	285 (243 - 302)	293 (260 -298)	0.02; 0.03[b]	0.553	0.25
S-osmolality post	282 (191 - 297)	288 (279 - 298)	0.003; 0.003[b]	288 (273 -307)	289 (282-299)	0.03; 0.03[b]	0.011	0.64
S-myoglobin post nmols/L	4.4 (2.1 - 10.3)	5.8 (2.3- 10.8)	<0.001; <0.001[b]					

[a] N varied for each variable ranging from 12 to 35 for each group.

[b]logged values used for hypothesis testing.

Table 5. Cross harvest, changes in serum creatinine or eGFR or both reported from five longitudinal investigations of sugarcane workers studied over the sugarcane harvest season.

Study	**Setting, subjects and study period**	**Cross season change in serum creatinine mg/dL and/or eGFR mL/min/1.73m^2**	**Increase in workers with eGFR < 60mL/min/1.73m^2**	**Comment**
Laws et al., 2015	Nicaragua. 284 sugarcane field and non-field workers. Pre-harvest to late harvest (4-6 months later)	eGFR mean-7.8 (95% CI -12.3 to -3.2) field vs non-field workers	7 All field workers	
et al., 2015	Brazil. 28 Sugarcane cutters. Pre-harvest to end harvest (8 months)	Not reported	Not reported	5 workers diagnosed with AKI defined as increase in post- vs pre-shift creatinine ≥ 0.3 mg/dL or increase ≥ 1.5 fold
Wesseling et al., 2016	Nicaragua. 29 sugarcane cutters and 25 referents (mainly office workers)	Mean, median for cutters Serum creatinine pre-shift 0.98, 0.91 to 1.18, 0.95 eGFR pre-shift 109, 111 to 99, 108	3. All cutters	
Butler-Dawson et al., 2018	Guatemala. 330 sugarcane cutters. Pre-harvest and end harvest (6 months). Active progarnme of hydration and breaks during work shifts reported by authors.	eGFR median (IQR) pre-season 111 (99-121; post-season 117 (103-125. Change %eGFR 2.97 (-2.35 to 13.34)	No increase (49 at pre-season vs 43 at post-season.)	eGFR declined in 37% of workers over the season, 6% of them by > 20%
Wegman et al., 2018	El Salvador. 80 cane cutters; 40 inland and 40 coastal. Pre-harvest and post-harvest (5 months). Intervention study: water, rest, shade programme for inland group.	eGFR mean (95% CI) Inland -3.4 (-5.5 to -1.3) Coastal -5.3 (-7.9 to -2.7)	Inland 0 Coastal 2	11 workers met AKI criteria defined as an increase in serum creatinine ≥ 26 µmol/L or increase 1.5 x reference value

7. Appendices

Appendix A: Information document for cane cutters. (Translated into Zulu.)
Dear Participant

Introduction

Good day, my name is Dr Mollen Magombo. l am a doctor at the National Institute for Occupational Health.

We are doing a study to find out whether the kidneys of sugarcane cutters are working well. We are asking you to take part in the study. This document explains the study.

The reason we want to do the study is because kidney damage has been found in sugarcane cutters in other countries. We do not know if kidney damage happens in South Africa. This study involves asking you questions, examining you and doing tests on your urine and blood. We will use this information to advise employers and employees on sugarcane farms about what should be done to protect employees and how they can check employees health over the long term.

The study will be done over 3 days of the sugarcane harvest season. The 3 days are the first day of the harvest, day six of the harvest and after nine weeks of harvesting.

Before you agree to take part it is important that you understand what is involved. If you have any questions that this information document does not fully explain please ask me. You can also contact Dr Mollen Magombo at any time. Her contact details are at the end of the document.

Should you decide to take part in the study you will be asked to give written consent.

What is involved in the study?

The study will include 60 sugarcane workers. 30 of the sugarcane workers will be cane cutters and the other 30 will be sugarcane workers who are not doing heavy work.

The details of what the study involves are:

1. During initiation time you will be asked to give written consent once you have agreed to participate in the study.
2. At the same time you will be asked to give a urine sample for testing. If your urine test result is normal you will take part in the study.
3. You will then be asked questions about your work and health.
4. You will be physically examined. The examination will include only measuring your weight, height and blood pressure.
5. On the first day of cane cutting in the morning, we will collect blood and urine from you. Two blood tubes and one urine bottle will be collected. Just over 5ml (equivalent to one teaspoon) will be drawn into each of the two blood tubes. This means about 10-15ml of blood (2-3 teaspoons) will be drawn. 25 ml of urine will be collected into a specimen bottle.
6. On the same day in the evening after work we will collect one tube of blood and one bottle of urine.
7. On day six of cane cutting in the morning we will collect one blood tube and one urine bottle.
8. On day six after work, we will collect one blood tube and one urine bottle.
9. On week nine in the morning we will collect two blood tubes and one urine bottle.
10. On week nine after work we will collect one blood tube and one urine bottle.
11. Please note that we collect blood and urine from you six times during the study.

12. The collected blood and urine will be sent at the end of each day to the laboratory where tests will be done to check the how your kidneys are working.
13. At the end of each study day you will be asked to give information about your rest breaks and how much water you took during your shift.
14. At each stage you will be informed of your test results. Should your result be abnormal you will referred to the doctor of your choice. Should the results be severely abnormal and show that you need to see a specialist you will be referred to the kidney specialists at the Inkosi Albert Luthuli Hospital. If the results are severely abnormal you will be asked to stop taking part in the study.
15. You will not pay for any medical costs.
16. No one else will know about your individual results except for yourself, the research team and the doctor that will treat you should there be a problem

Risks to you if you agree to be part of the study:

1. You may experience some pain and discomfort from tests involving a small needle prick when taking blood.
2. The test results will be looked at by medical doctors and the research team but kept confidential (see below). Your employer will not get your individual test results.

Benefits to you should you agree to be part of the study:

1. Although you may not benefit directly from the study, the information that will be gained from your participation will help us find out if sugarcane workers are at risk of kidney damage. If there is a risk of kidney damage while cutting cane we will be able to work with

employers and employees to find ways to reduce the risks. We will also develop a plan to monitor the kidneys of cane cutters over time.

2. If you have kidney damage already, we will find out during the study.
3. You will be informed of the results of the tests and assessments. Should doctors note anything from the assessments that requires further investigation and management, a referral will be made to a doctor.
4. You will not be paid for taking part in the study.

Confidentiality

All efforts will be made to keep personal information confidential. No personal information will appear on the questionnaire and blood results. Only data without your name will be used. Once the information has been analysed no one will be able to identify you.

No personal information will be made available to your employer. The results and interview reports will be made available to the research team only.

Your individual results will be made available and explained to you. If there is an abnormal result you will be referred to a doctor of your choice or to the company doctor if you prefer.

Referral to a specialist kidney doctor has been arranged for those who need it.

For additional information please contact Dr Mollen Magombo at NIOH, Occupational Medicine Department on (011) 712 6409,mollenzw@nioh.nhls.ac.za.
For any queries queries, concerns or complaints regarding anything you are unhappy about the study. Contact:
HREC (Medical) contact details: Prof P Cleaton Jones, Tel 011 717 2301, email peter.cleaton-jones1@wits.ac.za
Ms Z Ndlovu/ Mr Rhulani Mkansi/ Mr Lebo Moeng Administrative Officers 011 717 2700/2656/1234/1252 zanele.ndlovu@wits.ac.za; Rhulani.mkansi@wits.ac.za; and Lebo.moeng@wits.ac.za

Appendix B: Information document for Referents (Translated into Zulu)
Dear Participant

Introduction

Good day, my name is Dr Mollen Magombo. l am a doctor at the National Institute for Occupational Health.

We are doing a study to find out whether the kidneys of sugarcane cutters are working well. We are asking you to take part in the study. This document explains the study.

The reason we want to do the study is because kidney damage has been found in sugarcane cutters in other countries. We do not know if kidney damage happens in South Africa. This study involves asking you questions, examining you and doing tests on your urine and blood. We will use this information to advise employers and employees on sugarcane farms about what should be done to protect employees and how they can check employee's health over the long term.

The study will be done over 3 days of the sugarcane harvest season. The 3 days are the first day of the harvest, day six of the harvest and after nine weeks of harvesting. You will only take part in in the study on day one and week nine.

Before you agree to take part it is important that you understand what is involved. If you have any questions that this information document does not fully explain please ask me. You can also contact Dr Mollen Magombo at any time. Her contact details are at the end of the document.

Should you decide to take part in the study you will be asked to give written consent.

What is involved in the study?

The study will include 60 sugarcane workers. 30 of the sugarcane workers will be cane cutters and the other 30 will be sugarcane workers who are not doing heavy work.

The details of what the study involves are:

1. During initiation time you will be asked to give written consent once you have agreed to participate in the study.
2. At the same time you will be asked to give a urine sample for testing. If your urine test result is normal you will take part in the study.
3. You will then be asked questions about your work and health.
4. You will be physically examined. The examination will include only measuring your weight, height and blood pressure.
5. On the first day of cane cutting in the morning, we will collect blood and urine from you. Two blood tubes and one urine bottle will be collected. Just over 5ml (equivalent to one teaspoon) will be drawn into each of the two blood tubes. This means about 10-15ml of blood (2-3 teaspoons) will be drawn. 25 ml of urine will be collected into a specimen bottle.
6. On day one in the evening after work we will collect one tube of blood and one bottle of urine.
7. On week nine in the morning we will collect two blood tubes and one urine bottle.
8. On week nine after work we will collect one blood tube and one urine bottle.
9. Please note that we will collect blood and urine from you four times during the study.
10. The collected blood and urine will be sent at the end of each day to the laboratory where tests will be done to check the how your kidneys are working.
11. At the end of each study day you will be asked to give information about your rest breaks and how much water you took during your shift.

12. At each stage you will be informed of your test results. Should your result be abnormal you will referred to the doctor of your choice. Should the results be severely abnormal and show that you need to see a specialist you will be referred to the kidney specialists at the Inkosi Albert Luthuli Hospital. If the results are severely abnormal you will be asked to stop taking part in the study.
13. You will not pay for any medical costs.
14. No one else will know about your individual results except for yourself, the research team and the doctor that will treat you should there be a problem

Risks to you if you agree to be part of the study:

1. You may experience some pain and discomfort from tests involving a small needle prick when taking blood.
2. The test results will be looked at by medical doctors and the research team but kept confidential (see below). Your employer will not get your individual test results.

Benefits to you should you agree to be part of the study:

1. Although you may not benefit directly from the study, the information that will be gained from your participation will help us find out if sugarcane workers are at risk of kidney damage. If there is a risk of kidney damage while cutting cane we will be able to work with employers and employees to find ways to reduce the risks. We will also develop a plan to monitor the kidneys of cane cutters over time.
2. If you have kidney damage already, we will find out during the study.

3. You will be informed of the results of the tests and assessments. Should doctors note anything from the assessments that requires further investigation and management, a referral will be made to a doctor.
4. You will not be paid for taking part in the study.

Confidentiality

All efforts will be made to keep personal information confidential. No personal information will appear on the questionnaire and blood results. Only data without your name will be used. Once the information has been analysed no one will be able to identify you.

No personal information will be made available to your employer. The results and interview reports will be made available to the research team only.

Your individual results will be made available and explained to you. If there is an abnormal result you will be referred to a doctor of your choice or to the company doctor if you prefer.

Referral to a specialist kidney doctor has been arranged for those who need it.

For additional information please contact Dr Mollen Magombo at NIOH, Occupational Medicine Department on (011) 712 6409,mollenzw@nioh.nhls.ac.za.
For any queries queries, concerns or complaints regarding anything you are unhappy about the study. Contact:
HREC (Medical) contact details: Prof P Cleaton Jones, Tel 011 717 2301, email peter.cleaton-jones1@wits.ac.za
Ms Z Ndlovu/ Mr Rhulani Mkansi/ Mr Lebo Moeng Administrative Officers 011 717 2700/2656/1234/1252 zanele.ndlovu@wits.ac.za; Rhulani.mkansi@wits.ac.za; and Lebo.moeng@wits.ac.za

Appendix C: Informed consent
PARTICIPANT:

I understand the risks and benefits to me in being part (participating) in the study called **"**Kidney function changes in sugarcane workers in the south coast, Kwazulu Natal, South Africa**"** and I understand the information in the Information Document.

- I have had the opportunity to ask questions about the study.
- I know that the results of the study, including personal details regarding my age, date of birth and health information, will be anonymously processed into a study report.
- I may, at any stage, without prejudice, withdraw my consent and participation in the study.
- I consent to participate in this study.

Printed Name **Signature / Mark or Thumbprint** **Date and Time**

FIELD WORKER/DOCTOR:

I have given this research participant information about this study that I believe is accurate and complete. The participant has indicated that he or she understands the nature of the study and the risks and benefits of participating.

Printed Name **Signature** **Date and Time**

Appendix D: Study Questionnaire
QUESTIONNAIRE PART A

Survey Number:

Name: ☐ ☐ ☐

Surname:

Telephone:

I.D number

Tear off……

QUESTIONNAIRE A

Survey Number: ☐ ☐ ☐

A. SOCIO- DEMOGRAPHIC DATA	
What is your current age?	☐
2. What is your date of birth?	☐ / / **Dd/Mm/Yy**
3. Where do you live when you are not working on the sugar plantation?	
4. What is your highest level of education?	1 ☐ No formal education 2. ☐ Primary school level 3. ☐ High school level 4. ☐ Tertiary level

5. Do you smoke? 1 ☐ now 2 ☐ before 3 ☐ never – **SKIP TO QUESTION 8**	
6. If you smoke now or smoked in the past: for how many years have you smoked or did you smoke?	☐ Years
7. How many cigarettes did you or do you usually smoke in a day?	☐ Cigarettes

8. How many days per week do you drink alcohol? 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ Never 1-2 3-4 5-6 daily **IF NEVER SKIP TO QUESTION 10**	
9. On the days that you drink, how many alcoholic drinks do you usually take? 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐	Interviewer to note here if weekend days differ from weekends

0 1-2 3-4 5-6 >7	

B. EMPLOYMENT HISTORY	
10. What will be your main job during the harvest season?	☐ 1 Cane cutter ☐ 2 Seeder ☐ 3 Seed cutter ☐ 4 Factory worker ☐ 5 Pesticide applicator ☐ 6 Irrigator ☐ 7 Driver ☐ 8 Machinery operator ☐ 9 Other **(specif**y)_____
11. If you are a cane cutter how many years have you been cutting?	☐
12. How many months in a year do you perform this job?	☐ months
13. How many days per week will you perform the job?	☐ days

14.How many hours per day will you perform the job		☐ hours
15. How many breaks if any per shift do you take in a day?		☐ breaks
16. If you are currently not a cane cutter have you ever done any cutting before?		1 ☐ Yes 2 ☐ No
C. HEALTH		
17. Have you ever been diagnosed with any of these diseases?	a.) High blood pressure?	1 ☐ Yes 2 ☐ No
	b) Diabetes? (Sugar disease)	1 ☐ Yes 2 ☐ No
	c) HIV	1 ☐ Yes 2 ☐ No
18. Have you been diagnosed with a kidney, bladder or urine infection in the past 3 months		1 ☐ Yes 2 ☐ No
19. Have you had any of the following symptoms in the last three months?		1 ☐ Lower abdominal pain 2 ☐ Burning or pain when passing urine 3 ☐ Fever or chills

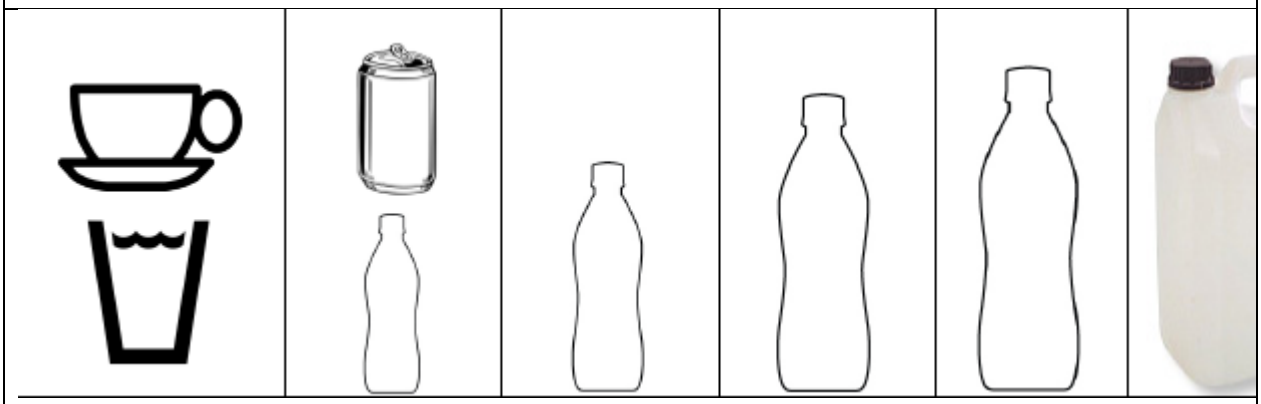
20. Do you take any tablets or medication for pain?	1 ☐ Yes 2 ☐ No
21. What is the name of the tablet or medicine?	a) ……. b)……. c)……..
22. How often do you take the pain tablet or medicine?	1=never 2=less than once/week 3=1-3x/week 4=almost everyday
23. How many months have you been taking the pain tablet or medicine?	☐ months
D. AGRICHEMICALS	
21. Do you work with any chemicals to kill weeds or animals?	1 ☐ Yes 2 ☐ No
22. Have you worked with any plant chemicals before?	1 ☐ Yes 2 ☐ No
23. How long have you been working with the chemicals?	☐ (Days /Months/ Years**)** **circle the appropiate**

24. Do you wear or use any protective clothing when using the plant chemicals?	1 ☐ Yes If yes list them 2 ☐ No

APPENDIX E: Questionnaire 2- Post shift hydration assessment

SURVEY NUMBER ☐☐☐

A. HYDRATION(to be completed at the end of the shift)



1. Please tell me how much water you have taken during the day:

Indicate how many of each you drank today (write amount in the box)

200ml	**350ml**	**500ml**	**1litre**	**2.5 litres**	**5 litres**

2. Did you drink any other liquids during the day besides water ?

1 ☐ Yes

2 ☐ No

if Yes specify_____

B. OBSERVATION OF FLUID INTAKE(to be done by field worker)

1. Type of fluid carried to the felds	
2. How do they carry the fluids	
3. Amount of fluids carried to the field (per person)	……litres

4. Visually confirmed /observed amount of fluids consumed during the entire shift	……..litres
5. Amount of fluids left	……litres
C.NUMBER OF BREAKS TAKEN	
6. Did you take any breaks in today's shift?	1. Yes ☐ 2 No ☐

Appendix F- University of Witwatersrand Postgraduate Education Committee Ethical Approval

R14/49 Mollen Magombo et al

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

CLEARANCE CERTIFICATE NO. M170153

NAME: (Principal Investigator)	Mollen Magombo et al
DEPARTMENT:	Division of Occupational Medicine National Institute of Occupational Health
PROJECT TITLE:	Kidney Function Changes in Sugarcane Workers, South Coast, Kwazulu Natal, South Africa
DATE CONSIDERED:	27/01/2017
DECISION:	Approved unconditionally
CONDITIONS:	
SUPERVISOR:	Prof David Rees and Spo Kgalamono
APPROVED BY:	Professor P. Cleaton-Jones, Chairperson, HREC (Medical)
DATE OF APPROVAL:	01/03/2017

This clearance certificate is valid for 5 years from date of approval. Extension may be applied for.

DECLARATION OF INVESTIGATORS

To be completed in duplicate and **ONE COPY** returned to the Research Office Secretary in Room 10004, 10th floor, Senate House/2nd floor, Phillip Tobias Building, Parktown, University of the Witwatersrand. I/We fully understand the the conditions under which I am/we are authorised to carry out the above-mentioned research and I/we undertake to ensure compliance with these conditions. Should any departure be contemplated, from the research protocol as approved, I/we undertake to resubmit to the Committee. **I agree to submit a yearly progress report**. The date for annual re-certification will be one year after the date of convened meeting where the study was initially reviewed. in this case, the study was initially review in [illegible] and will therefore be due in the month of [illegible] each year. Unreported changes to the application may invalidate the clearance given by the HREC (Medical).

Principal Investigator Signature

Date 28/01/2017

Appendix G- University of Witwatersrand Protocol approval

UNIVERSITY OF THE WITWATERSRAND JOHANNESBURG

Private Bag 3 Wits, 2050
Fax: 027117172119
Tel: 02711 7172076

Reference: Mrs Sandra Benn
E-mail: sandra.benn@wits.ac.za

09 January 2017
Person No: 1426085
PAG

Dr M Magombo
Box 4476 Kemptonpark
1620
South Africa

Dear Dr Magombo

Master of Medicine: Approval of Title

We have pleasure in advising that your proposal entitled *Kidney function changes in sugarcane workers in the South Coast, KwaZulu Natal, South Africa* has been approved. Please note that any amendments to this title have to be endorsed by the Faculty's higher degrees committee and formally approved.

Yours sincerely

Mrs Sandra Benn
Faculty Registrar
Faculty of Health Sciences

Appendix H: Approval from Illovo sugarcane estate

Dr EP Dorkin MBChB(Natal) DOH(Wits) CIME

Group Medical Consultant

Illovo Sugar,

1 Nokwe Drive, Umhlanga Ridge

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Telephone: +27 31 508 4468

Mobile: 083 625 2619

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edorkin@illovo.co.za

Fraud Hotline: 0800 201 988

Website: www.illovosugar.com

Reg. No. 1930/009032/06

7 December 2016

Letter of Support

This letter serves as official confirmation that Illovo Sugar is willing to provide access to its employees and workplace for the research proposal entitled: "Kidney function changes in sugarcane workers in South Africa", submitted by Mollen Magombo, student number: 1426085 for the degree: Master of Medicine (Occupational Medicine), subject to ethics approval and agreement by said employees to participate.

Regards,
Elton Dorkin

Appendix I: Plagiarism declaration

PLAGIARISM DECLARATION TO BE SIGNED BY ALL HIGHER DEGREE STUDENTS

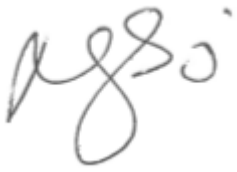
SENATE PLAGIARISM POLICY: APPENDIX ONE

I _____**MOLLEN MAGOMBO**_____ (Student number: ____1426085_____)
am a student

Registered for the degree of ____**MASTERS IN MEDICINE (OCCUPATIONAL MEDICINE)**_____ in the academic year _____**2019__.**

I hereby declare the following:

- I am aware that plagiarism (the use of someone else's work without their permission and/or without acknowledging the original source) is wrong.
- I confirm that the work submitted for assessment for the above degree is my own unaided work except where I have explicitly indicated otherwise.
- I have followed the required conventions in referencing the thoughts and ideas of others.
- I understand that the University of the Witwatersrand may take disciplinary action against me if there is a belief that this is not my own unaided work or that I have failed to acknowledge the source of the ideas or words in my writing.



Signature: ____ _____

Date: __18 October 2019_____

Appendix I- Research Protocol

Research Protocol Submission to the Postgraduate Research Committee.

Title: Kidney function changes in sugarcane workers in the south coast, Kwazulu Natal, South Africa.

Submitted by:

Mollen Magombo

Student number: **1426085**

Degree: Master of Medicine (Occupational Medicine)

Supervisors:

1. Professor David Rees
Professor Occupational Health

University of the Witwatersrand, Johannesburg

Head: Occupational Medicine and Epidemiology

National Institute for Occupational Health, National Health Laboratory Service

2. Dr Spo Kgalamono

Occupational Medicine Specialist

University of the Witwatersrand, Johannesburg

Head: Occupational Medicine Section

National Institute for Occupational Health, National Health Laboratory Service

Kidney function changes in sugarcane workers in the south coast, Kwazulu Natal South Africa

1. Introduction

1.1 Background

Chronic kidney disease of unknown origin (CKDu) has been discovered in some parts of the world amongst agricultural workers. CKDu was once called Mesoamerican nephropathy because it was originally reported in the Mesoamerican region but it has subsequently been reported in other areas globally. The disease is defined as kidney damage that is evidenced by structural damage or functional abnormalities of the kidney for more than three months with health implications [1]. The disease is characterised by the absence of known causes of kidney disease; young men being most affected. Based on current research findings there are some postulated risk factors, including prolonged strenuous work in a hot environment. There is consensus that sugarcane workers are the most affected agricultural population [2] .No research has been done on CKDu in sugarcane workers in South Africa and it is not known whether the disease occurs here. As with other sugarcane cutters and agricultural workers, the sugarcane cutters in South Africa undertake strenuous work in hot conditions.

If this kind of CKDu does exist in South Africa it is preventable using simple and affordable strategies [3, 4]. Measures can be implemented to prevent the disease [4].

1.2 Literature review

The focus of this literature review is on the reported prevalence globally, the currently identified risk factors for the disease and the features of the condition.

1.2.1 Epidemiology

Most regions that have been affected by CKDu are poor farming areas, probably explaining why cases of the chronic kidney disease have not been apparent until 2000[5]. Studies done since then have mainly focused on the prevalence as well as the possible risk factors. A high prevalence of CKDu has been reported in four countries: Sri- Lanka, Costa Rica, Egypt and India. In Sri Lanka 20% prevalence among agricultural workers was reported [6]. Costa Rica reported a prevalence ranging from 1-21% with an average of 17%. In Egypt a population study showed that 27% of renal patients had CKDu between 2002 and 2007[7] . The India kidney registry reported 165 CKD cases amongst the kidney patients [7]. Worldwide the prevalence is estimated between 8%-16% [8].

Other studies done mainly in Latin America, Sri Lanka, Egypt, and India have demonstrated that agriculture workers are the highest risk population, particularly sugarcane workers. The disease is characterized by absence of the traditional causes of kidney disease; and the disease is most prevalent in young healthy males [2, 8,9,10].

Of concern as well are the high mortality rates reported in patients with CKDu. In Central America, particularly Nicaragua and El Salvador, high levels of mortality have been reported in patients with the condition: 42.8% and 41.0% respectively. Mortality rates among the agricultural population in Central America have been found to be four times the global mortality.

The reported cases of CKD are of unknown etiology that is not attributable to the common cause of renal impairment such as diabetes and hypertension[8]. Since the cause is not known the disease is often known as chronic kidney disease of unknown etiology. CKDu unrelated to the traditional risk factors such as hypertension and diabetes has not been reported in Sub –

Saharan Africa. In Sub-Saharan Africa the most common causes of kidney damage are hypertension, infections (glomerulonephritis and schistosomiasis), diabetes and HIV. The highest prevalence of kidney disease is among people with HIV [11].

1.2.2 Features of CKDu

Studies done on men affected by the disease reported decreased kidney function (as defined by estimated glomerular filtration rate [eGFR] <60 mL/min/1.73m^2). CKDu is characterized by the progressive loss of kidney function. Urine tests show minor proteinuria (microglobulinuria) [9]. Several studies have found that proteinuria and albuminuria are uncommon in people with CKDu and when present is low grade[12]. In most kidney diseases albumin is the common type of protein found in the urine. For CKD and other tubulointerstitial diseases the predominant proteins are the low molecular weight proteins (macroglobulin) without albumin. An increased concentration of creatinine is seen in the blood, the plasma concentration of sodium is low, and hypokalemia is common[10] in advanced disease an insensitive and late marker of the disease. A histopathological examination of kidney biopsies reported interstitial fibrosis and tubular atrophy, glomerular sclerosis, glomerular collapse and features of vascular pathology[13]. Ultrasound of kidneys shows small kidneys with a narrow cortex[14].

CKDu can be classified by GFR (GFR in ml/min/1.73m^2 body surface area) [9]:

Stage 1, GFR >90 with renal damage markers;

Stage 2, 60-89 with renal damage markers;

stage3a, 45-59, 3b 30-44;

Stage 4, 15-29;

Stage 5, <15

A rapid decrease in GFR and increase in creatinine plasma levels is an indicator of acute renal damage. If acute kidney damage occurs repeatedly, animal studies have shown that it will progress to chronic kidney disease with glomerular damage[10].

1.2.3 Risk Factors

Many studies hypothesize that inadequate hydration with repeated exposure to heat and strenuous labor is responsible for CKDu[14, 15, 16]. Sugarcane cutters were said to be the most affected group as they are exposed to extreme ambient temperature during hard strenuous work. The reluctance to drink adequate volumes or unavailability of drinking water during the day may be responsible for the dehydration. Some workers have reported that drinking high volumes of water slows their pace of working and prevents them meeting their targets as set by employers[17]. However, these changes were different for the different jobs types with field workers being affected more, which is consistent with the heat stress hypothesis [18,19]. Significant changes in kidney function across shifts of work support the hypothesis that kidney damage in CKDu is as a result of recurrent dehydration and strenuous work in a hot environment [5, 18, 19, 20]. Agricultural workers in low lying areas are at higher risk as compared to the similar workers in high lying areas[14]. Heavy metals such as arsenic, lead, cadmium and mercury are nephrotoxic and have been suggested as causes of CKDu, arsenic being of most concern[21]. High levels of arsenic were found to be associated with decreased filtration rate [2]. Excess use of non-steroidal anti-inflammatory drugs (NSAIDS), fructose consumption and other nephrotoxic medications such as aminoglycosides are co-factors that interact with heat stress in causing kidney damage [5]. NSAIDS are also believed to cause ischemia due to dehydration as a result of chronic intake [22]. Based on animal experiments, fructose consumption has been

reported to cause dehydration resulting in acute kidney injury [23]. Sugarcane cutters may rehydrate themselves with fructose containing drinks that may cause dehydration and consequently potentiate acute kidney injury. Other factors such as pesticides, leptospirosis and hard water have also been considered amongst the etiological factors contributing to decreased kidney function. Drinking hard water has been reported to be associated with decreased kidney function [24] .Nephrotoxic medications are commonly used for urinary symptoms [17, 24]. These medications are usually poorly controlled and one can easily buy them over the counter. Leptospirosis infection has been reported in some studies to cause decreased kidney function in people who work outdoors including sugarcane cutters [15].

The epidemiology of CKDu is consistent with this hypothesis;

- Mortality is highest in lowland areas with high temperatures;
- Occupational groups most affected perform heavy manual labour in hot environments;
- Communities in cooler, high altitude areas have lower CKD prevalence than similar communities in hotter, low altitude areas[21].

1.3 Rationale for the study

South Africa is one of the world's sugarcane producing countries. The industry in South Africa employs a substantial number of workers, particularly young men. Production is undertaken in warm temperatures and utilizes strenuous manual labour. Sugarcane production is connected with CKDu with evidence pointing to strenuous work in hot environments and repetitive dehydration as the cause.

No studies have been done in South Africa to assess kidney function in sugarcane workers. This study therefore plans to identify whether work in similar conditions to those studied in other countries results in similar changes in kidney function here.

2. Aims and objectives

2.1 Aims

The main aim of the study is to assess kidney function changes in sugarcane workers in South Africa during the early part of the harvest season in 2017.

2.2 Objectives

- To determine cross shift renal function changes in sugarcane cutters and referents on a south coast of Kwazulu Natal sugarcane estate by measuring renal biomarkers three times during the first nine weeks of the harvest season .
- To compare renal function changes among Kwazulu Natal south coast sugarcane cutters and referents who are not doing strenuous physical work over the first nine weeks of the harvest season i.e. at the beginning and the end of the nine week period.
- To determine if a job category is an important determinant of renal function during the first nine weeks of the harvest season among Kwazulu Natal sugarcane cutters.

The measures of kidney function are explained in a table in appendix c.

3. Methods

3.1 Study design

A recent study on kidney function in sugarcane workers was done in Nicaragua [25]. The study provided valuable information on the risk of renal dysfunction related to work in this population. Our intention is to replicate the study in South Africa. The study will be a longitudinal cohort study to be done over a nine-week period during sugarcane harvesting or work of a similar nature. Appendix A shows the frequency and general nature of the information

to be obtained. Appendix B shows a table with a list of the tests to be conducted. The study procedure:

- obtain health, occupation and work practice data by administering questionnaire,
- Collect blood and urine samples for assessing kidney function pre and post shift on three occasions
- Undertake a physical examination.
- Measure ambient temperature on three occasions

Trained field workers will assist with the data collection and the interviewing of participants. All participants will be required to give a written consent to participate in the study.

3.2 Study setting

The study will include 60 sugarcane workers (30 sugarcane cutters and 30 non-field workers) employed by a sugarcane estate on the south coast of Kwazulu Natal 35km from Durban. The sugarcane estate is South Africa's biggest sugarcane producer. It is divided into four cane farms covering 42 hectors. The Sezela estate employees include cane cutters who are seasonal, seeders, irrigators, employees in the workshop, tractor drivers and milling factory workers [26]. The low lying area and the high temperatures make it suitable for sugarcane farming. The cane cutting season extends from April, just after Easter, to December. The expected maximum temperatures during the cutting season are typically 22 to 32 degrees Celsius [27], meaning that the study will be done in a cooler, less humid part of the year (April-June). The harvesting of the cane is done by workers manually cutting the cane with a machete. Shifts vary from four to eight hours per day, six days a week depending on how quickly an individual reaches their daily targets. Harvesters are paid according to how much they cut. Heat, unrelated to CKDu, has been documented as a hazard for sugarcane cutters at this farm.

3.3 Study population

Male sugarcane workers (sugarcane cutters and referents) who are less than forty years of age, at the south coast sugarcane estate of Kwazulu Natal.

3.4 Selection of study participants

An invitation to participate in the study will be made to the sugarcane workers from two different job categories: field workers preferably cane cutters; and a group of referents who are non-field workers not doing heavy work. Participants will be conveniently selected from volunteers. The screening tests for the participants will include:

1. *Cane cutters*

   Inclusion criteria - Must be 18- 40 years of age

   -Male sugarcane cutter

   - Absence of proteinuria (a urine dipstick will be done during the selection)

   - Voluntary participation

   Exclusion criteria- Workers reporting jobs other than sugarcane cutting and non-field work in hot conditions.

   -Workers performing more than one job

   Severe hypertension or Diabetes

   -Proteinuria> +1

2. *Referents*

   Inclusion criteria- Must be 18-40 years of age

   -Male

- Must be performing non-strenuous work in e.g. milling factory worker, driver, irrigator, pesticide applicator)

- Voluntary participation

-Absence of proteinuria

Exclusion criteria- Severe hypertension or Diabetes

-Proteinuria> +1

- Workers performing field work

The aim of the screening is to enroll young and healthy male workers.

3.5 Sampling method

The participants will be enrolled using convenience sampling, within two subgroups (field workers and non-field workers). The field workers will be the sugarcane cutters performing strenuous physical work in the hot conditions, whilst the non-field workers will be the workers performing non- strenuous work usually in cooler conditions. Permission will be sought from the sugarcane workers to enroll for the study. Those who agree to participate will be seen by the research assistants who will explain the nature of the study and ask them to give an informed consent.

3.6 Sample size

Our intention is to replicate the study which was done in Nicaraguan sugarcane workers [25]. The study was conducted on 60 participants, 30 field workers and 30 non-field workers. With this sample size they detected differences in renal function changes pre and post shift between the field workers and the referents. The study participants would be at least 30 male sugarcane workers, preferably cutters, and 30 male referent subjects who are not doing heavy work in hot conditions.

3.7 Study variables and data analysis

The researcher will do the analyses and consult a statistician for support.

OBECTIVES	**VARIABLES**	**DATA ANALYSIS**
1. To measure cross shift changes in renal function in Kwazulu Natal south coast sugarcane workers on the first, sixth day and after nine weeks into the cane harvesting season.	DEPENDENT VARIABLES: <u>Continuous variables</u> *Change in renal function assessed by:* 1.Reduced eGFR - single GFR < 60ml/min/1.73m^2 2. Blood biomarkers : S-creatinine, S-urea, S-uric acid, S-glucose,S-sodium	1. Distribution of each biomarker to determine if it's normally distributed will be examined using histograms, summary statistics and graphs. The change of biomarkers during harvest will be calculated by subtracting pre-harvest measurement from the corresponding late harvest measurement. 2.Cross-shift changes For outcome data (measures of renal function) which are normally distributed the actual data will be used and paired t-tests used to examine the changes. For non-normally distributed measures of renal function will be log-transformed and where this is

	Calcium, Phosphate, FBC Osmolality, Cystatin C, NGAL, Potassium Urinary biomarkers: Albumin, Albumin/creatinine ratio, Creatinine, Ketones, Leucocytes, Myoglobin, Nitrite, NGAL, Ph, Osmolality, Sodium, Urinary specific gravity, Urobilinogen	successful , paired t-tests will be used , but where transformation is not successful , Wilcoxon signed rank test will be used The three sets of data (cross shift changes on day one, six, week nine) will be examined separately for cross shift changes. 3. To evaluate renal function changes across the nine week period by comparing pre-shift day 1 and pre-shift week nine. And then post- shift day one (baseline renal function) with post-shift week nine.(post exposure renal function) Use either paired t-tests and Wilcoxon signed rank test as per above aspect.

2. To compare renal function changes among Kwazulu Natal south coast sugarcane cutters and referents who are not doing strenuous physical work in hot conditions.	DEPENDENT VARIABLES: <u>Continuous variables-</u> *change in renal function-* 1.Reduced eGFR 2. Urinary and blood biomarkers CONFOUNDERS 1.Positive HIV status 2.Severe hypertension, Diabetes 3. water- intake	1. An unadjusted comparison between cane cutters and referents is done using paired t-tests of the differences between pre-shift and post-shift day one and pre-shift week nine and post-shift week nine for cane cutters versus referents. Compare using Chi- square test, the percentage of cutters who moved from normal renal function at baseline to abnormal post-exposure renal function versus percentage of referents.
3. To determine if a job category is an important determinant of renal function during the first nine weeks of the harvest		1. HIV positive patients, diabetic and hypertensive workers with renal impairment with be excluded from the study if they are proteinuria from a urine dipstick test.

season among Kwazulu Natal sugarcane cutters.	4, NSAIDS use 5 number of breaks 6. alcohol use	2. Adjusting for confounders is job (cane vs. non-strenuous work) a risk factor for change in renal function. Job is the variable of interest, others are potential confounders (water intake, number of breaks taken, alcohol use, NSAIDS use, HIV status, Hypertension and diabetes). Regression analysis will be used.

3.8 Study procedure

Urine dipstick will be done on participants who have given written consent to take part in the study. The participants found to have proteinuria > 1 will be replaced and referred for further management. Those with no proteinuria will continue with the questionnaire interview.

Participants will be assigned a unique participant identification code which will be used for identification on questionnaires and for laboratory investigations throughout the study. The identification information will be torn off the questionnaire and kept the researcher (see appendix H).

Trained research assistants will assist with interviewing participants and completion of the questionnaire. The research assistants will be professional nurses, who will receive training on

how to take the blood and urine samples and storage for transportation. The assistants will also receive training on how to ask questions in a similar way and how to record the answers.

3.8.1) During initiation (before cutting season starts)

a)Questionnaire

The questionnaire was adopted from a study of sugarcane workers done in Nicaragua [19]. The questions were modified to suit the South African environment. The questionnaire took into consideration the prevalence of HIV. The participants will be asked about their HIV status and if they know it.

After giving a written consent participant will under-go a standard interview of questions on the questionnaire .The questionnaire will be in two parts; the first part will be administered during initiation before the cross shift study and will collect data on exposure as well as socio-demographic data (see appendix I). The second part of the questionnaire will be administered during the cross shift study post shift (see appendix J). They will also be asked about their exposure to pesticides and if they used any personal protective equipment. In addition, the questionnaire will have questions on age, date of birth, education, health diagnosed diseases, the use of pain medication particularly non-steroidal anti-inflammatory drugs, other medications that are nephrotoxic being taken and smoking and alcohol use. The workers will be asked to report on their current and previous work history particularly in the agricultural industry, how many years, and hours per day they had worked. The questionnaire will be written in English, the interviewer will translate to the local language which is Zulu as they will be interviewing the participants.

b)Physical examination

The physical examination will include height, weight, and blood pressure measurements. The workers will be asked to report on their current and previous work history particularly in the agricultural industry, how many years, and hours per day they had worked. The blood pressure will be measured by the research assistants, with the patient sitting and having rested for at least ten minutes using a digital automatic sphygmomanometer. The blood pressure will be measured on the right arm. The body weight will be measured using an electronic scale with minimal clothing. The height will be measure with a stadiometer.

The data for the examination and investigations will be entered into a data entry sheet (appendix K)

3.8.2) Day1, day 6 and nine weeks into harvest season

a) Cross shift evaluation of renal function

The research assistants will collect venous blood in three vacuum tubes, one tube for full blood count, and two tubes for renal serum biomarkers. The samples will be placed on ice and transported to the nearest NHLS laboratory for processing. Each participant will self-collect urine in a sterile polypropylene container. Blood samples will be measured for serum creatinine, sodium, uric acid, and the glomerular filtration rate (GFR) calculated. Urine dipstick analyses will be performed for measurements of the urine gravity, PH, blood, nitrites, leukocytes, bilirubin, ketones and urobilinogen (as shown in appendix B). The cross shift urine tests will be done on three days for the cane cutters i.e. On day one, day six and week nine into the harvest season and for referents only on day1 and week nine. Samples will be collected six times for the cutters and four times for the referents i.e. pre shift and post shift of the study days. . About 5ml will be drawn into each blood tube and 25 ml of urine collected into a specimen bottle.

For the cane cutters on day one pre shift, three blood specimen tubes will be collected and one urine specimen bottle. Post shift one blood specimen tube will be collected and 1 urine specimen bottle. On day six pre and post shift only one blood specimen tube and one urine specimen bottle will be collected. On week nine pre shift two blood specimen tubes and one urine specimen bottle will be collected. Post shift one blood tube specimen and one urine bottle specimen will be collected. All in all nine blood specimen bottles and six urine specimen bottles will be collected.

For the referents on day one pre shift, three blood specimen tubes and one urine specimen bottle will be collected. Post shift day one , one blood specimen tube and one urine specimen bottle will be collected. On week nine two blood specimen tubes and one urine specimen bottle will be collected. Post shift week nine one blood specimen tube and one urine specimen bottle will be collected All in all seven blood specimens and four urine specimen bottles will be collected.

b) Ambient temperature measurement

Wet bulb globe thermometer (WBGT) will be used to measure environmental heat stress on the three days of study. The thermometer will be placed in the open field, the device will automatically calculate and record WBGT index which is calculated from an equation of a combination of the three measurements (globe temperature, wet bulb temperature and the dry bulb temperature).

WBGT index = 0.7x wet bulb+ 0.2 x globe temperature+ 0.1x dry bulb temperature [4].

c) Fluid intake assessment

At the end of each shift a fluid intake questionnaire will be administered to ascertain how much water each worker consumed during the work shift (see appendix J). The observed water intake and remaining fluids will be recorded for each participant. The number of breaks taken will also be asked about at the end of the shift.

The data for the examination and investigations will be entered into a data entry sheet (appendix K)

4. Piloting

The questionnaire will be piloted to a group of farm workers similar to my target group who are exposed to strenuous work in hot conditions. The pilot study of the questionnaire will be done before the actual study. Trained data collectors will conduct interviews. The training will include a theoretical part, involving a detailed explanation of the questionnaire to the data collectors. The data collectors will then be asked to read and ask questions on the questionnaire. The data collectors will then be asked to interview at least two people and discuss any problems encountered Assessment will cover the feasibility of the study, how much time will be required for completion of an interview, staff training, the problems with the distribution and collecting of the questionnaires as well as predict an appropriate sample size based on the costs and time, Problems with the questionnaire will be identified, to check if the questions does measure what it is supposed to measure, are the words in the questionnaire understood and if the people interpret the questions the same. Verbal feedback from the participants will also be used to make improvements of the questionnaire.

5. Ethical considerations

Permission to conduct the study will be sought from the Research Ethics Committee of the University of Witwatersrand Medical School. Participation is voluntary and therefore participants will be requested to give a written consent before collection of data. The participant will be informed orally by the researcher or assistants on what the research is all about using the information document (appendix G). If the participant has understood and gives consent to participate, they will review the consent form (appendix H) together with the researcher or assistant and sign it. A copy shall be given to the participant to keep. Anonymity and confidentiality will be maintained throughout the study. Participants will be assigned a unique participant identification code which will be used for identification on questionnaires and for laboratory investigations throughout the study. The researcher will keep a copy with the names of participants and their assigned identification code. This list will be used to link abnormal laboratory results to the owner. Should an individual have abnormal results they will be identified and referred, depending on their choice, either to the Renal Unit at UKZN or to the company doctor for further investigations and management. The questionnaires and blood results will be locked-up and will only be accessible to the research team. The data will be stored for up to five years after the completion of the study after which the data will be destroyed. The blood and urine samples will be discarded after performing the tests.

6. Timing

The projected project timeframes are presented in Appendix D. Consequently, a multi-disciplinary team has been established to investigate CKDu in agricultural workers in South Africa. The team members are listed in Appendix F.

7. Funding

The funds will be provided by the Medical Research Council (MRC), grant as well as National Institute for Occupational Health (NIOH). A budget for the project is provided as Annexure E.

8. Limitations

Due to limited funds and time the study is not able to evaluate the progression to CKDu as the sugarcane workers will only be followed up for a short period of time. The repeated heat stress effects over a number of years could be missed. Another limitation to the study is the high prevalence of HIV which is also known to cause changes in renal function, should the participants not disclose their status during the interview. In addition the measurement of the water intake will also be a limitation as this will be self-reported, which may lead to misclassification of exposure. Potential health worker effect is also another limitation as those who are already ill will no longer be working. Another limitation is the fact that the study is being done during a cooler less humid part of the year (April- June, but if CKDu is present, then perhaps heat only may not be the major factor.

9. References

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Appendix J: Supplementary Material

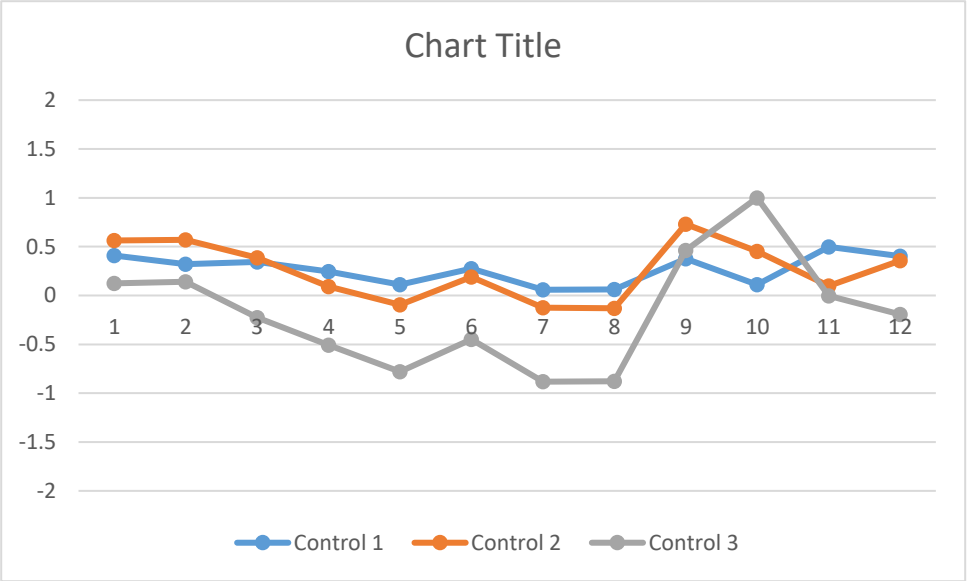
Supplementary Table 1.

CYSTATIN controls

	Ctl	Ctl2	Ctl3
Run MARCH	1.06	1.67	4
	1.05	1.66	4
	1.06	1.68	4
	1.06	1.64	3.9
	1.03	1.58	3.81
RUN APRIL	1.05	1.57	3.8
	1.02	1.61	3.89
	1.02	1.58	3.79
	1.03	1.58	3.81
	1.02	1.66	4.11
	1.08	1.68	4.29
	1.06	1.54	4.11
MEAN	**1.045**	**1.620833**	**3.959167**
SD	**0.020226**	**0.049627**	**0.155707**
CV	**1.935502**	**3.061845**	**3.932825**
Package insert RANGE (2SD)	0.86-1.16	1.39-1.87	3.73-4.57
Assigned mean	1.01	1.63	4.15
	0.075	0.12	0.21
[a]Uncertainty of measurement	0.040452	0.099255	0.311414

[a]Cystatin C controls shows a good performance of the test.

0.666667	0.25	-0.71429
0.533333	0.416667	-0.71429
0.666667	0.083333	-1.19048



Supplementary table 2. Comparison of initial creatinine measurements and repeat measurements.

S_creatinine	Initial measurement	[a]Repeat measurement	Difference in measurement
	84	89	5
	92	80	12
	83	91	8
	86	76	10
	83	80	3
	96	91	5
Mean	**87**	**84**	**7.1**

[a]The repeat creatinine measurements do not show that the initial measurements were too low.