Supplementary Material

Supplementary Methods (PDF)

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STROBE checklist (PDF)

Supplementary Methods

Study area

This cross-sectional study was conducted in farming communities in six selected Grama Niladhari (GN) divisions; Yudaganawa, Mahasenpura, Gonagan Ara, Rahathangama, Pelwatte and Kumaragama located within the Buttala Divisional Secretariat (DS) in Moneragala district in Sri Lanka (Supplementary Figure 1). Buttala is a highly agricultural area in area within the dry zone of Sri Lanka with a majority of the inhabitants involved in farming of paddy, sugarcane and several other major crops. In Buttala the average annual rainfall is 1047 mm, temperatures range between 24.5 ^{0}C (min) and 30.5 ^{0}C (max), and relative humidity is 76%. The area exhibits similar climatic factors to CKDu endemic Anuradhapura district where the average annual temperature, rainfall and relative humidity are 25 ^{0}C (min) to 31 ^{0}C (max), 1000 mm, and 69% respectively. People in this area also exhibit similar socioeconomic characteristics to individuals living in CKDu endemic regions of the north-central province.

Study design and sampling strategy

Six GN divisions of the Buttala DS, which consist of a total of 3,965 households, were selected based on previously reported high incidence of CKDu in these areas. 563 households were

excluded based on occupants' residency in the area of less than 10 years. Of the remaining households 567 were randomly selected for the study. Research team visited each household to recruit the study subjects. One adult resident from each household who is above 18 years of age and been a resident within the respective GN division for a period of at least 10 years was invited to attend the data collection session conducted at a local data collection centre on the next day. Consenting individuals were handed over a urine collection bottles to collect the first voided urine samples and bring these with them when attend the data collection. Subjects with institutional residency (clergy, those who are in foster homes), semi-permanent residents in the area and pregnant and lactating mothers were excluded. Of the 567 individuals selected, 352 (192 males and 160 females) consented and attended the data collection session (Supplementary Figure 2). Subjects were recruited to the study from 01.05.2017 to 04.11.2017.

Sample size was calculated using the formula; $n = [(z^2) P (1-P)]/d^2$. The standard normal variate (z^2) was taken as 2.58 at 1 % type 1 error ($P < 0.01$) and the absolute error (d) was assumed to be 5% ($d = 0.05$)[26]. The prevalence of CKD (P), in Moneragala district was considered as 9.7% ($P = 0.097$) based on the previously reported data. Accordingly, the estimated minimum sample size was 233.

Ethical clearance for the study was obtained from the ethics review committee of the Faculty of Medicine, University of Colombo, Sri Lanka (EC-16-158). Informed consent of the participants was obtained before enrolment for the study.

Data and sample collection

An interviewer- administered questionnaire was used to obtain data on demographic details, co-morbidities, water sources, habits, and the use of agrochemicals. A medical examination was

carried out and resting blood pressure, height, weight and waist circumference of the participants were measured using standard procedures. Medical history of the participants was explored using medical reports, hospital records and prescriptions that were available with the participants at the time of data collection. A first void morning urine sample was collected into a sterile container and a non-fasting blood sample was taken using a plain vacutainer tube.

Sample preparation, analysis and determinations

One portion of the blood sample collected was transferred to a sterile serum separator tube while the rest was transferred to a EDTA-coated vacutainer tube. The blood samples in serum separator tubes, were left for one hour for clotting at 37^0C and then centrifuged at 3500 rpm for 20 minutes at 37^0C before isolated serum was transferred into plain vacutainer tubes. A portion of the blood sample in EDTA coated tube was used for the assessment of glycated haemoglobin (HbA1c). The urine samples were collected into sterile containers (50 mL). Urine, blood and isolated serum samples were temporarily stored at 2 – 4 ^{0}C during the transit before immediate biochemical analysis upon reaching the lab. Portions of urine samples were stored in sterile cryogenic preservation tubes at -20^0C until the analysis for heavy metals and uβ_2M.

Urine samples for creatinine, albumin, and serum samples for creatinine were analysed using an automated biochemistry analyzer (Humastar 100, Human GmbH, Germany). Blood samples for glycated haemoglobin (HbA1c) were analysed using an automated HbA1c analyzer (HumaNex A1C, Human GmbH, Germany). Urine samples for cadmium, arsenic and lead were analysed using ICP-MS (Inductively coupled plasma mass spectrometry) at the SLINTEC (Sri Lanka Institute of Nano Technology). uβ_2M was assessed using ELISA (Enzyme-linked immunosorbent assay) method.

Diagnostic criteria

The diagnosis of CKD was made if an individual had an estimated GFR (eGFR) of <60 mi/min/1.72m^2 and/ or urine for albumin creatinine ratio (ACR) ≥30 mg/g as a one time measurement[S5]. CKD-EPI creatinine equation 2009[S6] was used for the calculation of eGFR. Among the individuals classified as potential cases of CKD, those who did not have a diagnosis of pre-existing diabetes or hypertension (based on their records and medications), newly diagnosed diabetes based on HbA1c above 48 mmol/mol (6.5 %) and newly diagnosed hypertension based on elevated systolic blood pressure (SBP) above 140 mmHg and diastolic blood pressure (DBP) above 90 mmHg, were classified as cases of suspected CKDu based on the criteria devised by the Sri Lanka Society of Nephrology[S7].

Statistical analysis

Continuous variables were reported as medians (IQR) whereas categorical variables were reported as proportions. Comparisons of urinary metal and metalloid contents among different CKD groups were performed using Kruskal–Wallis and Mann–Whitney test. Receiver-operator characteristic – Area under the curve (AUC-ROC) was used to determine the diagnostic accuracy of biomarkers. Correlation between urinary heavy metals, renal function and renal biomarkers was assessed with Spearman rank-order correlation coefficient. Urinary heavy metal and biomarker concentrations were adjusted by urine creatinine concentration. Odds ratios and confidence intervals were used to examine the associations of risk factors with CKDu. Data analysis was performed with SPSS (version 22.0; IBM Inc.) and Graphpad Prism (version 9; Graphpad software LLC).

Supplementary References

S1. Kim YD, Yim DH, Eom SY, et al. Temporal changes in urinary levels of cadmium, N-acetyl-β-d-glucosaminidase and β2-microglobulin in individuals in a cadmium-contaminated area. Environ Toxicol Pharmacol. 2015;39(1):35-41. doi:10.1016/j.etap.2014.10.016

S2. Satarug S, Vesey DA, Nishijo M, Ruangyuttikarn W, Gobe GC. The inverse association of glomerular function and urinary β2-MG excretion and its implications for cadmium health risk assessment. Environ Res. 2019;173:40-47. doi:10.1016/j.envres.2019.03.026

S3. Win-Thu M, Myint-Thein O, Win-Shwe TT, Mar O. Environmental cadmium exposure induces kidney tubular and glomerular dysfunction in the Myanmar adults. J Toxicol Sci. 2021;46(7):319-328. doi:10.2131/jts.46.319

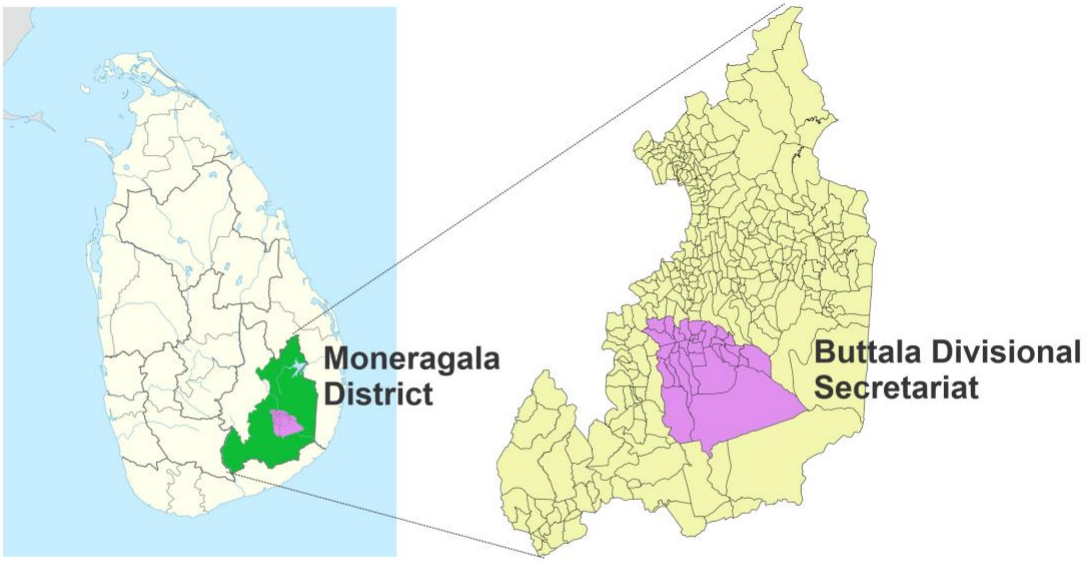
S4. Drüeke TB, Massy ZA. Beta2-microglobulin. Semin Dial. 2009;22(4):378-380. doi:10.1111/j.1525-139X.2009.00584.x

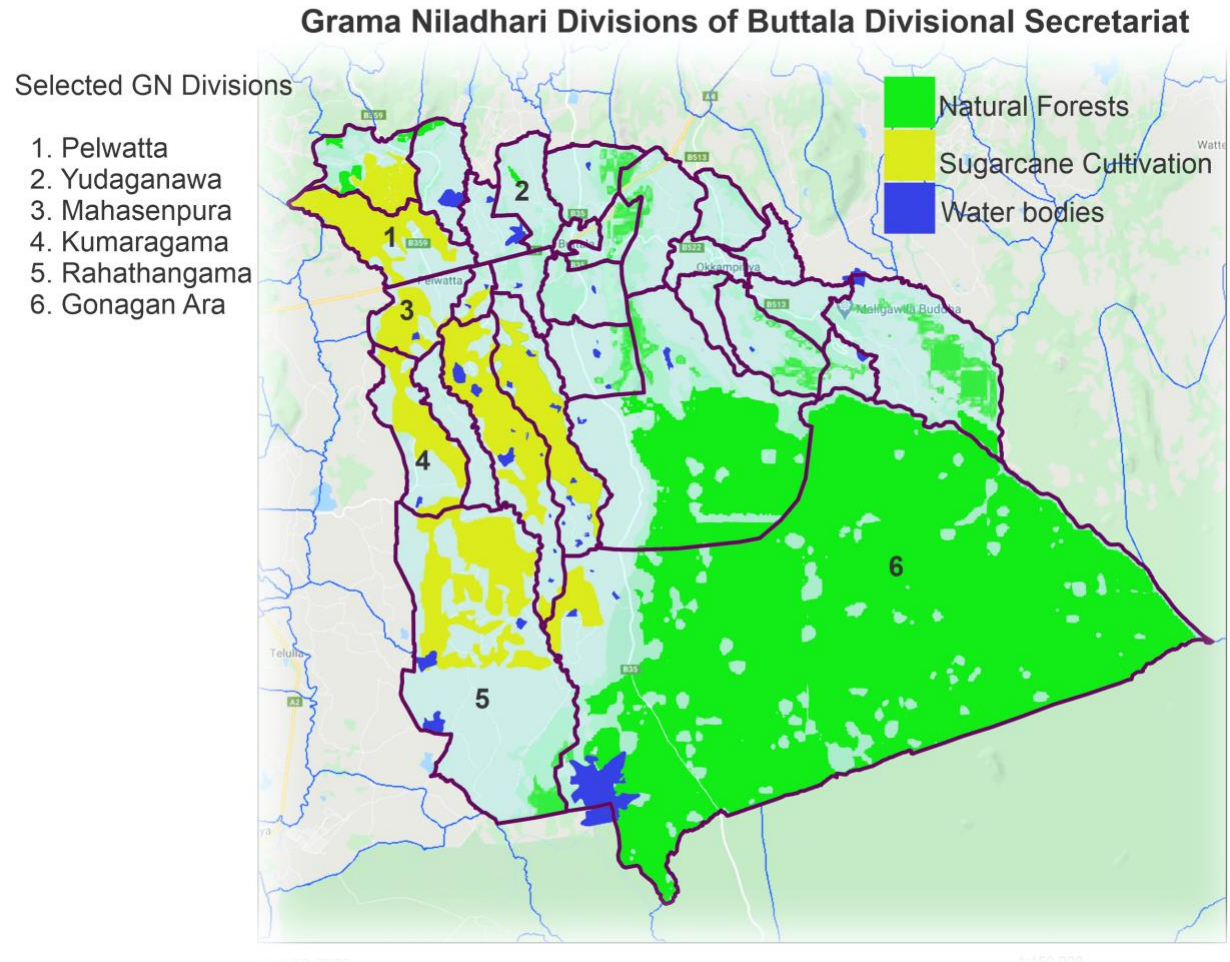
S5. Stevens PE, Levin A, Kidney Disease: Improving Global Outcomes Chronic Kidney Disease Guideline Development Work Group Members. Evaluation and management of chronic kidney disease: synopsis of the kidney disease: improving global outcomes 2012 clinical practice guideline. Ann Intern Med. 2013;158(11):825-830. doi:10.7326/0003-4819-158-11-201306040-00007

S6. Levey AS, Stevens LA, Schmid CH, et al. A new equation to estimate glomerular filtration rate. Ann Intern Med. 2009;150(9):604-612. doi:10.7326/0003-4819-150-9-200905050-00006

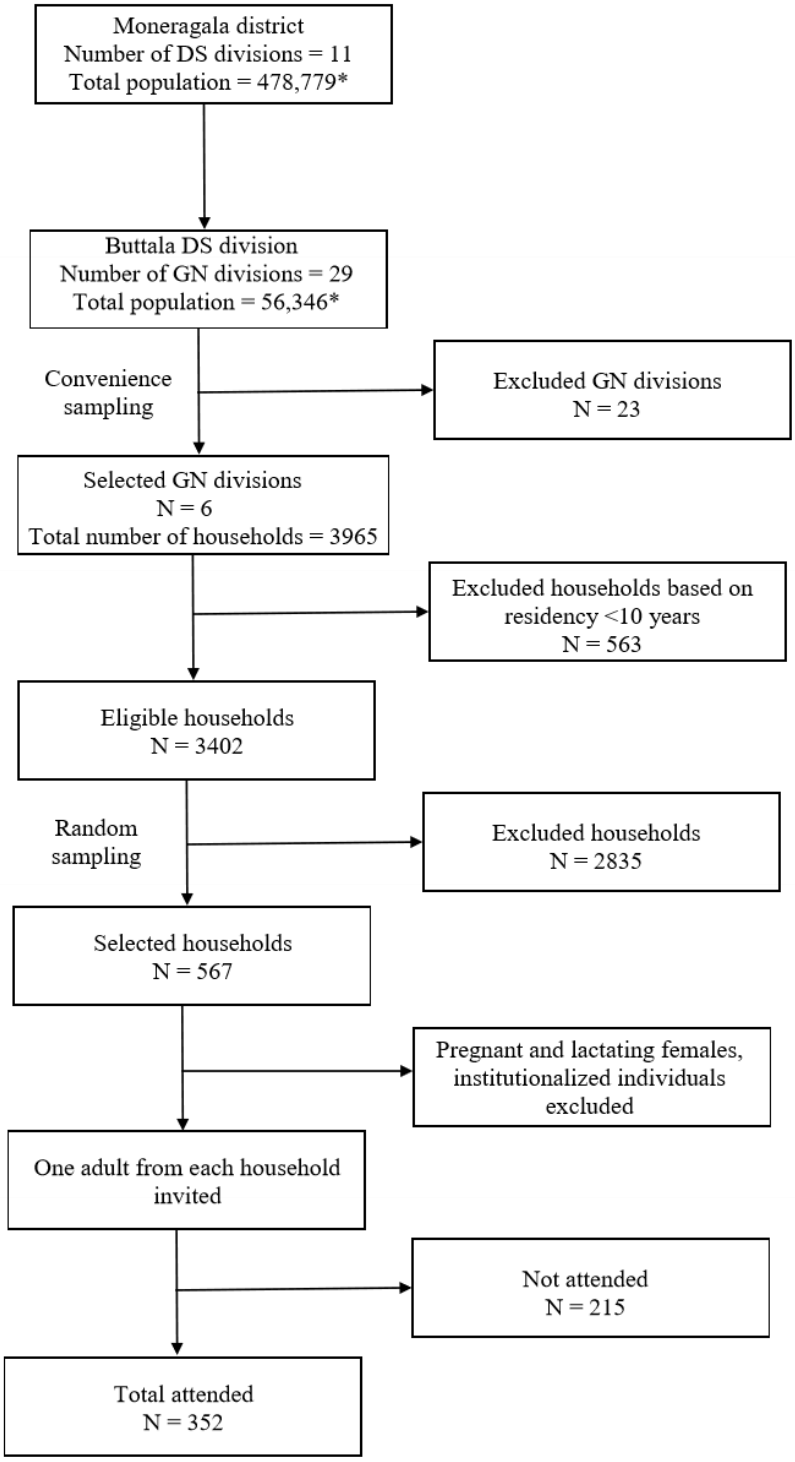
S7. Wijewickrama ES, Gunawardena N, Jayasinghe S, Herath C. CKD of Unknown Etiology (CKDu) in Sri Lanka: A Multilevel Clinical Case Definition for Surveillance and Epidemiological Studies. Kidney Int Rep. 2019;4(6):781-785. doi:10.1016/j.ekir.2019.03.020

Supplementary Figures

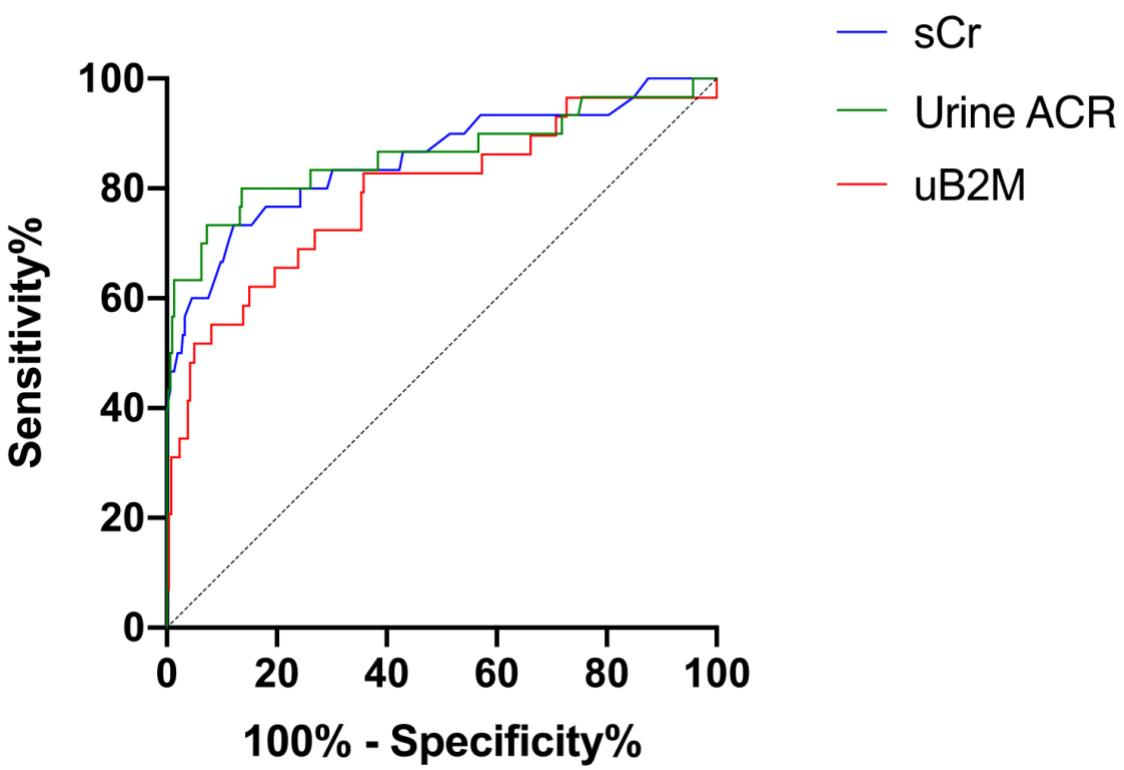




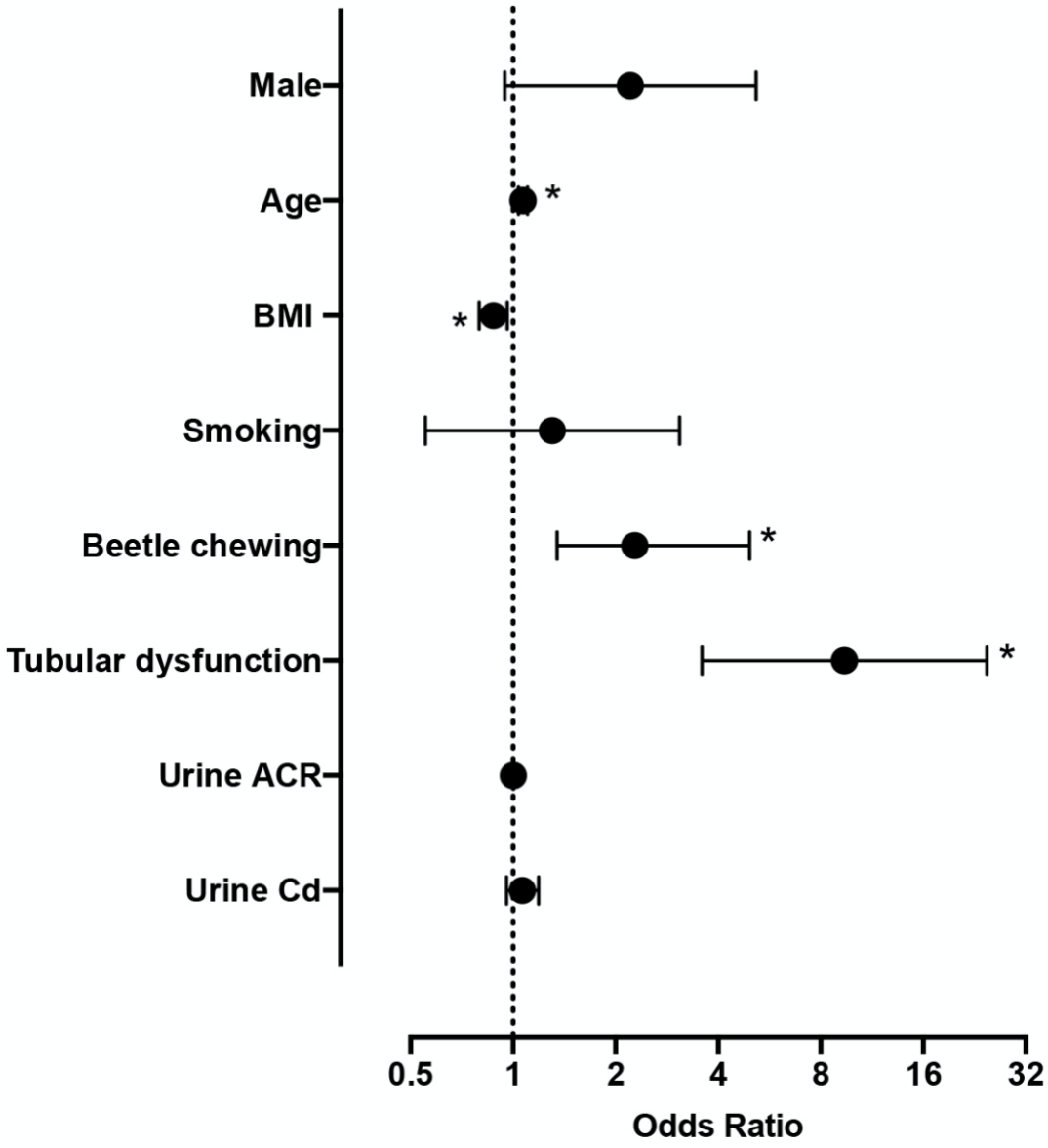
Supplementary Figure S1. Grama Niladhari (GN) divisions within Buttala Divisional secretariat. GN divisions selected for the study are shown with respect to distribution of cultivations within the study area. The map was created based on the data from the Department of Survey, Sri Lanka.


Moneragala district
Number of DS divisions = 11
Total population = 478,779*
Buttala DS division
Number of GN divisions = 29
Total population = 56,346*
Convenience sampling
Excluded GN divisions
N = 23
Selected GN divisions
N = 6
Total number of households = 3965
Excluded households based on residency <10 years
N = 563
Eligible households
N = 3402
Random sampling
Excluded households
N = 2835
Selected households
N = 567
Pregnant and lactating females, institutionalized individuals excluded
One adult from each household invited
Not attended
N = 215
Total attended
N = 352

Supplementary Figure S2. Flow diagram of the sampling strategy. *Population of Moneragala district and Buttala DS in 2017 estimated based on the 2012 census data considering annual percentage increase of 1.21%.



Supplementary Figure S3. Receiver operator characteristic curves for biomarkers of CKDu (sCr, serum creatinine; urine ACR, urine albumin: creatinine ratio; uB2M, urine β2 microglobulin).



Supplementary Figure S4. Risk factors associated with suspected CKDu. *Indicates significant associations.

Supplementary Tables

Supplementary Table S1. Demographic, clinical and exposure related data of the study population.

Characteristic	**Individuals without CKD (n=305)**	**Individuals with CKD (n=17)**	**Individuals with CKDu (n=30)**
Age, median (IQR, range)	43 (34-53; 12-78)	63 (56-69; 43-78)	57 (50-66; 29-83)
Gender (%)			
Male	164 (53.8)	6 (35.3)	22 (73.3)
Female	141(46.2)	11 (64.7)	8 (26.7)
Clinical Parameters Median (IQR, range)			
SCr / (mg/dL)	0.68 (0.58-0.86; 0.17-1.3)	1.1 (0.78-1.4; 0.47-8.4)	1.2 (0.92-2.3; 0.52-6.5)
eGFR / (mL/min/1.73m^2)	100 (87-118; 61-190)	50 (39-59; 7-123)	51 (30-86; 8-112)
uACR/(mg/g)	3.8 (2.3-6.2; 0.8-72)	8.1 (4.2-93; 2.6-3029)	55 (8.4-116; 1.2-1583)
BMI / (kg/m^2)	22 (19-25; 14-35)	21 (18-23; 15-31)	20 (18-22; 12-33)
uβ_2M / (mg/gCr)	20 (8.7-49; 1.3-391035)	209 (23-601; 1.8-342478)	154 (31-2833; 0.6-1428580)
Occupation (%)			
Paddy Farming	100 (32.8)	2 (11.8)	9(30.0)
Sugar Cane Farming	142 (46.6)	12 (70.6)	10(33.3)

Other types of farming	90 (29.5)	2 (11.8)	7(23.3)
Non farming	52 (17.0)	3 (17.6)	6(20)
Co-morbidities			
Diabetes	21 (6.9)	6 (35.3)	-
Hypertension	20 (6.6)	11 (64.7)	-
Habits*			
Alcohol	80(26.2)	2(11.8)	6(20.6)
Smoking	73(23.9)	2(11.8)	8(27.5)
Betel chewing	98(32.1)	10(58.8)	16(53.3)
Main water source during the preceding 10 years (%)*			
Deep well	99(32.5)	6(35.3)	12(40)
Shallow well	114(37.4)	8(47.1)	7(23.3)
Natural Spring	3(1.0)	-	-
Reservoirs	22(7.2)	-	-
River	24(7.9)	2(11.8)	7(23.3)
Tap water	46(15.1)	1(5.9)	4(13.3)
Filtered water	4(1.3)	-	-
Bowser water	1(0.3)	-	-
Pesticide use, years (%)*			
0-10	138 (45.2)	4 (23.6)	8 (26.7)
11-20	56 (18.4)	5 (29.4)	4(13.3)

21-30	13 (4.3)	2 (11.8)	6(20)
31-40	8 (2.6)	-	-
41-50	1 (0.3)	-	2(6.7)
51-60	-	2 (11.8)	-
Never used	89 (29.2)	6(35.3)	10(33.3)
Fertilizer use, years (%)*			
0-10	113 (37.0)	-	5(16.7)
11-20	59 (19.3)	8 (47.1)	7(23.3)
21-30	24 (7.9)	4 (23.5)	7(23.3)
31-40	13 (4.3)	-	-
41-50	3 (1.0)	-	2(6.7)
51-60	-	-	1(3.3)
Never used	93 (30.5)	5 (29.4)	8(26.7)

*Denotes self-reported data. For agrochemical usage the numbers of individuals who were involved in the application of pesticides and fertilizers for their cultivations, for the specified durations (years) are given.

Supplementary Table S2. Median urinary cadmium, arsenic and lead excretion in individuals with CKDu, non-CKDu and without CKD.

	Median (range) of concentrations in urine (µg/g creatinine)		
	Cd	**As**	**Pb**
CKDu (n=30)	1.478* (0.587-11.20)	24.24 (2.98-279.4)	19.86** (2.63-353.5)
CKD (non-CKDu) (n=17)	1.168 (0.761-3.64)	26.96 (9.93-90.88)	12.42 (3.78-33.55)
No CKD (n=305)	1.040 (0.16-31.44)	26.22 (1.03-765.3)	11.05 (0.93-558.9)

*Denotes P value <0.01 compared to individuals without CKD and ** Denotes P value <0.05 compared to individuals without CKD. Cd, cadmium; As, arsenic; Pb, lead.

Supplementary Table S3. Urine albumin and β_2 microglobulin excretion according to CKD stage in individuals with suspected CKDu

	Urine albumin to creatinine ratio, median (range) (mg/ g creatinine)	Urine β_2 microglobulin, median (range) (mg/ g creatinine)
CKD stage		
1 (n=6)	57 (31-104)	53 (31-383)
2 (n=4)	155 (45-351)	73 (10-154)
3A (n=7)	4 (2-8)	31 (0.6-182)
3B (n=7)	55 (1-1583)	1006 (13-629234)
4 (n=2)	38 (12-64)	32962 (14624-51301)
5 (n=4)	217 (124-781)	1428 (2-1428580)
Total (n=30)	55 (1-1583)	154 (0.6-1428580)

STROBE Statement—Checklist of items that should be included in reports of ***cross-sectional studies***

	Item No	Recommendation	Page No
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	NA
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	2
Objectives	3	State specific objectives, including any prespecified hypotheses	2
Methods			
Study design	4	Present key elements of study design early in the paper	S1
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	S1-2
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants	S1-2
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	S4
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	S2-3
Bias	9	Describe any efforts to address potential sources of bias	S2-3
Study size	10	Explain how the study size was arrived at	S2
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	S4
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	S4
		(*b*) Describe any methods used to examine subgroups and interactions	S4
		(*c*) Explain how missing data were addressed	NA
		(*d*) If applicable, describe analytical methods taking account of sampling strategy	NA
		(*e*) Describe any sensitivity analyses	S4
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	3 Fig S2
		(b) Give reasons for non-participation at each stage	S1-2
		(c) Consider use of a flow diagram	Fig S2
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	3 Table S1
		(b) Indicate number of participants with missing data for each variable of interest	NA
Outcome data	15*	Report numbers of outcome events or summary measures	3-4

Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	3-4
		(*b*) Report category boundaries when continuous variables were categorized	NA
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	NA
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	3-4
Discussion			
Key results	18	Summarise key results with reference to study objectives	4
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	5
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	4-5
Generalisability	21	Discuss the generalisability (external validity) of the study results	4-5
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	6

*Give information separately for exposed and unexposed groups.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.