Research Article

Exploring the Root Cause for Chronic Kidney Disease of Unknown Etiology (CKDu) via Analysis of Metal Ion and Counterion Contaminants in Drinking Water: A Study in Sri Lanka

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The introduction of elevated amounts of foreign ions into the blood may lead to impairment of the filtration membrane of kidneys and chronic kidney damage. In order to assess the risk of consumption of drinking water (dug well water) in chronic kidney disease of unknown etiology (CKDu), affected areas in Sri Lanka, trace metals, and other counterions in water samples obtained from dug wells were analyzed and compared with a reference area. Drinking water could be the major source that is responsible for entering nephrotoxic ion contaminants into the human body. To achieve the objective, drinking water samples were collected from dug wells in two CKDu endemic areas and a reference area where no CKDu patients were found in a dry season. In the Wewelketiya area (one of the endemic areas), Cd concentrations in 60% of water samples and Pb concentrations in 40% of water samples have exceeded the maximum limit given by Sri Lanka water quality standards. Fluoride concentrations also have exceeded the permissible limits of more than 80% of collected water samples in both CKDu endemic areas. However, none of the water samples in reference areas has reported that Cd, Pb, and fluoride are beyond their maximum permissible limits. Hence, people in the particular CKDu endemic areas are at risk of kidney tissue damage due to long-term exposure to drinking water with elevated levels of some metal ions and counterions.

1. Introduction

Chronic kidney disease of unknown etiology was first discovered in Sri Lanka in the mid-1990s and was mostly observed among the farmers in the North Central Province (NCP) of Sri Lanka, and since then, over two decades of a period, the disease spread dramatically up to the other farming areas of the country such as Northern, North-western, Eastern, Uva, and Central Provinces [1]. Chronic kidney disease (CKD) is a noncommunicable disease that is related to risk factors such as diabetes or hypertension, past snakebites, and urinary tract infections [2–4]. Another study has defined “chronic kidney disease” as either kidney damage or decreased kidney function (decreased GFR) for three months or more [5]. Chronic kidney disease (CKD) is a global public health concern, which is attracting increased

global attention because of the disease’s rapid spread. However, CKD of unknown etiology CKDu is also prevalent and is progressing rapidly in certain regions of the world, especially in Africa, Central America, and Asia [6].

The occurrence of CKDu within the same country will vary with the geographic area. An intimate relationship between water quality and the underlying geology has been recorded repeatedly in various geographical regions of the world. Since the CKDu exists in communities where groundwater is the primary source of drinking water, many risk factors are hypothesized, for example, unidentified environmental toxins leading to CKDu [7], chronic pesticide exposure and enhanced level of heavy metals in water and soil [8], high fluoride levels and potential impacts of AlFx in soil and water [9], and growth of cyanobacteria in water resources [10, 11].

CKDu was discovered mostly among males in the North Central Province (NCP) of Sri Lanka including Anuradhapura and Polonnaruwa Districts (Table 1), and lately, it has also been detected among women and children. Male patients are predominantly farmers and/or agricultural laborers, who are over the age of 40 years [12]. The distribution of the disease can be associated with some geographical and socioeconomic factors which have environmental and occupational origins. NCP of Sri Lanka is a part of the “dry zone” of Sri Lanka, with a rainfall of ~1750 to 1000 mm per annum [13], and some studies have shown that the rainfall map largely overlaps with the region affected by CKDu. Now, the disease has spread to nearby districts including Northwestern, Eastern, and Uva Provinces as well. It is a high burden to the country’s economy due to the high cost of treatments and the poor income of people in remote areas. Some cohort studies carried out using descriptive hospital records indicated that patients coming to nephrology clinics in Anuradhapura and Kandy were increasing during the period 2001-2002, and the majority of them were CKDu patients [14].

The causes and risk factors for the development of CKDu vary widely. As the CKDu prevails in households where groundwater or well water is the main drinking water source, several risk factors can be hypothesized: (a) chronic exposure to chemical pesticides and fertilizers and thereby increase of heavy metals (e.g., Cd, Pb, and As) in water and (b) presence of high levels of fluoride and other possible counterions such as phosphates and nitrates [15].

Ion imbalances of blood and the introduction of a high load of foreign ions or molecules into the blood cause impairment of the filtration membrane which can result in protein denaturation. Those protein molecules pass into the urine due to the absence of proper maintenance of pore size in the membrane. The glomerular capillaries progressively damage the filtration membrane with the high osmotic pressure gradients and negatively charged proteoglycans can also be affected by higher concentrations of cations [16–18]. Furthermore, toxins are not filtered out from the blood and accumulated in the body. When the dysfunction of kidneys occurs partially or completely, the normal body performances are altered resulting in some serious health issues with confusing etiology such as chronic kidney disease of unknown etiology (CKDu) [19–21].

The intention of the current study is to assess the drinking water quality by evaluating the levels of toxic metals and selected counterions in dug wells of CKDu endemic areas compared with a reference area (CKDu nonendemic areas) and comment on the risk of consumption of the drinking water in selected areas based on Sri Lankan water quality standards. Besides, the study aims to explore the evidence to the link of consumption of well water and the prevalence of kidney disease in selected CKDu endemic areas.

2. Methodology

2.1. Sample Collection. Based on the information obtained from the Ministry of Health, Sri Lanka, Wewalketiya Grama-Niladhari division (GND) and Ambagaswewa GND were

selected as CKDu endemic areas for water sampling which are located in Anuradhapura District and Polonnaruwa district, respectively, in North Central Province, Sri Lanka. Buddahangala GND in Ampara district was selected as the reference area for the present study. Sampling points for the drinking water sample collection were selected from shallow drinking water wells (dug wells) located in home gardens of the residents which are the main sources of water consumption in their daily life. Thirty dug wells were selected randomly for the water sampling, and all the sampling sites were located within the same climatic zone (dry zone of Sri Lanka). Sampling locations were recorded in the field using the global positioning system (GPS) (Figure 1). The sampling procedure was carried out within August 2019 (dry season) for all selected study areas. Triplicated 30 drinking water samples were collected from each sampling area into uncontaminated Teflon bottles (125 ml) and preserved by adding conc. nitric acid (0.10 mL) and stored at 4°C, and another triplicated 30 drinking water samples were collected into uncontaminated Teflon bottles (125 ml) without acidification and stored at 4°C.

2.2. Analysis of Water Samples. The concentration of trace elements including cadmium, lead, chromium, arsenic, zinc, copper, sodium, potassium, iron, manganese, cobalt, and nickel was determined using Inductively Coupled Plasma Mass Spectrometry (ICP-MS-7800-Agilent, Germany). Multielement ICP-MS standards (AccuStandard, USA) were used for instrumental calibration. Two calibration series (1 ppb–50 ppb and 10 ppb to 1000 ppb) were prepared using multielement standard. Acidified water samples (with conc. HNO₃) were filtered through 0.45 µm syringe filters before the insertion to the ICP-MS instrument. Phosphate and nitrate concentration in water samples were measured by Ion Chromatographic method according to the US-EPA standard procedures (Method 9056A). Sodium bicarbonate (CASRN 144-55-8) and sodium carbonate (CASRN-497-19-7) were used as an elution solution, and sulfuric acid (CASRN-7664-93-9) was used as a regeneration solution. ACS Reagent grade 1000 mg/L stock solutions of nitrate and phosphate were used for the standards for anions prepared for a range of concentrations (0.1 mg/L–10 mg/L). Each standard and collected samples were filtered using 0.22-micrometer nylon filters. Samples were introduced, under the flow rate of 0.7 mL/min into the Ion Chromatograph (Metrohm Eco IC). Fluoride concentrations of water samples were measured as on-site measurements using calibrated fluoride meter (Eutech Instrument, pH 510), and when taking the readings, TISAB (III) buffer was used with water sample in 1 : 1 ratio in order to stabilize the pH of the medium. Magnesium and calcium concentrations in water samples were determined using a flame atomic absorption spectrophotometer (GBC 5000). A series of standard metal solutions were prepared (10 ppm–500 ppm) separately using both Mg and Ca metal ion standards (1000 ppm, Bibby Scientific) to obtain the calibration curve, and the concentration of Mg and Ca of each water sample was determined.

TABLE 1: CKDu patients in high-risk areas for chronic kidney disease of unknown etiology (CKDu) in Sri Lanka.

District	Risk-AGA divisions	2014	2015
Ampara	Dehiattakandiya, Maha oya	493	468
Anuradhapura	All divisions	8903	8412
Polonnaruwa	All divisions	3483	5018
Badulla	Rideemaliyadda, Mahiyangana	1010	943
Kurunegala	Polpithigama	561	1660
Matale	Wilgamuwa	803	1107
Monaragala	Thanamalwila, Wellawaya, Buttala	246	794
Mullaitivu	Welioya	333	486
Vavuniya	Vavuniya south, Cheddikulam	163	1933
Trincomalee	Padavi siripura, Gomarakadawala	484	426
Hambanthota	Tissamaharama, Lunugamvehera	0	205
Total		16479	21452

Source: Ministry of Health, Nutrition and Indigenous Medicine, Sri Lanka, 2016.

FIGURE 1: The distribution of sampling points (Aquifer) which were located in the CKDu prevailed areas: (a) Ambagaswewa GND, Madirigiriya DSD, Polonnaruwa District and (b) Wewalketiya GND, Rambewa DSD, Anuradhapura District, Sri Lanka.

2.3. Geographical Data Treatment and Statistical Data Analysis. ArcGIS 10.2.2 software package was used to perform surface interpolation for all sampling sites using estimated mean values to measure the impact of water contamination in the study area. Statistical analysis was done using SPSS Statistics software. Descriptive statistics were performed on the data sets and a paired *t*-test was carried out to determine the differences or similarities of the chemical parameters recorded in each sampling location.

3. Results and Discussion

Chronic renal failure has recently shown a significant increase in some areas in the Anuradhapura and Polonnaruwa districts. Table 2 summarizes the results of trace metals and some of the counterion concentrations of selected well water

samples which are obtained from the Ambagaswewa GND (Madirigiriya DSD in Polonnaruwa district), Wewalketiya GND, (Rambewa DSD, in Anuradhapura district), and the reference site (Buddhangala GND in Ampara district), respectively, compared with the permissible limits [22]. When considering the causative factors of CKDu, toxic metals are the most important concern including cadmium, chromium, and lead. Cadmium traces naturally occur in phosphate and have been shown to get into water, soil, and also food through fertilizer application. Cadmium is present as an impurity in phosphate fertilizers and refined petroleum products [23]. According to Sri Lanka drinking water quality standards, the maximum permissible level (MPL) of cadmium (Cd) is $3.000 \mu\text{g/L}$ [22]. In the present study, 60% of the selected dug wells in Wewalketiya GND in the Anuradhapura district showed elevated Cd levels which exceed

TABLE 2: Descriptive statistics of the chemical composition of well water samples in all the sampling locations: Ambagaswewa GND in Polonnaruwa district, Wewalketiya GND in Anuradhapura district, and reference site (Buddhangala GND in Ampara district).

Concentration of variables	Ambagaswewa GND				Wewalketiya GND				Reference site (Buddhangala GND)				SLS standards SLS 614 : 2013	
	Mean	SD	Minimum	Maximum	Mean	SD	Minimum	Maximum	Mean	SD	Minimum	Maximum		
Cd ($\mu\text{g/L}$)	0.178	0.162	ND	0.660	3.440	2.072	1.120	9.350	0.124	0.008	0.030	0.340		3
Pb ($\mu\text{g/L}$)	1.229	1.537	ND	6.080	5.422	3.687	1.000	17.350	1.368	0.196	0.000	7.420		10
Cr ($\mu\text{g/L}$)	0.203	0.423	ND	1.880	11.510	36.860	1.000	48.000	ND	ND	ND	ND		50
As ($\mu\text{g/L}$)	0.336	0.414	ND	1.650	ND	ND	ND	ND	0.161	0.065	ND	2.850		10
Zn ($\mu\text{g/L}$)	3.138	5.451	ND	22.59	50.93	20.97	20.00	87.60	4.793	0.790	ND	33.44		3000
Cu ($\mu\text{g/L}$)	6.470	19.04	ND	175.0	10.61	25.82	3.210	250.0	3.990	1.230	0.210	106.0		1000
Ca (mg/L)	74.10	15.50	ND	135.0	90.7	72.1	11.0	288.0	23.90	14.64	ND	39.83		100
Mg (mg/L)	18.51	25.15	ND	89.57	41.46	31.68	10.00	112.6	16.14	11.90	90.00	156.5		30
Na (mg/L)	1266	1682	205.0	8905	1518	2099	126.0	8845	3684	5175	114.0	8382		200
K (mg/L)	505.4	379.6	0.000	1403	119.2	36.50	14.20	555.3	640.0	582.0	0.000	1832		N/A
Fe (mg/L)	6.814	3.957	2.250	17.46	20.27	13.26	6.680	64.38	28.10	95.00	1.000	526.5		0.3
Mn (mg/L)	21.36	44.56	0.130	166.3	11.09	8.540	1.080	41.59	29.80	79.40	0.200	340.2		0.3
Co (mg/L)	0.019	0.072	0.000	0.383	ND	ND	ND	ND	ND	ND	ND	ND		N/A
Ni (mg/L)	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND		0.02
Fluoride (mg/L)	1.260	0.654	0.320	3.160	1.370	0.658	0.220	3.020	0.505	0.081	0.100	3.200		1
Phosphate (mg/L)	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND		2.0
Nitrate (mg/L)	2.520	1.475	0.220	5.890	0.669	0.348	0.150	1.290	1.127	ND	0.110	3.330		50

ND: not detected; N/A: not available.

the MPLs. However, drinking water samples obtained from Ambagaswewa GND, Pollonnaruwa district, showed the low levels of Cd which have not exceeded the MPLs, and also none of the water showed Cd level exceeding the MPLs in the reference area.

However, previously published reports have interpreted that long-term exposure to Cd via drinking water persists in the kidney and can possibly cause kidney failure through several pathways [24, 25], because both renal proximal tubular damage and decline in glomerular filtration rate (GFR) in humans are due to chronic exposure to Cd [24, 25]. The cadmium distribution along the sampling sites in particular GNDs is shown in Figure 2 and cadmium concentrations were significantly ($p = 0.05$) higher in Wewalketiya GND with respect to the reference area. But the Cd concentrations were not significantly different from the reference in Ambagaswewa GND. The increase in geochemical mobility of Cd can be seen under acidic environmental conditions with the land application of fertilizers and pesticides which increases the total concentration of Cd in agricultural soils. Acidification of soils and surface waters increases the geochemical mobility of Cd. Cadmium (Cd) has been suggested as a possible factor contributing to the disease, and the source of contamination can be associated with triple superphosphate (TSP) application in paddy and other crop cultivations because TSP use is widespread throughout the agricultural sector in Sri Lanka and especially in the disease prevailed areas [26].

According to the results of the present study, the lead levels in Ambagaswewa GND are reported to be $6.080 \mu\text{g/L}$ as maximum value with an average of $1.229 \pm 1.537 \mu\text{g/L}$, and the lead levels in Wewalketiya GND ranged from $1.000 \mu\text{g/L}$ to 17.350 ppb with the average of $5.422 \pm 3.687 \mu\text{g/L}$ (Table 2). Even though dug well water samples in Wewalketiya GND showed a huge variation of Pb concentrations, 40% of collected samples have exceeded the MPLs of Pb, and in case of renal failure, it was reported that chronic exposure to Pb can lead to nephrotoxicity characterized by renal effects, such as glomerular sclerosis, interstitial fibrosis, and proximal tubular nephropathy [27] which have been commonly observed among the patients with CKDu in Sri Lanka [23].

Apart from that, ranges of Cr concentrations of selected dug wells in Ambagaswewa and Wewalketiya areas were reported to be $0.203\text{--}0.423 \mu\text{g/L}$ and $11.5\text{--}48.00 \mu\text{g/L}$, respectively, and all values were below the MPLs. But in the reference area, none of the selected water resources has been contaminated with Cr. In both humans and animals, chromium (III) is found as an essential nutrient that plays a valuable key role in fat, glucose, and protein metabolism, and it is done by the action of insulin [28]. Although chromium (III) has been found as an essential nutrient, both acute and chronic exposure to high levels via inhalation, ingestion, or dermal contact may result in adverse health effects. The kidney is, therefore, one of the main targeting organs for Cr in acute high doses and chronic cumulative exposure. In addition to that, renal damage and dysfunction caused due to chronic Cr exposure could involve both the glomerular and tubule [29].

The health effects due to hard water or bicarbonates and sulfates of calcium (Ca) and magnesium (Mg) are significant, and 100 mg/L [22] for Ca and 30 mg/L [22] for Mg are recommended for drinking water. The results which have been obtained from the sample analysis show variations in Ca and Mg concentrations in selected dug wells in Ambagaswewa and Wewalketiya, and most of the samples have reported that Ca and Mg concentrations are beyond the MPLs in those CKDu endemic areas. However, in the reference area, the mean concentrations of Ca and Mg have been reported to be $23.09 (\pm 14.63) \text{ mg/L}$ and $16.14 (\pm 11.90) \text{ mg/L}$, respectively, indicating the low values than CKDu endemic areas. Long-term exposure to the Ca and Mg ions via drinking water may adversely affect kidney dysfunctions modifying the risk for calcium stones and increasing the risk of calcium-containing kidney stones [30].

Fluoride can occur naturally in water above desirable levels. Fluoride also has been proposed as a cause of CKDu [9, 31, 32], and according to Sri Lanka drinking water quality standard, the maximum permissible level (MPL) of fluoride (F) is 1.0 mg/L [22]. However, the higher fluoride levels in the collected drinking water samples (Figure 3) were recorded in Wewalketiya GND (average: $1.370 \pm 0.658 \text{ mg/L}$) and Ambagaswewa GND (average: $1.260 \pm 0.654 \text{ mg/L}$) compared to the reference area (average: $0.505 \pm 0.765 \text{ mg/L}$), and both CKDu endemic areas have exceeded the MPLs of fluoride in the collected drinking water samples. The variations of fluoride levels in all sampling locations are shown in Figure 3. Furthermore, 80% of the samples in Ambagaswewa GND and 95% of samples in Wewalketiya GND were contaminated with fluoride which has exceeded the standard limits [22].

Excessive and long-term exposure to fluoride can be directly related to kidney tissue damage because high fluoride zones for groundwater overlap with CKDu-prevalent regions in NCP [31]. Patients with reduced glomerular filtration rate are having an increased risk of chronic fluoride toxicity because they have less ability to excrete fluoride via urine [33]. According to the dose-effect relationship between fluoride levels and CKDu [34], unexpected effects of fluoride on cellular systems have been investigated by Agalakova and Gusev [35] which clearly reveal that fluoride can affect oxidative stress, intracellular redox homeostasis, lipid peroxidation, protein synthesis inhibition, gene expression alteration, and apoptosis.

A range of geological factors such as dissolution rates and residence times of fluoride-bearing rocks can be related to higher fluoride levels in shallow wells in those areas. The minerals, namely, charnockite, granitic, hornblende, and biotitic gneisses [11, 36], and also fluoride-bearing minerals such as micas, pyroxene, fluorite, tourmaline, topaz, sphene, and apatite can enhance the fluoride levels in soil [37]. Fluoride in the water can be a potential causal factor in the development of the disease because of not only the impacts of fluoride itself but also its interaction with other ionic constituents such as Ca, Na, and possibly Mg that are present in the drinking water [11].

In particular, collected water samples were having high ionic content with the presence of high amounts of main

(a)

(b)

FIGURE 2: Continued.

FIGURE 2: Cadmium concentrations in drinking water samples collected from (a) sampling sites of Wewelketiya GND, in Anuradhapura district, (b) sampling sites of Ambagaswewa GND, in Polonnaruwa district, and (c) sampling sites of Buddangala GND (Reference) in Ampara district.

FIGURE 3: Variations of fluoride concentrations along the sampling points in the reference site (Buddangala) and disease prevalence areas (Ambagaswewa and Wewalketiya).

ions such as Na^+ and K^+ which are normally found in water. The increased ionicity of drinking water can influence the depletion of water molecules near the kidney membrane, changing water activity and ion activity, osmotic activity, and hydrophobic interactions. When ranking the ions by their capacity to denature proteins, the cations including

potassium, sodium, magnesium, and calcium and anions such as fluoride and phosphates play a major role which are present in higher contents in collected drinking water samples from sampling locations [21]. Fertilizer runoff which contains most of those ionic agents can contribute to the pollution of drinking water sources in that area.

Similarly, some anions such as fluoride and phosphates are the most active in protein denaturation, while nitrates are the least effective. Alteration of the composition and hydrology of the soil may lead to increased ionicity of adjacent water sources. The alternating reducing and oxidizing conditions (depending on the environmental and climate conditions of the areas) of soil promote the addition of iron (Fe) and manganese (Mn) into the soil solution which would get partly leached out into the water table which is indicated by the results of having high Fe and Mn contents in all sampling locations (Table 2). Those redox fluctuations of soil may decrease the pH of the solution due to some nonequilibrium ionic processes such as the conversion of carbonate to bicarbonate and reaction with ambient carbon dioxide (CO₂). pH reduction of the soil solution will lead to the release of soil-bound toxic heavy metals and those are added to the water sources in those areas. Intensive usage of chemical fertilizers and pesticides is also responsible for the reduction of soil pH levels [17, 21].

The kidney needs “good” drinking water [38–40]. It can be considered that the long-term exposure of the kidney to the drinking water that contains many ionic species and has high ionicity, which are going to persist in the kidneys, can adversely affect them. Such exposure is termed “chronic exposure”. Chronic exposure is when exposure occurs continuously with toxic substances over a long period of time [38]. Aquifers of those areas are constantly replenished by water with high ionicity from Mahaweli channels as well as frequent oxidation-reduction of those aquifers releasing and increasing quantities of ions to water table [21].

4. Conclusion

The persistence of exceeded levels of Cd, Pb, and fluorides which influence the kidney function in some selected dug wells in CKDu endemic areas indicates a risk of consuming the drinking water from those resources. Even though other analyzed trace elements and counterions in drinking water samples have not exceeded the permissible limits, long-term exposure of the kidney via drinking water with elevated levels of ions augments the occurrence of kidney failure. Contamination of drinking water sources with trace metals and fluoride mainly occurs by the release of ions bound to soil particles from the soil. Hence, it is worth investigating the sources and pathways of contamination of toxic metals and fluoride in the soil and further assessments should be carried out to understand the contribution of fertilizer application and other agricultural activities for those contaminations as well. Additionally, toxic metal concentrations of frequent use of fertilizers commonly used in Sri Lanka need to be evaluated continuously.

Data Availability

The data used to support the findings of this study are included within the article.

Conflicts of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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