Arsenic, cadmium, lead, and chromium in well water, rice, and human urine in Sri Lanka in relation to chronic kidney disease of unknown etiology

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ABSTRACT

Chronic kidney disease of unknown etiology (CKDu) is spreading gradually in Sri Lanka. In the current research, 1,435 well water samples from all 25 districts of Sri Lanka, 91 rice samples, and 84 human urine samples from both CKDu-endemic and non-endemic areas in Sri Lanka were analyzed for arsenic, cadmium, lead, and chromium to detect whether toxic elements could be a cause of CKDu. The liver-type fatty acid binding protein (L-FABP) concentration and arsenic, cadmium, lead, and chromium concentrations of the urine samples were analyzed to determine the relation of L-FABP with arsenic, cadmium, lead, and chromium. High concentrations of arsenic, cadmium, lead, and chromium were not detected in the well water samples from CKDu-endemic areas. Arsenic, cadmium, and lead contents in the rice samples from both CKDu-endemic and non-endemic areas were well below the Codex standard. There were no relationships between the L-FABP concentration and concentrations of arsenic, cadmium, lead, and chromium in urine. In addition, arsenic, cadmium, lead, and chromium concentrations in human urine samples from CKDu-endemic areas were not significantly different from those from non-endemic areas. These findings indicated that arsenic, cadmium, lead, and chromium could not cause CKDu.

Key words | arsenic, chronic kidney disease of unknown etiology, drinking water, human urine, rice

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INTRODUCTION

Sri Lanka is an island that covers an area of 65,610 square km. It is located in the Indian Ocean above the equator with a latitude and longitude of 6°–10° N and 79°–82° E. Sri Lanka is divided into three main climatic regions: the Dry Zone, the Intermediate Zone, and the Wet Zone, based on the rainfall. Well water is the main source of drinking water in the Dry Zone, including the Anuradhapura and Polonnaruwa Districts. Numerous wells supply people with water, since surface water is difficult to obtain in most of the Dry Zone, even though artificial canals have been constructed by successive dynasties since ancient times.

Chronic kidney disease of unknown etiology (CKDu) is one of the most serious health issues in Sri Lanka, as it has recently been increasing regularly in the North Central Province of the Dry Zone, which covers 16% (10,472 km^2) of the Sri Lankan landmass (Jayasumana *et al.* 2015). The North Central Province is inhabited by 6.2% of the Sri Lankan population; however, it has been reported that 73% of CKDu patients live in

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the North Central Province (Jayasumana *et al.* 2015). CKDu is an endemic disease that cannot be attributed to hypertension or diabetes (Jayasumana *et al.* 2013). The disease was first recorded in the early 1990s. It is more prevalent among males than females, with a ratio of 3:1, especially among males engaged in agriculture around the ages of 40–60 years (Nobel *et al.* 2014). It is worth noting that the disease is not confined to Sri Lanka. Similar cases have been recorded in India, Nicaragua, Costa Rica, Central American states (Nobel *et al.* 2014), Uddanam, China, Serbia, Bulgaria, Romania, Croatia, and Bosnia. It is believed that the hydrogeochemistry of the drinking water adversely affects CKDu, as the disease is highly endemic (Chandrajith *et al.* 2011).

Many hypotheses to explain CKDu have arisen, as follows: (1) high fluoride exposure through drinking water (Nanayakkara *et al.* 2012); (2) the use of aluminum containers for cooking (Jayasekara *et al.* 2013); (3) aflatoxins (Nobel *et al.* 2014); (4) ground water hardness (Jayasumana *et al.* 2014); (5) heavy metals contamination in rice and/or drinking water (Redmon *et al.* 2014); (6) vanadium in drinking water; (7) lead, chromium, and vanadium in the soil (Jayawardana *et al.* 2014); (8) arsenic in drinking water (Jayasumana *et al.* 2013); (9) toxic levels of cadmium in food and drinking water (Jayasumana *et al.* 2014); (10) various nucleotides; (11) uranium (Nobel *et al.* 2014); (12) pesticides (WHO 2012) and fertilizers (Jayasumana *et al.* 2015); (13) low water intake and exposure to high temperatures, resulting in significant dehydration (Jayasumana *et al.* 2014); (14) climate and hydrogeochemistry (Redmon *et al.* 2014); (15) soil geochemistry (Jayawardana *et al.* 2014); (16) the usage of ayurvedic medicine; (17) selenium deficiency (Nobel *et al.* 2014); and (18) genetic susceptibility (Redmon *et al.* 2014). Unfortunately, conclusive evidence has not yet been obtained, as detailed investigation was hindered by the civil war that lasted 30 years and the unavailability of analytical instruments in Sri Lanka.

In this research, heavy metals and arsenic concentrations in the well water from all Sri Lankan districts and in rice from Sri Lanka were determined in investigating the relationship between the prevalence of CKDu and heavy metals and arsenic. In addition, heavy metals and arsenic concentrations in urine from people living in both CKDu-endemic and non-endemic areas were analyzed for further confirmation.

MATERIALS AND METHODS

From 2010 to 2015, 1,435 water samples were collected from both dug and tube wells in all districts of Sri Lanka to determine the water quality. The study sites were selected randomly, and the locations were marked using GPS. Water samples were filtrated on site, using a membrane filter with a pore size of 0.45 μm to stabilize the water quality, and stored in polyethylene bottles until analysis. Collected samples were taken to Japan for the analysis of heavy metals: cadmium, lead, chromium, and arsenic. The metal concentrations in the well water were analyzed by inductively coupled plasma optical emission spectrometry (ICP-OES) (PerkinElmer Optima 5300) or inductively coupled plasma mass spectrometry (ICP-MS) (Agilent 7700). The water samples were transported from Sri Lanka to Japan within 1–4 days after collection.

Ninety-one rice samples were collected from Sri Lanka for analysis from 2014 to 2015. Samples were collected from the market and households in both CKDu-endemic and non-endemic areas in the Anuradhapura, Hambantota, Kandy, Matale, Ampara, Kegalle, Gampaha, Matara, and Colombo Districts in Sri Lanka. In total, 67 rice samples were from CKDu-endemic areas, and 24 rice samples were from CKDu non-endemic areas. Fifty-nine rice samples from CKDu-endemic areas were polished and eight rice samples from CKDu-endemic areas were unpolished. Eight rice samples from CKDu non-endemic areas were polished and 16 rice samples from CKDu non-endemic areas were unpolished. In total, 67 rice samples were polished, and 24 rice samples were unpolished. The rice samples were degraded with nitric acid by microwave (Analytik Jena TOPwave microwave system for pressure digestion) to determine the arsenic, cadmium, lead, and chromium levels of each sample by ICP-MS (Agilent 7700).

Eighty-four human urine samples were collected from the Anuradhapura, Polonnaruwa, Matale, and Kandy Districts in Sri Lanka for the analysis of heavy metals and arsenic concentrations, liver-type fatty acid binding proteins (L-FABPs), and creatinine concentrations from 2014 to 2015. Free and informed consent of the participants and their legal representatives were obtained for the collection of human urine samples, and the study protocol was approved by the appropriate committee for the protection of human participants (Toyama Prefectural University Committee for Ethical Research Screening Involving

Human Subjects), by Toyama Prefectural University, Imizu-City, Toyama, Japan, on 27th February 2016. Sixty human urine samples were collected from CKDu-endemic areas, and 24 human urine samples were collected from non-endemic areas. The samples were collected in sterilized plastic cups. Collected human urine samples were stored at 0 °C in an ice-cooled condition and brought to Japan also in an ice-cooled condition. Arsenic, cadmium, lead, and chromium concentrations were analyzed by ICP-MS, after acidifying the samples with 20% HNO_3. L-FABP and creatinine concentrations were determined by BML Inc., Tokyo, Japan. Heavy metals and arsenic in human urine were statistically analyzed using SPSS software after correcting the concentrations by using the creatinine concentration. The transportation of human urine samples from Sri Lanka to Japan took 1–4 days after collection.

RESULTS AND DISCUSSION

Arsenic, cadmium, lead, and chromium in well water in Sri Lanka

Arsenic

According to Sri Lankan standards (Sri Lankan Standard Institute 2013) and World Health Organization (WHO) guidelines (WHO 2011), the maximum permissible level of arsenic in drinking water is 10 μg/L. Table 1 shows the arsenic concentration in the well water of each Sri Lankan district. The maximum and average values and the standard deviation of each district are given. The Mannar, Puttalam, Batticaloa, and Mullaitivu Districts reported maximum arsenic concentrations that are higher than the Sri Lankan standard and the WHO guideline. Mannar District recorded the highest concentration, reaching 66 μg/L. These districts also exhibit high standard deviation values that show high variations in arsenic concentrations, even among wells within the same district. However, the average arsenic concentrations in all other districts were less than 10 μg/L (Herath et al. 2017).

As the incidence of CKDu has risen, it has been thought that arsenic is one cause of CKDu. Based on the analysis of 226 fertilizer samples collected from Padaviya, Medawachchiya, Mahawilachchiya, and Anuradhapura in the North Central Province of Sri Lanka, Jayasumana et al. (2015) found that phosphate fertilizers were a major source of inorganic arsenic in CKDu-endemic areas. Additionally, they reported that triple super phosphate contained the highest amounts of arsenic and rock phosphate produced at Eppawala, a mining area in the North Central Province, contained the second highest levels of arsenic.

Table 1 | Arsenic concentrations in the well water of each Sri Lankan district

District	Maximum conc. (μg/L)	Average conc. (μg/L)	Standard deviation (μg/L)	District	Maximum conc. (μg/L)	Average conc. (μg/L)	Standard deviation (μg/L)
Ampara	2	1	0.5	Kurunegala	1	0	0.1
Anuradhapura	3	0	0.4	Mannar	66	7	11.7
Badulla	1	0	0.3	Matale	2	0	0.3
Batticaloa	14	3	3.2	Matara	4	0	0.6
Colombo	0	0	0.1	Moneragala	2	0	0.4
Galle	0	0	0.1	Mullaitivu	13	3	3.7
Gampaha	0	0	0.1	Nuwara Eliya	0	0	0.0
Hambantota	3	1	0.6	Polonnaruwa	3	0	0.5
Jaffna	6	2	1.7	Puttalam	15	4	4.1
Kalutara	3	0	0.6	Ratnapura	0	0	0.1
Kandy	0	0	0.1	Trincomalee	9	1	1.7
Kegalle	0	0	0.1	Vavuniya	2	1	0.6
Kilinochchi	2	1	0.6				

Jayasumana *et al.* (2013) reported that abnormal spotty pigmentation observed in the palms and soles of CKDu patients in the Padavi Sri Pura Divisional Secretariat area of Trincomalee District in the Eastern Province of Sri Lanka was due to chronic arsenic toxicity; thus, arsenic could be a cause of CKDu. Furthermore, the authors have reported that pesticides and fertilizers used excessively in paddy farming are the most likely sources of arsenic in the study area. On the contrary, we have detected in our study that there was no high concentration of arsenic in the well water of CKDu-prevalent districts. High concentrations of arsenic that exceeded the Sri Lankan standard and WHO guideline were detected in well water in areas other than CKDu-endemic areas: Mannar, Puttalam, Batticaloa, and Mullaitivu. In addition, Wasana *et al.* (2012) assessed the water quality of 60 samples from areas with high and low prevalences of CKDu in Sri Lanka and reported that the arsenic level in the water was well below the WHO guideline of 10 μg/L. Water quality analysis of 234 different sources of water from CKDu-endemic and non-endemic areas of Sri Lanka conducted by the WHO demonstrated that arsenic concentrations were borderline in just four samples: 9.9 μg/L, 10.2 μg/L, 10.5 μg/L, and 13.4 μg/L in the endemic area (WHO 2012). These results coincide with our research, which shows low concentrations of arsenic in well water.

When we consider the soil map of Sri Lanka, that categorizes soil into 21 types, it is clear that all of the wells with arsenic levels higher than 10 μg/L were located on the soil type of 'sandy regosols on recent beach and dune sands' (Survey Department 1967), indicating that the arsenic had a geological origin (Herath *et al.* 2017).

Accordingly, we could conclude that arsenic in well water is not a cause of CKDu.

Cadmium

The Sri Lankan Standard for cadmium in drinking water is 3 μg/L (Sri Lankan Standard Institute 2013), which is similar to the WHO guideline for cadmium in drinking water (WHO 2011). Cadmium causes adverse health effects in humans due to its long half-life of 15–20 years in the human body (Averbeck & Bertin 2006) and its rapid uptake and accumulation through food chains. Cadmium poisoning's adverse effect on human health, itai-itai disease, was first reported in Japan (Roberts 2014) in 1912 (Liu *et al.* 2015) in subsistence farmers who had grown rice with water contaminated by mining waste. The disease resulted in the softening of bones and kidney failure (Roberts 2014).

Table 2 shows the concentrations of cadmium in the well water of each district in Sri Lanka as the maximum value, the average value, and the standard deviation. No

Table 2 | Cadmium concentrations in the well water of each Sri Lankan district

District	Maximum conc. (μg/L)	Average conc. (μg/L)	Standard deviation (μg/L)	District	Maximum conc. (μg/L)	Average conc. (μg/L)	Standard deviation (μg/L)
Ampara	0.1	0.01	0.02	Kurunegala	0.0	0.00	0.00
Anuradhapura	0.2	0.01	0.03	Mannar	0.1	0.02	0.07
Badulla	0.1	0.01	0.02	Matale	0.3	0.02	0.04
Batticaloa	0.2	0.01	0.03	Matara	0.1	0.01	0.01
Colombo	0.0	0.00	0.00	Moneragala	0.5	0.01	0.04
Galle	0.2	0.01	0.02	Mullaitivu	0.3	0.02	0.07
Gampaha	0.0	0.01	0.00	Nuwara Eliya	0.0	0.00	0.00
Hambantota	0.1	0.01	0.01	Polonnaruwa	0.0	0.01	0.01
Jaffna	0.1	0.02	0.04	Puttalam	0.0	0.00	0.00
Kalutara	0.0	0.00	0.00	Ratnapura	0.1	0.00	0.02
Kandy	0.0	0.00	0.00	Trincomalee	0.0	0.00	0.01
Kegalle	0.0	0.00	0.00	Vavuniya	0.1	0.01	0.01
Kilinochchi	0.1	0.01	0.02				

district exceeded the 3 μg/L cadmium concentration in well water; even the highest concentration recorded from the Moneragala District was 0.5 μg/L, well below the Sri Lankan standard and the WHO guideline. Therefore, according to the analytical results of 1,435 well water samples, there is no relationship between cadmium contamination in water and CKDu.

A study conducted by Jayatilake *et al.* (2013), based on water samples collected from CKDu-endemic and non-endemic areas in Sri Lanka, concluded that drinking water has not been contaminated by cadmium that causes CKDu. Wasana *et al.* (2012) reported that the cadmium level in drinking water was well below the WHO guideline of 3 μg/L, based on the water quality assessment of areas of high and low prevalence of CKDu in Sri Lanka (Wasana *et al.* 2012). In a study carried out by Chandrajith *et al.* (2011), drinking water samples collected from both CKDu-endemic and non-endemic regions of the North Central Province of Sri Lanka had very low levels of cadmium, well below the WHO guideline of 3 μg/L (Chandrajith *et al.* 2011). The WHO final report stated that cadmium concentrations in drinking water sources used by CKDu patients in Sri Lanka were within the normal range (WHO 2012). All findings support our conclusion that cadmium in drinking water should not be a cause of CKDu.

Lead

The Sri Lankan standard for lead in drinking water is 10 μg/L (Sri Lankan Standard Institute 2013), which is similar to the WHO guideline for lead in drinking water (WHO 2011). The maximum and average concentrations of lead in the well water of each district in Sri Lanka are shown in Table 3. Galle District showed an extremely high maximum concentration of lead in well water of 288 μg/L. All other districts reported maximum concentrations of lead below the Sri Lankan standard of 10 μg/L. No district exceeded the average lead concentration in well water than the Sri Lankan standard.

Lead is suspected to be one cause of CKDu, since lead damages the human kidney, immune system, circulatory system, and neurons, and causes joint diseases (Siraj & Kitte 2013). However, our results indicate that lead is not a cause of CKDu. The analysis of the WHO final report, which stated that lead concentrations in drinking water sources used by CKDu patients in Sri Lanka were within the normal range (WHO 2012), coincides with our analysis.

Chromium

The Sri Lankan standard for chromium in drinking water is 50 μg/L (Sri Lankan Standard Institute 2013). The WHO

Table 3 | Lead concentrations in the well water of each Sri Lankan district

District	Maximum conc. (μg/L)	Average conc. (μg/L)	District	Maximum conc. (μg/L)	Average conc. (μg/L)
Ampara	1.2	0.1	Kurunegala	0.1	0.0
Anuradhapura	4.6	0.1	Mannar	0.2	0.0
Badulla	0.9	0.1	Matale	2.8	0.1
Batticaloa	0.1	0.0	Matara	2.2	0.1
Colombo	3.3	0.3	Moneragala	4.3	0.1
Galle	288.0	3.8 (0.5)[a]	Mullaitivu	3.9	0.3
Gampaha	0.7	0.2	Nuwara Eliya	3.0	0.5
Hambantota	0.3	0.0	Polonnaruwa	0.1	0.0
Jaffna	0.0	0.0	Puttalam	3.6	0.3
Kalutara	1.0	0.1	Ratnapura	2.6	0.2
Kandy	0.0	0.0	Trincomalee	0.1	0.0
Kegalle	0.0	0.0	Vavuniya	1.3	0.3
Kilinochchi	0.6	0.1			

[a]The average concentration for lead in Galle District decreases from 3.8 μg/L to 0.5 μg/L when a single data with an extremely high concentration of 288 μg/L is eliminated.

guideline for chromium in drinking water is the same as the Sri Lankan standard of 50 μg/L (WHO 2011). Chromium affects human health in many ways, such as kidney and liver damage, skin rashes, respiratory defects, weakened immune systems, and alteration of genetic materials (Shanker & Venkateswarlu 2011). Table 4 shows the maximum and average chromium concentrations in well water of each Sri Lankan district. It is obvious that no district has recorded maximum or average chromium concentrations above the Sri Lankan standard and the WHO guideline. When we consider the average chromium concentration of each district, all districts showed average concentrations of 0 μg/L or 1 μg/L. Therefore, it is clear that Sri Lankan well water has not been contaminated with chromium, and that chromium is unlikely to be a cause of CKDu.

Arsenic, cadmium, lead, and chromium in rice in Sri Lanka

The arsenic, cadmium, lead, and chromium content in 91 rice samples (both polished and unpolished) collected from CKDu-endemic and non-endemic areas in the Anuradhapura, Hambantota, Kandy, Matale, Ampara, Kegalle, Gampaha, Matara, and Colombo Districts in Sri Lanka, are shown in Tables 5 and 6. Anuradhapura and Matale Districts are CKDu-endemic and Hambantota, Kandy, Ampara, Kegalle, Gampaha, Matara, and Colombo Districts are CKDu non-endemic.

The specific standards for the maximum level of heavy metals and arsenic in polished rice according to the Codex Alimentarius Commission, as set by the UN Food and

Table 4 | Chromium concentrations in the well water of each Sri Lankan district

District	Maximum conc. (μg/L)	Average conc. (μg/L)	District	Maximum conc. (μg/L)	Average conc. (μg/L)
Ampara	14	1	Kurunegala	3	0
Anuradhapura	3	1	Mannar	3	1
Badulla	2	0	Matale	3	0
Batticaloa	1	0	Matara	1	0
Colombo	0	0	Moneragala	6	0
Galle	6	0	Mullaitivu	2	1
Gampaha	0	0	Nuwara Eliya	0	0
Hambantota	2	0	Polonnaruwa	1	0
Jaffna	2	0	Puttalam	2	1
Kalutara	1	0	Ratnapura	4	1
Kandy	0	0	Trincomalee	0	0
Kegalle	3	0	Vavuniya	6	1
Kilinochchi	2	1			

Table 5 | Arsenic, cadmium, lead, and chromium in rice samples from CKDu-endemic areas ($n = 67$)

	Maximum value (mg/kg)		Average value (mg/kg)		Standard deviation (mg/kg)		
	Polished rice	Unpolished rice	Polished rice	Unpolished rice	Polished rice	Unpolished rice	CODEX[a] (mg/kg)
As	0.20	0.08	0.03	0.03	0.04	0.04	0.2
Cd	0.87	0.52	0.12	0.16	0.19	0.17	0.4
Pb	0.08	0.02	0.01	0.00	0.02	0.01	0.2
Cr	2.03	1.50	0.06	0.27	0.27	0.54	–

[a]Maximum level of the Codex standard for polished rice.

Table 6 | Arsenic, cadmium, lead, and chromium in rice samples from CKDu non-endemic areas ($n = 24$)

	Maximum value (mg/kg)		Average value (mg/kg)		Standard deviation (mg/kg)		
	Polished rice	Unpolished rice	Polished rice	Unpolished rice	Polished rice	Unpolished rice	CODEX[a] (mg/kg)
As	0.07	0.09	0.03	0.04	0.03	0.03	0.2
Cd	0.65	1.43	0.21	0.18	0.24	0.36	0.4
Pb	0.00	0.02	0.00	0.00	0.00	0.00	0.2
Cr	0.40	0.58	0.07	0.10	0.14	0.14	–

[a]Maximum level of the Codex standard for polished rice.

Agricultural Organization and WHO, are shown for comparison (Codex 2014).

It was observed that the average arsenic contents in polished and unpolished rice collected from both CKDu-endemic and non-endemic areas were well below the maximum level of the Codex standard for polished rice. Chandrajith *et al.* (2011) analyzed rice samples collected from the CKDu-endemic regions of Girandurukotte and Nikawewa in the North Central Province of the Dry Zone of Sri Lanka and found that the rice samples contained relatively high levels of arsenic, ranging between 0.09 and 0.26 mg/kg. The highest value was relatively higher than the maximum level of the Codex standard, 0.2 mg/kg.

The average cadmium content in polished and unpolished rice collected from both CKDu-endemic and non-endemic areas was also well below the maximum level of the Codex standard for polished rice. The study conducted by Bandara *et al.* (2008) to determine cadmium levels in rice from households of individuals with CKDu in the North Central Province of Sri Lanka where CKDu was prevalent showed the following results. All collected rice samples contained cadmium. Rice grains collected from Medawachchiya contained cadmium ranging from 0.001 to 0.093 mg/kg, with a mean value of 0.0444 mg/kg ±0.0165, while those from the Anuradhapura-Thuruwila area contained cadmium ranging from 0.001 to 0.194 mg/kg, with a mean value of 0.0404 ± 0.0196 mg/kg. The background value for cadmium in rice grains in Sri Lanka was found to be 0.001 mg/kg. Based on the analyzed results, Bandara *et al.* (2008) concluded that long-term exposure to dietary cadmium in rice over the years would adversely affect human kidneys. On the contrary, Chandrajith *et al.* (2011) analyzed the cadmium content in rice collected from CKDu-endemic regions of Girandurukotte and Nikawewa in the Dry Zone of Sri Lanka and found substantially lower values; rice samples from the Girandurukotte area ranged from 0.009 to 0.018 mg/kg, while those in the Nikawewa area ranged from 0.003 to 0.013 mg/kg. Chandrajith *et al.* (2011) concluded that cadmium in rice is not a possible risk factor for the occurrence of CKDu in Sri Lanka.

The lead levels in polished and unpolished rice collected from both CKDu-endemic and non-endemic areas were well below the maximum level of the Codex standard for polished rice. The chromium content in rice from both CKDu-endemic and non-endemic areas had lower recorded concentrations for both polished and unpolished rice, although there is no standard value set in the Codex standard for comparison.

The two-sample t-test, assuming unequal variances ($p < 0.05$), was performed to investigate whether there is a significant difference between arsenic, cadmium, lead, and chromium levels in rice collected from CKDu-endemic and non-endemic areas. Results indicated that there is no significant difference between the arsenic, cadmium, lead, and chromium levels in CKDu-endemic and non-endemic areas. Therefore, it is concluded that arsenic, cadmium, lead, and chromium in rice are not causing CKDu.

L-FABP, arsenic, cadmium, lead, and chromium in human urine in Sri Lanka

Urine L-FABP concentrations were analyzed to reveal the renal function of people in both CKDu-endemic and non-endemic areas. L-FABP is found in the cytoplasm of human proximal tubular cells of the kidney (Matsui *et al.* 2011). Human L-FABP binds to fatty acids and transports them into mitochondria or peroxisomes, where the fatty acids are β-oxidized, and contributes to intracellular fatty acid homeostasis. L-FABP has been detected as an early diagnostic of acute kidney injury and a biological indicator for the monitoring of chronic kidney disease (Yokoyama *et al.* 2009). Deterioration of renal function increases the L-FABP in the proximal tubules of the kidney and accelerates the excretion of L-FABP into the urine (Kamijo *et al.* 2006).

The safe limit of L-FABP in urine is 8.4 μg/g cr (μg/g creatinine). In our study, people with L-FABP concentrations of more than 8.4 μg/g cr were categorized as CKDu affected, while people with L-FABP concentration of 8.4 μg/g cr or less were categorized as CKDu non-affected. Since 1.5 μg/L is the minimum determination limit of L-FABP in urine, for the people with L-FABP concentration less than the limit, 1.5 μg/l was used as the L-FABP concentration for the categorization. Accordingly, people with L-FABP concentrations of less than 1.5 μg/L and more than 8.4 μg/g cr were categorized as 'unknown.' Ten percent of people in CKDu-endemic areas were categorized as CKDu affected, while all of the people in the non-endemic area were categorized as CKDu non-affected, based on their L-FABP values.

Figures 1–4, respectively, show the relationships between L-FABP concentrations and arsenic, cadmium, lead, and chromium concentrations in urine corrected by the creatinine concentration of both CKDu affected and non-affected people. Only the data showing L-FABP concentrations of more than 1.5 μg/l were used for the figures. It is obvious that there was no positive correlation between L-FABP concentrations and the arsenic, cadmium, lead, and chromium concentrations in urine. The arsenic, cadmium, lead, and chromium concentrations in urine of both CKDu affected and non-affected people are independent of L-FABP concentrations.

Two-sample t-tests that assumed unequal variances ($p < 0.05$) were performed to identify whether there is a significant difference between arsenic, cadmium, lead, and

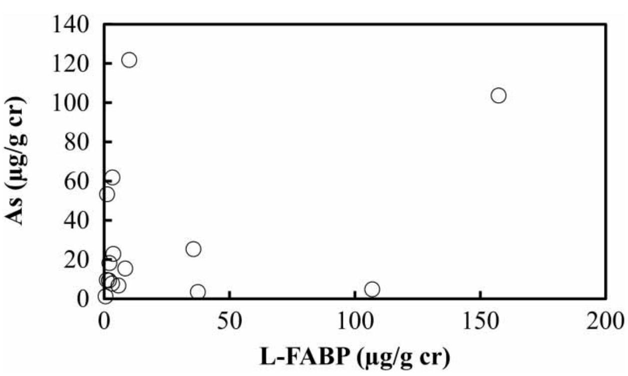

Figure 1 | Relationship between L-FABP and arsenic concentrations in human urine.

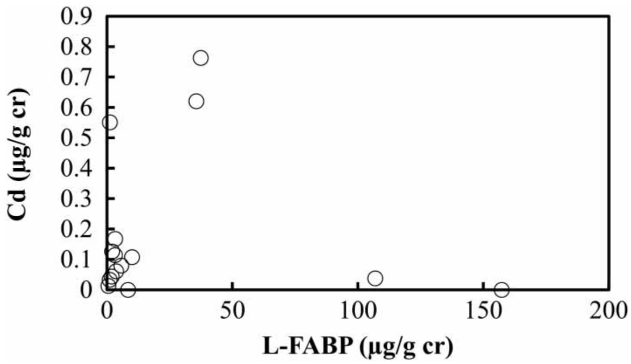

Figure 2 | Relationship between L-FABP and cadmium concentrations in human urine.

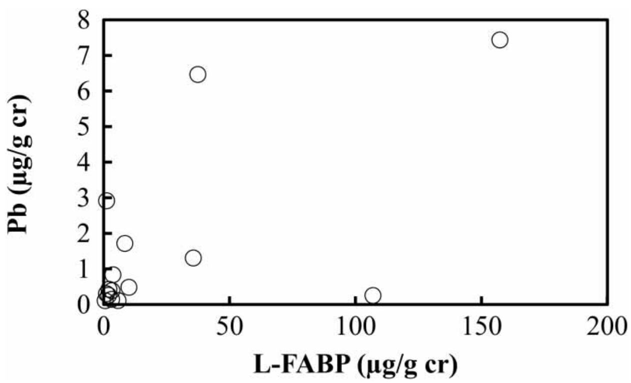

Figure 3 | Relationship between L-FABP and lead concentrations in human urine.

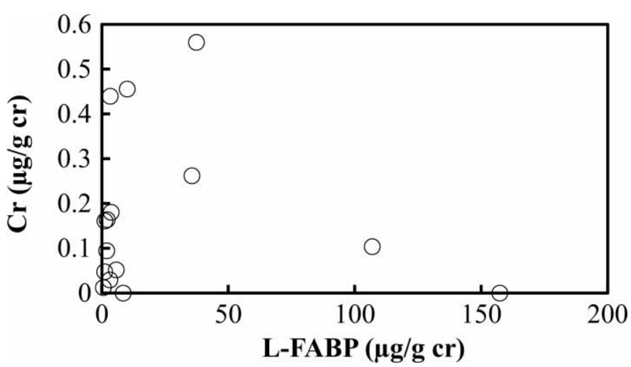

Figure 4 | Relationship between L-FABP and chromium concentrations in human urine.

chromium concentrations in the urine of people in CKDu-endemic areas and non-endemic areas. Analyzed results revealed that there were no significant differences between the groups.

After analyzing 745 samples, Jayatilake *et al.* (2013) reported that no significant differences were found in arsenic concentrations in the urine samples of CKDu-affected people as compared to controls in Sri Lanka. This coincided well with our analysis of arsenic. The WHO final report of 2012 (WHO 2012) revealed that there was significantly lower urine arsenic excretion in CKDu-affected people (mean 45.4, max. 617 μg/g cr) than in the urine arsenic excretion of non-affected people (mean 92.4, max. 966 μg/g cr) ($p < 0.01$) in endemic areas after analyzing 495 urine samples from CKDu-affected people and 250 urine samples from non-affected people. However, our study showed no significant difference between the arsenic concentrations in CKDu-affected people (mean 45.8, max. 122 μg/g cr) and non-affected people (mean 25.1, max. 100 μg/g cr) ($p < 0.05$) in endemic areas. The fact that there is no relationship between L-FABP and arsenic concentrations, as shown in Figure 1, indicates that arsenic excretion is not a possible indicator of CKDu.

Jayatilake *et al.* (2013) reported that the urine cadmium concentration in CKDu-affected people was significantly higher as compared to that of controls in both endemic and non-endemic areas of Sri Lanka. The WHO (2012) reported that considerably higher cadmium concentrations in urine were found in healthy people in endemic areas as compared to those in people in non-endemic areas (mean 0.35, max. 2.08 μg/g cr) ($p < 0.05$). The WHO (2012) also reported that significantly higher urine cadmium concentrations were detected in CKDu-affected people (mean 1.04, max. 8.93 μg/g cr) as compared to those of healthy people (mean 0.65, max. 5.13 μg/g cr) ($p < 0.05$) in endemic areas. On the contrary, we have detected that the urine cadmium concentrations in CKDu-affected people (mean 0.25, max. 0.76 μg/g cr) were not significantly different from those in non-affected people (mean 0.13, max. 0.55 μg/g cr) ($p < 0.05$) in CKDu-endemic areas. Urine cadmium concentrations in non-affected people in CKDu-endemic areas (mean 0.13, max. 0.55 μg/g cr) were significantly lower than those in non-endemic areas (mean 0.28, max. 0.96 μg/g cr) ($p < 0.05$).

The WHO (2012) revealed that there was no significant difference in the lead excreted in the urine of CKDu-affected people (mean 1.15, max. 8.53 μg/g cr) and healthy people (mean 1.02, max. 2.25 μg/g cr) in CKDu-endemic areas. In our study as well, the concentrations of lead in the urine of CKDu-affected people (mean 2.94, max. 7.44 μg/g cr) were not significantly different than those of non-affected people (mean 1.50, max. 10.82 μg/g cr) ($p < 0.05$) in CKDu-endemic areas.

CONCLUSIONS

Arsenic, cadmium, lead, and chromium in well water, rice, and human urine were measured to detect whether these elements could be a cause of CKDu prevalent in Sri Lanka. In addition, L-FABP in human urine was measured to detect the relationship with arsenic, cadmium, lead, and chromium in human urine.

Arsenic, cadmium, lead, and chromium concentrations in well water collected from 1,435 wells in all 25 Sri Lankan districts indicated that no district recorded maximum and average values of cadmium and chromium concentrations above the Sri Lankan standard or the WHO guideline, which shows that cadmium and chromium in well water are not possible causes of CKDu. Lead concentrations in well water were also recorded as being below the Sri Lankan standard and the WHO guideline, with the exception of one sample. The well water arsenic in the Mannar, Puttalam, Batticaloa, and Mullaitivu Districts had concentrations that were above the Sri Lankan standard and the WHO guideline of 10 μg/L. However, these areas have not yet been identified as CKDu-endemic areas. Therefore, it is clear that arsenic, cadmium, lead, and chromium in well water should not be the sole causative agents of CKDu.

Rice samples collected from CKDu-endemic areas and non-endemic areas were also analyzed for arsenic, cadmium, lead, and chromium. The rice samples showed arsenic, cadmium, and lead contents below the Codex standards, and the chromium content in rice also had a lower value. Furthermore, we found that there was no significant difference in the arsenic, cadmium, lead, and chromium contents in rice between CKDu-endemic and non-endemic areas. Thus, it is clear that arsenic, cadmium, lead, and chromium in rice are not possible causes of CKDu.

Furthermore, the L-FABP concentration in human urine was compared with arsenic, cadmium, lead, and chromium concentrations in human urine. No relationships were found between them. In addition, arsenic, cadmium, lead, and chromium concentrations in human urine samples from CKDu-endemic areas were not significantly different from those from non-endemic areas, indicating that arsenic, cadmium, lead, and chromium are not causes of CKDu.

Finally, it was concluded that arsenic, cadmium, lead, and chromium could not cause CKDu.

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