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Nephrotoxic Contaminants in Drinking Water and Urine, and Chronic Kidney Disease in Rural Sri Lanka

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Abstract

Chronic kidney disease of unknown ("u") cause (CKDu) is a growing public health concern in Sri Lanka. Prior research has hypothesized a link with drinking water quality, but rigorous studies are lacking. This study assesses the relationship between nephrotoxic elements (namely arsenic (As), cadmium (Cd), lead (Pb), and uranium (U)) in drinking water, and urine samples collected from individuals with and/or without CKDu in endemic areas, and from individuals without CKDu in nonendemic areas. All water samples—from a variety of source types (i.e., shallow and deep wells, springs, piped, and surface water)—contained extremely low concentrations of nephrotoxic elements, and all were well below drinking water guideline values. Concentrations in individual urine samples were higher than, and uncorrelated with, those measured in drinking water, suggesting

potential exposure from other sources. Mean urinary concentrations of these elements for individuals with clinically diagnosed CKDu were consistently lower than individuals without CKDu both in endemic and nonendemic areas. This likely stems from the inability of the kidney to excrete these toxic elements via urine in CKDu patients. Urinary concentrations of individuals were also found to be within the range of reference values measured in urine of healthy unexposed individuals from international biomonitoring studies, though these reference levels may not be safe for the Sri Lankan population. The results suggest that CKDu cannot be clearly linked with the presence of these contaminants in drinking water. There remains a need to investigate potential interactions of low doses of these elements (particularly Cd and As) with other risk factors that appear linked to CKDu prior to developing public health strategies to address this illness.

Keywords: nephrotoxic elements, urinary biomarker, chronic kidney disease, farming communities, Sri Lanka

1. Introduction

Over the last two decades, chronic kidney disease of unknown ("u") origin (CKDu) has progressively increased to epidemic levels in rural farming communities of Sri Lanka. This condition is therefore putting increasing strain on the country's scarce public sector health care resources (Nanayakkara et al., 2012; Wanigasuriya, 2012). CKDu is asymptomatic in its early stages; patients then experience kidney impairment in the proximal tubules and the interstitium as the disease progresses slowly to final stage renal failure (Wanigasuriya et al., 2009; Chandrajith et al., 2011; Nanayakkara et al., 2012). The disease mainly occurs in some areas of the dry zones, especially the North Central Province (NCP) of Sri Lanka, and disproportionately affects males from poor socioeconomic backgrounds who are engaged in paddy (rice) farming (Senevirathna et al., 2012; Jayasekara et al., 2013).

The exact prevalence and geographic scope of the problem is unclear at this time (de Silva, 2014). Some estimates suggest a 2 to 3% prevalence rate among those older than 18 years of age (Chandrajith et al., 2011), while other recent work suggests a prevalence closer to 15% (Jayatilake et al., 2013). Medical experts in Sri Lanka have estimated an affected population of 400,000 people and death toll of around 20,000 people (Perera, 2012; Gunawardena, 2012). The disease is also not unique to Sri Lanka; similar cases of tubular damage to the kidneys without a known cause have been found in other locations including Nicaragua (O'Donnell et al., 2011), El Salvador (Orantes et al., 2011; Peraza et al., 2012), Costa Rica (Cerdas, 2005), the Srikakulam District in Andhra Pradesh, India (Machiraju et al., 2009), and Egypt (Kamel and El-Minshawy, 2010).

Though several hypotheses have been advanced concerning the underlying causes of the CKDu epidemic in Sri Lanka and elsewhere, evidence in support of them remains limited. Existing research has reported that risk factors such as hypertension and diabetes are not the cause of the disease (Nanayakkara et al., 2012). Another hypothesis concerns the role of environmental exposures to high concentrations of nephrotoxicants (such as, Cd, Pb, and U), which may accumulate and cause functional and structural damage in the proximal tubule cells of the kidney (Sabolic, 2006). In Sri Lanka, previous studies using case-control methodologies have reported that CKDu is related to biomarkers for As (Jayasumana et al., 2013) and Cd (Wanigasuriya et al., 2011), and have identified farming and

the use of fertilizer or other agrochemicals as risk factors (Chandrajith et al., 2011; Jayatilake et al., 2013). Jayasumana et al. (2013) also report that high levels of As exposure in CKDu patients coincide with observable skin lesions.

Drinking water is often considered to be a major source of nephrotoxic contaminants that cause CKDu, and the contaminants of concern often come from natural (e.g., local geological materials) sources. In addition to the possibility of natural contamination, intensive use of agrochemicals in Sri Lanka since the 1960s may have polluted local water sources. Data on the distribution of such inorganic nephrotoxic elements (such as, Cd, Pb, and U) in water supplies is limited. Moreover, the geographic data coverage is patchy or limited to a few selected elements (e.g., Jayatilake et al., 2013). A systematic assessment of water quality is therefore needed and should be linked with biomonitoring (e.g., in urine) of the potential elements of concern, in order to more fully understand exposures—including the potential role of food sources of these contaminants—and assess their potential health effects (Wilhelm et al., 2004; Aguilera et al., 2008; Calafat, 2012; Karagas et al., 2001).

The study described in this paper is one of the first attempts to (1) systematically document the geographic distribution of nephrotoxic and other elements in drinking water sources of different types (i.e., dug wells, tube wells, springs, and pipewater), and their variation across endemic and nonendemic areas in Sri Lanka; and (2) link drinking water concentrations of these chemicals with biological levels in urine in individuals with (from endemic areas only) and without (from endemic and nonendemic zones) CKDu. We test the hypothesis that the concentrations of known nephrotoxicants (namely As, Cd, Pb, and U) in drinking water can be linked to cases of CKDu and urinary concentrations of these elements in Sri Lanka. It is important to note that we only measure the concentrations of inorganic elements in this study, using well-validated methodologies carried out on high-quality analytical equipment. We complemented our measures of inorganic element concentrations in water and urine with data on anthropometric and lifestyle factors to better assess the characteristics of patients with CKDu and compare to those populations from endemic and nonendemic locations. We also compared the levels of these elemental biomarkers with those from healthy populations from other countries with no known contamination problems, in order to provide perspective on the nature of CKD-related health risks from these contaminants in Sri Lanka. Such investigations are crucial for better interpreting the results we obtain, and for generating hypotheses regarding potential risk factors for CKDu.

2. Materials and methods

2.1. Site selection and study population

In order to assess how CKDu occurrence varies with the presence of contaminants in water and urine samples, our sampling strategy began with selection of a set of communities located in zones with and without CKDu (Fig. 1). Data collection took place in 20 communities located in 7 districts. These sites were identified based on a review of the previous literature (e.g., Jayasekara et al., 2013) and through consultations with experts on the geographic distribution of the disease in Sri Lanka. Within these two settings, we identified three groups of subjects: (1) individuals clinically diagnosed with CKDu (in endemic areas

only); (2) individuals without clinically diagnosed CKDu (also in endemic areas); and (3) individuals without clinically diagnosed CKDu (in nonendemic areas). The individuals with clinically confirmed CKDu (group 1) were purposively selected for inclusion in the study based on information provided by local communities and health centers. All individuals in Groups 2 and 3 (the comparison groups) were selected randomly from the set of other households living in the sample communities. In most cases, only one such randomly selected subject was enrolled from a sample household, except for 14 households from which 2 subjects were recruited (one parent and one child over the age of 9). The inclusion of children is particularly important in order to identify the potential for future kidney damage among individuals who may be particularly sensitive to contaminant exposures.

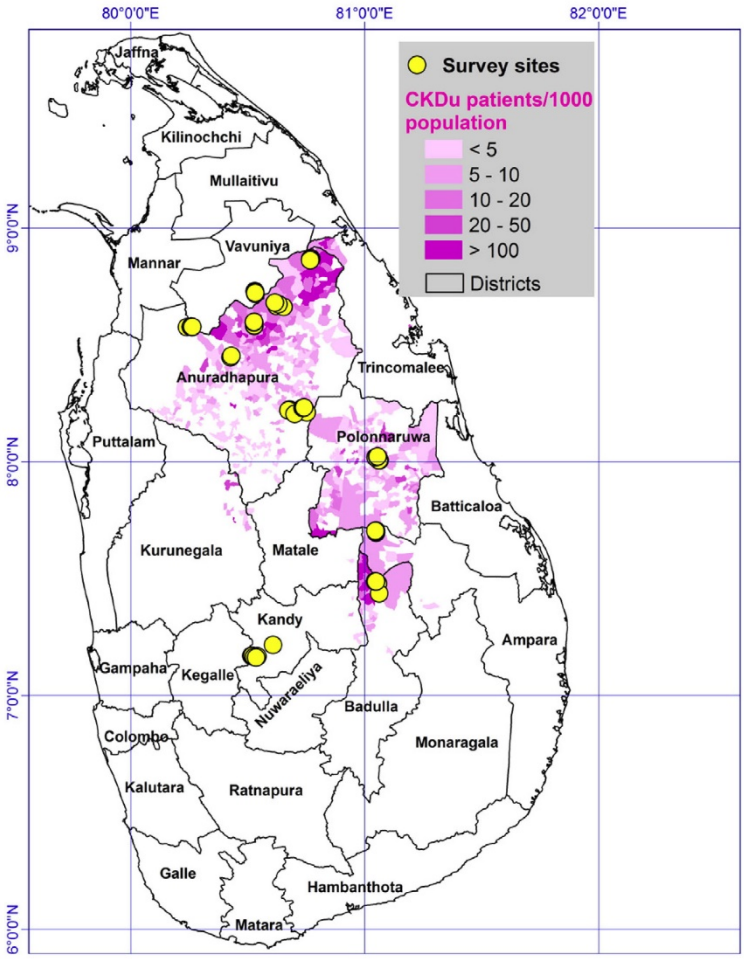

Figure 1. The sampling locations in endemic areas were Anuradhapura, Polonnaruwa, Badulla, and Ampara districts; Kandy district is in the nonendemic area. Map of survey sites (yellow circle; this study), and distribution of CKDu patients (shaded) in NCP adopted from Jayasekara et al. (2013).

The final study population includes 134 individuals, 109 of whom are adults (over the age of 18) and 25 of whom are children between 10 and 18 years of age. The majority of those enrolled come from the most heavily affected areas in the NCP (61 in Anuradhapura and 11 in Polonnaruwa districts). The other sampling sites include Kandy District in the Central Province (n = 27), Northern Badulla District in Uva Province (n = 14), Ampara District in Eastern Province (n = 15), and Vavuniya District in Northern Province (n = 6) (Supplementary material Table S1). Among the 134 sample individuals, 26 were from endemic areas (NCP, Northern, Uva, and Eastern Provinces) and had previously been clinically confirmed to have CKDu; the other 79 and 29 were healthy subjects from endemic and nonendemic (Central Province only) areas, respectively. None of these 108 other subjects had previously been clinically tested for CKDu.

The research design was conducted with the ethical approval (Protocol No. B0983) of the Institutional Review Board (IRB) at Duke University. Permission to carry out the survey was also obtained from University of Peradeniya in Kandy, Sri Lanka, and from local health authorities in the study region. All children (and their parents) who willingly participated in the survey provided signed informed consent prior to enrollment and participation in the study. The anonymity of investigated subjects has been maintained.

2.2. Water sampling and analytical methods

Water samples were collected from 61 private and community water supplies identified as drinking/cooking water sources by study respondents at the time of the study (38 dug wells, 9 tube wells, 9 cold springs, 2 reservoirs, and 3 pipes; Fig. 1), between October 27 and November 15, 2013. Rainfall is generally high during this period over most of Sri Lanka. The average depths of the dug and tube wells were 5.7 ± 3.5 m and 57.8 ± 20.5 m, respectively. The springs were sampled at the mouth of the source, and reservoir samples were taken 50–100 m away from the lakeshore; these sources are mainly used for drinking and irrigation purposes, respectively. Prior to sampling, in-situ measurements were conducted for pH, redox potential (Eh), dissolved O_2 (DO), temperature, and electrical conductivity (EC) using measurement instruments that were calibrated daily. Water samples were filtered in the field using 0.45 μm filters. Samples allocated for cation/trace metal analyses were filtered directly into 60 ml polyethylene bottles that had been cleaned with trace metal grade ~1N HCl and ~1N HNO_3, and then rinsed with deionized water having resistivity N18 MΩ/cm. The samples were immediately acidified with high-purity HNO_3 (Fisher Optima). Unfiltered and unacidified water samples were also collected into 60 ml and 30 ml polyethylene bottles for measurement of alkalinity (as bicarbonate (HCO_3^-)).

All lab analyses were conducted in the analytical facilities at Duke University (USA). Concentrations of major cations of calcium (Ca^{2+}), magnesium (Mg^{2+}), sodium (Na^+), and silica (SiO_2) were measured using a Fisons Spectraspan 7 direct-current plasma spectrometer (DCP). This instrument was calibrated using solutions prepared from plasma-grade single-element standards. Major anions of chloride (Cl^-), sulfate (SO_4^{2-}), and nitrate (NO_3^-) were analyzed using an ion chromatograph (IC). The fluoride (F^-) concentration in groundwater was measured in-situ and determined electrochemically using the Thermo Scientific Orion Ion-Selective Electrode (ISE) (results were confirmed using the Ion Chromatography method) following a procedure reported by Singh et al. (2007) and Ruiz-Payan et al. (2005).

Samples were mixed in a 1:1 volume ratio with a total ionic strength adjustment buffer (TISAB) of pH 5–5.5, which allows for optimal analyses of F^- in the aqueous solution. Alkalinity (as HCO_3^-) was measured using titration techniques to pH 4.5. All trace elements including As, Cd, U, and Pb were analyzed via a VG Plasmaquad 3 inductively coupled plasma–mass spectrometer (ICP–MS) calibrated using serial dilutions of National Institute of Standards and Technology (NIST) 1643e standard spiked with U and Th.

2.3. Urine sampling and analysis

We collected first morning void urine samples from the 134 study participants for assessment of chemical elements exposure. These samples were collected in acid-washed 60 ml ultra-cleaned polyethylene bottles. A semi-quantitative urinalysis (using Siemens microalbustix reagent strips)was used to measure albumin and creatinine; the strips are read visually and their color is compared with a color chart that displays different ranges of concentrations. The F^- concentration in urine was also measured using ISE following a procedure similar to that used for the water samples. Quality control was conducted using freeze-dried urine reference material (SERO210705; LGC Standards, and NIST SRM 2668 low level). The accuracy of ISE F^- measurements for both urine and water standards ranged from 98% to 102.5% relative to the LGC standard. Repeatability of other trace element measurements in urine was tested in a lab at the Research Triangle Institute (RTI) International (Research Triangle Park, USA) for 25 samples, and results from these reanalyses were consistent with our measurements. For example, the repeatability of As, Pb, and Mo was 103.8%, 95%, and 102.7%, respectively. The recovery for As, Cd, Pb, and U in urine samples with respect to the NIST standard was 105%, 90%, 94.4%, and 78.4%, respectively. No F^- concentrations were below the detection limit (DL) of the F^- electrode (0.02 mg/L). Arsenic, Cd, Pb, and U concentrations were measured below the DLs. DLs of As, Cd, Pb, and U were 0.05, 0.04, 0.008, and 0.006 μg/L respectively.

2.4. Survey data

Additional data—household demographics (age, gender), health (anthropometry, various symptoms, chronic diseases (hypertension, diabetes), use of traditional medicine, years of residence in the study location, and socioeconomic status (occupation and education), as well as nutritional and lifestyle factors—were collected through a household survey. These data also include information on drinking-water sources (used to identify water sampling locations), estimated amount of water consumption per day, agrochemical (pesticide, fertilizer, and herbicide) use (yes/no), use of protective gear (yes/no), type and frequency of major dietary consumption, and tobacco and alcohol consumption (frequency, start and stop age). Body mass index (BMI; weight (kg)/height^2 (m^2)) was derived from measured height and weight. Standard international cutoff points were used to classify participants into underweight (< 18.5), healthy weight (18.5–24.9), and overweight (≥ 25.0) categories (WHO, 2013).

3. Results

3.1. Characteristics of study population

The average age of study individuals (n = 134) was 37.5 ± 16.6 (range: 10.3–74.5) years; females and males represent 38.8% (n = 52) and 61.2% (n = 82) of the respondents, respectively (Table 1). The majority of the subjects are Sinhalese (96.3%; n = 129), with the rest being Tamil (3.7%; n = 5). Most participants (60.4%; n = 81) had attended or were enrolled in high school at the time of the survey (9–12 grade), followed by those with primary education only (36.6%; n = 49) (1–8 grade). Only 2 participants had no formal education. The mean BMI of the participants was 21.3 ± 3.9 kg/m^2 (range: 13–31 kg/m^2). Based on the WHO classification, most participants were categorized as normal weight (53.7%; n = 72) or underweight (25.4%; n = 34). Twenty percent (n = 27) of respondents were overweight, and only one was obese.

Table 1. Sociodemographic and other characteristics of the study participants

Characteristics	Total number of individuals	Number of individuals in each group		
		With CKDu in endemic areas (n = 26)	Without CKDu in endemic areas (n = 79)	Without CKDu in nonendemic areas (n = 29)
Gender				
Male	82 (61%)	25	42	15
Female	52 (39%)	1	37	14
Education level				
No education	2 (1.5%)	1	0	1
1–8 grade	49 (36.6%)	17	24	8
9–12 grade	81 (60.4%)	8	55	18
Other	2 (1.5%)	0	0	2
Occupation				
Paddy farming	72 (53.7%)	24	43	8
Nonfarming	19 (14.1%)	0	4	12
Student	27 (20.1%)	0	21	5
Others (housewife, fishing, driving, etc.)	17 (12.3%)	2	11	4
Agrochemical use				
Pesticide, fertilizer, and herbicide	71 (53%)	23	44	4
Fertilizer and herbicide	2 (1.5%)	1	1	0
One of the agrochemicals	4 (2.9%)	2	0	2
Nonusers	57 (42.5%)	0	34	23
Use of protection				
Yes	19 (14.2%)	3	15	1
No	115 (85.8%)	24	64	25
Elevation: range	43–630	44–127	43–154	286–630
(mean ± s.d.) m.a.s.l.	(180 ± 166)	(82 ± 22.4)	(105 ± 30.8)	(471 ± 125)

Years in agriculture				
0	44 (32.8%)	0	25	19
< 5	13 (9.7%)	2	9	2
5–10	16 (11.9%)	0	15	1
11–20	18 (13.4%)	4	13	1
21–30	21 (15.7%)	7	9	5
> 30	22 (16.4%)	13	8	1
BMI: mean +/– SD (range)	21.3 ± 3.9 (14.7–31.3)	21.1 ± 3.6 (14.9–28.6)	21.4 ± 3.7 (13.8–29.8)	21.5 ± 4.8 (12.7–31.3)
Albumin/creatinine (ACR)				
< 30 mg/g	58 (43.6%)	5	68	25
≥ 30 mg/g	75 (56.4%)	21	11	4
Number of individuals with ACR ≥ 30 mg/g by age (total individuals)				
10–20	3 (28)	0 (0)	3 (21)	0 (7)
> 20–35	6 (29)	1 (1)	4 (21)	1 (7)
> 35–50	9 (41)	5 (7)	4 (26)	0 (8)
> 50–65	15 (30)	12 (13)	0 (11)	3 (6)
> 65–75	3 (6)	3 (5)	0 (0)	0 (1)

Participants reported consuming up to 7 L of water per day (mean: 2.82 ± 1.44 L). A majority of study subjects reported regular use of agrochemicals; about half (53%; $n = 71$) of the participants responded that they used pesticides, fertilizer, and herbicides simultaneously, and 4.4% ($n = 7$) used only one of these agrochemicals, while 42.5% of the subjects ($n = 57$) did not report use of such chemicals.

There are a number of important differences between subjects from the endemic and nonendemic areas, and between those with and without clinically diagnosed CKDu. We investigated these differences by comparing mean values for the various subsamples as well as using multivariate logit models. We note that all but one of the CKDu patients is male, and their education levels are lower than those of the randomly selected respondents. Another notable difference is that CKDu patients have spent longer on average working in agriculture than the other groups; half of these had been farming for more than 30 years at the time of the survey, compared with just 10% and 3% among the non-CKDu patients in endemic and nonendemic areas, respectively. In multivariate logit regressions, we found that CKDu patients were more likely to be male, older, longer term residents of their current location, using tube wells or dug wells as a primary drinking water source (rather than springs), using fertilizers in farming, and more likely to have been consumers of alcohol in the past. At the same time, they were less likely to be currently drinking alcohol (Table 2). These logit models are used solely for the purpose of examining the issue of selection into our sample; the differences should not be interpreted as well-identified risk factors given that CKDu subjects were not matched with controls at the time of sample construction.

Table 2. Differences among individuals in the sample groups

	Has CKDu				In sample from endemic CKDu area			
Variable	Basic		Extended[a]		Basic		Extended	
Male	2.7*	(1.38)	3.6	(2.9)	0.80	(0.97)	0.46	(1.92)
Age	0.066*	(0.039)	0.20**	(0.094)	−0.028	(0.034)	−0.016	(0.075)
Education level	−0.64	(0.52)	0.31	(0.94)	−0.66	(0.90)	−0.44	(1.45)
Household head	−0.033	(0.91)	−3.1**	(0.94)	−1.91	(1.54)	−9.8*	(5.5)
Farming household	−1.21	(1.92)	6.3	(4.5)	−1.11	(0.81)	−0.34	(4.2)
Paddy farming	0.58	(1.19)	−2.5	(2.7)	3.9***	(0.83)	7.1	(5.5)
Drink from tube well	3.3*	(1.9)	6.6**	(3.2)				
Drink from dug well	2.2	(1.8)	5.0**	(2.5)	4.8***	(1.19)	15.9**	(6.4)
Use agricultural chemicals	2.2	(2.8)			0.30	(1.13)		
Use fertilizer			5.1*	(2.5)			2.2	(2.8)
Use pesticide			0.058	(1.75)			−9.0	(6.2)
Use herbicide			−4.3	(4.1)			13.9***	(3.9)
Use protective gear	−0.60	(0.99)	1.18	(1.45)	1.00	(1.83)	6.3*	(3.8)
Use tobacco now	0.46	(0.64)	1.18	(1.50)	−0.22	(0.78)	−15.0**	(7.3)
Consume alcohol now	−1.27*	(0.77)	−4.4***	(0.99)	−1.53*	(0.93)	−32.1***	(7.3)
Ever used tobacco			−1.31	(2.2)			3.0	(3.2)
Ever consumed alcohol			3.1*	(1.8)			32.0***	(8.3)
Years at current residence	0.15**	(0.066)	0.044	(0.20)				
Time using water source			−0.22	(0.074)			−0.18	(0.15)
Other controls[b]	No		Yes		No		Yes	
N	134		118		134		118	
Pseudo-R[b]	0.45		0.66		0.55		0.78	

Notes: Reported values are coefficients from a logit regression with standard errors in parentheses; significance levels are indicated by *** 1%; ** 5%; * 10%. Education level is a categorical variable that ranges from 0 (no education) to 4 (post-secondary). Use agricultural chemicals is a dichotomous variable that is equal to 1 if the household uses at least one of the following: fertilizer, pesticide, or herbicide. Spring is the omitted drinking water source.

a. In the extended model, 16 individuals are omitted due to missing dietary data.

b. Other controls that were included (but not statistically significant) were self-reported daily water consumption and non-rice food consumption frequency (other grains, fish, and meat).

Comparing the endemic area and nonendemic area samples, we also note differences. First, endemic and nonendemic areas in this study are at different altitudes; the former are located at lower altitude (44 to 154 meter above sea level) than the latter (286 to 630 m.a.s.l.). The primary occupation of the majority (82%) of study adults (and of over 90% of CKDu patients) in endemic areas is paddy farming; adults from nonendemic sites are much less

likely to be either paddy farmers (32%) or involved in agricultural occupations. Again using multivariate logit regression, we found that respondents from endemic areas were less likely to be currently consuming alcohol or tobacco, but were more likely to be practicing paddy farming, drinking from wells, using herbicides and protective equipment in farming, and to have previously consumed alcohol (Table 2).

3.2. Physicochemical water parameters

The pH of the water samples taken from the study area ranged from moderately acidic to alkaline (range: ~5–8). Temperature and EC varied from 23 to 31.7°C (mean: 27.7°C ± 1.6°C) and 34.2 to 1589 μS/cm (mean: 538 ± 330 μS/cm), respectively. Positive Eh values were measured in the samples (range: 14 to 143 mV; mean: +75.9 ± 31.5 mV). These analyses suggest that the samples were under slight to moderately oxidizing conditions.

Most of the water samples, taken from various sources, were Ca^{2+} (Mg^{2+})–HCO_3^- dominated. The range of SiO_2 concentrations across all samples was 1.0 to 127 (mean: 77.9 ± 35.3),with the spring and irrigation samples having particularly low SiO_2, indicating weak water-rock interactions. The concentration of major and trace elements was generally similar across the different water types (i.e., dug wells, tube wells, and springs) (Supplementary Material Table S2). Thus, the differences in water sourcing among sample groups shown cannot be clearly linked to differences in water quality, an issue that we explore further in Section 3.3.

3.3. Distribution toxic elements concentrations in water and urine samples

3.3.1. Water quality analyses

The water samples collected in this study all contained low concentrations of toxic elements such as, Cd, Pb, B, U, V, Mo, and F^- (Supplementary Material Table S2). In addition, none of the nephrotoxicants exceeded the permissible limits set by WHO, 2011, the U.S. EPA, 2009, and the EU Council, 1998 (Supplementary Material Table S3). The concentration of As, Cd, Pb, and U were below the analytical DL in many samples (e.g., As in 50 samples (82%), Cd in 11 samples (18%), Pb in 38 samples (62%), and U in 18 samples (25.6%) of the 61 samples). Below we summarize these water quality results in additional detail.

The concentration of As in all of the 61 water samples was less than 1 μg/L (mean 0.05 ± 0.16 μg/L, range below DL to 0.86 μg/L), which is well below the World Health Organization guideline of 10 μg/L in drinking water (WHO, 2011). In addition, speciation modeling revealed that the As(V) species [$HAsO_4^{2-}$ and $H_2AsO_4^-$] in the groundwater samples predominates the more toxic As (III) species under the oxidizing conditions. The cadmium (Cd) level in the samples varied from below DL and 0.05 μg/L, with a mean of 0.01 ± 0.01 μg/L; these levels are well below the WHO recommended limit of 3 μg/L of Cd. Lead (Pb) concentrations in the samples ranged from below DL to 1.6 μg/L (mean of 0.11 ± 0.29 μg/L), again well below the WHO recommended limit of 10 μg/L for drinking water. All uranium (U) concentrations were below the WHO drinking water standard of 15 μg/L; levels in the water samples varied from below DL to 0.96 μg/L with a mean of 0.18 ± 0.24 μg/L. Finally, F^- concentrations in all tested water samples were below2 mg/L (except for one dug well sample with F^- concentration of 4.3mg/L), with a mean of 0.66 ± 0.66mg/L. Only 4 (6.6%)

water samples contained F^- exceeding the WHO recommended limit of 1.5 mg/L in drinking water.

Concentrations of other trace elements (such as B, V, Mo, Se, Cr, Sb, Zn, Ni, Cu, and Ba) were also found to be generally low in the water samples, and below specified WHO guidelines (Supplementary Material Table S3).

3.3.2. Distribution of trace elements in urine

Measurements of the chemical composition of urine can help in monitoring recent and/or long-term exposures through ingestion via drinking water and other food sources. We measured urinary concentrations of a wide spectrum of elements, including the known nephrotoxicants discussed above—As, Cd, Pb, and U (Table 3). Here we describe these results and compare them with reference concentrations measured in healthy unexposed individuals and/or populations from other countries. While these reference values are useful for the purposes of comparisons, they may not imply a safe level of exposure for Sri Lankan population.

Table 3. Summary of elemental concentrations in urine samples of individuals in the study area

Inorganic elements	Min	Max	Percentiles			Mean	Reference range in healthy and unexposed population in studies in other countries	
			25	50	75		Ranges[a,b,c,e]	Ranges[d]
As	2.70	313	16.9	26.4	48.6	39.5 ± 40.2	< 0.5–48.2[a]; 15[b]	5.3–11.7
Cd	bd	2.30	0.20	0.40	0.60	0.49 ± 0.44	0.05–1.64[a]; 0.8[b]	0.005–0.46
Pb	bd	8.80	0.40	0.90	1.73	1.40 ± 1.53	0.3–30[a]	0.12–2.9
U	bd	0.20	bd	bd	bd	0.003 ± 0.02	0.01–0.35[e]	0.0007–0.019
B	123	2858	447	771	1204	929 ± 627	490–3290[c]	380–3600
Mo	2.90	541	23.0	48.7	95.2	76.2 ± 87.6	2.8–288[a]	12–108
Al	bd	114	2.15	5.15	10.6	8.65 ± 13.7	1.2–168	0.6–5.1
Mn	bd	140	bd	bd	0.20	1.50 ± 12.4	< 0.09–7.8[a]	< 0.05–0.24
Li	0.80	54.4	6.35	9.60	15.7	11.9 ± 8.24	0.5[e]	5.2–23
Be	bd	bd	bd	bd	bd	bd	0.04–0.76[c]	< 0.01–0.018
Co	bd	4.90	0.30	0.60	1.10	0.86 ± 0.78	< 0.12–2.05[a]	0.04–0.81
Ni	bd	25.8	2.38	4.80	8.43	6.00 ± 4.87	< 0.3–59[a]	0.24–2.7
Cu	bd	96.2	4.58	7.75	12.4	10.9 ± 11.5	4.6–40.4[a]	1.9–15.9
Zn	20.6	2562	154	250	476	370 ± 365	8–767	40–430
Rb	97.7	6016	725	1150	2048	1600 ± 1311	284–4096[c]	500–5500
Sr	21.8	1270	88.3	157	297	228 ± 216	220[e]	18–260
Ag	bd	1.20	bd	bd	bd	0.01 ± 0.11	0.04–0.88[c]	< 0.01–0.02
Sb	bd	0.70	bd	bd	bd	0.02 ± 0.09	0.19–1.1[c]	0.022–0.104
Ba	bd	55.9	bd	1.60	5.50	4.75 ± 8.10	0.03–5.7[e]	0.22–2.7
Tl	bd	1.30	0.10	0.20	0.40	0.30 ± 0.28	0.07–0.7[c]	0.05–0.54
Th	bd	0.70	bd	bd	bd	0.02 ± 0.10	0.01–0.28[e]	0.0005–0.005
Mg	9.40	301	33.7	62.4	95.8	75.7 ± 55.9	–	–
Ca	5.30	383	30.3	57.3	117	83.5 ± 77.2	–	–
F	0.10	8.80	0.60	0.95	1.63	1.46 ± 1.43	–	–
Cl	904	14,589	2906	4308	6887	5258 ± 3137	–	–

Notes: bd = below detection. All concentrations are in μg/L except Ca, Mg, and F^-(mg/L). For each element, the range of reference values reported for healthy populations in different geographical regions is also presented.

a. White and Sabbionia (1998)—UK population
b. Wilhelm et al. (2004)—German population
c. Minoia et al. (1990)—Italian population
d. Rodushkin et al. (2001)—Sweden population
e. Caroli et al. (1994)—Sweden population

We found considerably higher concentrations of nephrotoxic and other trace elements in sample individuals' urine samples than in the drinking-water samples. This suggests that individuals' additional exposure to these elements comes from other food sources. Urinary As concentrations ranged from 2.7 to 313 μg/L (mean: 39.5 ± 40.2). The majority (77%; n = 103) of individuals had urinary As concentrations below 50 μg/L, while 17% (n = 23) and 6% (n = 8) had urinary As of 50–100 μg/L and 100–313 μg/L, respectively. Cadmium concentrations were found to range from below DL to 2.3 μg/L (mean: 0.49 ± 0.44). The majority of individuals (91%; n = 122) had urinary Cd concentrations below 1 μg/L. Lead concentrations were found to range from below DL and 8.8 μg/L (mean: 1.4 ± 1.5). Uranium concentrations ranged from below DL to 0.2 μg/L (mean: 0.003 ± 0.02), and fluoride concentrations were between 0.1 and 8.8 mg/L (mean: 1.5 ± 1.4 mg/L). Overall, the low levels of each of these elements in drinking water and the lack of correlation of these elements in drinking water and urine sample concentrations suggest that drinking water is not an important source of exposure. And though the majority of the groundwater and urine F^- concentrations (80%; n = 107) lie below 2 mg/L, we found higher correlations between F^- in drinking water and individuals' urinary F^- concentration, suggesting that water is an important source of F^- (Fig. 2). Individuals in the CKDu group also appear to have systematically lower urinary excretion of F^- than healthy individuals, which is likely suggestive of the inefficiency of kidney in excreting F^-.

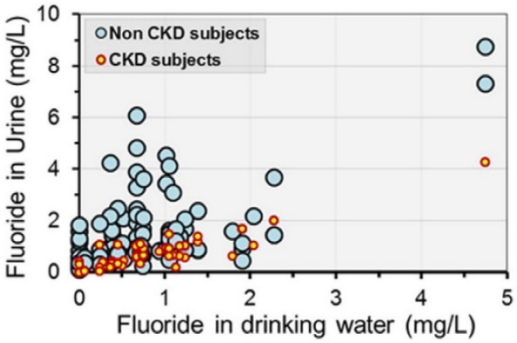

Figure 2. The F^- concentration in drinking water and urinary F^- concentration in individuals with and without CKDu.

As summarized in Table 3, the concentrations of As, Cd, Pb, and U are not outside the ranges found in other reference populations. The concentrations of these contaminants are positively correlated in these urine samples (Pearson correlation of Cd vs As was 0.36 (p = 0.01); Cd vs Pb was 0.39 (p = 0.01); Cd vs U was 0.15 (p = 0.08)); this suggests that these contaminants may come from similar sources, and that their interactions may be important to consider in future studies.

3.4. Comparison of elemental concentrations in water and urine of individuals with and without CKDu

The drinking water and urinary characteristics of the sample individuals were compared across the three identified sample groups. In general, we found that the mean concentrations of As, Cd, Pb, and U in the drinking water samples across the three groups were similar, as were the majority of other elements (Supplementary Material Table S4). Although the concentrations (mostly from fresh spring waters) in the nonendemic area are relatively lower for most of the elements, they are all very low, and the small differences observed are not expected to account for the differential CKDu rates across these areas.

Interestingly, mean urinary concentrations for individuals with clinically diagnosed CKDu were consistently lower than individuals without CKDu both in endemic and nonendemic areas, despite the very similar water concentrations (Table 4). For example, the mean concentration of As in CKDu patients was 29.9 ± 22.1 μg/L, which is lower than the concentration measured in individuals without CKDu in endemic (42.4 ± 43.4 μg/L) and nonendemic (39.9 ± 42.8 μg/L) sites. Rather than necessarily pointing to lower exposure, this result likely stems from the fact that the kidneys in CKDu patients may be inefficient in excreting these toxic elements via urine. In fact, if this excretion of these toxins is inefficient among CKDu patients, they may further accumulate in the kidney, and subsequently aggravate the disease.

Table 4. Summary of elemental concentrations in urine samples of individuals in the three groups

	Individuals with CKDu in endemic areas (n = 26)					Individuals without CKDu in endemic areas (n = 79)					Individuals without CKDu in nonendemic areas (n = 29)				
		Percentiles					Percentiles					Percentiles			
	Mean	25	50	75	Range	Mean	25	50	75	Range	Mean	25	50	75	Range
As	28.9 ± 22.2	15.4	22.8	35.1	2.72–89.4	42.9 ± 43.5	18.0	28.5	55.0	5.05–313	39.9 ± 42.8	13.7	27.6	41.1	2.67–164
Cd	0.36 ± 0.4	0.15	0.23	0.45	0.06–2.11	0.44 ± 0.35	0.19	0.34	0.59	0.015–1.59	0.73 ± 0.60	0.32	0.59	0.89	bd–2.34
Pb	0.79 ± 1.19	0.05	0.33	0.77	bd–4.96	1.43 ± 1.35	0.56	0.97	1.76	bd–5.30	1.9 ± 2.06	0.85	1.16	2.23	0.021–8.75
U	0.004 ± 0.02	bd	bd	bd	bd–0.09	0.003 ± 0.02	bd	bd	bd	bd–0.2	0.0007 ± 0.004	bd	bd	bd	bd–0.02
F^-	1.26 ± 1.47	0.51	0.67	1.19	0.11–5.8	1.84 ± 1.53	0.87	1.38	2.18	0.27–8.75	0.63 ± 0.37	0.34	0.58	0.83	0.103–1.59
B	567 ± 375	336	470	691	207–1900	1025 ± 638	570	886	1275	174–2858	992 ± 675	444	821	1409	123–2635
Mo	49.5 ± 69.3	14.4	34.5	57.2	5.1–360	81.8 ± 89	26.7	49.9	105	9.5–541	85 ± 95.7	20.3	55.5	112.9	2.94–427
Cr	61.8 ± 33.3	40.2	56.8	73.8	20.2–164	74.6 ± 40.6	42.9	66.8	96.6	10.5–196	93.4 ± 58.7	48.2	81.3	125.5	24.1–268
Al	10.8 ± 23.0	0.69	3.9	8.15	bd–114	7.8 ± 11.5	1.25	4.36	10.6	bd–83.4	8.9 ± 7.08	4.31	7.12	11.5	1.25–33
Mn	0.38 ± 0.95	bd	bd	0.35	bd–4.5	1.94 ± 15.7	bd	bd	0.13	bd–140	1.3 ± 6.17	bd	bd	0.43	bd
Li	7.6 ± 3.1	5.90	7.4	9.8	2.33–13.8	13.6 ± 9.3	6.7	10.8	17.6	2.9–54.4	11.2 ± 6.8	5.93	10.2	16.3	0.82–26.9
Be	bd	bd	bd	bd	bd	bd	bd	bd	bd	bd	bd	bd	bd	bd	bd
Co	0.35 ± 0.27	0.17	0.25	0.49	0.06–1.06	0.97 ± 0.86	0.39	0.76	1.21	0.05–4.94	0.99 ± 0.69	0.50	0.82	1.35	0.11–2.84

Ni	3.3 ± 3.4	1.23	2.2	4.5	0.43–16.1	6.8 ± 5.14	2.91	5.28	8.9	0.01–25.8	6.25 ± 4.46	2.46	6.19	8.78	0.015–17.9
Cu	13.2 ± 10.6	5.72	8.85	19.6	2.85–38.5	10.6 ± 13	4.13	7.1	11.8	0.43–96.2	10.0 ± 6.93	4.62	7.81	16.2	bd
Zn	306 ± 206	125	229	506	59.7–693	371 ± 343	158	267	435	54.5–2133	424 ± 512	154	189	553	20.6–2562
Rb	1220 ± 931	629	1019	1450	339–4006	1560 ± 1295	742	1146	1978	290–6016	2051 ± 1538	915	1633	2746	97.7–5812
Sr	137 ± 134	54.4	99.2	153	26.8–558	270 ± 244	102	188	340	25.6–1271	195 ± 163	73.6	154	261	21.8–665
Ag	0.01 ± 0.02	bd	bd	0.01	bd	0.02 ± 0.14	bd	bd	0.01	bd–1.22	0.008 ± 0.01	bd	bd	0.01	bd
Sb	0.03 ± 0.11	bd	bd	bd	bd–0.47	0.02 ± 0.1	bd	bd	bd	bd–0.67	0.0009 ± 0.01	bd	bd	bd	bd
Ba	1.67 ± 3.49	bd	0.18	1.94	bd–16.6	6.1 ± 9.4	0.01	2.64	8.9	bd–56	3.79 ± 6.47	bd	2.04	3.97	bd
Tl	0.15 ± 0.2	0.01	0.07	0.23	bd–0.84	0.28 ± 0.24	0.10	0.23	0.43	bd–1.12	0.46 ± 0.34	0.23	0.40	0.61	bd
Th	bd	bd	bd	bd	bd	0.024 ± 0.11	bd	bd	bd	bd–0.68	0.022 ± 0.12	bd	bd	bd	bd
Mg	49.8 ± 25.6	31	46.2	67.8	14–113.5	78.4 ± 59	40	63.4	96.4	9.37–301	91.5 ± 60	43.9	73.2	121.7	13.6–223
Ca	46.9 ± 40.9	18.8	32.6	63.8	8.9–167	97 ± 87	35.3	77.9	117	5.3–383	80 ± 62.3	32.2	57.7	134	6.2–221

Looking more closely at the distribution of individuals' urine concentrations of As, Cd, Pb, and F^- in these groups, we do not note large differences across groups. The majority of individuals in the CKDu group have As concentration between 20 and 40 μg/L, Cd below 0.25 μg/L, Pb below 1 μg/L, and F^- between 0.5 and 1.5 mg/L. In comparison, a large proportion of non-CKDu individuals in nonendemic areas have As concentrations between 20 and 40 μg/L, Cd between 0.5 and 1 μg/L, Pb between 1 and 3 μg/L, and F^- between 0.5 and 1.5 mg/L (Fig. 3). The mass of the frequency distribution in each of the three groups lies toward the lower end of these concentration ranges, and a somewhat larger proportion of subjects identified with CKDu have low urinary concentrations of Cd and Pb.

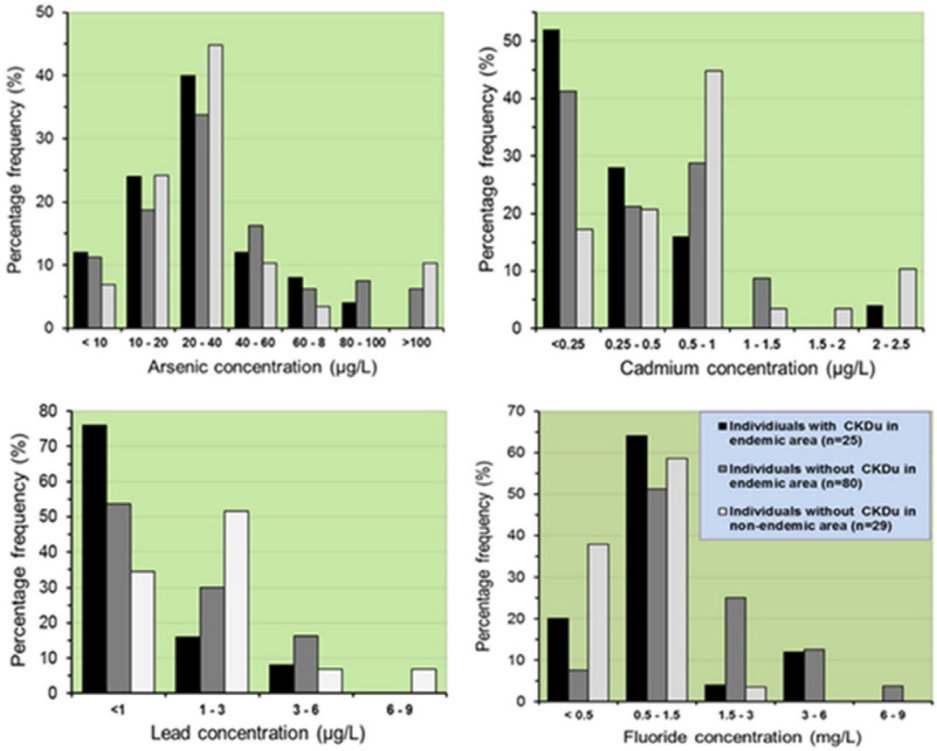

Figure 3. Percentage of urinary As, Cd, Pb, and F^- distribution in the urine of subjects with CKDu (n = 25) in endemic areas, and non-CKDu subjects in endemic (n = 80) and nonendemic areas (n = 29).

3.5. Microalbuminaria in urine

The prevalences of microalbuminuria (albumin-creatinine ratio; ACR of 30–300 mg/g), and macroalbuminaria (ACR of N300 mg/g) were used to further investigate the possibility of kidney damage (via increased glomerular permeability to proteins) in study individuals (National Kidney Foundation, 2002). Albumin is the most common circulating protein in blood and is not normally present in urine. The presence of albumin in urine can also indicate tubular dysfunction, reflecting inability of the proximal tubules to fully reabsorb it. When the amount of albumin leaking across the glomerular capillaries exceeds the ability

of the tubules to reabsorb, albumin may leak into urine, leading to albuminuria. Nephrotoxic elements such as Cd may also affect glomerular function subsequent to the primary tubular effects (Nogawa, 1984; Järup et al., 1995; Åkesson et al., 2005).

We measured significantly higher albumin and lower creatinine in CKDu subjects than in non-CKDu individuals. Nearly all individuals with clinically confirmed CKDu in our sample (22 of 25 subjects) were found to have ACR ≥ 30 mg/g (Fig. 4), and macroalbuminuria was detected in 40% (n = 10) of the subjects. Among non-CKDu subjects in the endemic and nonendemic areas, we found lower rates with ACR ≥ 30 mg/g (11 of 52 (21%) in non-CKDu subjects in endemic areas, and 4 of 18 (22%) in nonendemic areas), and no instances of macroalbuminaria were detected among non-CKDu individuals. Among the 25 CKDu subjects, 8 and 5 subjects were also reported to have hypertension and diabetes, respectively; whereas only 4 of 108 subjects among the other two subsamples reported being hypertensive or diabetic. The co-occurrence of hypertension, diabetes, and macroalbuminaria could be a consequence of tubular damage on glomerular filtration or the direct effect of environmental agents or lifestyle factors on glomeruli. The high rates of hypertension and diabetes in CKDu subjects are likely consequences of the kidney damage from the disease.

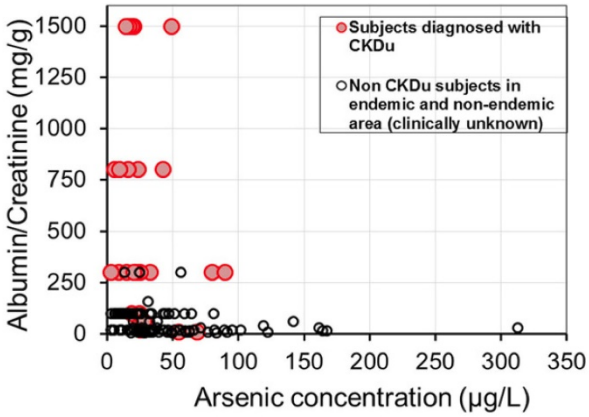

Figure 4. Relationship between As concentration and albumin-creatinine ratio in urine of CKDu subjects and non-CKDu individuals in endemic and nonendemic areas. [**Note:** Samples with 10 mg/L of albumin and 0.1 g/L of creatinine measurements (i.e., ACR of 100 mg/g) are considered as too dilute to accurately determine ratios. These samples include 40 subjects in endemic and nonendemic areas (2 of them are CKDu subjects).]

We note that the majority of CKDu subjects (n = 26) had a long-term (over several decades) habit of consuming alcohol (85%; n = 22) and tobacco (78%; n = 20). As shown previously in Table 2, many of these patients also reported that they had stopped consuming alcohol or tobacco products by the time of the survey (perhaps due to medical advice), and rates of consumption of both were also high among male adults in the comparison groups not affected by CKDu. Alcohol consumption is primarily of a local drink called "arack," which is known for its high alcohol content, while tobacco consumption takes the form of

chewing and cigarette smoking. Both of these likely speed the progression of the CKDu, leading to faster end-stage renal failure.

Many studies in Sri Lanka have reported that CKDu predominantly affects males. The aforementioned risk factors—chronic alcohol consumption, cigarette smoking, and betel chewing—were generally more common among the randomly selected sample of men (excluding CKDu subjects due to nonrandom selection of CKDu cases) in our sample (37% of males smoked or chewed tobacco) than among women (only 6%). In this group, we also found that Cd concentrations in urine of cigarette smokers (0.61 ± 0.48 μg/L; n = 39) were higher than those of nonsmokers (0.48 ± 0.43 μg/L; n = 69) (p = 0.107). Among the non-CKDu subjects, we also found significantly higher urinary concentrations (p = 0.001) of Cd (0.65 ± 0.5 μg/L; n = 57) in male subjects than females (0.38 ± 0.32 μg/L; n = 51). The higher Cd excretion found in men in our study can perhaps be explained by additional Cd intake from chronic cigarette smoking and tobacco chewing. This is in contrast to other study findings that have found higher urinary Cd in women than in men (Jarup et al., 1998; Vahter et al., 2007; Ferraro et al., 2010).

4. Discussion

Previous studies related to CKDu in Sri Lanka have suggested the possibility that water resources are the main source of toxic elements that contribute to the development of this disease (e.g., Jayasumana et al., 2013;Wanigasuriya et al., 2011). This study explored the concentrations of inorganic contaminants in drinking water sources from CKDu endemic and nonendemic areas, and we were unable to establish a clear link between nephrotoxic contaminant levels and the presence of CKDu. The concentrations of the most important nephrotoxic elements (As, Cd, U, and Pb) from drinking water sources in all sample areas were below 1.6 μg/L, and were all below the water quality guideline limits for these contaminants suggested by the WHO, US EPA, and EU. Though we do not expect that the concentrations of these elements would vary greatly over time, it should be noted that we collected samples at only one point in time, and that future studies might more carefully track whether they vary across seasons.

In addition, there was no significant difference between the water and urinary concentrations of As, Cd, Pb, and U from residents in endemic and nonendemic regions, and we found little relationship between these elements in water and urine, which suggests the possibility of other important sources (e.g., food) of these elements in the study population. In fact, the mean urinary concentrations of As, Cd, Pb, U, and a range of other inorganic elements in individuals with CKDu were consistently lower than individuals without CKDu in both endemic and nonendemic areas. This systematic difference likely indicates declines in the kidney's ability to remove toxics in CKDu patients, which leads to bioaccumulation of such toxics in kidney, and therefore more rapid progression of the disease towards end-stage renal failure.

The urinary levels of As, Cd, Pb, and U measured in this study were similar to reference values found in healthy populations in areas without known exposure risks. In a study conducted in Germany (n = 4741 urine samples), a reference value of 15 μg/L of As was used as a benchmark concentration (Wilhelm et al., 2004, 2005). Reimann and de Caritat

(1998) reported the mean As concentrations of 16.7 μg/L in the urine of individuals from uncontaminated areas of Lombardy, Italy. The American Conference of Governmental Industrial Hygienists (ACGIH) provides an occupational biologic effect index (BEI) of 35 μg/L for urinary inorganic As plus metabolites (ACGIH, 2001). Similarly, NRC (1999) reported international values of As concentrations below 50 μg/L. In our study, about 77.6% (n = 104) individuals excreted urinary As above the 15 μg/L reference level from the German study, while 35% (n = 47) and 23% (n = 31) of the study subjects had As above 35 (BEI value) and 50 μg/L (international value), respectively.

In our study, the As concentrations measured in drinking water were all below 1 μg/L. Thus, it is not surprising that we also found urinary As levels to be uncorrelated with levels of As in drinking water. In regions with low As exposure via drinking water, diet is the primary exposure route (European Food Safety Authority, 2009). In Sri Lanka, and other Asian countries, foods such as rice and other grains are commonly consumed, and may contribute to inorganic As exposure in the population. Fish consumption is also a well-recognized source of the organic form As (e.g., arsenobarite) (Cullen and Reimer, 1989; Heinrich-Ramm et al., 2002), and was a common element in the diet of our sample of Sri Lankan households. Arsenobetaine is a nontoxic arsenic species that is rapidly excreted unmetabolized via the kidneys, which contributes to total urine As (Le et al., 1993; Sabbioni et al., 1991; Navas-Acien et al., 2011). To more fully characterize the inorganic and organic components of As in this sample, As speciation analysis of urine and major food items would be required.

While we measured urinary As concentrations reaching 313 μg/L among individuals living in the North Central and other provinces in Sri Lanka, no symptoms of As toxicity (e.g., skin lesions) were observed during field examinations. In studies from West Bengal and Bangladesh, arsenic skin lesions were found among adults when the As concentrations in drinking water rose above 300 μg/L, and urinary concentrations ranged between 10 and 3147 μg/L (mean: 180 μg/L; n = 9700) and 24 to 3086 μg/L (mean: 495 μg/L; n = 1000), respectively (Chowdhury et al., 2000). The levels we observed in Sri Lanka were well below these levels, though some individuals had urinary As levels that were higher than reference ranges.

Nonetheless, emerging evidence indicates the potential for adverse health effects from inorganic As exposure at relatively low exposure levels common to many populations worldwide, including an increased risk of cancer, cardiovascular and respiratory conditions, and diabetes mellitus (Ettinger et al., 2009; European Food Safety Authority, 2009; Karagas et al., 2001; Leonardi et al., 2012; Navas-Acien et al., 2011; Sohel et al., 2009). Chen et al. (2011) and Zheng et al. (2013) have reported As exposure and its association with proteinuria in chronic kidney disease patients. Similarly, Hsueh et al. (2009) have shown that increased urinary As is linked with decreased glomerular filtration rate. In their study, the concentration of As in the drinking water ranged from bd to 4 μg/L (average of 0.7 μg/L), and total urinary As (~ the majority had below 100 μg/g) was comparable with what we found in our work.

The urinary Cd measured in this study is consistent with that found in other studies conducted in healthy unexposed populations. Reference ranges of Cd in urine from the literature include 0.38–1.34 μg/L (Minoia et al., 1990), 0.05–1.64 μg/L (White and Sabbionia,

1998), 0.005–0.46 μg/L (Rodushkin et al., 2001), and < 0.8 μg/L (Wilhelm et al., 2004). In our data, about 20% of 134 subjects exceeded 0.8 μg/L Cd in urine, although a considerable number of the subjects in our study are chronic tobacco consumers, which is a common source of exposure to Cd. The kidney is the main target organ for Cd toxicity, and Cd has been linked to kidney damage (Jarup and Akesson, 2009; Nordberg, 2009; WHO, 2011). Concern about environmental Cd first emerged with the outbreak of Itai-itai disease in Japan earlier in the 1950s (Chaney et al., 1998). Itai-itai disease was shown to be due to exposure to Cd in runoff from mining tailings into paddy fields, and subsequent ingestion of contaminated rice by humans (Kabata-Pendias and Mukherjee, 2007). The disease resulted in severe bone malformations in elderly women and kidney damage.

Urinary Cd concentration is mainly influenced by the burden of Cd accumulated in the kidney, and is considered to be a reliable biomarker of long-term Cd exposure. Cadmium is efficiently retained in the kidney and has a long biological half-time of around 10–30 years. Urinary Cd concentration is proportional to the concentration in the kidney. A safe intake of 7 μg Cd/week/kg body weight was set based on the critical renal Cd concentration of between 100 and 200 μg/g wet weight that corresponds to a urinary threshold limit of 5–10 μg/g creatinine (i.e., a urine-to-kidney ratio of 1:20) (WHO, 1993). An important new study by Akerstrom et al. (2013b) reported that a kidney cadmium content of 25 μg/g corresponded to a urinary cadmium of 0.42 μg/g creatinine (i.e., a urine-to-kidney ratio of 1:60). This ratio suggests a higher Cd accumulation at low urinary cadmium excretion than what has been estimated previously.

The effect of Cd on kidney at high exposure is well recognized; however, considerable uncertainty remains on the clinical significance of the renal effects of Cd at low levels of exposure. In fact, many studies have shown early adverse health effects at much lower levels of Cd exposure than previously anticipated. In a cross-sectional study conducted in Swedish women (53–64 years old; n = 820), Åkesson et al. (2005) showed low Cd levels (< 1.3 μg/L) associated with clear effects on tubular and glomerular. Other studies have also revealed adverse kidney effects at urinary Cd levels < 0.5 μg/g creatinine (Satarug and Moore, 2004). In contrast, a recent study by Thomas et al. (2014) found no association between low levels of Cd exposure (for the average intake of 13 μg/day Cd in women, and 19 μg/day in men), and increased renal dysfunction in adults more than 44 years old in Sweden. In a subsample of women from their study, the median urinary Cd concentration was 0.34 μg/g creatinine (Engstrom et al., 2011),with only 1.7% having concentrations > 1 μg/g creatinine. Similarly, Akerstrom et al. (2013a) and Chaumont et al. (2012) suggest that coexcretion of Cd with proteins in urine may be indicative of normal physiology among individuals with kidney damage, though a toxic effect of Cd cannot be ruled out. There appears to be a need for additional research to clarify the role of exposure to low doses of Cd in causing kidney injury.

Two other contaminants of concern were also found at low concentrations in the urine samples we collected: Pb (below8.8 μg/L and mean 1.4 ± 1.5 μg/L) and U (below 0.2 μg/L and mean 0.003 ± 0.02 μg/L). These levels are comparable to what has been reported in other healthy and uncontaminated areas (White and Sabbionia, 1998; Rodushkin et al., 2001). The level of Pb exposure, which may be associated with early adverse renal effects, is uncertain because of the lack of appropriate blood or urinary biomarkers reflecting an

early adverse effect on the kidney (Skerfving et al., 1998). With regard to U, sample individuals' urinary levels were generally significantly lower than those from other studies of patients with altered tubular function (e.g., Kurttio et al., 2002).

There are also many locations globally where drinking water contains much higher levels of the main inorganic nephrotoxic contaminants of concern than what we found in Sri Lanka. In the Main Ethiopian Rift (MER), for example, most drinking groundwater wells have high levels of F^-, As, and U (Rango et al., 2012, 2014; Merola et al., 2014). Despite these high concentrations, the exposed MER communities, which are characterized by a semi-arid climate, do not show similar cases of CKDu (as documented in field observations and local communication with medical experts in the local hospital). Even so, it would be premature to rule out the possibility that low-dose interactions among contaminants could be responsible for the high CKDu rates found in some parts of Sri Lanka.

One of the important limitations of this study is that organic contaminants in water and non-water-related risk factors for CKDu could not be thoroughly investigated. Therefore, the role of exposures to agrochemicals and ingestion of both inorganic and organic contaminants related to use of these chemicals deserves additional study. In fact, the primary occupation in the CKDu endemic areas is rice farming, and this labor intensive work is done under hot and humid conditions. People in low-elevation rice farming areas may also be vulnerable to excessive work, sweating, and dehydration, which could potentially put them at greater risk of kidney injury (Roncal-Jimenez et al., 2014), particularly if work patterns and habits have been changing over time as a result of intensification of farming. Finally, we observed that CKDu patients reported more frequent alcohol and cigarette consumption in the past than the comparison groups, suggesting possible risk factors for CKDu progression. Other research from the same region has also found similar patterns (e.g., Senevirathna et al., 2012; Jayatilake et al., 2013).

5. Conclusion

This is one of the first studies conducted to better understand the concentrations of a range of inorganic contaminants in drinking water and urine of individuals from rural farming communities in Sri Lanka where CKDu is endemic and nonendemic. The measured levels of inorganic nephrotoxicants such as, Cd, Pb, and U in all water sources were found to be well below the guideline values for drinking water. In addition, no significant difference in the concentrations of As, Cd, Pb, U, and other elements were found across drinking water sources from endemic and nonendemic zones. Inorganic contamination of drinking water sources therefore seems unlikely to be the cause of CKDu in this region. We also found that urinary As, Cd, Pb, and U concentrations in rural Sri Lankan communities were comparable with those in healthy and uncontaminated populations in the world. The lack of correlation between the levels of these elements in water and urine suggests the presence of nonwater sources of these toxic chemicals in the region, presumably from food and other sources such as tobacco consumption. We also highlight that previous literature has suggested an association between As exposure and CKD, and between Cd exposure and kidney disease, even at low doses. Although the clinical relevance of these findings is controversial

and remains unclear, similar low exposures were found in this study, and interactions between low doses of these and other contaminants (such as Pb and U) cannot be ruled out. Thus, further cohort studies with larger sample sizes encompassing both endemic and non-endemic areas will be required to evaluate low-exposure effects of As and Cd on biomarkers of tubular damage. Such studies should also devote greater attention to the potential role of other risk factors—namely alcohol and tobacco consumption, and agrochemical use and diet, but also other sociodemographic and lifestyle factors—in contributing to kidney disease. Differences among such factors were found between CKDu and non-CKDU individuals included in this study.

6. Limitations

Because this analysis used data from a single cross-section and in a relatively small sample, changes over time (with respect to drinking water sourcing and quality) and low statistical power may affect the results. CKDu patients in this study were not randomly enrolled (i.e., they were identified with the help of local communities and health officials) and were at advanced stages of the disease, and thus they are likely not representative of the sample of CKDu patients in endemic areas. Also, several potentially useful measurements could not be included in this study: (1) dietary intake of nephrotoxic elements through food, which would be necessary to better assess total exposures, (2) analyses of tubular proteinuria as biomarkers of tubular damage, which would be needed to evaluate the association between low-to-high exposure of nephrotoxicants and kidney effects; (3) measures of exposure to agrochemicals in water, food, and biological samples; (4) Arsenic (As) speciation measurement (analysis of inorganic As and its metabolites = monomethylarsonic acid and dimethylarsinic acid); and (5) precise measures of urinary creatinine. All of these aspects are worthy of further investigation in future studies.

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Supplemental Material

Supplementary Material Table S1: Distribution of individuals in endemic and nonendemic locations in this study. All except the Central Province in Kandy are endemic areas.

Village name	District	Prov-ince	Endemic CKDu area	Confirmed CKDu cases	Total number of subjects en-rolled
B-Yaya	Anuradhapura	NCP	Yes	3	9
Huruluwewa	Anuradhapura	NCP	Yes	1	2
Iluppukanniya fishing village	Anuradhapura	NCP	Yes	0	7
Kodiyabendawewa	Anuradhapura	NCP	Yes	1	8
Mahadiwulwewa	Anuradhapura	NCP	Yes	0	7
Mahahalmillewa	Anuradhapura	NCP	Yes	2	6
Padikaramaduwa	Anuradhapura	NCP	Yes	0	4
Parasangaswewa	Anuradhapura	NCP	Yes	1	2
Ruwangama	Anuradhapura	NCP	Yes	0	1
Thanthirimale	Anuradhapura	NCP	Yes	4	10
Siyambalagaswewa	Polonnaruwa	NCP	Yes	0	3
Weerapura	Polonnaruwa	NCP	Yes	4	7
Lankapura	Vavuniya	NCP	Yes	1	4
Mahamailankulama	Girandu-rukotte	Northern	Yes	2	6
Ulhitya	North Badulla	Uva	Yes	0	8
Rathkinda	Ampara	Uva	Yes	2	6
Dolakanda	Kandy	Eastern	Yes	5	15
Kaikatuwa	Kandy	Central	No	0	2
Kalugamuwa	Kandy	Central	No	0	11
Ambuluwawa (Watte Kade)	Kandy	Central	No	0	16

Supplementary Material Table S2: Summary of pH, EC, Eh, DO and major and trace elements in waters sampled from ground-water samples (dug and tube wells), cold springs, and irrigation in the study area (mainly from NCP). Note: bd = below detection.

	Min - Max	Percentiles			Mean ± SD	Min - Max	Mean ± SD	Min - Max	Mean ± SD	Mean ± SD	Mean ± SD
		25	50	75							
Water types	**DUG WELLS (n=37)**					**TUBE WELLS (n=10)**		**SPRINGS (n=9)**		**IRRIGATION (n=2)**	**PIPE WATER (n=3)**
pH	5.45 - 8.0	6.6	7.00	7.50	7.0 ± 0.55	6.06 - 7.63	6.83 ± 0.61	4.99 - 7.21	6.27 ± 0.86	7.88 ± 0.13	7.46 ± 0.14
EC (µS/cm)	188 - 1029	382	560	848	597 ± 252	369 - 1064	679 ± 248	34.2 - 140.2	75.1 ± 44.2	368 ± 22.1	855 ± 726
DO (mg/L)	1.20 - 7.30	2.2	2.50	3.50	2.82 ± 1.06	1.6 - 3.9	2.53 ± 0.65	0.24 - 3.35	2.0 ± 0.8	2.65 ± 0.27	4.28 ± 2.97
Eh (mV)	14.0 - 123	52.0	72.0	87.0	71.1 ± 26.2	24 - 131	80.2 ± 37.9	20 - 143	101 ± 39.5	37 ± 5.66	52 ± 2.0
Temp. (^{O}C)	26.1 - 30.2	27.0	27.7	28.5	27.8 ± 1.03	26.9 - 30.2	28.9 ± 1.02	23 - 26.6	25.2 ± 1.57	29.8 ± 2.69	27.7 ± 1.27
Major elements (mg/L)											
F^-	**0.095 - 4.3**	**0.36**	**0.49**	**0.98**	**0.76 ± 0.73**	**0.49 - 1.78**	**0.92 ± 0.37**	**bd - 0.19**	**0.05 ± 0.05**	**0.26 ± 0.01**	0.71 ± 0.52
Cl^-	7.7 - 168	16.4	26.3	57.7	41.5 ± 35.8	6.67 - 84.7	37 ± 26.9	0.83 - 17.2	5.13 ± 6.73	35.7 ± 0.65	87.5 ± 120
NO_3^-	bd - 20.6	bd	bd	0.48	1.19 ± 3.68	bd - 16.4	1.84 ± 5.16	bd - 3.71	0.63 ± 1.32	bd	7.76 ± 10.0
SO_4^{2-}	bd - 48.2	9.92	14.3	18.9	15.3 ± 9.33	5.1 - 32.6	18.3 ± 9.05	bd - 1.56	0.49 ± 0.47	4.3 ± 0.05	17.2 ± 4.45
Ca^{2+}	8.3 - 104	27.98	45.5	74.0	51.5 ± 27.0	21.6 - 92.3	60.8 ± 25.9	0.35 - 10.0	4.5 ± 3.4	23.6 ± 1.72	47.6 ± 31.7
Mg^{2+}	2.0 - 81	12.66	17.2	25.8	20.5 ± 14.7	10.5 - 40.3	22.2 ± 9.06	0.24 - 4.8	2.6 ± 1.57	11.0 ± 0.39	37.3 ± 40.5
Na^+	bd - 88.7	14.40	30.5	47.6	31.9 ± 21.6	bd - 77.3	35.4 ± 29.3	1.48 - 27.3	8.48 ± 8.01	29.3 ± 1.14	25.7 ± 40.3
SiO_2	33.4 - 122	78.6	96	107	90.5 ± 22.9	76.6 - 127	96 ± 14.1	14.5 - 49.6	24.2 ± 12.4	1.1 ± 0.11	73.6 ± 53.7
HCO_3^-	83.5 - 502	183.8	269	317	262 ± 103	198 - 494	332 ± 109	9.16 - 77.6	33.7 ± 23.6	139 ± 0.78	294 ± 232
$CaCO_3$	68.5 - 411	150.6	220	260	215 ± 84.5	162 - 405	272 ± 89.5	7.50 - 63.6	27.6 ± 19.4	114 ± 0.64	241 ± 190

Trace elements (µg/L)											
As	*bd - 0.86*	*bd*	*bd*	*bd*	*0.05 ± 0.16*	*bd - 0.62*	*0.09 ± 0.2*	*bd*	*bd*	*bd*	0.15 ± 0.26
Cd	*bd - 0.04*	*bd*	*bd*	*0.01*	*0.007 ± 0.01*	*bd - 0.05*	*0.02 ± 0.01*	*0.001 - 0.03*	*0.001 ± 0.01*	*0.01 ± 0.01*	0.01 ± 0.01
Pb	*bd - 1.04*	*bd*	*bd*	*bd*	*0.037 ± 0.17*	*bd - 1.59*	*0.34 ± 0.46*	*bd - 1.10*	*0.15 ± 0.36*	*bd*	0.15 ± 0.24
U	*bd - 0.96*	*0.001*	*bd*	*0.32*	*0.17 ± 0.24*	*bd - 0.7*	*0.3 ± 0.26*	*bd - 0.14*	*0.016 ± 0.05*	*0.17 ± 0.01*	0.39 ± 0.36
Se	bd - 1.2	bd	bd	0.10	0.12 ± 0.27	bd - 0.78	0.16 ± 0.33	bd	0.02 ± 0.06	0.06 ± 0.08	0.9 ± 1.0
B	3.03 - 133	12.5	32.8	61.4	38.6 ± 30.2	12.4 - 95	57 ± 30.4	2.80 - 41.0	12.6 ± 16.1	26.9 ± 1.79	61.4 ± 49.7
V	0.50 - 36.8	5.6	12.9	22.6	13.6 ± 9.54	1.75 - 32.7	19.9 ± 12.0	0.14 - 2.6	0.78 ± 0.79	1.15 ± 0.02	31.9 ± 30.5
Mo	bd - 3.7	bd	0.16	0.70	0.6 ± 0.94	bd - 5.2	1.16 ± 1.71	bd - 0.05	0.01 ± 0.02	bd	0.57 ± 0.53
Cr	0.11 - 5.4	0.54	0.90	1.93	1.39 ± 1.17	0.23 - 3.8	1.64 ± 1.31	bd - 0.75	0.28 ± 0.30	0.85 ± 0.14	2.79 ± 3.84
Al	4.1 - 80	8.8	9.87	11.5	12.4 ± 12.3	bd - 26.2	10.6 ± 6.8	1.16 - 27.0	16.9 ± 7.80	16.8 ± 4.77	22.1 ± 24.6
Mn	0.07 - 237	2.1	8.10	57.7	39.3 ± 60.5	0.33 - 153	34.9 ± 49.6	0.51 - 13.0	4.04 ± 4.10	25.6 ± 31.1	0.45 ± 0.42
Li	0.21 - 10.8	0.84	1.95	3.54	2.68 ± 2.44	1.13 - 8.84	3.73 ± 2.65	0.007 - 3.9	1.05 ± 1.45	0.04 ± 0.01	1.58 ± 0.52
Be	bd - 0.06	bd	bd	bd	0.0016 ± 0.01	bd	bd	bd - 0.12	0.03 ± 0.05	bd	bd
Fe	29.7 - 211	47.2	76.0	129	91.8 ± 49.9	28.6 - 214	116 ± 62.7	0.19 - 30	10.5 ± 8.84	32.3 ± 3.0	78.6 ± 57.8
Co	0.08 - 0.47	0.14	0.21	0.27	0.22 ± 0.09	0.14 - 1.15	0.32 ± 0.31	0.02 - 1.27	0.33 ± 0.47	0.21 ± 0.05	0.14 ± 0.07
Ni	0.94 - 6.0	2.28	3.13	3.84	3.05 ± 1.26	1.47 - 4.63	3.27 ± 1.24	0.11 - 3.41	1.01 ± 1.34	1.23 ± 0.06	1.92 ± 1.18
Cu	bd - 8.73	bd	bd	0.16	0.42 ± 1.46	bd - 17.1	5.54 ± 5.43	bd - 4.75	0.59 ± 1.56	0.31 ± 0.16	1.34 ± 0.68
Zn	2.08 - 41.4	4.98	7.87	10.5	8.66 ± 6.55	4.13 - 36.5	13.5 ± 9.52	1.01 -131	30.2 ± 43.6	2.88 ± 0.16	10.1 ± 10.1
Rb	0.10 - 8.18	0.39	0.52	0.84	1.11 ± 1.76	0.14 - 9.6	1.6 ± 2.86	0.65 - 9.08	3.10 ± 2.73	6.73 ± 0.03	1.19 ± 1.56
Sr	30.5 -1921	165.0	269	472	354 ± 324	77.4 - 1180	546 ± 418	3.45 - 70.2	24.9 ± 21.9	149 ± 0.54	594 ± 640
Ag	bd - 0.02	bd	bd	bd	0.0005 ± 0.003	bd - 0.001	0.00011 ± 0.00034	bd - 0.01	0.001 ± 0.002	bd	bd
Sb	bd	bd	bd	bd	bd	bd - 0.22	0.02 ± 0.07	bd	bd	bd	bd
Ba	23.5 - 440	45.1	97.8	198	137 ± 114	13.5 - 471	191 ± 173	11.1 - 57.2	25.2 ± 18.8	106 ± 2.09	168 ± 129
Br	bd - 0.31	bd	bd	0.14	0.065 ± 0.09	bd - 0.16	0.06 ± 0.07	bd	bd	bd	0.19 ± 0.24
Tl	bd - 0.02	bd	bd	bd	0.0005 ± 0.003	bd	bd	bd - 0.05	0.01 ± 0.02	bd	bd
Th	bd - 0.06	bd	bd	bd	0.0017 ± 0.01	bd - 0.03	bd ± 0.01	bd	bd	bd	bd

Supplementary Material Table S3: Summary of pH, EC, Eh, DO and major and trace elements concentrations in 61 water samples

	Min	Max	Percentiles			Mean	Water quality standards			Samples Exceeding the standards
			25	50	75		WHO	U.S.EPA	EU	
pH	4.99	8.04	6.53	6.90	7.46	6.92±0.68				
EC (µS/cm)	34.2	1589	331	461	809	538±330				
DO (mg/L)	0.24	7.70	2.10	2.48	3.15	2.72±1.17				
Eh (mV)	14.0	143	50.5	77.0	100	75.9±31.5				
Temp. (^{O}C)	23.0	31.7	26.7	27.8	28.6	27.7±1.6				
Major elements (mg/L)										
F^-	0.0003	4.26	0.27	0.49	0.95	0.66±0.66	1.5	4.0	1.5	4
Cl^-	0.83	225	12.5	24.8	52.4	38.0±40.8	–	–	–	–
NO_3^-	0.00	20.6	0.00	0.00	0.92	1.52±4.27	50	45	50	none
SO_4^{2-}	0.00	48.2	5.70	13.3	18.9	13.6±9.94	–	–	–	–
Ca^{2+}	0.37	106	20.2	36.3	65.1	42.4±29.4	–	–	–	–
Mg^{2+}	0.24	82.4	8.40	16.5	23.4	18.7±16.1	–	–	–	–
Na^+	0.00	88.7	10.4	28.4	44.4	28.6±23.4	200[a]	–	–	–
SiO_2	1.03	127	51.1	92.4	102.3	77.9±35.3	–	–	–	–
HCO_3^-	9.2	502	140.6	230	323	237±136	–	–	–	–
$CaCO_3$	7.51	411	115	189	265	195±112	–	–	–	–

Trace elements (µg/L)

As	**bd**	**0.86**	**bd**	**bd**	**bd**	**0.05±0.16**	**10**	**10**	**10**	none
Cd	**bd**	**0.05**	**0.0016**	**0.01**	**0.01**	**0.01±0.01**	**3**	**5**	**5**	none
Pb	**bd**	**1.59**	**bd**	**bd**	**0.06**	**0.11±0.29**	**10**	**15**	**10**	none
U	**bd**	**0.96**	**bd**	**0.05**	**0.33**	**0.18±0.24**	**15**	**30**	–	none
Se	bd	2.01	bd	bd	0.10	0.15±0.35	40[a]	50	10	none
B	2.81	133	8.66	30.9	66.7	38.5±31.5	2,400	–	1,000	none
V	0.14	62.1	2.06	9.46	22.6	13.3±12.7	–	–	–	–
Mo	bd	5.20	bd	0.05	0.68	0.58±1.04	–	–	–	–
Cr	bd	7.20	0.49	0.77	1.85	1.32±1.37	50	100	50	none
Al	bd	80.3	8.89	10.1	13.8	13.4±11.6	–	200[a]	–	–
Mn	0.07	237	1.08	5.06	37.0	31.0±52.8	400	50[a]	–	–
Li	0.01	10.8	0.79	1.92	3.41	2.47±2.4	–	–	–	–
Be	bd	0.12	bd	bd	bd	0.01±0.02	–	4	–	none
Fe	0.19	214	34.1	68.6	120	81.2±57.5	–	–	–	–
Co	0.02	1.27	0.14	0.20	0.27	0.25±0.23	2,000	13,000	2,000	none
Ni	0.11	6.04	1.54	2.72	3.65	2.67±1.46	70	–	20	none
Cu	bd	17.1	0.00	0.07	0.99	1.33±3.10	2000[a]	–	–	–
Zn	1.01	131	4.46	7.87	12.6	12.5±18.9	5000[a]	–	–	–
Rb	0.10	9.61	0.39	0.65	1.69	1.67±2.35	–	–	–	–
Sr	3.5	1921	112	244	472	342±360	–	4000	–	–
Ag	Bd	0.02	bd	bd	bd	0.0005±0.0025	–	–	–	–
Sb	Bd	0.22	bd	bd	bd	0.0036±0.03	20	6	5	none
Ba	11.1	471	32.9	91.1	200	130±123.2	700	–	–	–

Br	Bd	0.46	bd	bd	0.11	0.06±0.09	700	2,000	–	none
Tl	Bd	0.05	bd	bd	bd	0.0014±0.01	–	2	–	none
Th	Bd	0.06	bd	bd	bd	0.0016±0.01	–	–	–	–

Notes: bd = below detection; [a] Secondary standards.
Regulatory limits for inorganic chemicals in drinking water established by the WHO (2011), the U.S. EPA (2009), and the EU Council (1998).

Supplementary Material Table S4: Summary of major and trace elements in drinking water samples for the three study groups.

Drinking Water Quality	Individuals with CKDu in endemic areas				Individuals without CKDu in endemic areas				Individuals without CKDu in nonendemic areas			
	Mean ± SD	Percentiles 25	50	75	Mean ± SD	Percentiles 25	50	75	Mean ± SD	Percentiles 25	50	75
Parameter												
pH	7.1±0.57	6.6	7.20	7.43	6.9±0.48	6.55	6.83	7.28	6.08±0.98	4.99	6.74	6.88
EC (µS/cm)	634±241	422	606	837	649±286	408	595	855	76.8±50.3	46.3	46.3	117
DO (mg/L)	3.16±1.48	2.50	2.70	3.50	2.84±0.89	2.20	2.50	3.50	1.36±0.89	0.24	1.90	2.10
Eh (mV)	64.2±24.7	48.0	56.0	84.0	75.3±25.2	52.0	74.0	86.0	68.1±41.0	20.0	86.0	98.0
Temp. (^{O}C)	28±0.88	27.2	28.0	28.6	28±1.16	27.0	28.1	28.6	26±0.99	26.1	26.5	26.5
Major elements (mg/L)												
F^-	1.0±0.85	0.44	0.80	1.43	0.91±0.79	0.44	0.65	1.04	0.05±0.06	0.00	0.03	0.06
Cl^-	31.4±24.8	12	29.3	36.4	47±48.8	19.4	28.4	54.1	3.32±4.83	0.85	0.86	3.19
NO_3^-	0.51±0.93	0.00	0.00	1.15	1.33±3.98	0.00	0.00	0.92	1.62±2.44	0.00	1.27	1.27
SO_4^{2-}	14.3±7.23	7.65	14.9	18.9	14.2±8.16	8.41	14.2	18.9	0.59±0.66	0.32	0.40	0.52
Ca^{2+}	52.2±24.7	32.5	55.0	66.0	49.9±25.9	29.8	51.4	66.0	4.86±6.78	0.37	2.74	4.90
Mg^{2+}	20.6±14.3	12.1	18.4	25.4	22.9±17.8	13.2	18.4	25.8	1.78±1.53	0.24	1.88	2.64
Na^+	38.6±25.5	14.4	35.2	52.5	33.9±22	22.6	32.8	48.4	8.3±7.55	3.66	3.66	11.3
Si	42.7±11.2	38.9	45.8	49.2	43.5±10.3	41.7	45.8	49.4	11.2±5.12	8.35	8.83	12.5
HCO_3^-	275±107	186	289	342	287±107	188	289	342	30.6±27.3	9.16	20.3	34.6
$CaCO_3$	226±88	152	237	280	236±87.8	154	237	280	25.1±22.4	7.51	16.6	28.3

Trace elements (µg/L)												
As	0.061±0.21	bd	bd	bd	0.026±0.08	bd	bd	bd	bd	bd	bd	bd
Cd	0.008±0.008	bd	0.01	0.02	0.009±0.01	bd	0.01	0.01	0.01±0.007	0.01	0.01	0.01
Pb	0.17±0.26	bd	bd	0.42	0.134±0.31	bd	0.003	0.098	0.45±0.52	bd	0.15	1.10
U	0.18±0.24	0.003	0.07	0.35	0.23±0.23	0.00	0.14	0.40	0.056±0.07	bd	0.01	0.14
Se	0.17±0.32	bd	0.04	0.13	0.14±0.38	0.00	0.00	0.05	0.069±0.09	bd	bd	0.18
B	40.1±36	9.80	27.8	74.6	42.4±32.7	12.2	30.9	68.5	6.69±9.58	3.43	3.66	5.54
V	16.3±10.5	5.41	21.9	23.7	16.1±12.9	6.85	12.7	24.6	0.58±0.72	0.14	0.48	0.61
Mo	0.46±0.54	0.004	0.53	0.68	0.62±0.81	0.02	0.48	0.69	0.006±0.02	bd	bd	bd
Cr	0.96±0.67	0.51	0.81	1.22	1.53±1.58	0.57	0.92	1.87	0.29±0.28	0.09	0.15	0.62
Li	2.30±1.86	1.02	1.95	2.85	3.22±3.11	0.95	2.18	3.77	0.93±1.24	0.15	0.36	1.92
Be	bd	bd	bd	bd	bd	bd	bd	bd	0.06±0.06	0.00	0.06	0.12
Al	10.6±8.56	7.96	9.68	10.1	10.6±8.89	7.20	9.68	11.8	18.3±4.26	16.9	17.9	21.4
Mn	28.9±59.2	0.35	2.52	15.8	39.7±60.8	1.39	5.80	55.6	6.23±5.62	0.73	2.91	12.9
Fe	97±46.1	59.8	95.7	126	96.5±51	56.3	93.0	126	11.1±13.6	0.19	6.45	16.9
Co	0.21±0.09	0.16	0.20	0.28	0.21±0.08	0.16	0.20	0.26	0.26±0.31	0.07	0.09	0.35
Ni	3.06±1.24	2.11	3.13	4.11	3.06±1.21	2.27	2.99	3.48	0.56±0.82	0.11	0.31	0.65
Cu	0.95±1.86	bd	0.01	2.11	1.72±3.69	0.00	0.11	2.05	0.78±1.64	0.00	0.02	0.64
Zn	9.10±5.61	5.35	7.69	10.8	14.4±11.4	6.64	10.7	17.0	20.3±30.2	3.50	10.9	12.1
Rb	1.17±2.18	0.32	0.48	0.68	0.82±0.86	0.33	0.51	0.93	5.45±3.46	2.05	5.00	9.08
Sr	492±441	226	396	528	421±401	155	299	503	22.3±24.4	3.45	11.9	26.4
Ag	0.00012±0.0004	bd	bd	bd	0.0001±0.0004	bd	bd	bd	0.0002±0.0013	bd	bd	bd
Sb	bd	bd	bd	bd	bd	bd	bd	bd	bd	bd	bd	bd
Ba	169±150	48.1	130	200	154±119	51.9	130	210	23.9±16.4	14.7	16.6	31.6
Br	50±80	bd	bd	108	78±110	bd	bd	124	bd	bd	bd	bd
Tl	bd	bd	bd	bd	bd	bd	bd	bd	0.022±0.024	bd	0.01	0.05
Th	0.0025±0.01	bd	bd	bd	0.0036±0.013	bd	bd	bd	bd	bd	bd	bd

Note: bd = below detection.