REVIEW ARTICLE

Groundwater fluoride as a geochemical   marker in the etiology of chronic kidney disease of unknown origin in Sri Lanka

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Abstract: The correlation of chronic kidney diseases of unknown etiology (CKDu) in some dry zone areas of Sri Lanka has led to several research investigations into the association of groundwater fluoride with chronic kidney diseases. Environment related kidney diseases in the tropics are clearly multi-factorial with drinking water expected to play a major role in addition to diet, heat stress and severe dehydration. The geochemistry of groundwater in such regions is marked by the presence of fluoride in significant quantities and is receiving increasing attention as a major risk factor in the prevalence of the chronic kidney disease in view of its geographic distribution pattern similar to that of the CKDu, nephrotoxicity and its very high reactivity with other substances. The dual cytoprotective and cytotoxic nature of fluoride depending on the dose and its competitive interactions with Na, Ca and Mg has raised some interesting possibilities for Mg to act as a protective agent for CKDu. This may be applicable, in general, to tropical arid regions with high amounts of Na, Ca, Mg and F in the groundwater. A case study from such regions in Sri Lanka shows that Mg-rich groundwater in the hard water terrains points to a lesser CKDu prevalence. This is indicative of a protective role for Mg against heart ailments caused by CKDu and more detailed investigations on the mechanisms of geo-biochemical interactions of F, Na, Ca and Mg in the etiology of CKD are clearly warranted.

Keywords: cytotoxicity; dry zone; Sri Lanka; kidney diseases, fluoride; water hardness.

INTRODUCTION

Chronic kidney diseases (CKD) are prevalent in many parts of the world and it has now become a global health issue (El Nahas and Bello, 2005). Among the well-known causes for CKD are hypertension, diabetes and chronic glomeronephritis. However, it is now known, notably in tropical arid regions, that there could be other causes for CKD as shown by several studies in Sri Lanka where CKD of unknown etiology (CKDu) has been discovered (Athuraliya *et al*., 2011; Chandrajith *et al*., 2011a). Large populations in tropical arid regions, notably those intimately associated with agriculture and farming is mostly at risk (Jayatilake *et al*., 2013). A very important and noteworthy feature is that these farming communities consume groundwater as their drinking water and at the same time they suffer from severe dehydration, bearing in mind the long hours of work under extremely hot and humid conditions. While agrochemicals and fertilizers laden with heavy metals notably Cd, Pb are implicated as causative agents of the CKD of "unknown etiology" in Sri Lanka, the geographic distribution of the disease clearly indicates that only some regions in the dry zone are affected while the wet zone is free of the disease (Figure 1). This is indicative of a unique and constantly changing hydrogeochemistry brought about perhaps by a significant climatic change within the last two decades. Further, the fact that the common causes such as hypertension and diabetes have been ruled out as the initiators of CKDu in these regions, a more geo-oriented natural cause associated with a critical set of chemical parameters unique to the geology and the hydrogeochemistry of the regions mostly in tropical countries is indicated. The first Hydro-geochemical Atlas of Sri Lanka was published by Dissanayake and Weerasooriya (1985) and one of their major findings was that high concentrations of groundwater fluoride were distributed in the dry zone and that the wet zone had minimal fluoride. This map was later updated by Chandrajith *et al*. (2012) and the disease pattern of the CKD of unknown etiology coincided with that of the fluoride rich regions. This fact had shown that groundwater fluoride could well be a risk factor in the etiology of the kidney disease. Based on the geographical distribution of ground-water fluoride in the dry zone of Sri Lanka, Dissanayake (2005) first suggested that ground-water fluoride and its

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interactions with other elements could be associated with CKDu in Sri Lanka. Studies from several other countries of the tropical belt have clearly shown the importance of groundwater fluoride as a major risk factor for kidney diseases (Bober *et al.*, 2006; Chandrajith *et al.*, 2011a; Chandrajith *et al.*, 2011b; Chouhan and Flora, 2008). The geochemical significance of some diseases in tropical countries have been highlighted by Dissanayake and Chandrajith (1999) and they have shown that some diseases such as dental and skeletal fluorosis, iodine deficiency disorders, selenium-related diseases among others could also be classified as diseases of "geochemical origin". The new discipline "Medical Geology" therefore is of particular importance to tropical countries where extremely large populations live intimately associated with the immediate physical environment with their unique geochemical characteristics.

Figure 1: Distribution of Chronic Kidney Diseases with uncertain etiology (CKDu) in the dry zone of Sri Lanka.

GEOCHEMISTRY OF GROUNDWATER FLUORIDE

It is a well-known fact that there is a strong relationship between fluoride geochemistry and the incidence of dental and skeletal fluorosis. While the essentiality of fluoride for human health is still being debated, its toxicity when present in excessive quantities has caused concern in many lands, particularly in dry arid zones where fluoride is found in groundwater in excessive quantities (Dissanayake and Chandrajith, 2007). Even though food is the main source of many trace elements entering the human body, much of the fluoride entering the body however, is from water (Dissanayake, 2005). The hydro-geochemistry of fluoride, particularly ground-water which is the main source of drinking water for millions of people in the tropical arid regions, is therefore of extreme importance.

Fluorine is the most electronegative element. Due to its very high reactivity, fluoride is a marked geochemical reactant in ground-water. Fluoride, as an ion, therefore does not act alone and its extreme reactivity makes it combine easily with metals such as Cd, Pb, Hg and also with metalloids such as arsenic, implicated by some authors (Jayasumana *et al*., 2013) as being the cause of the CKDu in Sri Lanka. Fluoride therefore, is an excellent geochemical carrier and a marker for a large number of possible causative agents. It is noteworthy that with the exception of the pineal gland, it is the kidney which accumulates fluoride most in the human body (Massy and Drüeke, 2012). The fact that the ionic radius of the fluoride ion (0.133 nm) is very similar to that of the hydroxyl ion (~0.110 nm), makes fluoride an excellent aquaphile. Due to the replacement of hydroxide ion with fluoride ion, in dental and bone tissues it becomes geochemically and biochemically very active. The passage through the geo-bio barrier is ensured and once inside the human body, fluoride may affect several important enzyme-related metabolic functions. Fluoride crosses cell membranes by simple diffusion, enters soft tissues and causes their impairment (Chattopadhyay *et al*., 2011). A kidney when damaged for whatever reason will accumulate fluoride fast, thereby damaging the kidney even further. Farmers who are severely dehydrated may have damaged kidneys and the consumption of fluoride-rich water will almost certainly aggravate the CKD condition.

In addition, climate change has had a marked effect on the elevated concentrations of fluoride in groundwater in tropical countries over the last two decades. Recent investigations have shown that even within a few years, groundwater wells in the dry zone of Sri Lanka have shown a marked increase of fluoride changing from safe levels to "not recommended" levels (Chandrajith *et al*., 2012). The climate change and the consequent evapotranspiration have been very high in these tropical lands with the resulting elevations of fluoride, Na, Ca and Mg in the groundwater. Jacks *et al.* (2005) studied the controls on the genesis of fluoride rich groundwaters in India where there was an increase in the incidence of dental and skeletal fluorosis with 62 million people at risk. They observed that fluoride concentrations are governed by adsorption equilibria and by fluorite stability. Evapotranspiration lead to a precipitation of calcite, a lowering of calcium activity and increase of Na/Ca ratios which allowed an increase of fluoride levels in the solution. A significant feature was that due to irrigation and farming practices, there was an increase in the extent of the sodic soils which contributed to an increase of fluoride in groundwater and hence dental fluorosis (Jacks *et al*., 2005). This is also applicable to the dry zone of Sri Lanka where due to the irrigation practices and water impounded in reservoirs, the lower reaches tend to be very rich in sodic soils thereby affecting the geochemical balance of groundwater fluoride, a fact which will have an impact on the kidney disease etiology. Therefore, the geochemical scenario of the arid tropical regions is of immense importance in that it changes constantly and rapidly while disease patterns also change accordingly. One cannot therefore arrive at too simplistic inferences by stating that fluoride and water hardness among others have been there for decades and that there had been no noticeable kidney diseases of uncertain etiology in Sri Lanka (Wimalawansa, 2016). The geochemical environment is in a continuous state of flux and subtle changes of geochemical ratios, particularly in groundwater therefore, could well have a major impact on changing patterns of health and disease. This fact has to be taken into cognizance by those who attempt to use total quantities, rather than chemical species and ratios of chemical species

in nature for correlation with disease patterns. Unfortunately, many researchers investigating environment-related diseases do not consider the subtle temporal and spatial geochemical changes in the geo-environment.

FLUORIDE AS A NEPHROTOXIN

Fluoride has consistently been underestimated for its toxic effects mainly because of the proponents of water fluoridation emphasizing the supposed benefit of fluoride to dental health. While an optimum range may have benefits in dental health, excessive amounts will cause several negative physiological effects notably in the kidneys (Cittanova *et al*., 1996; Harinarayan *et al*., 2006). However, toxicity of fluoride was largely ignored due to its "good reputation" for preventing caries via topical application and in tooth pastes (Barbier *et al*., 2010). Even at low doses, fluoride could interact with cellular systems inducing oxidative stress and modulation of intracellular redox homeostasis among several others resulted in serious concerns about its toxicity (Cittanova *et al*., 1996).

Fluoride is more genotoxic at lower doses than at higher doses when ingested through drinking water (Podder *et al.,* 2011). The determination of the exact dose of fluoride that is effective in the triggering of physiological imbalances and how other interactive chemical species such as Na^+, Ca^{2+} and Mg^{2+} control the critical dose needs further extensive research. Zager and Iwata (1997) carried out some experiments in order to characterize some subcellular determinants of fluoride cytotoxicity and to determine whether sub-toxic fluoride exposure affects tubular cell vulnerability to superimposed ATP depletion and nephrotoxic attack. They concluded that;

(a) fluoride induces dose-dependent cytotoxicity in cultured human proximal tubular cells,

(b) this occurs via Ca^{2+} and phospholipase A2-(PLA2) dependent mechanisms,

(c) partial cytosolic PLA2 depletion subsequently results and

(d) sub-toxic fluoride exposure can acutely increase cell resistance to further attack.
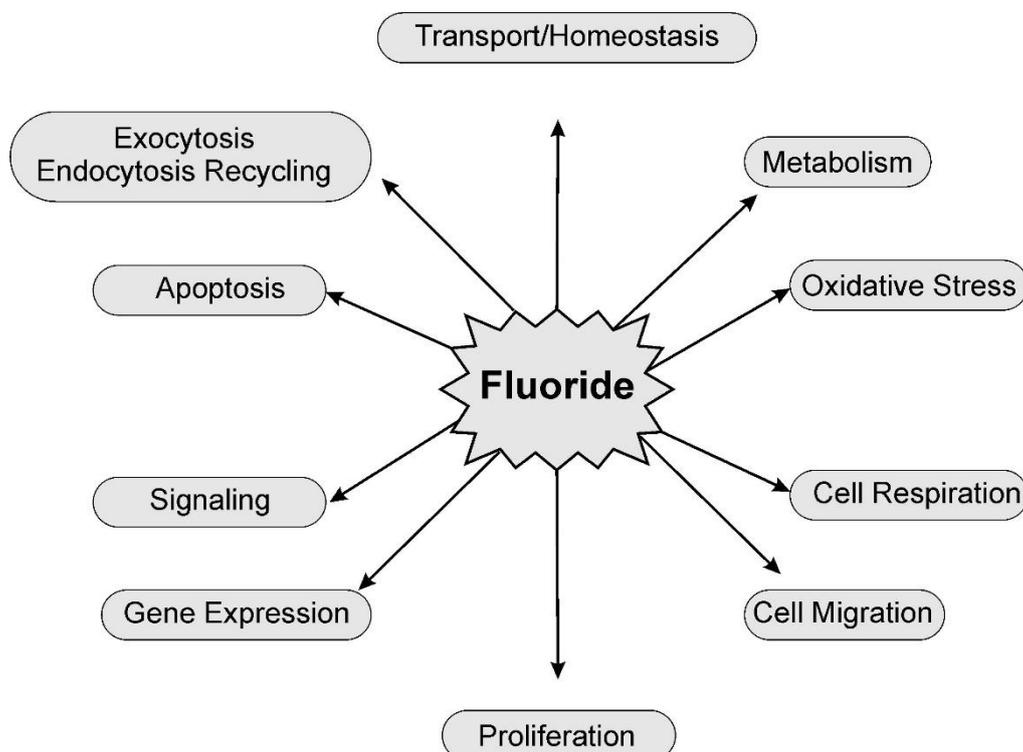
Figure 2: General scheme of the biological consequences of fluoride exposure on mammalian cells (modified after Barbier *et al*., 2010).

Although dental fluorosis is the common symptom associated with high fluoride in drinking water, the liver and kidney functions can also be affected by high fluoride (>2 mg/L) in children (Xiong *et al.*, 2007). Moreover, co-exposure of cadmium and fluoride could increase the liver and kidney damage compared with that exposed to individual ions alone as indicated using rat models (Zhang et al., 2013). These observations clearly highlight the need to place more emphasis on the critical doses and how these doses may be influenced by other factors in the physical environment, notably in groundwater. In the case of "CKD of unknown etiology" in the dry zone of Sri Lanka, the geochemical ratios of F⁻ with Ca^{2+}, Mg^{2+} and Na^+ and their collective impact on the biochemical mechanisms leading to the disease may provide useful clues as to its actual cause. The effect of fluoride on a variety of cell functions becomes complex due to the fact that it is related to dose and concentration (hormesis effect) and fluoride has to be considered as a potent toxic compound in toxicology, epidemiology/ ecological research and in both fundamental and applied research (Barbier et al., 2010). The general scheme of the biological consequence of fluoride exposure on mammalian cell is illustrated in figure 2.

WATER HARDNESS AND FLUORIDE IN GROUNDWATER – ROLE OF MAGNESIUM

One of the noteworthy features of the areas with CKDu in the dry zone of Sri Lanka, is the high prevalence of water with high hardness often exceeding 75 mg/L (Chandrajith *et al.*, 2011b). The fact that these regions also superimpose, in many cases, on the high fluoride areas brings into focus a further complicating factor and possibly both water hardness and fluoride play a role in the etiology of the kidney disease. Hard water, by itself may not pose a serious threat to human health but its main components, Ca^{2+} and

Mg^{2+} may interact with other species to form complexes that may be of significance in health issues. When such water with high fluoride levels is consumed in large quantities, as in the case of the dry zone population of Sri Lanka, the interactions of Ca^{2+}, Mg^{2+}, Na^+ and F⁻ may, in all probability, have an impact on the physiological functions. Numerous studies have shown an inverse relationship between drinking water magnesium level and heart diseases (Jiang *et al.*, 2016; Knezović *et al.*, 2014; Kousa, 2013; Leurs *et al.*, 2010) although its effect on kidneys is still poorly understood. However, the increase of Ca^{2+}/Mg^{2+} ratio in drinking water was attributed to the occurrence of urolithiasis (Yang *et al.*, 2016). Dharma-Wardana *et al.* (2015) highlighted the importance of groundwater ionicity, emphasizing the suggestion that F⁻ may contribute to the CKDu by its high rank in the Hofmeister Series for denaturing proteins of the kidney membrane. They also suggest that while the increase of hardness (below a threshold) correlates with good health, ionicity increases above a threshold would correlate with ill health. One of the main elements present in hard water is magnesium. While calcium has received great attention in issues pertaining to health and disease, notably the heart, magnesium however has not been considered adequately in medical research. Magnesium is the activator of more than 300 enzymes (Gröber, 2009), while fluoride is known to act as an inhibitor. Their geochemical and subsequent biochemical interactions are of importance in tracing the pathways to kidney disease. A further complication is the fact that some enzymes are known to be activated by fluoride (Strochkova and Zhavoronkov, 1983). The interference of F⁻ with the biological activity of the Mg^{2+} stresses the importance of studying the critical F⁻-Ca^{2+} and the F⁻-Mg^{2+} ratios in the groundwater of the hard water terrains on which the population with CKDu lives.
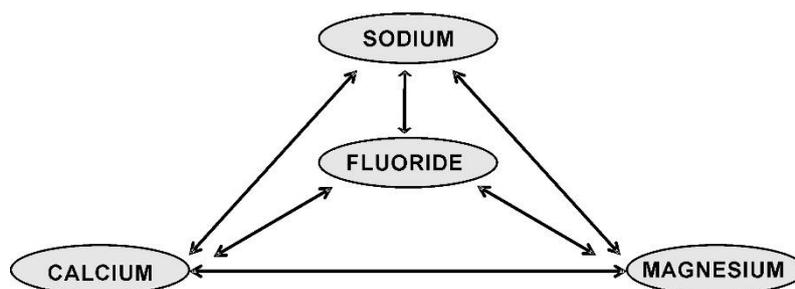

Figure 3: Antagonistic relationship between of Ca, Mg, Na and fluoride

The effects of Ca^{2+}, Mg^{2+}, and F^- supplementation on tissue mineralization in Ca-Mg deficient rats had been studied and it has been noted that the toxic effect of fluoride plays a key role in Mg deficiency and that the kidneys are the probable place for Mg-F interaction (Ericsson *et al*., 1986). They showed that F^- clearly interferes with the biological activity of the Mg^{2+}. Since hard water contains both Ca^{2+} and Mg^{2+} though in different amounts, their ratios will clearly have an impact on their distribution in tissues. In areas such as the dry zone of Sri Lanka, where the farming community is at most risk from the CKD of unknown origin, diet clearly plays a major role. It may be expected that, due to the poor diet, the intake of elements from water may also have significant physiological effects. The biochemical antagonism between Ca^{2+} and Mg^{2+} is known and there could be mutual dislodging from cells. In interactions of F^- with Ca^{2+} and Mg^{2+}, it is the Ca ions rather than Mg ions that intensify mineral- ization. Calcium accumulation is much more pronounced in the kidneys than in the heart or aorta but both Mg and fluoride have inhibitory effects. It has been found that moderately high chronic doses of fluoride prevent renal accumulation while higher acute doses increase calcinosis (Luoma *et al*., 1983; Suketa *et al*., 1977). The dual role of fluoride as a cytotoxic and cytoprotective agent has to be taken into serious consideration when optimum levels of fluoride are recommended in health issues. In the case of the CKDu in Sri Lanka, most researchers ignore this fact and attempt to correlate total water fluoride values with disease prevalence thereby leading to erroneous conclusions. Renal fluorosis and nephrocalcinosis therefore, becomes a possibility in areas where the groundwater has Ca^{2+}, Mg^{2+}, F^-, and PO_4^{3-} in abundance such as in arid regions of tropical regions where phosphate fertilizers are also used in abundance. Although calcification is mainly extracellular, intracellular calcification has been reported and may contribute to pathologic calcification of soft tissues (Azari *et al*., 2008). The kidneys could therefore be such a location (figure 2). As shown in figure 3, Ca^{2+} and Mg^{2+} show interact with each other, calcium being the dominant ion for biomineralization. It combines with fluoride and phosphate to produce nanocrystals of fluoro-apatite in the soft issues in the kidney, notably in the tubules which could cause clogging. This is mitigated by magnesium and fluoride, depending strictly on the dose.

Azari *et al*. (2008) experimentally found that hydroxyapatite crystals nucleate within intracellular membrane–bounded compartments. The discussion thus far emphasizes the extreme importance of the critical ratios of F^-, Ca^{2+}, Mg^{2+} and Na^+ that activate chronic kidney diseases not related to diabetes, hypertension and any other known factors. The fine dividing line between cytotoxicity and cytoprotectivity of fluoride depends strongly on the effect of the above-mentioned ions of the ingested water on F^- metabolism (Chandrajith *et al*., 2011b). The unique feature of fluoride as both a cytotoxic and cytoprotective agent was highlighted by Chouhan and Flora (2008) in their study on the effects of fluoride on the tissue oxidative stress and apoptosis in rats. They noted that fluoride is biologically active even in lower concentrations and that at low levels it exerts more deleterious effects while at higher concentrations the toxic effects are less pronounced. The dose and concentration dependency is once again emphasized. High fluoride, hardness and salinity prevalent in the disease stricken areas of the dry zone of Sri Lanka therefore appear to be contributory factors. It should be noted that areas below irrigation schemes, tanks and reservoirs where water is impounded, as seen in many parts of the dry zone of Sri Lanka, are clearly sodic with the consequent increase of F^- and Ca^{2+}. These areas are best known for the high incidence of CKDu. In such areas, Mg could play a protective role. It was also reported that there was a significant reduction of fluorosis in Mg-fed animals presumably due to a decrease in the intestinal absorption of fluoride (Chouhan and Flora, 2008; Khandare *et al*., 2011). A noteworthy observation is that patients with CKD have a high prevalence of vascular calcification and cardiovascular disease is the leading cause of death in CKD populations (Massy and Drüeke, 2012). Observational studies had shown that low serum Mg may be an independent risk factor for premature death in CKD patients and patients with mildly elevated serum levels could have a better chance of survival over those with lower Mg levels. It has been suggested that Mg supplementation might be useful in reducing the progression of atherosclerosis in chronic dialysis patients (Turgut *et al*., 2008).

Table 1: Summary statistics of some chemical parameters in drinking water wells from CKDu and Non-CKDu regions of Sri Lanka.

Area	CKDu Status	No. of Samples		Hardness	F⁻	Na⁺	Ca⁺²	Mg⁺²
Giradurukotte	Endemic	46	min	47	0.02	1.54	1.02	2.26
			max	1921	2.14	108	37.1	79.6
			Avg	226	0.64	22.8	13.8	19
Nikawewa	Endemic	39	min	122	0.02	15.2	2.38	0.98
			max	1380	5.3	540	256	313
			Avg	426	1.21	135	40.3	54.6
Medawchchiya	Endemic	10	min	208	0.52	8.02	13.5	11.4
			max	676	4.9	80.7	53.5	785
			Avg	324	1.42	47.7	33.3	98
Padaviya	Endemic	34	min	80	0.02	2.09	2.35	2.08
			max	816	1.33	188	113	41.6
			Avg	454	0.62	58.3	35	20.1
Vaunniya	Endemic	18	min	88	0.23	8.50	4.6	0.67
			max	352	1.27	183	32.3	33.5
			Avg	185	0.71	48.5	12.8	11.9
Vaunniya	Non-Endemic	14	min	120	0.38	8.66	1.6	0.73
			max	556	1.37	209	69	51.6
			Avg	356	0.97	92.6	30.4	26.2
Huruluwewa	Non-endemic	29	min	96	0.02	38.5	3.57	9.63
			max	590	1.68	1910	58	1280
			Avg	285	0.72	561	29.6	189
Wellawaya	Non-endemic	38	min	140	0.80	19.2	16.0	10.6
			max	580	7.0	101	110	94.7
			Avg	280	2.31	48.7	49.2	38.4
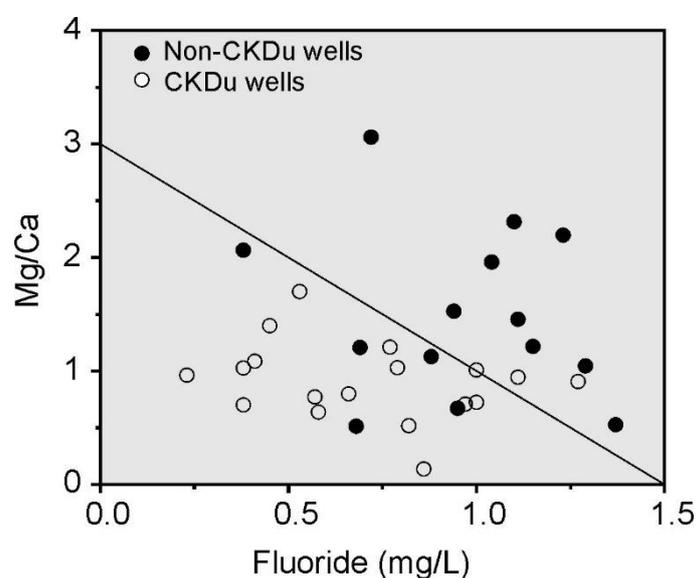
Figure 4: Discrimination between fluoride and Mg2+/Ca2+ ratio in drinking water wells used by CKDu and non-CKDu people in Vavuniya, Sri Lanka.

MAGNESIUM DEFICIENCY AS A RISK FACTOR FOR CKDu

During the last few years, many studies had considered drinking water geochemistry as a causative factor for CKDu in the dry zone of Sri Lanka. Some of these investigations showed a clear difference in Na, Ca and fluoride activities in drinking water between affected and non-affected regions (Chandrajith *et al*., 2011a; Chandrajith *et al*., 2011b; Dharma-Wardana *et al*., 2015). It is evident that elevated fluoride in drinking water along with high Ca-hardness could be a possible cause for CKDu since the

total hardness is always high in most of the dry zone regions of Sri Lanka (table 1). It is also seen that Mg^{2+} is higher in non-CKDu regions compared to the endemic regions. A recent study carried out in a CKDu prevalent village in Vavuniya in which the water samples were collected from wells used by families with CKDu patients and healthy families (Thilakerathne, 2012), indicated that the groundwater hardness in the study village varied from 88 to 352 mg/L in CKDu wells, while in non-CKDu wells were 120 to 556 mg/L. Therefore high hardness is common in the areas under investigation. However, the mean Mg^{2+} in drinking water wells of CKDu and non-CKDu groups were 12 and 26 mg/L. respectively with the fluoride concentration in all studied samples ranging from 0.23 to 1.37 mg/L with a mean value of 0.82 mg/L. It was observed that 72% of the samples had fluoride above 0.6 mg/L recommended for tropical countries (WHO, 2004). The mean values of fluoride in CKD region and non-CKD regions were 0.71 and 0.97 mg/L, respectively and are higher than that of the WHO recommended levels. A clear discrimination between the fluoride and Ca/Mg ratios can be observed for the CKDu and non-CKDu drinking water wells (figure 4). Although higher hardness (Ca + Mg) and fluoride is a common factor in most of the dry zone of Sri Lanka (Chandrajith *et al.*, 2012), the Mg-factor is extremely important in the case of CKDu foci in the dry zone of Sri Lanka. While calcium has been given very high prominence, magnesium has been grossly underestimated even though it plays a major role in cardioprotectivity and its calcium antagonistic properties. The complex biochemical interaction among Ca, Mg, Na and fluoride ions is once again highlighted. The variation of the Ca/Mg ratio in the groundwater of a region and its possible link to the prevalence of CKDu needs to be studied in depth.

Xiong *et al.* (2007) indicated that high levels of drinking water fluoride can increase the levels of two kidney damage markers, N-acetylglucosaminidase (NAG) and γ-glutamyl transferase (γ-GT) in urine in children. The CKDu in dry zone of Sri Lanka is known as a slow progressing disease and the investigations on histopathology of kidney of patients with CKDu indicated a tubulointerstitial disease present mainly as focal to diffuse fibrosis. This is accompanied by varying degrees of sclerotic vascular lesions resulting in high frequency of glomerular sclerosis and collapse more indicative of a toxic etiology (Nanayakkara *et al.*, 2014). Therefore initial damage can occur even in the younger cohorts who drink water with high hardness, high fluoride in which Ca is the dominant ion for the hardness.

CONCLUSION

Groundwater geochemistry plays a major role in the etiology of some diseases such as dental and skeletal fluorosis, chronic kidney disease among others in arid regions of tropical countries. Fluoride, when abundant in such terrains acts a geochemical carrier and as a strong marker for most heavy metals such as Cd, Hg, Pb and metalloids such as As, generally implicated for chronic kidney diseases not attributed to diabetes and hypertension. The very high reactivity of the fluoride ion enables many insoluble environmental toxicants to enter pathways that may ultimately enter the human body causing the disease. Magnesium, commonly present in hard water in the tropical arid regions plays a protective role against the disease while fluoride shows both cytoprotectivity and cytoxicity depending on the dose, bioavailability and other factors affecting its biochemistry. Climate change within the last two decades has brought about marked geochemical changes in the groundwater of arid regions prone to kidney diseases, with subtle changes in the ratios involving Ca, Mg, F, and phosphate ions. Consideration of Ca-hardness, Mg-hardness and temporary hardness is more important than total hardness when correlating disease patterns with geochemical parameters. Fluoride, by virtue of its extreme reactivity acts as a potent geochemical carrier for a large number of chemical species that are often classified as nephrotoxic as exemplified by Cd, Pb and Hg.

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