8.6 DOSE DEPENDENT Na AND Ca IN FLUORIDE –RICH DRINKING WATER – ANOTHER MAJOR CAUSE OF CHRONIC RENAL FAILURE IN TROPICAL ARID REGIONS

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Dose-dependent Na and Ca in fluoride-rich drinking water —Another major cause of chronic renal failure in tropical arid regions

Rohana Chandrajith [a,*], C.B. Dissanayake [a,b], Thivanka Ariyarathna [a], H.M.J.M.K Herath [a,c], J.P. Padmasiri [b]

[a] *Department of Geology, University of Peradeniya, Peradeniya, Sri Lanka*
[b] *Institute of Fundamental Studies, Hantana Road, Kandy, Sri Lanka*
[c] *Postgraduate Institute of Science, University of Peradeniya, Peradeniya, Sri Lanka*

ARTICLE INFO

Article history:
Received 10 June 2010
Received in revised form 22 October 2010
Accepted 27 October 2010
Available online 24 November 2010

Keywords:
Chronic kidney diseases
Fluoride
Dry zone
Na–Ca ratio
Groundwater
Hydrogeochemistry
Medical geology
Sri Lanka

ABSTRACT

Endemic occurrence of chronic kidney disease with unknown etiology is reported in certain parts of the north central dry zone of Sri Lanka and has become a new and emerging health issue. The disease exclusively occurs in settlements where groundwater is the main source of drinking water and is more common among low socio-economic groups, particularly among the farming community. Due to its remarkable geographic distribution and histopathological evidence, the disease is believed to be an environmentally induced problem. This paper describes a detailed hydrogeochemical study that has been carried out covering endemic and non-endemic regions. Higher fluoride levels are common in drinking water from both affected and non-affected regions, whereas Ca-bicarbonate type water is more common in the affected regions. In terms of the geochemical composition of drinking water, affected households were rather similar to control regions, but there is a large variation in the Na/Ca ratio within each of the two groups. Fluoride as shown in this study causes renal tubular damage. However it does not act alone and in certain instances it is even cytoprotective. The fine dividing line between cytotoxicity and cytoprotectivity of fluoride appears to be the effect of Ca^{2+} and Na^{+} of the ingested water on the F^{-} metabolism. This study illustrates a third major cause (the other two being hypertension and diabetes) of chronic kidney diseases notably in tropical arid regions such as the dry zone of Sri Lanka.

1. Introduction

Globally, chronic kidney disease (CKD) is an emerging and increasing health issue in many populations, caused mainly by an epidemic of diabetes mellitus (Nahas and Belle, 2005). Chronic glomeronephritis, hypertension and diabetes are the significant contributing factors for CKD in developing countries (Codreanu et al., 2006). In Sri Lanka, the prevalence of CKD has been increasing over a period of 8–10 years, particularly in some geographically discreet regions. However, the disease is not associated with any known risk factors, i.e., diabetes and hypertension or chronic glomeronephritis. The "Chronic Kidney Disease of unknown etiology" (CKDu) appears to be more prevalent in certain parts of the dry zone regions of the country where the annual rainfall is less than 1000 mm and is restricted to a few months of the year (Fig. 1). About 4–10% of the CKDu prevalence rate was recorded in some regions in the dry zone, namely, Medawachchiya, Girandurukotte, Padaviya, Medirigiriya and Nikawewa (Aturaliya et al., 2006). The studies on endemic CKDu in Sri Lanka are therefore mainly focused on geoenvironmental factors in view of their exclusive geographic distribution. The disease commonly manifests in young male farmers of low socio-economic class and most affected patients belong to the 30–60 year age group (Aturaliya et al., 2009). Detailed histopathological investigations indicated that the CKDu in Sri Lanka exhibits focal to extensive tubulo-interstitial fibrosis accompanied by glomerular sclerosis and collapse. Their pathological observations also suggested that focal renal tissue ischemia might play a major role in the pathogenesis of CKDu. The most striking feature was the focal nature of the histopathological lesions in the kidney tissue, which is more in favor of vascular injury than toxic nephropathy caused by heavy metals such as Cd and U (Dr. Shanika Nanayakkara, Kyoto University, Personal Communications).

The quality of drinking water in relation to CKDu is a subject of increasing interest as shallow and deep wells are the main source of potable water in almost all the affected regions. The high CKDu prevalent regions overlap with the high groundwater fluoride zone of Sri Lanka suggested by Dissanayake (1996), indicating that at least to some extent, the fluoride content of drinking water might contribute to the CKDu (Dissanayake and Chandrajith, 2007). Interestingly, in some other parts of the dry zone, particularly in the eastern and southern sectors and also within endemic CKDu foci, the disease is not recorded even though the drinking water fluoride

* Corresponding author. Tel.: +94 77 781 2392; fax: +94 81 238 8018.
E-mail addresses: rohanac@pdn.ac.lk, rohanac@hotmail.com (R. Chandrajith).

0048-9697/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.scitotenv.2010.10.046

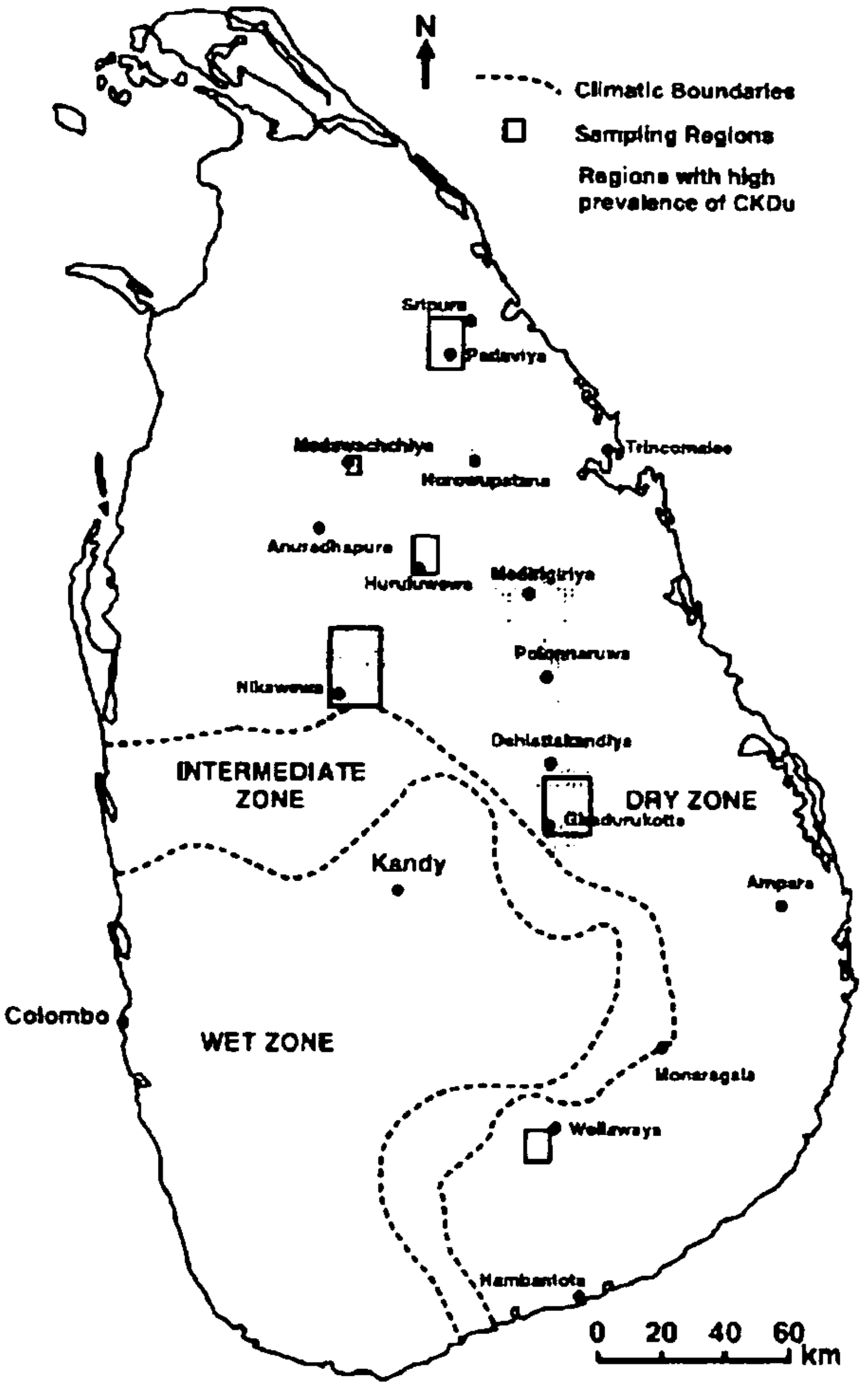

Fig. 1. Map of Sri Lanka showing the clusters of endemic CKDu villages and sampling locations.

content is significantly higher, indicative of the fact that fluoride is not acting alone. In the dry zone areas, more than 85% of the population depend on groundwater from shallow (<10 m) and deep wells. Data obtained from a large number of drinking water wells indicate that a large part of the dry zone of Sri Lanka is fluoride-rich, causing high incidences of dental and skeletal fluorosis. This has been highlighted by several workers (e.g.: Dissanayake, 1996; Tennakoon, 2004). For instance, the rate of occurrence for fluorosis in the north central part of Sri Lanka is 89.8%, with a Community Fluorosis Index of 1.69 (Tennakoon, 2004). However in most cases, the fluoride contents are well below the WHO recommended levels of 1.5 mg/L. Previous studies on water quality and the incidence of CKDu considered single, or a few suspected elements as the causative agents of the disease. In this paper, hydrogeochemical data collected from endemic regions are interpreted in order to highlight the importance of element/s excess or deficiency in drinking water as the causing agent of CKDu. The hydrogeochemistry of CKDu regions will be compared with non-endemic regions located within the high fluoride zone. Up to now, the geochemistry of tropical arid regions where millions of people who depend directly on groundwater for their drinking water, has received only scant attention, even in view of its relation to increasing trends in CKD due to the overwhelming emphasis on diabetes and hypertension. This study from the dry zone in Sri Lanka presents one of the first such case studies illustrating the significance of the geochemistry of fluoride-rich groundwater as a major cause of chronic renal failure.

2. Materials and methods

Well water samples were randomly collected from CKDu prevalent regions of Giradurukotte (n = 46), Nikawewa (n = 52), Medawachchiya (n = 10) and Padaviya (n = 34) while for the non-endemic regions, Huruluwewa (n = 29) and Wellawaya (n = 8) regions were selected (Fig. 1). From among these, Huruluwewa is located in an area within the high prevalent north central region, but with extremely low prevalence of CKDu (Aturaliya et al., 2006). Wellawaya is another region, located away from the known CKDu foci, but which has higher fluoride levels in the potable water. Only wells in use for more than 10 years were selected for sampling in order to obtain information on the effect of long-term human consumption. In endemic regions, samples were taken randomly, but in Medawachchiya all samples were taken from wells used by CKDu patients. The samples were collected in duplicate from each site into pre-cleaned high-density polypropylene bottles, one of which was acidified with concentrated nitric acid. Standard sampling and analytical methodologies were adopted throughout the study (APHA, 1998; Hach, 2002). pH, electrical conductivity (EC) and alkalinity were measured in-situ. Fluoride concentration was determined by the SPADNS method in which the detection limit is 0.02 mg/L. Nitrate, phosphate and sulfate contents of the samples were measured spectrophotometrically whereas chloride and total hardness were determined by titrimetric methods. The acidified sample was used for the analysis of cations by Atomic absorption spectrophotometry. Bicarbonate and carbonate contents were calculated using alkalinity and pH values. The analytical accuracy calculated using the cation–anion balance was found to be within ±5%.

3. Results and discussion

The summary hydrogeochemical characteristics of studied water samples are presented in Table 1. The pH of all water samples was neutral to alkaline in the studied regions and the lowest pH (5.71) was reported in the Nikawewa region. Extremely large variations in EC were observed in water samples, with values ranging from 52.5 to 3400 μS/cm. The very low EC value is possibly due to mixing of well water with surface water, mainly from irrigation water diverted from the wet zone regions. Low alkalinity and low hardness were also observed in these samples. High hardness is common in most of the water samples except for the water from the Wellawaya region where higher alkalinity was prominent compared to the other studied regions. It was observed that the water collected from Nikawewa had higher hardness as compared to the other regions. Except for a sample from Medawaxhxhlya, the nitrate-nitrogen content is well below the WHO recommended limit of 10 mg/L.

Fig. 2 illustrates the Piper trilinear diagram plotted for endemic and non-endemic CKDu regions. Geochemically, water from the dry zone region is abundant in HCO_3^- and cations of Na^+ and/or Ca^{2+}. It is interesting to note that Ca-bicarbonate type water is predominant in endemic CKDu regions whereas Na-K-non dominant anion type water is common in the non-endemic regions. In the cation triangle, most water samples from CKDu regions are plotted towards the Ca and Mg ends whereas non-CKDu regions show more affinity towards the Na + K axis. Vithanage et al. (2010) also showed that groundwater from the Hambantota region, which is considered to be a non-endemic CKDu region, was predominantly of the Na/K–Cl type.

Table 1
Summary of hydrogeochemical results obtained from endemic and non-endemic regions (all concentrations are expressed in mg/L unless otherwise specified; nd—not detected)

Area	Giradurukotte			Nikawewa			Medawchchiya			Padaviya			Huruluwewa			Wellawaya		
CKDu status	Endemic			Endemic			Endemic			Endemic			Non-endemic			Non-endemic		
No. of samples	46			52			10			34			29			8		
	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean
pH	6.10	8.10	6.88	6.15	8.11	7.08	6.69	7.74	7.23	6.21	7.93	6.94	6.25	8.06	7.02	6.57	7.11	6.84
EC (μS/cm)	52.5	672	330	50	3400	1008	500	1600	852	254	2180	834	230	1129	706	317	950	604
Alkalinity	8.8	366	101	34.4	864	280	188	348	250	39.6	420	238	33.2	530	199	252	439	322
Hardness	11	1921	217	11.2	1380	336	208	676	324	70	816	443	15	590	276	25.2	59.6	39.5
Cl^-	2.75	105	25	13	281	83	nd	nd	nd	27	688	175	14.5	498	145	92.5	293	216
NO_3^-	0.10	9.00	2.77	0.10	17.3	2.55	0.40	25.60	6.19	0.50	9.50	2.99	0.10	3.90	1.07	0.50	2.10	1.17
SO_4^{-2}	4.0	49.0	12.7	5.0	560	47.5	7.00	75.0	39.5	0.01	130	34.6	1.00	47.0	21.4	1.00	62.0	30.7
F^-	0.02	2.14	0.64	0.02	5.30	1.21	0.52	4.9	1.42	0.02	1.33	0.62	0.02	1.68	0.72	0.45	2.2	1.05
PO_4^{-3}	0.07	1.6	0.41	0.10	8.40	1.35	0.14	0.61	0.36	0.09	1.74	0.58	0.06	0.65	0.31	0.19	1.96	0.79
Na^+	1.54	108	22.8	15.2	540	135	8.02	80.7	47.7	2.09	188	58.3	38.5	1910	561	19.2	101	48.7
K^+	0.10	7.89	1.37	0.01	31.6	4.51	0.94	60.3	13.08	0.08	3.28	0.62	0.64	9.80	2.25	0.97	4.6	2.53
Ca^{+2}	1.02	37.1	13.8	2.38	256	40.3	13.5	53.5	33.3	2.35	113	35.0	3.57	58	29.6	0.04	1.43	0.53
Mg^{+2}	2.26	79.6	19.0	0.98	313	54.6	11.4	785	98.0	2.08	41.6	20.1	9.63	1280	189	14.1	30.5	23.0
Fe^{+3}	0.02	1.14	0.15	LD	1.03	0.23	nd	nd	nd	0.01	0.31	0.07	0.01	0.52	0.09	0.02	0.10	0.06
Mn^{-2} (μg/L)	10.0	700	74.8	LD	270	19.3	13	333	113	LD	319	49	10	340	27.6	0.01	0.53	0.11

Fluoride, on account of its chemical similarity to the hydroxyl ions, is easily taken up by the water when in association with fluoride-bearing minerals in rocks and soils. The dry zone of Sri Lanka is no different from the wet zone as far as the types of rocks and minerals are concerned. However, the climate and the hydrological conditions are markedly different and these appear to play a major role in the geochemical cycling of fluoride in the groundwater. The mean content of fluoride is 0.66, 1.21, 1.03 and 0.62 (in mg/L) in endemic CKDu areas of Giradurukotte, Nikawewa, Medawachchiya and Padaviya respectively, whereas non-endemic Huruluwewa and Wellawaya showed 1.42 and 1.05 mg/L of mean fluoride levels, respectively. These values exceed 0.6 mg/L, the limit recommended for tropical countries by WHO (WHO, 1994). People in the dry zone regions consume higher volumes of water daily to regulate the water balance, and it should be borne in mind that the fluoride in groundwater varies remarkably from very low to extremely high values even within a small area. In contrast to the dry zone, the wet zone region in which the mean annual rain fall exceeds 2500 mm/year, contains very low levels of fluoride. For instance, the Kandy region showed a mean fluoride content of 297 μg/L (range 44–770 μg/L)(Dissanayake and Chandrajith, 1996), clearly indicative of the major climatic influence on hydrogeochemistry of fluoride.

Geologically over 90% Sri Lankan landmass is covered with metamorphic rocks of Precambrian age. Suites of metasedimentary and metavolcanic rocks formed under granulite and amphibolite facies conditions consist of various fluoride-bearing minerals such as micas, pyroxene, hornblende and apatite. Fluorides in the groundwater are derived mainly from the leaching of rocks rich in fluoride-bearing minerals; however, high-fluoride-bearing groundwater is distributed mostly over the comparatively dry regions. Under the tropical climate conditions, weathering of rocks and minerals is intense and fluoride shows a higher tendency to enter the aqueous medium. Climatic effects, notably high evaporation due to the prevailing high ambient temperature and the low dilution affect can increase the fluoride concentration in groundwater. Intensive and long-term irrigation in the dry zone areas is probably another important factor that causes the leaching of fluoride from the soils and weathered rocks. However in some studied locations, fairly low levels of fluoride were reported, probably due to mixing of groundwater with the irrigation water which was diverted from the central wet zone of the country, having remarkably low levels of fluorides.

In the study regions most groundwater is alkaline, leading to removal of more fluoride ions from the solid phase to the aqueous

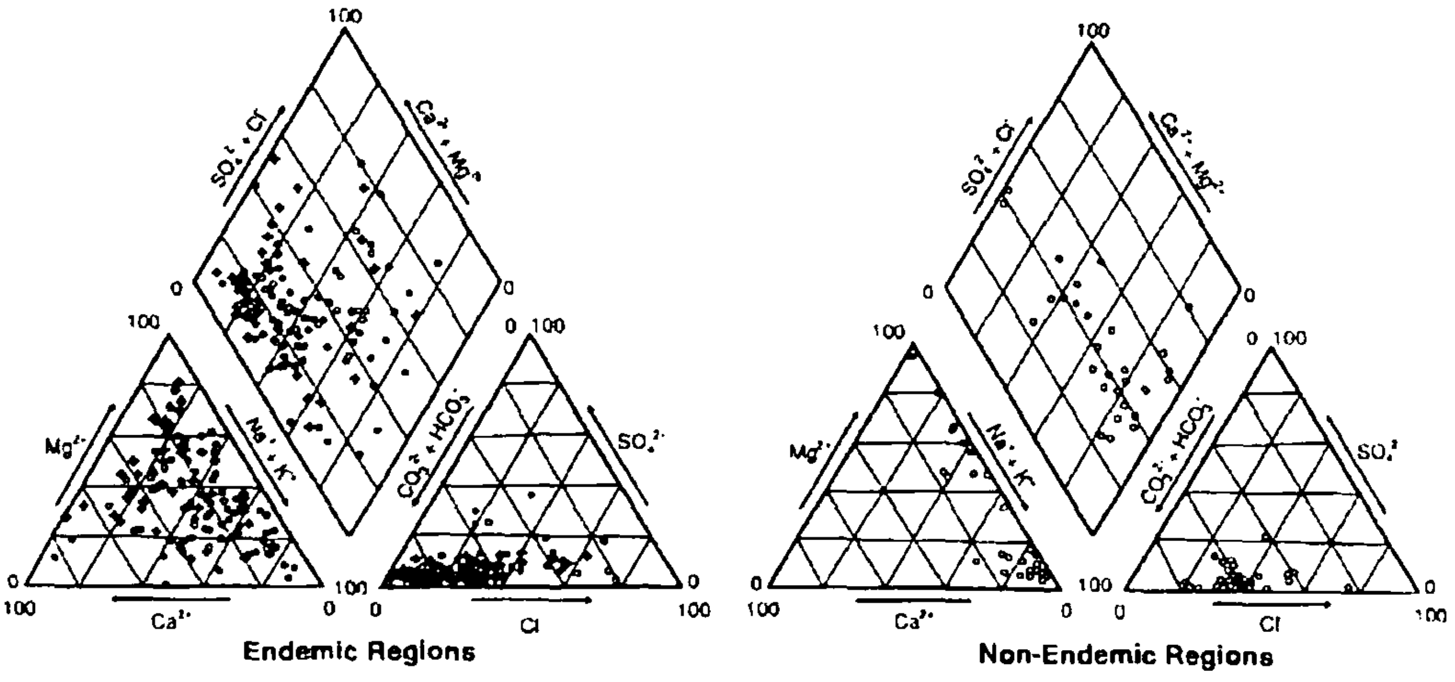

Fig. 2. Piper trilinear diagrams plotted for endemic and non-endemic regions.

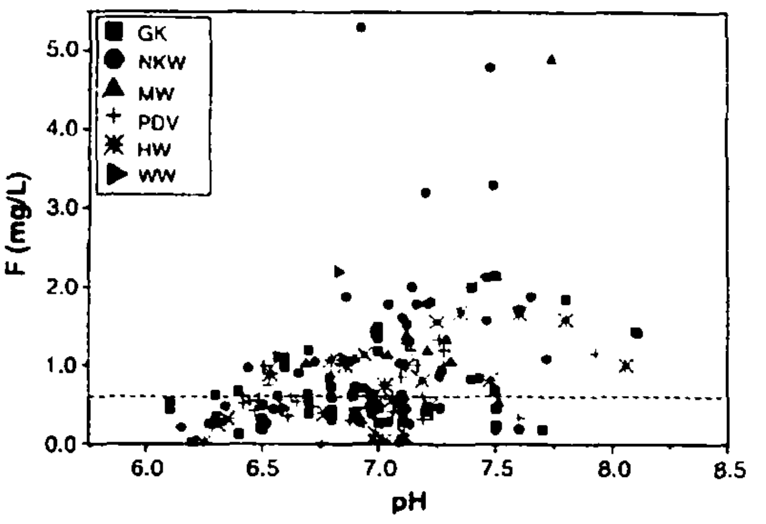

Fig. 3. Variation of fluoride with pH: dotted line indicates the WHO recommended fluoride level for tropical countries (GK–Giradurukotte; NKW–Nikawewa; MW–Medawachchiya; PDV–Padaviya; HW–Huruluwewa; WW–Wellawaya).

phase. Fig. 3 illustrates the variation of fluoride contents in sampled water with respect to their pH values. Saxena and Ahmed (2003) also showed that the alkaline nature of the groundwater could increase the fluoride levels as the alkaline water can mobilize fluoride from minerals. Their experimental results indicated that an alkaline medium (pH 7.6 to 8.6), high HCO_3^- concentration (ranging from 350 to 450 mg/L) and moderate EC (ranging from 750 to 1750 µS/cm) are favorable for fluoride dissolution. However this influence is true for sodium-rich alkaline waters as indicated by Chae et al. (2007).

Fig. 4 shows the discrimination of fluoride and Na/Ca ratio for the study regions. The mean Na/Ca ratio was observed to be in the range of 1.6 to 6.6 for the CKDu regions; whereas it was 34 to 469 for the non-endemic regions (Table 2), indicative of a major geochemical difference. In the water from Medawachchiya, in which all water samples were obtained from wells used by clinically identified CKDu patients, the Na/Ca ratio varies from 0.22 to 2.89 with the mean of 1.6. The mean Na and Ca ratios within an endemic region and between endemic and non-endemic regions were compared using the Kruskal Wallis test. There was no significant difference in mean Na/Ca within the endemic regions ($P>0.05$) while the ratios showed a significant differences between endemic and non-endemic regions ($P<0.05$).

The increase in the Na/Ca ratio in non-CKDu regions is probably due either to a lowering of the calcium activity or a high sodium activity. The Na/Ca ratio in groundwater generally increases with the

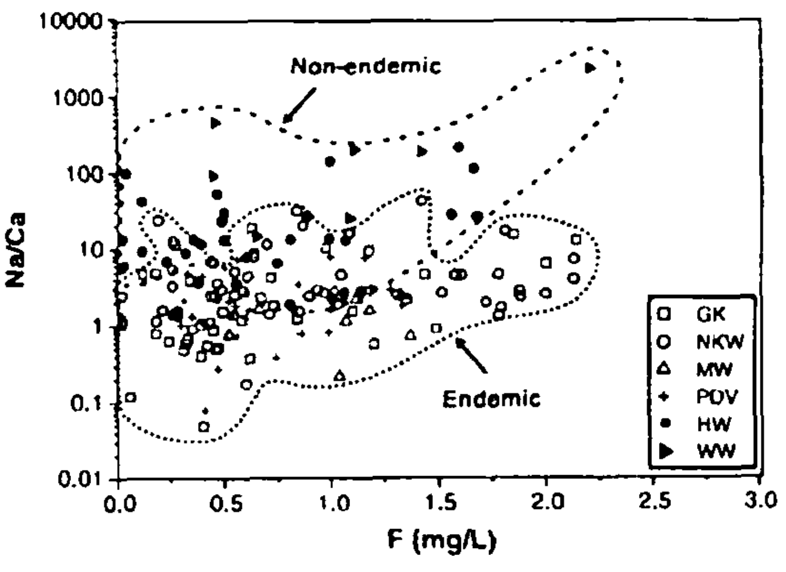

Fig. 4. Groundwater fluoride content vs. Na/Ca ratio in endemic and non-endemic CKDu regions (GK–Giradurukotte; NKW–Nikawewa; MW–Medawachchiya; PDV–Padaviya; HW–Huruluwewa; WW–Wellawaya).

increasing fluoride content (Fig. 4) because of the differences in solubility between sodium and calcium fluorides (Salve et al., 2008). Gao et al. (2007) showed that in a solution with an increased Na/Ca ratio, the chemical conditions are advantageous to the complexation of F^- with Na^+, regardless of the water type. As indicated by the dissociation constants, the complexation of Na^+ and F^- to form NaF will occur as the Na^+ concentrations increase (Gao et al., 2007). They also observed that with the increase in Na^+ activity, for instance by adding NaCl as a Na source, the increase of NaF^0 concentration was rapid at first and then became slower, but the concentrations of HF, HF^{2-}, CaF^+, and MgF^+ were continuously decreasing at invariable Ca^{2+} concentration in a natural system. Therefore, the intake of high sodium water could lead to an increased loss of calcium whereby the CaF^- formation is restricted. Spencer et al. (1969) observed a 30% decrease of plasma Ca^{2-} levels during the intake of sodium fluoride in human subjects, indicating that there is a regulation of Ca^{2-} due to high intake of Na^+. On the other hand, as observed in endemic CKDu regions where Ca^{2+} activity is more prominent compared to the sodium activity, F^- shows more affinity towards Ca^{2+}. In areas with high Ca^{2+} activity and with high contents of fluoride, even endemic fluorosis problems are very common as exemplified by Yong and Hue (1991). The chemical activity of fluoride makes it physiologically more active than any other elemental ion and the geochemical occurrence of fluoride in the natural system governs its behavior. Therefore in areas with high Ca^{2+} activity and resulting lower Na/Ca ratios with increased fluoride in groundwater, it could enhance the incidence of CKDu.

3.1. Dose-dependent F^-, Na^+ and Ca^{2+}: renal implications

The fact that the CKD of yet unknown etiology is found distributed only in the fluoride-rich groundwater terrains in the dry zone of Sri Lanka makes fluoride a major candidate among the causative agents. Yet, the fact that the CKDu is not prevalent in some parts of the fluoride-rich dry zone seems to indicate that high fluoride in the groundwater may not be acting alone as a causative agent. Even within the endemic CKDu areas, there are some regions (e.g.: Huruluwewa as described earlier) relatively free of the disease.

Fluoride is known to be a widely distributed nephrotoxin, potentially resulting from environmental pollution and from fluoridated anesthetic use. Zhan et al. (2006) from animal experiments showed that fluoride causes various histological structural changes of the kidney including extensive indication of cell apoptosis resulting in impairment of renal function and metabolism. Although the exact mechanism of fluoride-induced renal impairment is not known, it is apparently related to the concentration of fluoride within the renal medulla. This may be a requisite factor in the development of fluoride nephropathy. Zager and Iwata (1997) showed the divergent effect on human proximal tubular cell viability by inorganic fluoride. They showed that fluoride induces dose-dependent cytotoxicity in cultured human proximal tubular cells that occurred via Ca^{2+} with phospholipase-A_2 (PLA_2) dependent mechanisms. On the other hand, sub-toxic concentrations may demonstrate a cytoprotective effect. A marked effect is shown by clinically relevant fluoride levels on PLA_2 expression, and this is an important observation in view of the fact that this enzyme has strong cell signaling and homeostatic effects. Fluoride, by acutely decreasing cytosolic PLA_2, actively exerts cytoprotective effects and, at sub-lethal doses, it can even protect proximal tubular cells from a superimposed attack. The point of emphasis here is that fluoride's unique duel role as both a cytoprotective and a cytotoxic agent is heavily dose-dependent (Zager and Iwata, 1997).

The biochemical pathways and mechanisms by which fluoride acts are therefore strongly influenced by the presence of Ca^{2+} and Na^+ ions. NaF for example could protect agent cell injury by interfering with free Ca^{2+}, thereby inhibiting Ca^{2-} dependent PLA_2 activity.

Table 2
Summary statistics for Na/Ca ratio values in endemic and non-endemic regions (SD—Standard deviation).

Na/Ca	Giradurukotte	Nikawewa	Medawachchiya	Padaviya	Huruluwewa	Wellawaya
	Endemic	Endemic	Endemic	Endemic	Non-endemic	Non-endemic
Min	0.1	0.2	0.2	0.1	10 0	14 8
Max	18 9	42.4	2.9	7.8	207.6	2300
Median	1.14	3.37	1.62	1.96	13.33	188
Mean	3.3	6.6	1 6	2.3	33.6	469.1
SD	4.3	8.1	0.9	2.0	48.3	821.4

Borke and Whiteford (1999) were also of the view that both acute and chronic exposures to elevated levels of fluoride have negative effects on several Ca-dependent processes, including kidney glomerular and tubular function. They suggested that chronic high fluoride ingestion producing high plasma fluoride levels may occur in humans and may affect Ca^{2+} homeostasis by increasing the turnover or breakdown or decreasing the expression of plasma membrane and endoplasmic reticular Ca^{2+} pump proteins.

In the case of CKDu in the dry zone, it has been shown by Chandrajith et al. (2010) that nephrotoxic metals such as Cd, U and Al could be eliminated as causative agents on account of their very low presence in water. Furthermore their observation that histopathological lesions in the kidney tissue exist leads credence to the view that chronic fluoride ingestion probably is responsible for the tubular damage. The subtle variations of the Na/Ca ratios in the groundwater consumed as drinking water within a fluoride-rich background could thus explain both the presence and absence of the CKDu in the dry zone of Sri Lanka. The marked variations in the endemicity of the diseases and their correlation with the Na/Ca ratio further emphasizes the different biochemical mechanisms followed by Ca^{2+} and Na^{+} in their influence of fluoride-induced tubular damage.

4. Conclusions

The detailed hydrogeochemical investigation carried out in this study shows a clear difference in Na and Ca activities in drinking water between affected and non-affected regions as evidenced by their Na/Ca ratios. Although high fluoride levels are common in all studied regions, non-endemic areas are characterized by much higher Na/Ca ratios. With an increased Na/Ca ratio, the geochemical behavior of drinking water is favorable for the complexation of fluoride with Na^{+}, which reduces both the toxicity of fluoride ions in the human body and the absorption of Ca^{2+}. Conversely, higher Ca^{2+} activity aggravates the damage caused by fluoride, resulting in possible lesions on tubular cells leading to their death. This study provides new insights that inorganic fluoride intake can cause a considerable nephrotoxic effect on human proximal tubular cells, but this toxicity depends strongly on Na^{+} and Ca^{2+} activities. The nutritional states of the individuals and genetic predisposition also play a major role in the CKDu in the north central dry zone of Sri Lanka where only susceptible members of the family or families are affected, although all of them are exposed to similar geoenvironmental conditions. The farming community in such regions, who mostly depend on their immediate natural environment, become more vulnerable for the disease as they drink more water (obtained from the ground) when working under warm humid conditions that prevail in the dry zone of Sri Lanka.

The conclusions arrived at in this study are of special importance to millions of people living in poverty in arid zones of some tropical countries. The groundwater used for drinking and domestic purposes may have unique fluoride, sodium and calcium ion concentrations, which could lead to fatal chronic kidney diseases. Apart from diabetes and hypertension, this may be another major cause for CKD in the arid zones of tropical countries where high levels of groundwater fluoride are present. Such terrains cover a vast area in Southeast Asia and in the equatorial belt of Africa.

Acknowledgements

Authors gratefully acknowledge the Alexander von Humboldt (AvH) Foundation for granting an Atomic Absorption Spectrophotometer that was used for the water analysis in this project. Special thanks to Drs. Nimmi Aturaliya and Thilak Abeysekera, and all team members of the Research Program on Chronic Kidney Disease at the University of Peradeniya for their help in various ways.

References

APHA (American Public Health Association). Standard methods for the examination of water and wastewater. 19th edn. Washington DC: American Public Health Association; 1998.

Aturaliya TNC, Abeysekera DTDJ, Amerasinghe PH, Kumarasiri PV, Bandara P. Towards understanding of chronic kidney disease of North Central Province. Proc of Annual Scientific Sessions of Sri Lanka Medical Association, 2006.

Aturaliya TNC, Abeysekera DTDJ, Amerasinghe PH, Kumarasiri PVR, Dissanayake V. Prevalence of chronic kidney disease in two tertiary care hospitals: high proportion of cases with uncertain aetiology. Cey Med J 2009;54(1):23–5.

Borke JL, Whiteford GM. Chronic fluoride ingestion decreases 45Ca uptake by rat kidney membranes. J Nutr 1999;129:1209–13.

Chae GT, Yun ST, Mayer B, Kim KH, Kun SY, Kwon JS, et al. Fluorine geochemistry in bedrock groundwater of South Korea. Sci Total Environ 2007;385:272–83.

Chandrajith R, Nanayakkara N, Itai K, Aturaliya TNC, Dissanayake CB, Abeysekera T et al. Chronic Kidney Diseases of Uncertain Etiology (CKDue) in Sri Lanka geographic distribution and environmental implications. Environ Geochem Health 2010. doi:10.1007/s10653-010-9339-1.

Codreanu I, Perico N, Sharma SK, Schieppati A, Remuzzi G. Prevention programmes of progressive renal disease in developing nations. Nephrology 2006;11:321–8.

Dissanayake CB. Water quality and dental health in the dry zone of Sri Lanka. In Appleton JD, Fuge R, McCall GJH, editors. Environmental geochemistry and health, 113. U.K.: Geological Society Special Publication; 1996 p. 131–40.

Dissanayake CB, Chandrajith R. Iodine in the environment and endemic goitre in Sri Lanka. In: Appleton JD, Fuge R, McCall GJH, editors. Environmental geochemistry and health, No 113. U.K.: Geological Society Special Publication; 1996. p. 213–22.

Dissanayake CB, Chandrajith R. Medical geology in Sri Lanka. Environ Geochem Health 2007;29:155–62.

Gao X, Wang Y, Li Y, Guo Q. Enrichment of fluoride in groundwater under the impact of saline water intrusion at the salt lake area of Yuncheng basin, northern China. Environ Geol 2007;53:795–803.

HACH. Water analysis handbook. 4th Edition. Colorado, USA: Hach Company; 2002.

Nahas AMEI, Belle AK. Chronic kidney disease: the global challenge. Lancet 2005;365:331–40.

Salve PR, Maurya A, Kumbhare PS, Ramteke DS, Wate SR. Assessment of groundwater quality with respect to fluoride. Bull Environ Contam Toxicol 2008;81:289–93.

Saxena VK, Ahmed S. Inferring the chemical parameters for the dissolution of fluoride in groundwater. Environ Geol 2003;43:731–6.

Spencer H, Lewin I, Fowler J, Samachson J. Effect of sodium fluoride on calcium absorption and balances in man. Am J Clin Nutr 1969;22:381–90.

Tennakoon TMMH. Dental fluorosis in Anuradhapura District, Sri Lanka. Proceedings of the 4th International Workshop on Fluorosis Prevention and Defluoridation of Water. Colombo, Sri Lanka; 2004. p. 19–22.

Vithanage M, Ramanayake RMAC, Rajapakshe A, Jayarathna L, Kumara C, et al. Hydrogeochemical characteristics of groundwater in Hambantota District Sri Lanka 26th FebruaryProceedings of the 26th Annual Sessions on "Geology for National Development" Geological Society of Sri Lanka; 2010. p. 10 Colombo, Sri Lanka.

WHO. World Health Organization: fluorides and oral health. Report of a WHO Expert Committee on Oral Health Status and Fluoride Use. Geneva, 1994.

Yong L, Hue ZW. Environmental characteristics of regional groundwater in relation to fluoride poisoning in North China. Environ Geol Water Sci 1991;18:3-10.

Zager RA, Iwata M. Inorganic fluoride divergent effects on human proximal tubular cell viability. Am J Pathol 1997;150:735–43.

Zhan X-A, Wang M, Xu Z-R, Li J-X. Toxic effects of fluoride on kidney function and histological structure in young pigs. Fluoride 2006;39:22–6