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# Drinking water quality and chronic kidney disease of unknown etiology (CKDu): synergic effects of fluoride, cadmium and hardness of water

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**Abstract** High prevalence of chronic kidney disease of unknown etiology (CKDu) in some regions of the world is suspected mainly due to a toxin-mediated renal failure. We examined the incidence of CKDu and potable chemical water quality in a CKDu-affected region. This region has been identified as a high-risk zone for CKDu (location: latitude: 8.3500°–9.0000°, longitude: 80.3833°–81.3000°, North Central Province, NCP, Sri Lanka) by the World Health Organization (WHO). However, within this macro-region, small pockets of CKDu non-prevalence zones do exist; notably, the residents in those pockets consume spring water. Therefore, the drinking water quality of four areas, namely high-CKDu-prevalence areas (zone I), low-CKDu-prevalence area (zone II), the CKDu-free isolated pockets (zone III) and control areas (controls) were examined for F, Al, Cd, and As, and hardness and the statistical analysis were carried out to probe possible correlations among these parameters. The fluoride and hardness concentrations of water in zone III and control areas are much lower compared to zones I and II, and the water hardness is ~61 mg/L $CaCO_3$. In zones I and II, the harness of drinking water is ~121–180 mg/L $CaCO_3$; however, Al, Cd and As concentrations are almost comparable and below WHO recommendations. In most of the locations in zones I and II, the F concentration in drinking water is higher than the WHO recommendations. The peculiar distribution patterns of CKDu point to a synergic effect of trace elements in water for etiology of the disease.

**Keywords** Chronic kidney disease · Fluoride toxicity · $AlF_x$ complex · Metal toxicity · CKDu · Synergistic effect

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## Introduction

Chronic kidney disease (CKD) is a worldwide public health problem, and it is receiving increased global attention because of the rapid spread of the disease. Aging, diabetes, hypertension and nephrotoxic drugs usage are known to be primary risk factors for CKD. However, CKD of unknown etiology (hereafter

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CKDu) is also prevalent and rapidly progressing in some regions of the world notably in Africa, Central America, and Asia (Codreanu et al. 2006; Nahas and Belle 2005). The term "unknown etiology" refers, because the disease is not associated with any known risk factors. If not properly treated, CKD can lead to end-stage renal disease and often death (Dalrymple et al. 2011). Recent studies revealed that there is an increasing trend in mortality due to chronic kidney disease of unknown etiology (Abeysekara 2006; Aturaliya et al. 2009; Gooneratne et al. 2008). Although precise epidemiological data are lacking to date in most cases, it has been identified occupational and environmental causes as the main reason for prevalence of CKDu (Cerdas 2005; Soderland et al. 2010). The natural substances aristolochic (Debelle et al. 2008) acid and ochratoxin A (Hassen et al. 2004) have been related to CKDu outbreaks, and long-term exposures to trace elements have been revealed as possible causes of renal damage (Barbier et al. 2005). In South Asian countries like Sri Lanka, it is noted for an alarming high incidence of CKDu during last three decades. The disease is characterized by a slow, progressive, asymptomatic development, frequently starting at a younger age group. A steady increase of this disease has been observed during the last twenty years. At present, CKDu is a severe health problem in Sri Lanka (WHO 2012).

Risk factors/hypotheses for CKDu

The causes and risk factors for the development of CKD vary widely. The CKDu is not related to any of the known causes, such as diabetes, mellitus, hypertension, and infection. However, histopathology of the affected kidneys showed tubule-interstitial nephritis which is suggestive of a toxic etiology (Dissanayake et al. 2012). The incidence of CKDu can vary with geographic area within the same country. An intimate relationship of water quality and the underlying geology has repeatedly been reported in different geographic regions of the world (for example Stamatis et al. 2011 and references therein; Gamvroula et al. 2013). Furthermore, Custodio (www report 1) reviewed the spatial distribution of selected trace elements in water and their relationship to drinking water quality. As the CKDu occurs in settlements where groundwater is the main drinking water source, several risk factors are hypothesized: (a) Unrecognized environmental toxins and occupational exposures may lead to the chronic kidney disease (Wanigasuriya et al. 2011); (b) chronic pesticides exposure and thereby increase of heavy metals (e.g., Cd, Pb) in soil and water (Bandara et al. 2010; Jayatilake et al. 2013); (c) presence of high levels of fluoride and possible implications of $AlF_x$ in soil and water (Ileperuma et al. 2009); (d) cyanobacterial toxins in water (especially in reservoirs) (Dissanayake et al. 2011, 2012); and (e) genetic as well as social factor (Nanayakkara et al. 2013). Though all these risk factors can be categorized into water quality, there is no indisputable evidence to distinguish the possible environmental causative factors that could lead to a nephrotoxin responsible for the disease to date. During preliminary investigation of water quality and the prevalence of CKDu, we noticed that even among high-CKDu-prevalence areas, there are small areas or villages that are not affected by the disease (Wasana et al. 2012). In addition to water source and quality, those areas are different from the affected areas (Dissanayake 2005). In these areas, the water source and quality are distinctly different from the CKDu-affected areas. In this research, we systematically examined the quality of potable waters in these locations to assess possible relationships between water quality and the incidence of CKDu. This study was undertaken in a high-risk CKDu region (location: latitude: 8.3500°, longitude: 80.3833°, NCP, Sri Lanka) as identified by WHO (WHO 2008).

## Materials and methods

As identified by WHO, for our investigation, the high-risk CKDu region under examination was further divided into three sub zones: zone I, high-CKDu-prevalence areas where the majority of the people living in the region are affected by the disease; zone II, low-CKDu-prevalence areas where the majority of the people living in the region are not affected by the disease; and zone III, CKDu non-prevalence areas where the people are not affected by the disease, although these areas are located within high-CKDu-prevalence region and control areas. As identified by WHO, the control areas are disease free. In zone I, the main water resources are either dug or tube well water, while in the zone II, it is mostly dug well water. However, in the zone III, the residents used spring

water. We tested all potable waters for common contaminants such as fluoride and trace metals that have previously been considered to be the main causative agents for CKDu disease.

Drinking water samples were randomly collected from the aforementioned zones (I, II, & III) and control areas (details are given in Table S1 in SI). The GPS coordinates and other details of the sampling locations are given in Figure S1 (support documentation). The water sources were selected for sampling, only if they have continuously been used by the residents for over 10 years. The water samples were collected from the sources which are used by the CKDu patients regularly. The samples were collected in duplicate from each site into pre-cleaned high-density polypropylene bottles. Each water sample was well mixed and filtered through a 0.45-μm membrane filter. For cation analysis, one set of sample was acidified with concentrated nitric acid. Filters and filter device were rinsed with 50 ml deionised water as described in method 3030B (Greenberg et al. 2005). From each filtrate, two 20-mL portions of samples were obtained randomly and stored in 25-mL glass vials at 4 °C until analysis for trace elements. The temperature and pH values of the samples were measured in situ in the field. The details of the CKDu patients' at each sampling locations were also recorded for future research.

Water samples were analyzed for F, Al, Cd, As, Ca and Mg. Fluoride concentrations were analyzed potentiometric method by a Bench top pH/ISE meter (Thermo Orion 4-star$^{TM}$ meter, USA) using combination fluoride ion selective electrode (ISE, 0.01 μg/L and 2–5 %CV) (Thermo Orion 9609BNWP, USA). The total Al and Cd in drinking water samples were determined using Graphite Furnace Atomic Absorption Spectroscopy (GF-AAS), (AAS GBC 933AA;GF GBC GF 3000; auto sampler GBC PAL 3300, Australia). Detection of total As in a selected set of drinking water samples were carried out by a continuous flow hydride generator AAS (GBC 933AA, Australia). Total Ca and Mg in the drinking water samples were analyzed using flame atomic absorption spectroscopy. For the determination of accuracy of AAS measurements, a standard reference material (SRM) 1643e was used. The estimated accuracy was within the 95 % confidence interval. The detection limits of Al, Cd, As, Ca and Mg by AAS method are 0.05 μg/L, 0.015 μg/L, 0.10 mg/L, 0.0005 mg/L and 0.0003 mg/L, respectively. The relevant experimental details are given in supplementary file. For quality control and quality assurance of results, the following method was employed: Calibration and reagent blanks were prepared and analyzed to establish a zero baseline and a background value, respectively, two independent sets of high-quality calibration standards were employed, calibration standards were prepared daily for AAS (Al, Ca, Mg, Cd, As) and ISE methods (for F) in diluted nitric acid (0.2 %) by serial dilution of concentrated stock solutions (e.g.,1000 μg/mL), and standard reference material (SRM) 1643e was used to analyzed with the real samples to assess accuracy. The standard errors for As, Cd, Al, Ca, Mg and F were −0.01, −0.56, −0.71, 2.70, 0.05 and 0.40 %, respectively.

Statistical analyses of data were performed by using Vegan package in R software (www report 2) to identify whether there is any significance evidence of the concentrations of chemicals among the given areas. The mean, range and the $p$ values of normality test for each element were obtained. There was no significant evidence that the data follow a normal distribution. Therefore, nonparametric statistical techniques were used for data analysis. Adonis test was carried out to compare the water quality parameters between selected areas including the control areas. Then, Wilcoxon signed-rank test was performed using Vegan-R package to test whether the median of the each element (F, Al, Cd and hardness) is larger than or equal to the recommended WHO value. In the case of F, both the lower (0.5 mg/L) and upper (1.5 mg/L) WHO limits were tested separately and the lower WHO fluoride limit was designated as adjusted WHO fluoride level. Thereafter, a biplot was drawn to understand the relationship of water quality parameters to the areas selected, and factor analysis was done to identify the number of significant factors and their respective factor loadings on water quality parameters.

## Results and discussion

In following sections, the water quality data in zones I, II, III and control areas are presented. Adonis test showed a significant difference ($p$ value = 0.001) of water quality parameters among selected areas. The mean, range and the corresponding $p$ values from the Wilcoxon signed-rank test, and a 95 % confidence

**Table 1** Statistical analysis of F, Cd, Al and hardness levels in drinking water samples in zones I–III and control areas with the maximum and minimum recommended WHO values (the values in bold indicate that the corresponding null hypothesis is not rejected for the given group at 5% significance level)

| Area | Mean (mg/L) ± SE | Range (mg/L) | *p* value to test the maximum recommended WHO value (1.5 mg/L) | 95 % Confidence interval | *p* value to test the minimum recommended WHO value (0.5 mg/L) | Mean (μg/L) ± SE | Range (μg/L) | *p* value | 95 % Confidence interval |
|---|---|---|---|---|---|---|---|---|---|
| A (HP) zone I | 0.554 ± 0.037 | 0.164–1.530 | <0.0000 | 0, 0.559 | **0.6228** | 0.492 ± 0.051 | 0.032–2.498 | <0.0000 | 0, 0.500 |
| B (HP) zone I | 1.020 ± 0.094 | 0.231–3.270 | <0.0000 | 0, 1.030 | **1.0000** | 1.405 ± 0.257 | 0.268–7.468 | <0.0000 | 0, 1.143 |
| C (HP) zone I | 1.337 ± 0.135 | 0.176–3.130 | **0.0837** | 0, 1.555 | **1.0000** | 1.388 ± 0.192 | 0.296–7.468 | <0.0000 | 0, 1.409 |
| D (HP) zone I | 1.050 ± 0.134 | 0.238–1.780 | 0.0054 | 0, 1.314 | **0.9910** | 1.873 ± 0.365 | 0.586–4.994 | 0.0148 | 0, 2.787 |
| E (LP) zone II | 0.411 ± 0.084 | 0.121–0.883 | 0.0001 | 0, 0.572 | **0.2490** | 1.992 ± 0.311 | 0.510–3.876 | 0.0050 | 0, 2.600 |
| F (LP) zone II | 0.412 ± 0.086 | 0.064–1.030 | <0.0000 | 0, 0.568 | **0.1877** | 2.277 ± 0.506 | 0.546–6.588 | **0.0770** | 0, 3.110 |
| G (LP) zone II | 0.464 ± 117 | 0.245–1.130 | 0.0078 | 0, 0.748 | **0.1484** | 1.090 ± 0.317 | 0.370–2.486 | 0.0078 | 0, 1.692 |
| H (LP) zone II | 1.122 ± 0.179 | 0.190–2.080 | 0.0273 | 0, 1.430 | **0.9961** | 1.036 ± 0.105 | 0.568–1.587 | 0.0019 | 0, 1.254 |
| I (NP, cont) | 0.046 ± 0.001 | 0.020–0.060 | <0.0000 | 0, 0.049 | <0.0000 | 0.578 ± 0.042 | 0.121–1.520 | <0.0000 | 0, 0.630 |
| J (NP, cont) | 0.257 ± 0.021 | 0.110–0.760 | <0.0000 | 0, 0.265 | <0.0000 | – | – | – | – |
| K (NP, cont) | 0.055 ± 0.006 | 0.030–0.100 | 0.0008 | 0, 0.065 | 0.0008 | – | – | – | – |
| L (NP, cont) | 0.170 ± 0.031 | 0.012–0.465 | <0.0000 | 0, 0.002 | <0.0000 | 0.802 ± 0.184 | 0.208–4.876 | <0.0000 | 0, 0.805 |
| | F | | | | | Cd | | | |

| Area | Mean (μg/L) ± SE | Range (μg/L) | *p* value to test the secondary standard WHO value | 9 95 % Confidence Interval | Mean (mg/L) ± SE | Range (mg/L) | *p* value to test the secondary standard WHO value | 95 % Confidence Interval |
|---|---|---|---|---|---|---|---|---|
| A (HP) zone I | 16.38 ± 2.45 | 3.28–120.43 | <0.0000 | 0, 13.50 | 215.29 ± 11.62 | 62.95–481.66 | **0.9950** | 0, 24.75 |
| B (HP) zone I | 56.42 ± 6.65 | 9.29–248.64 | <0.0000 | 0, 56.82 | 263.12 ± 27.72 | 48.41–978.95 | **0.9990** | 0, 269.25 |
| C (HP) zone I | 56.54 ± 11.18 | 4.32–239.57 | <0.0000 | 0, 61.58 | 222.60 ± 14.30 | 109.0–483.50 | **0.9830** | 0, 38.95 |
| D (HP) zone I | 51.17 ± 16.91 | 17.08–261.15 | 0.0001 | 0, 52.75 | 179.04 ± 44.63 | 69.39–664.42 | **0.0969** | 0, 48.95 |
| E (LP) zone II | 362.46 ± 59.61 | 21.21–633.82 | **0.9915** | 0, 489.49 | 143.77 ± 19.65 | 71.94–279.02 | **0.0467** | 0, 176.98 |
| F (LP) zone II | 56.48 ± 62.87 | 11.64–226.57 | <0.0000 | 0, 73.50 | 149.70 ± 15.95 | 48.41–233.93 | 0.0290 | 0, 76.96 |
| G (LP) zone II | 24.45 ± 1.94 | 18.71–31.98 | 0.0078 | 0, 28.93 | 252.96 ± 52.80 | 48.74–398.38 | **0.9219** | 0, 64.67 |
| H (LP) zone II | 22.89 ± 7.18 | 8.90–73.62 | 0.0019 | 0, 42.01 | 157.60 ± 13.80 | 105.60–252.20 | **0.0483** | 0, 178.91 |
| I (NP, cont) | 24.00 ± 3.13 | 10.00–130.00 | <0.0000 | 0, 20.00 | 35.22 ± 1.70 | 12.00–63.00 | <0.0000 | 0, 38.49 |
| J (NP, cont) | 76.25 ± 6.18 | 14.00–171.00 | <0.0000 | 0, 86.00 | 56.33 ± 3.90 | 16.00–120.00 | <0.0000 | 0, 62.00 |
| K (NP, cont) | 26.15 ± 5.60 | 10.00–90.00 | 0.0007 | 0, 25.00 | 21.54 ± 2.31 | 14.00–47.00 | 0.0007 | 0, 22.99 |
| L (NP, cont) | 57.65 ± 3.74 | 19.60–100.50 | <0.0000 | 0, 64.05 | 58.42 ± 5.64 | 24.20–106.80 | <0.0000 | 0, 67.95 |
| | Al | | | | Hardness | | | |

HP-high CKDu prevalence, LP-low CKDu prevalence, NP-CKDu non-prevalence and cont-control areas). Details of areas are given in supporting information. (SE-Standard error)

interval are shown in Table 1. In all these cases, the null hypothesis is that the median value of the respective element is greater than or equal to the WHO recommended value. The results showed some evidence for a close relationship between the prevalence of CKDu and the water quality.

## Fluorides in water

It is postulated that fluoride is one of the substances believed to be responsible for the occurrence of CKDu in the region (Ileperuma et al. 2009). Because the kidney accumulates more fluoride than all other soft tissues (with the exception of the pineal gland) (Inkielewicz and Krechniaka 2003), there is a concern that excess fluoride exposure may contribute to kidney disease. In a recent study, it was shown that elevated consumption of fluoride during childhood can cause kidney damage when the fluoride level in drinking water exceeds over 2.0 mg/L (Xiong et al. 2007). Table 1 presents the fluoride concentrations in drinking water of zone I, zone II and control areas. The quality of the spring waters, i.e., zone III is also shown in Table 2. According to WHO recommendations, the optimal fluoride level in drinking water is 1.5 (mg/L); however, due to adverse climatic conditions prevail in tropical countries, people consume more water than normal intake resulting in a high fluoride intake. Therefore, when assessing biological activity, the maximum level of fluoride concentration in these countries should be lowered (Warnakulasuriya et al. 1992, WHO 1994). It is evident from the data shown in Table 1 that the average fluoride levels in zone I are always higher than 0.5 mg/L, while in zone II, it is compatible with the WHO recommendations. Interestingly, in zone III, the average fluoride level is 0.066 (mg/L) which is much lower than the WHO recommendations. As shown in Table 1, the significance comparison test of fluoride levels in water was evaluated against WHO recommended limits (upper fluoride limit) and lower fluoride limit separately [recommendation level F 1.5 mg/L (upper fluoride limit) and adjusted level F 0.5 mg/L (lower fluoride limit)]. According to the results shown, when WHO level 1.5 (mg/L) is considered, most of the locations in zones I and II, a significance is not noted with respect to WHO fluoride level ($p < 0.05$) except area C. Note that area C is a high-CKDu-prevalence area, and according to the results, it has a significantly higher fluoride level than the WHO upper fluoride limit. When WHO lower fluoride limit was chosen for the calculations, a significance is noted in almost all locations found in zones I and II ($p > 0.05$). However, in control areas (areas I, J, K, L), the fluoride level is significantly lower ($p < 0.05$) than the WHO lower fluoride limits. These results imply that there could be a relationship between the fluoride concentration in drinking water and the prevalence of the CKDu in the areas under examined (Wasana et al. 2015).

Though the average value of fluoride concentration in zone II is 0.44 mg/L, around 30–40 % of water sources exceed WHO recommendations (Fig. 1). This implies that the residents who consumed this water in zone II are prone to CKDu disease. In agreement with previous data (Dissanayake and Chandrajith 2007), the zones I and II overlap with the high groundwater fluoride terrains of Sri Lanka. The risks of fluoride exposure of people living in the zone I to III were evaluated based on the exposure assessment calculation proposed by EPA, USA (www report 3), and details of calculation methods are given in the SI. According to EPA assessment calculation (SI), daily intakes of fluoride by the people living in zone I (HP), zone II (LP) and zone III/control areas (NP) are 0.103,

**Table 2** Water quality of different spring water collected in the subject region (zone III). Details of areas are given in supporting information

| Spring area | F (mg/L) | Al (μg/L) | Cd (μg/L) | Hardness (mg/L) | As (μg/L) |
|---|---|---|---|---|---|
| M—zone III | 0.061 | 49.180 | 0.700 | 31.475 | ND |
| N—zone III | 0.069 | 79.538 | 0.750 | 26.864 | ND |
| O—zone III | 0.066 | 72.434 | 0.296 | 16.527 | ND |
| P—zone III | 0.068 | 58.228 | 0.548 | 26.112 | ND |

Statistical analysis has not been performed for the chemical analysis results of the spring water samples since the number of observations is only 4

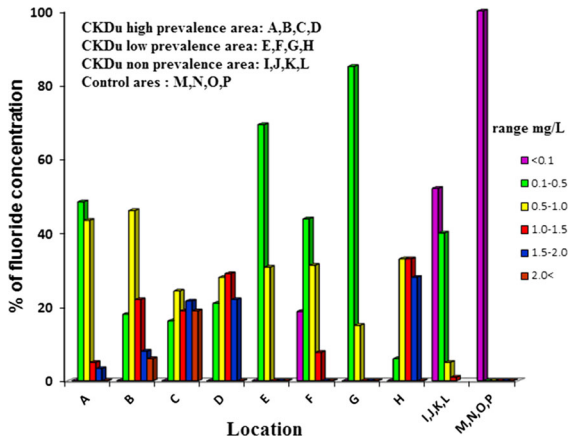


**Fig. 1** Distribution patterns of fluoride concentrations of potable water in CKDu prevalence and CKDu non-prevalence areas

0.034 and 0.008 mg/kg, respectively, against the WHO recommended value of 0.077 mg/kg. These data clearly indicate that people living in zone I consume more fluoride than WHO recommended value, and therefore, the presence of the fluoride in drinking water for the prevalence patterns of CKDu incidence is noteworthy. The confirmatory evidence in this respect needs to be obtained by animal trials (presently in progress in our laboratories).

## Trace elements in water

Although controversial, the high concentrations of Cd, Al, Pb and As in drinking water, soils or food are suspected agents for high incidence of CKDu. The following sections report the distribution of these elements in zones I, II, III and control areas.

### *Cadmium*

Table 1 summarizes Cd concentrations in drinking water collected from zones I, II, III and control areas. In zone I, the average Cd concentration ranged from 0.43 to 1.52 μg/L, while in zone II, it varies from 0.83 to 2.27 μg/L. Interestingly, in zones I, II and control areas, the Cd levels are very low (0.57–0.64 μg/L). The lowest Cd concentrations were recorded from spring waters (zone III). According to data shown in Table 1, only selected locations in zone II (zone II area F) showed significantly high values of Cd against WHO recommendations ($p > 0.05$) (see Table 1 for detail). The Cd concentration in all other locations in zones I, II, III and control areas is not significant against WHO levels. However, previous reports have shown that long-term exposure to Cd via the food, water or air, builds up in the kidneys and can possibly cause kidney disease and kidney failure via a number of pathways (Järup et al. 2000; Thijssen et al. 2007). For example, chronic exposure to Cd can cause both renal proximal tubular damage and decline in glomerular filtration rate (GFR) in humans which has been confirmed in experimental models (Järup et al. 2000; Thijssen et al., 2007). In a recent study, Bandara et al. (2010) reported high Cd concentrations in water (0.03–0.06 mg/L), sediments (1.78–2.45 mg/kg), fish and some vegetables in the same of high-risk area and designated Cd as a causative factor for CKDu. However, in the same region, many researchers (Chandrajith et al. 2011a, 2011b) showed over hundred-fold lower concentrations of Cd in surface water (0.003–0.008 mg/L) and rice grains of the same region, debating the role of Cd as a causative agent for the incidence of CKDu. In the present case, Cd levels in drinking water are within or well below the WHO recommended levels in both in high and non-prevalence CKDu areas. Therefore, it is unlikely that it alone to be considered as a potential risk factor for high CKDu incidence. However, in a recent study, it was suggested a relationship between CKD and urine cadmium concentration where cadmium is considered as a risk factor for the pathogenesis of CKDu (Ferraro et al. 2010).

### *Arsenic*

The latest hypothesis is that arsenic in pesticides combined with the hardness in water that results in formation of calcium arsenate crystals that are bound to arsenic transporters in the liver and transport to kidneys causing kidney damage (Jayasumana et al. 2011). Calcium arsenate precipitates at pH 4–8, it exists as free $Ca^{2+}$ and $AsO_4^{3-}$ at pH below 3.0, and the overall process is kinetically controlled. Considering the low As content in drinking water and the calculated free energies of calcium arsenates formation (−3787.87 kJ/mol) and solubility (2.9 to $4.8 \times 10^{-2}$ mol/L at pH 5.0) (Zhu et al. 2006), there is a high tendency that calcium arsenate exists as free ions. In all cases, the total As concentration in drinking water was lower than 2 μg/L (well below the WHO

**Table 3** Statistical analysis of arsenic levels in drinking water in zones I–III and control areas with the recommended WHO value 10 μg/L

| Area | Mean (μg/L) | Range (μg/L) | *p* value | 95 % Confidence interval |
|---|---|---|---|---|
| A (HP) | 0.8219 | 0.023–1.605 | 1.863e−09 | 0.9582 |
| B (HP) | 0.2728 | 0.117–0.263 | 0.000047 | 0.3554 |
| E (LP) | 0.190 | 0.018–0.610 | 0.0156 | 0.2449 |
| I (NP) | ND | ND | – | – |
| Spring area (zone III) | ND | ND | – | – |

(HP-high CKDu prevalence, LP-low CKDu prevalence, NP-CKDu non-prevalence and cont-control areas). Details of areas are given in supporting information

level of 10 μg/L total As). Our total As data which is shown in Table 3 are much lower than the values reported by Jayasumana et al. (2011). Accordingly, it can be concluded that none of the areas have a significant higher As level than the WHO recommended levels. Therefore, total As in water cannot be considered as the causative factor for the incidence of CKDu. As reported by Johnson et al. (2012), "Heavy trace metals in drinking water are not related to chronic kidney disease in Sri Lanka. If heavy trace metal is responsible, then there is a different source for it than drinking water and that should be explored."

*Aluminum*

Table 1 shows the Al concentration in drinking water from zones I, II, III and control areas. Only the area E (zone I area E) has a significantly higher Al levels ($p > 0.05$) than the WHO recommendations. The WHO secondary standard value for Al was used in the statistical analysis. This area represents a small proportion of total population (<1 %) who have accessed to alum-treated water. The Al concentrations in all other samples are comparable and well below the WHO recommendations; hence, it is unlikely that Al alone be a potential risk factor for the incidence of CKDu. However, Al in combination with fluoride may be a potential risk factor for CKDu. Aluminum readily forms complexes with fluoride to $AlF^{2+}$, $AlF_2{}^{+}$, $AlF_3$, $AlF_4{}^{-}$, $AlF_5{}^{2-}$ and $AlF_6{}^{3-}$ complexes [$AlF_x^y$] (Ileperuma et al. 2009, Jin et al. 2011). Previous workers showed that the deposition of aluminum in the kidneys of rats and postulated the role of aluminofluoride complex in the transport and the penetration of aluminum across the blood–brain barrier causing a potential factor for the development of Alzheimer's disease (Lubkowska et al. 2002, Varner et al. 1998). Ileperuma et al. (2009) postulated the same mechanism to the toxicity of same species to the kidney since it also involves the transport of aluminofluoride across biological membrane. Though this postulation has not been proven yet, investigation of the effect of aluminofluoride complex in CKDu may lead to answer the mystery of this disease. Further Ileperuma et al. (2009) observed the dissolution of Al from the aluminum utensils in the presence of high fluoride concentrations forming aluminofluoride complexes. They have observed leaching of 25 and 56 mg/L Al at basic and acidic pHs, respectively, in the presence of 6 mg/L fluoride in solution. However, under same conditions, at neutral pH, the observed dissolved Al amount is 1.2 mg/L and it was less than 0.1 mg/L at fluoride concentration of 2 mg/L. These results together with our Al and F results (30–40 % of drinking water in zones I and II contains 0.1–0.2 mg/L Al, except in the area A) suggest that if aluminofluoride complex is formed, aluminum source could also be naturally occurring rather than aluminum coming from utensils as suggested by Ileperuma et al. (2009). Furthermore, even though people living in the subject region begun to use clay pots for a considerable time, (more than 5 years) a number of new CKD patients are alarmingly increasing, suggesting further that the source of aluminum might not be the aluminum utensils but a naturally occurring aluminum source.

The effects of naturally occurring aluminum and fluoride in water on CKDu etiology are examined, and the data are shown in Fig. 2. From the data, it can be concluded that: (a) in zone I, where prevalence of CKDu is high, water contained high fluoride (0.6–1.2 mg/L) and low aluminum (~100 μg/L) concentrations (zone I), (b) in zone II, where prevalence of CKDu is moderate, water contained low fluoride and high aluminum concentrations and (c) in

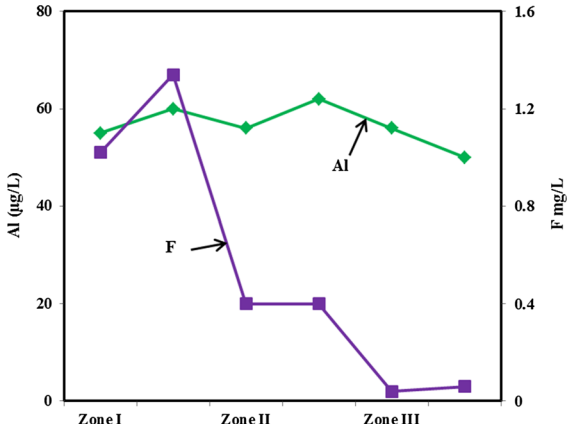


**Fig. 2** The variation of Al and F concentrations in potable waters with the prevalence of CKDu: zone I—high CKDu prevalence, zone II—low CKDu prevalence and zone III—No CKDu prevalence

zone III, where CKDu is non prevailing, water contained low fluoride and low aluminum concentrations. There appears that the levels of fluoride and aluminum in water are related to the prevalence of CKDu disease. However, these results required scientific verifications by animal trials (Wasana et al. 2015) and further investigations are currently in progress.

*Hardness*

Hardness of water is not considered causative for the prevalence of CKDu in this region. It should also be noted that there is no WHO recommendation for drinking water hardness and the reason for not establishing a guideline value is that the hardness levels observed in drinking water is of no apparent health concerns (WHO drinking water quality guidelines 2011). However, water with a hardness of <61, 61–120, 121–180 and more than 180 mg/L is considered soft, moderately hard, hard and very hard, respectively (www report 4). The presence of hardness may modify chemical forms of fluoride and trace elements in water; therefore, it may act as a secondary factor for the disease etiology. In the presence of high fluoride in water, Chrandrajith et al. (2011a, 2011b) showed the importance of $[Na^+]/[Ca^{2+}]$ ratio and $Ca^{2+}$ saturation index) for the assessment of CKDu etiology. In zones I and II, when $[Ca^{2+}] \gg [Na^+]$, the $F^-$ shows enhanced affinity to $Ca^{2+}$ (Chrandrajith et al. 2011a, 2011b). Further, these zones are mainly characterized by Ca–$HCO_3$ water type. However, high fluoride levels are common in both CKDu-affected and non-affected regions (zones I, II and III). Tables 1 and 2 show the variations of hardness in drinking water in CKDu-affected areas as compared to springs. The highest hardness (average ~230 mg/L as $CaCO_3$) in water is reported in zones I, and the water hardness decreases to ~150 mg/L $CaCO_3$ in zone II (except in zone II, area G). High hardness values were not reported ("soft water," average ~25 mg/L) in zone III and control areas. According to the results in Table 1, areas A, B and C in zone I and area G in zone II have a significantly very high hardness levels than the maximum WHO secondary standards. Also areas E, F and H in zone II have a comparable hardness level when compared to WHO recommended level, while control areas have very low hardness. Accordingly, the *p* values shown in Table 1 at 10 % level, in areas A (HE), B (HE), C (HE) and G (LE), have a significantly very high hardness than the maximum WHO recommended level. Areas E (LE), H (LE) and D (HE) show comparable hardness values to the maximum WHO recommendations, while control areas have very low hardness values. Similarly, it has been reported earlier (Dissanayake 2005) that, fluoride levels are higher in CKDu prevalence areas than in CKDu non-prevalence areas and we have noted the lowest F levels in spring waters where CKDu is absent. These results and observations that high $Ca^{2+}$ activity with increased fluoride in groundwater suggest that CKDu may link to combined effects of high fluoride and hardness factors. To further access this claim, we have plotted the fluoride and hardness of drinking water against the CKDu prevalence in Fig. 3a. As shown in the Fig. 3, in zone I where CKDu is prevalence, high fluoride and high hardness are observed in drinking water sources indicating a possible link between CKDu to combined effects of high fluoride and high hardness factor which needs to be confirmed by an animal trial. Further, as shown in Fig. 3b, where CKDu prevalence is plotted against aluminum and hardness of drinking water, does not show clear correlation between hardness and presence of aluminum to CKDu prevalence levels.

According to the results so far shown, it is not possible to demarcate a single causative factor (from among F, Al, Cd, hardness) for the etiology of CKDu. Therefore, a multi-faceted nature of possible causative factors is assumed. This behavior was examined by a multivariate scatterplot (known as biplot) as illustrated

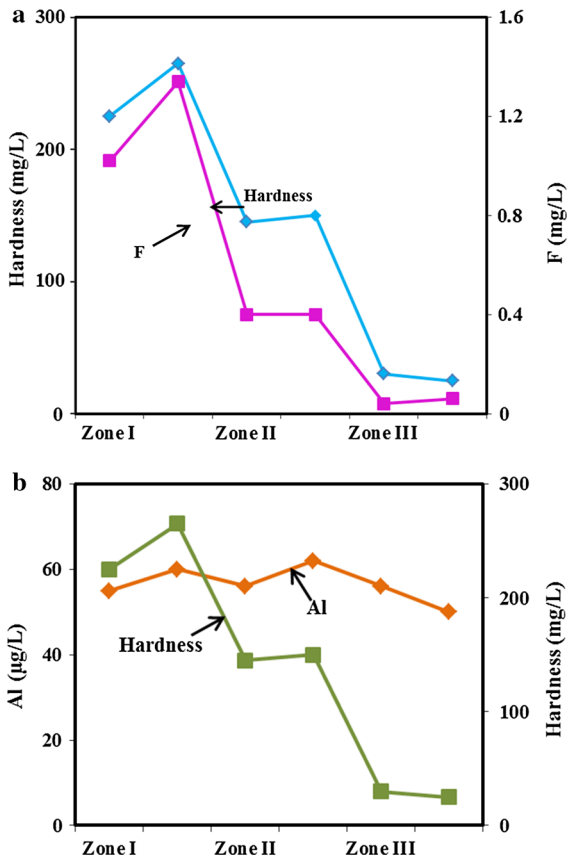


**Fig. 3** **a** The variation of total hardness and F of potable waters with the prevalence of CKDu. **b** The variation of total hardness and Al of potable waters with the prevalence of CKDu: zone I—high CKDu prevalence, zone II—low CKDu prevalence and zone III—no CKDu prevalence

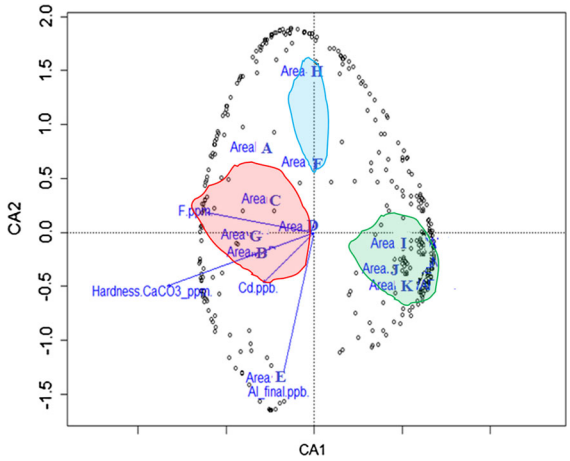


**Fig. 4** Multivariate scatterplots of water quality parameters in zones I–III and control areas

in Fig. 4. The biplot is drawn from the correlation matrix used in principal component analysis. As expected, the control areas (I, J, K and L, marked in green color in Fig. 4) are distinctly separated from other areas, showing contrasting differences in water quality. The water quality of two locations in zone II (H and F, marked in blue color in Fig. 4) are also different in comparison with zone I and control areas. In zone I (B, D and C, marked in red color in Fig. 4), the occurrence of CKDu is highly correlated with F and hardness (and to a lesser extent with Cd). In other words, effects of F and hardness are marked in zone I. Further, the area G (which shows growing trends as zone I) is highly correlated with F and hardness. As mentioned earlier, the effect of Cd in zone I and II (B, D and C, marked in red color in Fig. 4) and the area G is considerable even though the effect of Cd is not that marked as of F and hardness, suggesting a possibility of existing of synergy among hardness, F and Cd with the etiology of CKDu. Further, as shown in Fig. 4, the acute angles between F and hardness, F and Cd, and hardness and Cd vectors indicate strong positive correlations. Adachi et al. (Adachi et al. 2007) reported liver and renal toxicity in animal models following acute CdF administration and if a synergic effect does exist within F, Cd and hardness, there is a strong possibility of formation of toxic substances such as $CaF_2$ and $CdF_2$ which can damage liver and kidney.

Data presented in Fig. 4 clearly indicate a strong correlation between CDKu and presence of F, Cd and hardness in drinking water. However, data analysis also revealed some exception in area A which is a known high-CKDu-prevalence area but slightly deviated from other high-prevalence areas in Fig. 4. This may indicate a different effect(s) to this region compared to other high-prevalence regions. Also, water quality of the area A is clearly separated from the control areas as well. It is also interesting to note the behavior of Al where the effect of Al is weak in all considered areas, while it is high in the area E (which is a low-prevalence area) due to treated water (higher dose of Alum addition).

The overall results presented a clear correlation between prevalence of CKDu and the presence/absence of certain elements in drinking water. The correlation analysis showed a synergistic effect of hardness, F, Al and Cd with the incidence of CKDu. To extract the number of significant factors, parallel

analysis, optimal coordinates and acceleration factor methods were used and the results have pointed to a single significant factor. Then, an orthogonal factor model was fitted by using standardized vales of Al, Cd, F and hardness. Based on the uniqueness values derived by this analyses, approximately 99 % of the variance in "Al," 87 % of the variance in "hardness," 69 % of the variance in "Cd" and 51 % of the variance in "F" were not shared with other variables in the overall factor model. This implies that Al, hardness, Cd and F contribute approximately 1, 13, 31 and 49 % of the variance to this significant factor, respectively. Based on the proportion of variance, 24 % of the total sample variance can be explained by factor 1, and the standardized variables of Cd, F and hardness are highly loaded on factor 1 and the loading of Al is negligible. When comparing the loadings of each element, it follows the order F > Cd > hardness where F has the highest effect.

## Conclusions

In spring waters, the concentrations of F, Cd, As and hardness showed lowest values. Aluminum levels are comparable (except in treated water) in other areas and in the spring water. No CKDu incidence was reported where people consumed spring water for a considerable period of time (more than 10 years). It is suspected that the high quality of the spring water and the lack of possible causative CKDu agents may keep the people healthy. In all areas examined, the average Cd and As levels are below the WHO recommendations (Cd 3 μg/L and As 10 μg/L); hence, these elements alone cannot be causative factors for the disease etiology. A positive correlation between the fluoride concentration in drinking water and the prevalence of the CKDu was noted. However, the fluoride in water cannot be considered causative since low-CKDu-prevalence areas were also located in high fluoride zones. Therefore, it is conferred that fluoride in water is not acting alone and it is triggered by other factors. The triggering factor/s could be either hardness or Cd or it could be synergic effects of F, Cd and hardness. It is inferred that both mechanisms are actively responsible for triggering CKDu. The multifaceted nature of possible causative factor for CKDu was confirmed by the multivariate scatterplot analysis which shows a strong correlation of synergic effect of F, Cd and hardness for the prevalence of CKDu. This observation needs further validations by animal trial experiments which are currently underway. It is recommended to utilize either treated or spring water as a preventive measure of CKDu in the regions examined.

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