Groundwater as a potential cause of Chronic Kidney Disease of unknown etiology (CKDu) in Sri Lanka: a review
Sachithra Imbulana and Kumiko Oguma

ABSTRACT
The cause of Chronic Kidney Disease of unknown etiology (CKDu) in the rural dry zone of Sri Lanka remains unidentified, despite vast research efforts that brought about an extensive list of potential risk factors. Among these, the long-term exposure to various nephrotoxic elements through drinking groundwater was widely suspected owing to the unique geographical distribution of the disease. This review focuses on such well-known hypotheses suspecting the relations with fluoride, hardness, major ions, heavy metals, metalloids, organic matter, agrochemical residues, pathogens, and bacterial toxins in the groundwaters of the CKDu-endemic region. It was comprehensively discussed why each of these constituents was considered a risk factor of CKDu, how could they possibly trigger the pathogenesis of the disease, what was the evidence that supported or failed each hypothesis, and whether providing safe drinking water had been effective at mitigating the progression of the disease. Although plenty of circumstantial evidence supported an etiology related to groundwater for CKDu, it was impossible to elucidate the cause-effect relationships between drinking impaired groundwater and the occurrence of the disease. Future research should be effectively designed to clarify the role of groundwater in the onset of CKDu by taking into account the gaps in past research.

Key words | Chronic Kidney Disease of unknown etiology, drinking water, groundwater, water quality

HIGHLIGHTS
- Most updated review on the underlying topic to cover all relevant original studies up to 2020.
- An intensive discussion focusing on groundwater as a potential cause of CKDu.
- Useful for identifying the gaps in previous research and seeking new methods to elucidate the cause of CKDu.
- Highly relatable to those who are interested in exploring health implications of drinking water.

INTRODUCTION
Chronic Kidney Disease (CKD) is a global health problem of ‘non-communicable’ nature, which accounts for nearly 700 million active cases and 1.2 million deaths per year (GBD Chronic Kidney Disease Collaboration 2020). The commonly known risk factors of CKD include diabetes, hypertension, and chronic glomerulonephritis (Noble et al. 2014). However, an increasing incidence of a different form of CKD that is not associated with any of these traditional risk factors has been reported from many parts of the
globally, especially from developing countries of the tropical-arid regions of Central America, Eastern Europe, and South Asia (Gifford et al. 2017). Because the cause of this disease is devoid of commonly known risk factors of CKD, it is termed CKD of unknown etiology, or ‘CKDu’.

Balkan Endemic Nephropathy (BEN) in countries around the Danube river basin, Mesoamerican nephropathy (MeN) among sugar cane cutters from El Salvador and Nicaragua, Uddanam endemic nephropathy in Andhra Pradesh of India, and CKDu in the rural dry zone of Sri Lanka set best examples for famous CKDu cases across the world. A striking similarity of these nephropathies is their clustered distribution among rural agricultural communities subjecting to strenuous labor under hot climatic conditions (Weaver et al. 2015; Levine et al. 2016; Gifford et al. 2017; Johnson et al. 2019). Decades of research revealed that BEN occurred due to chronic dietary exposure to aristolochic acid (AA) via ‘Aristolochia clematitis’, a weed grown in the wheat fields of the endemic region (Stiborová et al. 2016). Nevertheless, the causes of CKDu elsewhere remain unidentified despite immense research attempts.

CKDu in Sri Lanka was first reported from the North Central Province (NCP) of the rural dry zone (temperature: 25–30 °C; mean annual rainfall: <1,500 mm) in the 1990s. The primary victims of the epidemic are male paddy-farmers of 30–60 years belonging to substandard socio-economic backgrounds (Athuraliya et al. 2011; Jayatilake et al. 2013; Noble et al. 2014). Early socio-demographic studies on CKDu identified 2–3% prevalence of CKDu in the NCP (Athuraliya et al. 2006), but the figures have escalated to around 15–23% in the recent past (Rajapakse et al. 2016). Moreover, the disease keeps rapidly propagating to other farming areas of the dry zone that are located outside the NCP (Noble et al. 2014).

Multi-disciplinary research of nearly three decades has postulated a large number of risk factors for the etiology of CKDu in Sri Lanka. Interestingly, the confined nature of observed geographical and socio-economic disease patterns implied a cause associated with geo-environmental and occupational factors (Chandrajith et al. 2011a; Jayasumana et al. 2017). Some of those popular risk factors included chronic exposure to nephrotoxic elements such as toxic heavy metals and agrochemical residues during farming activities (Jayatilake et al. 2013; Jayasumana et al. 2014, 2015a, 2015b); long-term consumption of untreated groundwater that is rich in harmful chemical and microbiological contaminants (Chandrajith et al. 2011a, 2011b; Jayasekara et al. 2013; Dharma-wardana et al. 2015; Jayasumana et al. 2015a; Wasana et al. 2016; Dissanayake & Chandrajith 2017); occupational exposure to heat stress under the hot climatic conditions prevail in the dry zone, and subsequent low water intake leading to dehydration of kidneys (Herath et al. 2018; Jayasekara et al. 2019).

Unfortunately, none of the said risk factors alone could logically explain the concomitance of the unique geographical, clinical, and socio-economic characteristics of the disease; thus, the synergistic involvement of two or more risk factors is suspected from the onset of CKDu. Meanwhile, a substantial number of past studies postulated at least the partial intervention of groundwater, because it is the main source of drinking water in the disease-endemic areas. The review herein intends to build up a comprehensive discussion on drinking impaired groundwater as a potential cause of CKDu. The findings of past research were collectively reviewed to understand whether the major pollutants detected in groundwater of the CKDu-endemic region might have contributed to the origin or progression of the disease.

RELATIONSHIP BETWEEN CKDu AND THE SOURCES OF DRINKING WATER

The dry zone of Sri Lanka suffers prolonged dry periods and limited rainfall (temperature: 25–30 °C and mean annual rainfall: <1,500 mm) (Department of Meteorology Sri Lanka 2000); thus, supplying enough water to fulfill daily water needs is challenging. NCP, the origin and the geographic hotspot of CKDu in Sri Lanka, is the home for an ancient tank–cascade system that supplies water for irrigation-based agriculture in the dry zone for nearly two millennia (Madduma Bandara 1989). As the population kept growing, the subsequent growth in agricultural activities started stressing the limited water resources of the dry zone. In order to meet this increased demand, exploiting groundwater was commenced in the dry zone around the 1960s (Panabokke & Perera 2005). The groundwater resources in the hard metamorphic rock regions of the
North Central dry zone are primarily found in two aquifer forms, namely the ‘shallow regolith aquifer’ (~10 to 20 m) and the ‘deeper fracture zone aquifer’ (~30 to 40 m). The agro-wells and domestic dug wells in this region are typically associated with the regolith aquifers that are in close contact with the tank cascade systems, whereas the tube wells are constructed in the fractured rock zone (Pathmarajah 2002).

Due to the limited accessibility to drinking water through regular pipe water supplies, dug wells and tube wells are adopted as the primary sources of drinking and cooking water in these areas. According to Kafle et al. (2019), more than 98% of the population in CKDu highest-affected areas of Sri Lanka had been solely dependent on well water to fulfill their potable water needs for at least five consecutive years over the last two decades. Past research suggested that the quality of groundwater in this region was unfit for drinking, yet most of the consumers did not undertake the slightest treatment before consuming their well water (Chandrajith et al. 2019a). This inferred that groundwater might be interfering with the upkeep of health condition of the inhabitants in the CKDu-endemic region.

Some small communities in these areas relied on natural springs to fulfill their potable water needs. Surprisingly, a very low (1.5%) or zero prevalence of CKDu was reported from those who consumed this spring water continuously for more than a decade while living amidst the affected groups (Jayasekara et al. 2013; Wasana et al. 2016). It is recognized that spring water is of better quality compared with groundwater and does not require any treatment prior to consumption. Interestingly, anecdotal evidence suggested a reduction in the incidence of CKDu with the provision of safe drinking water through bowser supplies, service extensions, and community-type reverse osmosis (RO) water treatment plants (Wimalawansa 2019). Although the beneficial effects of safe drinking water on the health condition of CKDu patients have not yet been elucidated through proper scientific research, this circumstantial evidence hinted at a close association between the consumption of untreated groundwater and the occurrence of CKDu in the dry zone.

A substantial number of studies attempted to bring about a relationship between the incidence of CKDu and the hydrological characteristics and dynamics of groundwater resources used by the patients. GPS (Global Positioning System) data depicted that CKDu patients are clustered toward the lower elevations of the irrigational tanks and canals, implying a hydro-geochemical relationship for CKDu (Jayasekara et al. 2013; Ranasinghe et al. 2019). Using an isotopic approach, Edirisinghe et al. (2017) explained that the occurrence of CKDu seemed to be noticeable with groundwater resources only recharged by direct rain (but not by surface water inputs) or maintained in stagnation. Pollutants and chemical constituents in groundwater were thought to be maintained at high concentrations in stagnant groundwater resources used by CKDu patients, whereas the toxicity was reduced due to mixing with surface water in non-CKDu groundwaters. Subsequently, Balasubramanya et al. (2020) noted that CKDu was significantly associated with deep wells where water was held in pockets and extracted by pumps, but no such relationship was noted with shallow wells, where water was in contact with the irrigational schemes.

On the other hand, a great deal of inconclusive evidence from past studies supported that the quality of groundwater might be playing an important role in the origin or development of CKDu. A large number of water quality studies to date investigated a range of harmful constituents in these groundwaters as potential causative agents of the disease. Fluoride, total hardness and ionicity (mainly associated with Ca\(^{2+}\), Mg\(^{2+}\), and Na\(^{+}\)), some toxic heavy metals and metalloids (e.g., cadmium, lead, arsenic, and silica), agrochemical residues, organic matter, bacterial toxins, and certain viruses set best examples for such contaminants. Not only the individual effect of each of these elements, but also the synergistic effects of two or more of them have been broadly investigated so far. A comprehensive discussion on the possible links between each of these constituents in groundwater and the occurrence of CKDu is presented below.

**SUSPECTED RELATIONSHIPS BETWEEN GROUNDWATER QUALITY AND OCCURRENCE OF CKDu**

**Fluoride**

Fluoride is one of the most ubiquitously occurring natural elements in the Earth’s crust, and it is listed among the
very few substances to interfere with human health through drinking water. Although low doses of fluoride are essential for human health, excessive intake could lead to numerous detrimental effects such as dental and skeletal fluorosis (Fawell et al. 2006). The environmental exposure to fluoride and its nephrotoxic effects are not well documented to date, and the global literature presented a very limited evidence on renal failure attributed to chronic fluoride intoxication. However, kidney plays a key role in the excretion and retention of fluoride; hence, it is highly vulnerable to chronic fluoride intoxication (Shashi et al. 2002). As explained by Dharma-Wardana et al. (2015), fluoride may likely affect kidney tissues due to its high rank in the Hofmeister Series for denaturing proteins of the kidney membrane. Dose–effect relationships between fluoride levels in drinking water and the occurrence of kidney failure have been widely reported in international research (Reggabi et al. 1984; Lantz et al. 1987; Ludlow et al. 2007; Xiong et al. 2007); therefore, potential exposure to high fluoride concentrations through drinking groundwater gained a lot of attention with regard to the incidence of CKDu in Sri Lanka.

The Hydro-geochemical Atlas of Sri Lanka well illustrated that the groundwater–fluoride concentrations in the dry zone are relatively high (>0.5 mg/L) compared with the wet zone (<0.5 mg/L), where the disease is absent (Dissanayake & Weerasooriya 1985). Subsequently, Chandrajith et al. (2010a) recognized that the endemic areas of CKDu overlapped these high groundwater fluoride zones (1.0 mg/L to levels exceeding 3.0 mg/L), indicating a potential correlation between the exposure to high fluoride through drinking water and the occurrence of CKDu. Weathering of fluoride-bearing minerals under the hot climatic conditions prevails in the dry zone, and subsequent high evaporation is considered the main pathway via which fluoride is brought into these groundwaters (Dissanayake 1991; Chandrajith et al. 2010a).

Apart from CKDu, a high incidence of dental fluorosis has also been reported from the NCP with a community fluorosis index exceeding 1.66 (Dissanayake 1991). People tend to consume relatively high volumes of water in a daily basis under the hot tropical climatic conditions exist in Sri Lanka, thus they are exposed to relatively high fluoride intakes compared with those from the non-tropical regions (Chandrajith et al. 2011a). To regulate this excess intake, the national standards for drinking water, i.e., Sri Lanka Standards for Potable Water Quality (SLS 614: 2013), set the maximum allowable limit (MAL) for fluoride at 1.0 mg/L, a level less than the universal maximum guideline value suggested by the World Health Organization (WHO), i.e., 1.5 mg/L. Warnakulasuriya et al. (1992) suggested a MAL of 0.6 mg/L for Sri Lanka. Meanwhile, some scientists suggested that this maximum limit should be further lowered, in line with the recommendation of WHO to maintain a maximum fluoride level of 0.5 mg/L for tropical countries, as being practiced in Hong Kong and Gulf countries (WHO 1994).

Literature reported fluoride levels as high as 13.0 mg/L in groundwaters used by CKDu-affected communities. Nevertheless, the concentrations largely varied within close vicinities due to the undulating geological characteristics of this region (Dissanayake & Weerasooriya 1985; Ranasinghe et al. 2019), and the mean concentrations (0.5–1.6 mg/L) reported from most of the locations were less than or comparable with 1.5 mg/L, but exceeded 0.5 mg/L (Herath et al. 2005; WHO 2011; Chandrajith et al. 2011b; Wasana et al. 2016; Dissanayake & Chandrajith 2017; Wickramarathna et al. 2017; Nanayakkara et al. 2019; Imbulana et al. 2020). Wasana et al. (2016) reported significantly high fluoride concentrations in wells used by CKDu patients compared with those of controls, when 0.5 mg/L was regarded as the MAL. However, no such significance was noted with respect to a MAL of 1.5 mg/L. Meanwhile, spring water consumed by non-affected communities also contained fluoride concentrations less than 0.5 mg/L.

When all these observations were taken into account, 0.5 mg/L could be considered a better reference value than 1.0 or 1.5 mg/L to compare the fluoride intakes between CKDu-affected and non-affected parties. Nevertheless, groundwater with fluoride concentrations exceeding 0.5 mg/L can be commonly found in many tropical regions elsewhere, including North Africa, Middle East, Central Asia, and South America (Fawell et al. 2006; Kimambo et al. 2019). Therefore, if such low concentrations of fluoride could give rise to a detrimental health issue like CKDu, similar incidents must have been frequently reported from all over the world. Impaired groundwater quality was a suspected risk factor of Uddanam endemic nephropathy in India as well, but fluoride levels in the endemic regions...
were remarkably lower than that in the Nalgonda area where fluorosis was prominent, and CKD was absent (Reddy & Gunasekar 2015).

Coming back to the case of Sri Lanka, fluoride, as a sole causative factor, would not explain why CKDu is absent in certain neighboring settlements located amidst the endemic regions of the disease; and in certain areas of the dry zone with similar geographic and socio-economic characteristics (e.g., Eastern and Southern regions of Sri Lanka), and similar fluoride concentrations in groundwater. On the other hand, CKDu has been diagnosed even with individuals who used to drink low fluoride water. Meaning that, the geographic distribution of CKDu cases is not ideally correlated with the presence of high fluoride in groundwater. Concrete evidence therefore is lacking to confirm a relationship between these two occurrences. Nonetheless, it should not be overlooked that the exposure conditions, total intake, and pathways of exposure to fluoride could widely vary among different regions and subjects.

Chandrajith et al. (2011b) pointed out the importance of considering the total intake of fluoride rather than the input from drinking water only, when comparing the fluoride exposure by CKDu patients and non-patients. Wasana et al. (2016) showed that the total intake of fluoride in the endemic areas (0.103 mg/kg) was higher than that in the control areas (0.008 mg/kg) and exceeded the WHO recommended maximum daily intake (0.077 mg/kg). Negligence of the dietary exposure through secondary sources of fluoride such as black tea, which plays a vital part of the diet in the rural dry zone, is considered a limitation of the past research (Chandrajith et al. 2011b).

While it is impossible to explore all possible exposure routes of fluoride, clinical observations are known to provide more reliable evidence on exposure. Measuring serum fluoride levels is considered an appropriate indicator of fluoride exposure (Kono et al. 1984). Significantly high serum fluoride levels were found in CKDu subjects, compared with the healthy controls from the same endemic areas as well the subjects from foreign countries, indicating higher fluoride exposure in CKDu (Fernando et al. 2020; Nanayakkara et al. 2020). The serum fluoride levels measured by Nanayakkara et al. (2020) were positively correlated with the development of CKDu (fluoride levels increased with increasing stages of the disease), whereas negatively correlated with the estimated glomerular filtration rate (eGFR). This inferred the accumulation of fluoride in renal tissues with continuous exposure to fluoride over a long period.

Apart from the real-world cases, plenty of animal studies worldwide indicated that kidney damages could occur due to the chronic exposure to fluoride. Several mice studies conducted within the Sri Lankan context revealed that long-term exposure to the typical fluoride levels in CKDu groundwaters could lead to impaired renal functions with similar histopathological changes as CKDu (Thammitiyagodage et al. 2017; Wasana et al. 2017; Perera et al. 2018). Even if the initial damage to kidneys in CKDu may not occur due to fluoride, a damaged kidney causes fast accumulation of fluoride and thereby exacerbates its condition (Fawell et al. 2006). Moreover, aggravated renal failure is suspected when fluoride interacts with major cations (Ca$^{2+}$, Mg$^{2+}$, and Na$^{+}$) and metals such as cadmium (Zhang et al. 2013; Wasana et al. 2017). Further explanation about the synergistic effect between fluoride, hardness, major ions, and metals on kidney functions is presented below.

**Hardness and major cations**

In terms of the measured concentrations, the major anions and cations in the groundwaters of CKDu-endemic region occurred in the order: HCO$_3^-$ > Cl$^-$ > SO$_4^{2-}$ > F$^-$ > NO$_3^-$ > PO$_4^{3-}$ and Ca$^{2+}$ > Na$^+$ > Mg$^{2+}$ > K$^+$ (Wickramaratna et al. 2017; Cooray et al. 2019). Past research reported mean electrical conductivity (EC) levels spanning between 315 and 1,008 μS/cm (range: 50–3,400 μS/cm), indicating high ionicity of the wells. Intensified weathering of mineral-bearing rocks and redox processes taking place in the stagnant shallow wells of the CKDu-endemic areas were known to bring plenty of ions into the solution (Chandrajith et al. 2011b). The concentrations of major ions in groundwater complied with the drinking water standards in most of the wells. However, several studies reported excessive magnesium concentrations (mean 38–98 mg/L) exceeding the MAL for drinking (30 mg/L) (Chandrajith et al. 2011b; Dissanayake & Chandarajith 2017; Wickramaratna et al. 2017; Imbulana et al. 2020; Nikagolla et al. 2020).

High hardness is a key characteristic of this groundwater, owing to the abundant presence of Ca–Mg–HCO$_3$
ion combinations (Chandrajith et al. 2017; Wickramarathna et al. 2017; Cooray et al. 2019; Imbulana et al. 2020). Guidelines or regulations are not available for optimum hardness in drinking water as water-hardness poses no apparent health concerns (WHO 2011). However, as per the WHO classification of water-hardness, 61–120, 121–180, and >181 mg/L ranges are named moderately hard, hard, and very hard, respectively (WHO 2010). Additionally, Sri Lanka Standards for potable water define a non-health-based standard of 250 mg/L as CaCO₃ for hardness, considering its impact on water palatability.

Groundwater-hardness showed some positive correlation with the geographic distribution of CKDu cases in the endemic region. Dissanayake & Weerasooriya (1985) reported hardness levels exceeding the SLS maximum permissible level in these groundwaters. Wasana et al. (2016) reported significantly high hardness concentrations (~230 mg/L as CaCO₃) in CKDu highest-prevalent areas, in comparison to lower-prevalent (~150 mg/L as CaCO₃) and control areas (~25 mg/L as CaCO₃). According to Jayasumana et al. (2014), 96% of the CKDu patients they examined had consumed hard or very hard water drawn from shallow wells for nearly 5 years. Moreover, a high incidence of CKDu was reported from certain areas where people tend to abandon their wells due to the increased unpalatability of water caused by increased hardness (Jayasumana et al. 2015b). Similar to the case of fluoride, a low prevalence of the disease was reported with those who consumed spring water containing hardness levels less than 31 mg/L, while living amidst the same endemic areas (Jayasumana et al. 2014; Wasana et al. 2016).

Though this evidence superficially hinted a connection between water-hardness and CKDu incidence, health concerns have been rarely discussed in relation to hardness of drinking water. Nevertheless, adequate intake of calcium (>50 mg/L) and magnesium (10–30 mg/L in drinking water), the major cation causing hardness is thought to be crucial for human health, especially in terms of providing protection against vascular diseases and oxidative stress. Adverse effects are not indicated from increased intakes beyond the recommended levels in healthy people because the excess will be effectively excreted through kidneys. However, this is rather problematic for those who are experiencing renal impairments, as it decreases the ability of kidneys to excrete excessive minerals (WHO 2010). The increased magnesium levels in well water might exacerbate the damage to kidneys of CKDu patients, yet no other evidence claimed that hardness, calcium or magnesium, would give rise to chronic kidney ailments.

In addition, CKDu is absent in areas where groundwater is worst-affected by hardness and high EC, for instance, the Jaffna peninsula of Sri Lanka inferred that none of these elements alone would trigger the disease. Nonetheless, it is presumed that hardness cations might interfere with other chemical constituents in water to form various complexes that are harmful to human health, as explained below.

Synergistic effect of fluoride, hardness cations, and other ions

Fluoride in drinking water plays a dual role by exhibiting dose-dependent cytotoxic and cytoprotective properties. The fine line between these two roles is thought to be governed by the presence of major cations in solution (Na⁺, Ca²⁺, and Mg²⁺) (Chandrajith et al. 2017; Dissanayake & Chandrajith 2017). Chandrajith et al. (2017) identified a division between CKDu-endemic and non-endemic areas based on Na⁺/Ca²⁺ ratios in groundwater. While high fluoride was a common characteristic of groundwater throughout the dry zone, the endemic areas of CKDu marked significantly lower Na⁺/Ca²⁺ ratios (1.6–6.6) compared with the non-endemic areas (34–469). Higher ratios in the non-endemic areas favored the complexation of fluoride (F⁻) with Na⁺, leading to reduced fluoride toxicity and reduced absorption of Ca²⁺ in the human body. On the other hand, lower ratios in CKDu-endemic areas promoted Ca²⁺ activity, which enhanced fluoride toxicity, and thereby exacerbated the damage to kidney tissues. However, none of the subsequent studies identified such a relationship between Na⁺/Ca²⁺ ratios in groundwater and the occurrence of CKDu.

Wasana et al. (2016) using multivariate scatterplot analysis demonstrated that the co-occurrence of elevated fluoride and hardness along with relatively high cadmium concentrations in groundwater could give rise to the incidence of CKDu. This assumption was later validated in a follow-up study based on mice experiments (Wasana et al. 2017). They showed that fluoride, hardness, and cadmium when
present altogether at concentrations equal to WHO-MALs for drinking could give rise to histopathological changes in kidneys similar to those of CKD. The damage to mice kidney tissues was aggravated when the dose was further increased. Interestingly, fluoride alone, when present at concentrations as high as 10 mg/L, did not indicate any harmful effects on kidneys, implying the enormity of fluoride toxicity when present with the right types and right amounts of counter-ions in drinking water. Dharma-wardana (2018) used the Hofmeister concept and Gibbs-free energy theory for ion–pair formation to theoretically explain the observations by Wasana et al. (2017). He explained that fluoride along with magnesium-hardness (but not calcium-hardness) and/or cadmium could pose more nephrotoxic effects than fluoride ions alone. Cadmium which is a well-known nephrotoxicant is reported only at minute quantities in groundwater in the CKDu region, but the mutual presence of fluoride and cadmium in solution may intensify the toxicity of both elements. This will be further elaborated in the forthcoming discussion.

In agreement of the hypotheses presented by Wasana et al. (2017) and Dharma-wardana (2018), Balasooriya et al. (2019) observed significantly high magnesium and fluoride concentrations in well water consumed by CKDu patients, compared with the controls, and hence concluded that fluoride- and magnesium-hardness might be linked with the etiology of CKDu. Imbulana et al. (2020) also pointed out that magnesium predominant hardness and high ionicity of groundwater in the CKDu-endemic region might be linked with the geographic occurrence of the disease. These findings suggested a magnesium- and fluoride-induced root cause for CKDu.

In contrast to the afore-mentioned views, Dissanayake & Chandrajith (2017) observed higher total hardness concentrations, but relatively low magnesium concentrations in groundwater from CKDu-endemic areas, compared with the non-endemic areas. Hence, assumed calcium-hardness alone with fluoride would trigger CKDu, while magnesium-hardness provides protection against such harmful effects, due to its calcium-antagonistic properties. Similarly, Paranagama et al. (2018) statistically illustrated that Na–F ion combinations were more prominent in groundwater consumed by CKDu patents, whereas mixed combinations between Mg$^{2+}$, Ca$^{2+}$, F$^-$, and Cl$^-$ were typical in non-CKDu groundwaters.

Although the findings of the above studies remain divided, most of the researchers were in the common view that the synergistic effect of fluoride, hardness, and ionicity seems to play a significant role in the development of CKDu. Meanwhile, it is suggested that the critical doses of chemical constituents such as fluoride for drinking must be decided based on its counter-ions in solution, especially considering the chronic exposure to such toxicity levels via long-term consumption of the same type of groundwater.

It is arguable that groundwater with similar characteristics may occur in other parts of the world, where CKDu is absent. However, making such comparisons between the groundwater quality in different regions is not meaningful, owing to a large number of factors associated with CKDu-epidemiology, such as geography, climate, socio-economic status, and health condition of the inhabitants, genetic predisposition, and lifestyle habits. For instance, the irrigational schemes and regolith aquifers are very unique to NCP, the hotspot of CKDu in Sri Lanka; thus, the groundwater chemistry as well as its dynamics may possess certain features that are unique to this region, making it difficult to be compared with the groundwater quality elsewhere in the world.

As explained before, groundwater associated with CKDu mostly appeared as stagnant and discrete water bodies, where longer residence times in hard rock aquifers lead to high ionicity. Simultaneously, the perennial dry weather conditions that exist in the dry zone demanded large water intakes, yet adequate consumption was restricted due to the unpalatability of groundwater caused by high hardness. Many scientists were in the view that this reduced water consumption causes dehydration of kidneys, mainly among farmers who are constantly exposed to extremely dry weather conditions. Even the little amount of water they consume contained high concentrations of ions leading to deterioration of the kidneys. Constructive scientific evidence is still necessary to prove the validity of these hypotheses.

**Aluminum and silica**

An early hypothesis suggested that using aluminum utensils for cooking and storage of drinking water would increase
the dissolution of aluminum in fluoride-rich groundwater, and the aluminum–fluoride complexes formed in this manner were suspected a possible cause of CKDu (Herath et al. 2005; Ileperuma et al. 2009). However, the absence of the disease in certain high fluoride zones in the country where similar types of utensils were used; CKDu being asymptomatic of Alzheimer, a prominent symptom of aluminum toxicity; and the innocuous nature of aluminum–fluoride complexes dissuaded this view (Dharma-wardana et al. 2017). Interestingly, some in vitro experiments by Mascarenhas et al. (2017) revealed that silica would be nephrotoxic to humans at 100–120 mg/L concentrations in drinking water. With reference to CKDu in Sri Lanka, Nikagolla et al. (2020) reported an average of 46 mg/L silica in groundwater, which was 2.5–3 times higher than the levels reported from the wet zone and spring water from the dry zone itself. Water quality data gathered by authors also indicated similar silica concentrations ranged between 52 and 66 mg/L (Imbulana et al. 2020). These levels did not exceed the nephrotoxic limit defined by Mascarenhas et al. (2017). Moreover, the alkaline nature of groundwater in the CKDu-endemic region of Sri Lanka, together with its high calcium and magnesium contents, could restrict the bioavailability of silica due to the formation of nontoxic precipitates (Mascarenhas et al. 2017). Nevertheless, more groundwater studies should be encouraged within the context of Sri Lanka to elucidate whether silica might be playing a role in CKDu.

**Nephrotoxic heavy metals and metalloids**

There are international reports on the potential nephrotoxic effects of environmental exposure to heavy metals, including but not limited to cadmium (Cd), mercury (Hg), lead (Pb), chromium (Cr), uranium (U), gold (Au), platinum (Pt), cobalt (Co), nickel (Ni), vanadium (V), and thallium (Tl); and metalloids such as arsenic (As) and antimony (Sb) (Barbier et al. 2005; Jaishankar et al. 2014; Rehman et al. 2018). These elements are nonessential for human health, toxic even at very low doses, and non-biodegradable due to very long biological half-life. The kidney, as the first target organ in the human body to reabsorb and accumulate divalent metals, has an increased the chances of being damaged due to heavy metal toxicity (Barbier et al. 2005). Several studies explained below reported substantial quantities of Cd, As, Pb, and Hg in the biological samples of CKDu patients, and thus chronic exposure to these elements was suspected in the onset of CKDu.

Arsenic was considered a potential risk factor of the disease in the early attempts to identify its etiology. Jayatilake et al. (2013) measured significantly high arsenic levels in the hair samples of CKDu cases (median = 0.159 μg/g), compared with the controls (median = 0.103 μg/g). Jayasumana et al. (2015b) reported urinary arsenic concentrations (89.3 μg/g creatinine) exceeding the US reference range (0–35 μg/g creatinine) in CKDu patients. Nevertheless, the existing evidence seldom supported an etiology related to arsenic as it primarily appeared in a nontoxic form in CKDu patients (Rango et al. 2015). Meanwhile, CKD cases attributed to arsenic have not been reported from countries like Bangladesh, where the population is exposed to higher doses, primarily through drinking groundwater (Karim 2000).

Owing to the well-known nephrotoxicity of cadmium, and the similarities with the farming background and the rice-based staple diet in the cadmium nephropathy named ‘itai-itai disease’ originated in Japan, a substantial number of exploratory studies on CKDu in Sri Lanka focused on a cadmium-induced nephrotoxicity. Cadmium was found in the biological samples (serum, urine, hair, and nails) of CKDu patients at levels exceeding the US reference values and higher than the levels detected in non-patients (Jayatilake et al. 2015; Levine et al. 2016).

Additionally, Pb and Hg levels exceeding the US reference values were reported in the blood and hair of CKDu patients, respectively (Levine et al. 2016). Moreover, Jayasumana et al. (2015b) noted significantly high Cd, Cr, Pb, Sb, Co, Ni, and V concentrations in the urine samples of CKDu cases, compared with those in the controls from both endemic and non-endemic areas (p < 0.001, Chi-square test). On the contrary, Chandrajith et al. (2013), Rango et al. (2015), Diyabalanage et al. (2017), Nanayakkara et al. (2019), and Gunawardena et al. (2020) emphasized that exposure to heavy metals could hardly be a likely risk factor...
of CKDu considering the biological and pathological evidence.

Different research groups reported largely varying concentrations of heavy metals/metalloids (specifically Cd, As, and Pb) in drinking water sources used by CKDu patients. Nonetheless, the amounts detected in almost all the studies were far less than the MALs recommended by the WHO. Moreover, the concentrations did not show considerable variations between the endemic and non-endemic areas (Chandrajith et al. 2011; Jayatilake et al. 2013; Jayasumana et al. 2015b; Rango et al. 2015; Levine et al. 2016; Wasana et al. 2016; Diyabalanage et al. 2017; Wickramaruthna et al. 2017; Herath et al. 2018; Balasooriya et al. 2019; Nanayakkara et al. 2019; Nikagolla et al. 2020). Due to such insignificant levels of heavy metals detected in drinking water sources, and the failure to observe a correlation between the levels of these elements found in biological samples and the levels measured in drinking water, heavy metal contamination through drinking water or groundwater was often ruled out as a potential cause of CKDu. Instead, the possible exposure through multiple sources such as agrochemicals, rice, certain types of vegetables, inland fish, alcohol, and tobacco were highly debated (Bandara et al. 2008; Jayatilake et al. 2013; Rango et al. 2015; Levine et al. 2016). However, the chronic exposure to minute quantities of heavy metals through drinking water and food by CKDu patients may require deep examination, considering the ability of heavy metals to bioaccumulate within human body tissues.

At the same time, the presence of even small amounts of heavy metals is thought to be a concern when drinking water is rich in fluoride. Due to its very high reactivity, fluoride easily combines with heavy metals to act as an excellent geochemical carrier for a multitude of nephrotoxicants. For instance, mice studies have shown that co-exposure to cadmium and fluoride could increase the damage to liver and kidney, beyond the damage caused by their individual presence (Zhang et al. 2013). Once again, combined clinical and environmental evidence are much needed to clarify whether such interactions could trigger the pathogenesis of CKDu.

Agrochemicals

Agrochemicals that are extensively used in the farming activities in the CKDu-endemic region are considered a major source of heavy metals. Triple Super Phosphates and Rock Phosphates that are extensively used in agriculture in the dry zone are found to be rich in toxic heavy metals like Cd, As, Cr, Co, Ni, Hg, Pb, V, and U (Bandara et al. 2010; Chandrajith et al. 2011a; Jayasumana et al. 2015a). Soil analysis indicated that Cu, Hg, Ni, Se (selenium), Fe (iron), and Mn (manganese) levels reported from the disease-endemic areas exceeded the levels in US soil (Levine et al. 2016). Nevertheless, Cd and As which are highly suspected as possible causative agents of CKDu were found within the acceptable levels.

The direct exposure to agrochemicals itself is considered a primary risk factor of CKDu. Pesticide residues such as 2,4-dichlorophenoxyacetic acid (2,4-D), pentachlorophenol (PCP), chlorpyrifos, carbaryl naphthalene, glyphosate, and aminomethylphosphonic acid (AMPA) were spotted in biological samples of CKDu patients, and some of them exceeded the reference levels suggested by the WHO (Jayatilake et al. 2013). A famous supposition suggested that the cascade irrigation systems of the disease-stricken region convey and accumulate fertilizer runoff from the plantations in the hill country toward the reservoirs and agricultural fields downstream, where most of the CKDu incidents occurred (Jayasekara et al. 2013; Jayasumana et al. 2017). However, it was poorly acknowledged due to the facts that fertilizers are widely applied at a similar rate in the wet zone of Sri Lanka as well as in many other parts of the world where CKDu is completely absent; and the amounts of heavy metals (mainly cadmium) found in the rice grown in the wet zone were 40–60% higher than the levels reported from the endemic areas of the dry zone (Diyabalanage et al. 2016 cited in Dharma-wardana 2018).

Jayasumana et al. (2015a) claimed that CKDu is prominent among those who sprayed glyphosate as a pesticide and had a history of drinking water from abandoned wells that contained glyphosate at levels exceeding 1 μg/L. They hypothesized that glyphosate creates a nephrotoxic compound when combined with hardness cations. However, Dharma-wardana (2018) argued that glyphosate–metal complexes would hardly create any toxic effects to humans as they form insoluble precipitates in water. Meanwhile, Gunarathna et al. (2018) pointed out that the levels of glyphosate in groundwaters of CKDu-endemic areas (1–4 μg/L) were far less than the maximum contaminant level.
(MCL) defined by United States Environmental Protection Agency (700 mg/L), and thus were not high enough to create detrimental health issues like CKDu.

**Organic matter**

Few studies discussed whether the etiology of CKDu is linked with concentrations and characteristics of dissolved organic matter (DOM), the soluble fraction of organic matter in groundwater. The concentrations of DOM (DOC) measured by Makehelwala et al. (2019), Cooray et al. (2019), and Imbulana et al. (2020) were not noticeably different among CKDu high-risk and low-risk areas. However, the levels observed by Cooray et al. (2019) (3.7–6.4 mg/L) and Imbulana et al. (2020) (2.51–5.49 mg/L) were rather high compared with Makehelwala et al. (2019) (1.35–2.08 mg/L). DOC concentrations in natural groundwaters typically fall below 4 mg/L, and thus the levels measured above indicated a glimpse of an anthropogenic influence or natural contamination that could potentially jeopardize water safety (Regan et al. 2017). Agricultural activities vastly undertaken in the CKDu-endemic region could be the likely source of such high DOC inputs in groundwater, yet it needs to be proven through further research.

Failure to observe a dose–effect relationship between DOC and CKDu does not imply that exposure to organic matter through drinking water should be discarded as a potential risk factor of CKDu. Organic matter is very complex by nature and the characteristics rather than concentrations should be taken into account when evaluating the harmful effects. Makehelwala et al. (2019) characterized DOC in CKDu–HR areas by highly aromatic, non-biodegradable, or recalcitrant-type DOC. They hypothesized that aromatic DOC in CKDu–HR–groundwater might interact with toxic pesticide residues like 2,4-D and PCP, which had been detected in the biological samples of some CKDu patients. Furthermore, they noted statistical correlations between DOC and Mg$^{2+}$, Ca$^{2+}$, and SO$_4^{2-}$ ions in groundwaters from CKDu high-risk areas. These correlations reflected the propensity to produce DOC–sulfone–Ca$^{2+}$ complexes, which, in the presence of gut microbiota, could act as uremic toxicants to generate oxidative stress in CKD (Makehelwala et al. 2020). Nevertheless, the validity of this supposition in relation to CKDu should be supported with concrete clinical evidence.

Even if DOM may not be directly involved with the causation of CKDu by producing any toxic complexes, they might influence the solubility, mobility, toxicity, and bioavailability of heavy metals in the soil–water environment (Vaughan et al. 1993). As explained before, bioaccumulation of heavy metals or toxic elements in human kidneys over decades was debated as a plausible cause of CKDu, considering the chronic nature of the disease. In such a scenario, DOM could be playing a critical role by acting along with the heavy metals in the soil–water environment of the endemic region to influence their bioavailability and bioaccumulation.

Strong inner-sphere complexation or chelation by fulvic acid-type organic matter is a major process by which heavy metals like Cu, Pb, Fe, and Al are naturally leached from soil to water (Linehan 1985). Because aromatic fulvic acids are typically abundant in the groundwaters of the CKDu-endemic region, high amounts of DOM–metal interactions can be anticipated within the soil–water environment associated with the agricultural lands of the CKDu region. As chronic exposure to cadmium have been extensively studied in relation to CKDu in Sri Lanka, it would be worthwhile to explore DOM–heavy metal interactions in natural aquatic environment in the efforts to establish the onset of CKDu.

**Pathogenic bacteria and cyanotoxins**

A substantial amount of recent research focused on the possible involvement of pathogens and bacterial toxins in groundwater in the genesis of CKDu. The composition of the human–gut–micobiome is partly reflected by the total microbial composition of drinking water, and some of the phyla indicated positive correlations between the occurrence of CKD (through their detection in feces) and presence in household wells used by the patients (McDonough et al. 2020). However, such detailed studies have not yet been conducted in relation to CKDu in Sri Lanka. Cooray et al. (2021) showed that the composition of microbial community varied among the differently demarcated CKDu zones with statistical significance, using one-way ANOSIM test based on Bray–Curtis ($R = 0.1525$, $p = 0.0237$), and it is important to investigate whether the
known human pathogens in this groundwater would trigger a condition like CKDu.

Recent studies investigated the microbial content in groundwater of CKDu-endemic areas of Sri Lanka. Some of them revealed the abundant presence of cyanotoxin-producing bacteria, or cyanobacteria in wells of CKDu high-prevalent areas (Liyanage et al. 2016; Abeyseri et al. 2018; Manage 2019; McDonough et al. 2020; Cooray et al. 2021). Chronic exposure to cyanotoxins, mainly microcystin (MCs), nodularin (NOD), and cylindrospermorpsin (CYL), can impose detrimental effects on human liver and kidneys. Piyathilaka et al. (2015) investigated the nephrotoxicity of Microcystin-LR (MC-LR) in vitro at laboratory scale. The concentrations of MC-LR used in their study did not usually occur in natural groundwater, but there is a possibility of exposing to such high doses following chronic bioaccumulation of much smaller levels.

Owing to the detrimental health hazards of MCs, the WHO has set a MAL of 1 μg/L for MC-LR in drinking water (WHO 2011). Abeyseri et al. (2018) determined higher cyanobacteria concentrations (MC-LR up to 2.6 μg/L; CYN up to 7.6 μg/L) in groundwater of the CKDu region that exceeded the MAL. Moreover, a positive correlation was observed between the geographical occurrence of CKDu and cyanobacteria (MC-LR and CYN) concentrations in wells, with none reported in the control wells. Additionally, a positive relationship was noted between MC-LR and CYN concentrations in wells and in urine of CKDu patients.

In addition, Gamage et al. (2017), Sayanthooran et al. (2018), Yang (2018), and Yoshimatsu et al. (2019) discussed the influence of Hantavirus infection and leptospirosis as plausible risk factors of CKDu. Occupational exposure to such viruses via multiple pathways was suspected, and in the meantime, the presence of Leptospira bacteria was detected in well waters in the CKDu-endemic region (McDonough et al. 2020). Nevertheless, no statistical relationships were established among the bacteria levels detected in groundwater and the levels in biological samples of CKDu patients.

The above-mentioned findings shed light on a microbiology-induced etiology for CKDu, yet more comprehensive research is needed to attest how the clinical features of CKDu can be described with the exposure to pathogenic bacteria/viruses/bacterial toxins present in drinking water.

The major findings of the literature that were reviewed so far are summarized in Table 1.

SAFE DRINKING WATER INTERVENTIONS TO MITIGATE CKDu

Although the involvement of groundwater in CKDu is still unclear, it is assured that direct consumption of groundwater in the CKDu-endemic region should be avoided owing to its impaired quality. Safe drinking water interventions are thus advocated for these areas to maintain good health of the inhabitants.

The current accessibility to pipe-borne water is as low as 32% in the CKDu-endemic NCP, and it may take another two to three decades to attain full coverage by centralized schemes (NWSDB 2015). For the time being, some temporary water supply solutions such as RO water treatment plants operated at the community scale have been introduced to the disease-stricken villages to produce safe drinking water for the inhabitants (Jayasumana et al. 2016; Imbulana et al. 2020). Additionally, safe drinking water supplies through bowser services, household rainwater harvesting, and domestic water filtration methods are also being practiced. Anecdotal evidence suggested that safe drinking water, especially RO-treated water, has likely reduced the progression of CKDu and, at certain occasions, has reversed the disease condition among the early-stage patients (Wimalawansa 2019). The Ministry of Health reported a considerable reduction of CKD/CKDu cases in Sri Lanka after 2016, which they attributed to the introduction of safe water interventions (Ranasinghe et al. 2019).

To date, only Siriwardhana et al. (2018) have attempted to verify whether safe drinking water ameliorates the progression of CKDu. They conducted a case–control study by replacing the habitual drinking water of CKDu subjects with bottled water, while the controls continued to use their old sources. A diminished disease progression was noted among the patients in terms of some biological indicators (serum creatinine, hemoglobin, GFR, and urinary protein levels) and thus inferred positive impacts of safe drinking water on disease mitigation. Nevertheless, the time duration of the study (18 months) was not long enough, and the sample size (15 cases versus 15 controls)
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\(^{a}\)Cadmium.  
\(^{b}\)Arsenic.  
\(^{c}\)Lead.  
\(^{d}\)Uranium.  
\(^{e}\)Chromium.  
\(^{f}\)Dissolved organic carbon.
was not adequate. Positive outcomes of this study inspired further scientific research to assess the effectiveness of safe drinking water interventions on mitigation of CKDu.

Finally, Jayasumana et al. (2016) and Imbulana et al. (2020) highlighted some key issues related to the operations and maintenance of the existing community RO plants in the rural setting of the CKDu region despite their high popularity among the local people and water supply authorities. Such shortcomings could worsen the disease burden in the CKDu-endemic areas by leading to numerous social and health problems in future unless they are properly handled without delay.

CONCLUSIONS

A significant number of studies investigating the etiology of CKDu in Sri Lanka have postulated that the unique geographic occurrence of the disease could be explained with the long-term exposure to one or more nephrotoxic constituents through ingestion of groundwater. This review comprehensively discussed how each potential contaminant detected in these groundwaters might possibly give rise to the CKDu condition. The different types of constituents and complexes discussed herein indicated positive as well as negative attributes toward the hypotheses that they are triggering the disease, and hence the direct involvement of any of them could not be assured in the genesis of CKDu.

A majority of the past studies adopted descriptive statistics to compare the water quality between CKDu-endemic and non-endemic (or control) areas, and the relationships between water quality and disease prevalence were often hypothesized based on the statistical significance. Instead, localized data on water pollutants and disease prevalence should be frequently monitored and thoroughly analyzed over time to understand the trends between the propagation of the disease and variation of groundwater quality. The absence of a proper national registry to report CKDu cases; absence of databases to keep records on groundwater quality and quantity; unavailability of a proper case definition and geographic risk categorization for CKDu; and lack of knowledge on nephrotoxic levels of water contaminants made it difficult for the scientists to arrive at definitive conclusions regarding the disease etiology. At the same time, there were many incompatibilities between the findings of various research groups.

The lack of consistency between clinical evidence on CKDu and the findings of groundwater quality studies was another common issue associated with past research. Future studies must try to elucidate whether the clinical features of CKDu could be explained with the exposure to any of the groundwater pollutants discussed above. In the meantime, such efforts should be collaborated by multi-disciplinary experts. To enhance the reliability and accuracy of results, higher number of samples should be involved; and repetition of experimental trials, replication of experimental conditions, and validation of the results should be encouraged. Meanwhile, more case–control type research, and in vitro and in vivo experiments, should be utilized to clarify how these groundwater pollutants could give rise to the pathogenicity of CKDu.

Finally, it is a timely need to verify the beneficial effects of safe drinking water on controlling the disease progression through proper scientific approaches. At least, it would be helpful to narrow down the risk factors of CKDu and utilize the available resources effectively to set up mitigatory measures for CKDu.

DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

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