

Possible link of Chronic arsenic toxicity with Chronic Kidney Disease of unknown etiology in Sri Lanka

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Abstract

In recent years a significant increase in number of patients of Chronic Kidney Disease of unknown etiology (CKDu) has been observed in some parts of Sri Lanka, especially in the North Central Province. A case control study has been performed with the intention of determining the prevalence of clinical features of Chronic Arsenic Toxicity (CAT) among CKDu patients in Padavi Sripura divisional secretarial area in Trincomalee District, Sri Lanka. Clinical assessment were done in diagnosed CKDu patients (n=125) and non-CKDu persons (n=180) as the control group. Hair and urine samples collected from both CKDu patients and controls were analyzed for presence of arsenic using Atomic Absorption Spectrometry equipped with Hydride generator (HG-AAS). The results revealed that 68% of CKDu patients and 28% of the controls had urine arsenic levels above 21 µg/g creatinine, which is considered the point of threshold for manifestation of early renal changes that can be developed in to chronic kidney disease. Among the CKDu patients, 48% and 17.4% of the subjects in the control group have fulfilled the criteria to be diagnosed CAT, indicating the potential link between CAT and CKDu. Agrochemicals could be the possible source for this contamination of arsenic since no reported work is available to indicate the presence of arsenic in the bedrocks of Sri Lanka.

Key words: Chronic kidney disease, arsenic, agrochemicals, Sri Lanka

01.Introduction

1.1 Chronic Kidney Disease of unknown etiology (CKDu)

During the last two decades, noteworthy numbers of patients with Chronic Kidney Disease were reported from Rajarata area of Sri Lanka. Rajarata was one of the three administrative divisions of ancient Sri Lanka and it was the epicentre of Sinhala Buddhist civilization where two ancient capitals, Anuradhapura and Polonnaruwa are located. Geographically, Rajarata area encompasses the present day North Central Province and parts of adjacent Provinces in Sri Lanka.(Fig 01)

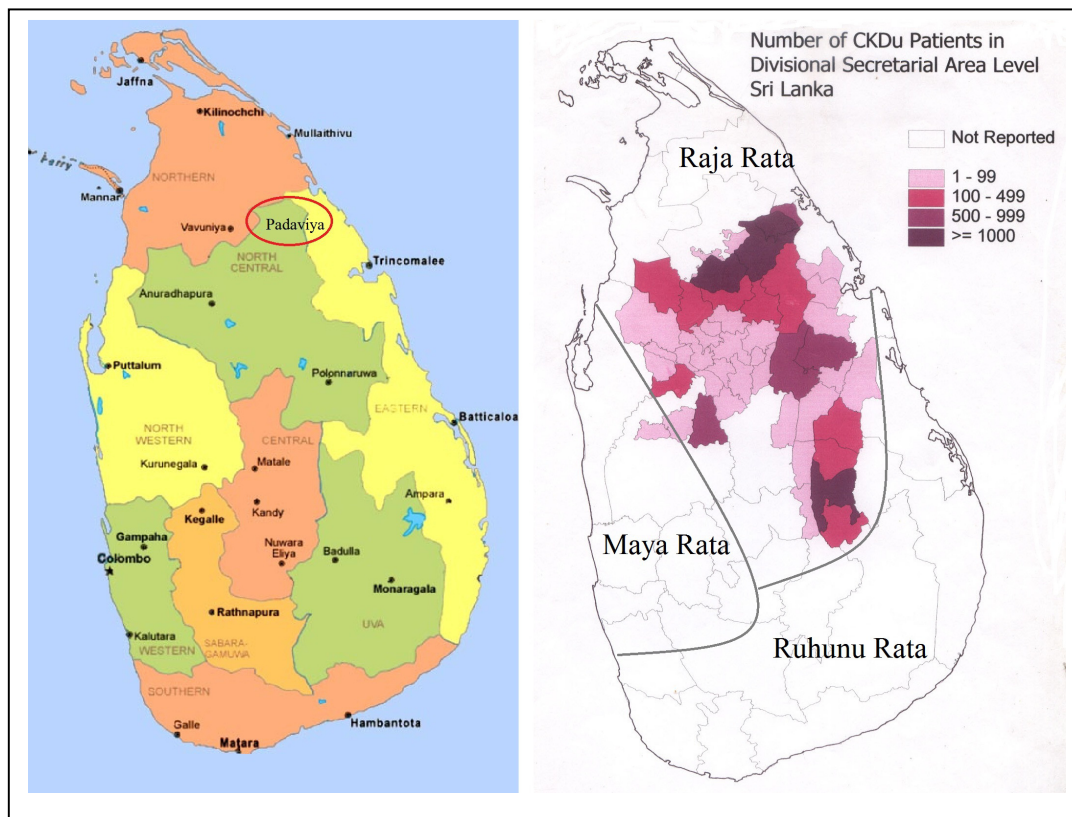


Fig. 01 Provinces of Sri Lanka, Geographical distribution of CKDu patients and study area

Causality of CKDu appears to be different to that of other renal failures, which result from diabetes, high blood pressure, past snake bites or urinary tract infections. This disease therefore is named as Chronic Kidney Disease of unknown etiology (CKDu) based on recommendations of the National Research Programme for CKDu of the Ministry of Health in Sri Lanka. (Circular No 01-10/2009, Ministry of Health, Sri Lanka.) This has been first identified in the 1990s, to be endemic to certain geographical areas of Sri Lanka including Medawachchiya, Padaviya, Kebithigollawa (North Central Province), Nikawewa, Giribawa, Nickaweratiya (North Western Province), Dehiattakandiya (Eastern Province) and Giradurukotte (Uva Province) (Jayasumana 2010).

The CKDu is a slowly progressive disease. Patients are asymptomatic during most part of the disease course and there are no specific clinical features. The disease onset is in adolescence and the proportion of cases developing chronic renal failure increases with increasing age indicating the progressive nature of the disease. (Athuraliya et al. 2009) Histopathological findings have shown tubular interstitial nephritis with or without nonspecific interstitial mononuclear cell infiltration associated with glomerular atrophy and glomerular loss. Disease is characterized by tubular proteinuria usually alpha-1 and beta-2 microglobulinuria, high urine NGal levels (>300ng/mg creatinine/dL) and the absence of edema. (Jayasumana 2012).

The primary medico-legal drawback associated with the disease is that deceased patients are not subjected to medico-legal autopsies and the bodies are released giving the cause of death as chronic kidney disease of unknown etiology. This has caused an enormous loss of valuable autopsy evidences which are of utmost importance to reveal its etiology.

In the past, several researchers have attempted to explain the etiology of CKDu. Herath et al. (2005) reported that high fluoride content in drinking water may be the cause of CKDu, nevertheless, they have not been able to explain the reasons for absence of CKDu in places where drinking water contains extremely high content of fluoride. Toxicity was hypothesized to be caused by aluminium fluoride formed by the reaction between fluoride in water with

aluminium in low-quality utensils that are in wide use in these areas. It has been reported that aluminium fluoride is a neurotoxic which cause Alzheimer's disease (Foncin et al.1986) and it is not observed among CKDu patients. Although the usage of low quality aluminium utensils was discouraged by the government in CKDu endemic areas, the statistics of CKDu patients indicate that the rapid increase in number of CKDu patients in Rajarata. Bandara et al. (2008) reported that presence of high concentrations of cadmium in drinking water and food as a potential cause of CKDu. Chandrajith et al. (2010) have shown that no such high cadmium levels were detected in drinking water and food from the CKDu endemic areas. It is most unlikely therefore that fluoride and cadmium are the sole causative agents of CKDu. Later in 2010, it was reported that presence of toxins produced by cyanobacteria in surface waters, particularly in the freshwater reservoirs was the reason for the disease, nevertheless results could not bring out a plausible explanation for prevalence of CKDu only among those who drink groundwater. As such, none of the previous work on the subject could adequately explain etiology of CKDu.

Being an agrochemical (Chemical fertilizers and Pesticides) using male paddy farmer who drinks water with high hardness has been identified to be of highest risk for CKDu (Jayasumana 2012). Majority of people in north central, north western and eastern Sri Lanka are paddy farmers and they are heavily exposed to pesticides and fertilizers. Agrochemicals are freely and irrationally used and very little attention is given to hazardous effects on human health.

Prevalence of diseases comparable to CKDu has been reported to occur among young male sugarcane farmers in El Salvador, Central America, resulting in substantial morbidity and mortality. (Orantes et al 2011). Symptoms are most consistent with tubulointerstitial disease. A similar scenario has also been reported from Upper Egypt where investigators have noted abnormal increase in CKD patients among male farmers who use pesticides (Kamel et al.2010).

Arsenic is an infamous toxin which has caused major human health problems in several parts of the world and concern over such incidents has prompted investigations into the nephrotoxic effects of arsenic. Early studies have shown acute or sub acute arsenic poisoning can cause tubulointerstitial nephritis. (Prasad et al.1995) But no detail study is available to show the nephrotoxic effect of chronic arsenic poisoning and present study is the first study conducted on potential link of arsenic for prevalence of CKD.

1.2 Arsenic

Arsenic (As) is considered an extremely toxic element and a "non threshold carcinogen" found to occur in the environments around the globe. (Meharg 2010). Arsenic is a naturally occurring metalloid and it can exist in inorganic or organic forms. Since most of the organic or inorganic arsenic compounds are white or colorless, without a smell or taste, it is difficult to detect its presence in food, water and air. The inorganic forms of arsenic are more toxic than its more complex organic compound. Toxicity of arsenic has been reported to be as Arsenine > Arsenite > Arsenoxide > Arsenates > Arsenic. These toxic arsenic compounds, particularly, copper, lead, sodium, calcium and zinc arsenates were in use to control insect pests, weeds, molds, bacteria, and rodents.

It has been well-established that arsenic compounds have detrimental effects on health of living beings. Incidents of arsenic contamination in groundwater have been reported from many countries (Henke 2009). Arsenic toxicity in drinking water and food remains a problem in many parts of the world. Among the various sources of arsenic in the environment, drinking water probably poses the greatest threat to human health, especially for arsenic concentrations that have been observed in ground water. (Bhattacharya et al. 2002) In water, arsenic is frequently found in inorganic species such as arsenite (AsO_3^{3-}) and arsenate (AsO_4^{3-}), referred to as As(III) and As(V), respectively, which have reported to cause harmful effects on humans, plants and animals (Henke 2009). Based on human health data, World Health Organization (WHO) has recommended a maximum permissible contaminant level for arsenic as $10\mu\text{g/L}$ for drinking water. (WHO guidelines for Drinking water quality,1996). Ill effects of chronic arsenic toxicity have drawn attention of scientists since early 1980s and extensive studies on the subject therefore have only been reported during the last two decades.

Diagnosis of arsenic toxicity, especially from low doses of inorganic arsenic is very complicated as there are no distinct symptoms since people exposed to it respond differently, depending on the extent of exposure and the nature of the source. Arsenic can be inhaled, ingested or absorbed through skin. Arsenic which remains in body is stored in

the liver, kidney, brain, and other tissues and causes serious damage to them as it is able to bio-accumulate within living cells. Long term arsenic exposure, even at low levels, can result in a range of symptoms (Muzumder 2000). Contaminations of arsenic in drinking water, food, air or soil have not been studied in Sri Lanka comprehensively. There are no records available therefore on occurrence of arsenic in bedrocks, soil and water of Sri Lanka.

Initial clue for the present study has emerged while the first author was engaged in his PhD research on etiology of CKDu. Abnormal spotty pigmentation observed in palms and soles of CKDu patients during clinical examinations of CKDu patients, that were subsequently confirmed by Medical officer of Padavi Sripura hospital was a basis for the hypothesis that chronic arsenic toxicity could be a cause of CKDu. Objectives of the present study therefore was to determine the prevalence of clinical features and analysis of urine and hair samples for arsenic levels, with the intention of assessing chronic arsenic toxicity (CAT) among CKDu patients in Padavi Sripura divisional secretarial area in Trincomalee District, Eastern Province of Sri Lanka.

02. Study design, setting and methods

2.1 Study area

Paddy farming areas irrigated with water from Padaviya reservoir and associated agricultural settlements were included in the study area.

2.2 Clinical assessments of CKDu patients

A reconnaissance survey was conducted in the study villages by the medical doctors in this research team followed by a door-to-door survey to gather background information on CKDu patients. Then, the selected individuals diagnosed to suffer from CKDu were requested to attend medical camps organized at Government Hospital at Sripura and biological samples (hair and urine) were collected from them.

Study was conducted during December 2010 to July 2011 with the concurrence of Ethics Committee of the Faculty of Medicine and Allied Sciences of Rajarata University of Sri Lanka. Written informed consent was obtained from all the individuals who have participated in the study.

During the clinical observations of patients (n=125), signs and symptoms of chronic arsenic poisoning such as hyperpigmentation, keratosis in palms and soles, generalized body weakness, headache, burning eyes, anemia, nausea, mild to moderate hepatomegaly and splenomegaly, epigastric pain and paresthesia were observed and recorded. Control group (n=180) were selected from those individuals from the study area who have not been diagnosed to have CKDu and some of them were selected randomly from family members of CKDu patients.

2.3 Sample collection and preparation

Urine samples were collected from selected CKDu patients from the study area into acid-washed (10% nitric acid) polypropylene bottles. Immediately after collection, the samples were stored in salt-ice mixture and brought to the laboratory where they were kept at 4°C until measurement of arsenic content.

Hair (300–500 mg) samples were collected using a cleaned pair of scissors from the same individuals from whom urine samples were collected and kept in plastic bags. They were brought to the laboratory, washed with de-ionized water, followed by acetone for 10 minutes each and finally were dried in an oven at 50°C.

2.4 Apparatus

GBC 932 plus Atomic Absorption Spectrometer-AAS (Australia) equipped with a hydride generation system (GBC 3000) and Graphite Furnace (GF 3000) with the background corrector was used to detect arsenic content in all the samples. Arsenic hollow cathode lamp was utilized to detect arsenic in the samples.

2.5 Chemicals and reagents

Reagents of analytical grade and deionized water (Bransstead E Pure, Model 4832-33) were used for the analyses.

Stock solution of arsenic trichloride (AsCl_3) with concentration of 1000 mg/L in 1M HCl (VWR International LTD, England) was used for the standard solution preparation for atomic absorption spectrometer. Initially a standard solution of 10 mg/L was prepared from the stock solution (1000 mg/L) using a mixture solution of nitric acid (10%). Then, a standard solution of 1 mg/L was prepared, directly from the 10 mg/L standard, using the same mixture of solutions. Finally, solutions of 1 $\mu\text{g/L}$, 5 $\mu\text{g/L}$, 10 $\mu\text{g/L}$, 20 $\mu\text{g/L}$,30 $\mu\text{g/L}$ and 100 $\mu\text{g/L}$ were prepared. Stock solutions were kept in polyethylene bottles and kept at 4 °C. Standard solutions were prepared daily for analyses. The calibration curves were established with the standard solutions and the square of correlation coefficient (R^2) was in the range of 0.987 - 0.999 in most occasions while AAS was in use.

2.6 Digestion of samples

Digestion of samples was carried out with concentrated nitric acid (70% AR) (Sigma Aldrich Pvt. Ltd), concentrated sulfuric acid (98% AR) (Sigma Aldrich Pvt. Ltd), perchloric acid (70% AR) (Sigma Aldrich Pvt. Ltd), HCl (37% AR) (Sigma Aldrich Pvt. Ltd), and high purity hydrogen peroxide (35.5%) (Sigma Aldrich Pvt. Ltd), Laboratory glassware was kept overnight in 10% (v/v) nitric acid. Before use, the glassware were rinsed with de-ionized water and dried in a dust free environment.

03. Results

Percentage abnormal skin manifestations (Fig 02) observed in the sample of CKDu patients and with individuals of the control group are presented in Table 01. More than half the patients (54.4%) have shown hyper pigmentation in palms and 49% in soles although prevalence of keratosis was relatively low among them. Furthermore, nearly 10 to 25% of the individuals of control group too manifested the manifestations. Some individuals in the control group also showed other symptoms manifested in CKDu patients, nevertheless in a lesser degree (Table 2).

Burning of the eyes, parasthesia and epigastric pain are not common symptoms in patients with chronic kidney disease unless a patient is at the final stages of renal failure or the patient is affected by the adverse effects of a drug. Hence these three symptoms can be identified to a certain extent, as characteristic to CKDu patients. Even to a lesser extent, these symptoms were observed among individuals of the control group too.

Table 1: Percentage dermal manifestation observed in the case and control groups

Dermal manifestation	Number of patients (n=125)	%	Number of controls (n=180)	%
Hyper pigmentation of palms	68	54.4	34	18.8
Hyper pigmentation of soles	49	39.2	26	14.4
Keratosis of palms	29	23.2	19	10.5
Keratosis of soles	22	17.6	15	08.3

Table 2: Other clinical symptoms manifested by CKDu patients and individuals in control group

Other clinical symptoms observed among the subjects of the study	CKDu patients (%)	Controls (%)
Generalized body weakness	95	35
Headache	91	44
Burning of eyes	84	12
Nausia	76	18
Mild to moderate hepatomegaly and splenomegaly	25	04
Epigastric pain	63	16
Paresthesia	51	12



Fig 02- Abnormal skin manifestations of CKDu patients encountered in the study area

This substantiates the observations made on incidence of keratosis and hyper-pigmentation among the population in this study area. Since it is evident that with the exception of dermal manifestations, other clinical features of chronic arsenic poisoning can occur under some other medical conditions too, history of exposure to arsenic, arsenic content in hair and urine have to be considered along with clinical features to arrive at a conclusive judgment on chronic arsenic poisoning. Previous studies have shown urinary excretion of arsenic and its concentration in hair and nail are most important biomarkers of internal exposure.(Hindmash 1998). In general, blood concentrations of arsenic are too low and transient; hence it is not considered a biomarker for chronic arsenic poisoning (Muzumder 2000). Total arsenic amount in urine has been in use as an indicator of recent arsenic exposure because kidney is the main route of the excretion of many arsenic species (Buchet et al.1981). Half life of inorganic arsenic in humans is about 4 days. In European population, average urinary arsenic concentration is less than 10 µg/L (Apeal and Stoeppler 1983; Trepka et al.;1996 and Kavanagh et al.,1998). Urinary arsenic levels detected among the subjects of the present study are presented in Table 3 while table 4 shows calculation of Odds ratios.

Toxic excretory level for arsenic in urine for south Asian population is 35µg/L. (Muzumder 2000). Among the patients, 68% and 28% among the controls had urine arsenic levels above 21 µg/g creatinine. Urine arsenic levels of 21 µg/g creatinine is considered as the threshold for commencing early renal changes which can potentially lead to chronic kidney disease.

Table 3: Urinary arsenic contents of CKDu patients and individuals of the control group

Urinary arsenic Concentration ($\mu\text{g/L}$)	CKDu patients (%) (n=125)	Controls (%) (n=180)
0-10	4.0	10.0
10-20	4.8	13.3
20-30	6.4	26.6
30-40	12.0	21.2
40-50	32.0	14.4
50-60	25.6	10.0
>60	15.2	4.5

Table 4: Urinary arsenic contents and Odds ratios

Urinary Arsenic Conc.	CKD patients n = 125	Controls n = 108	Odds Ratios
30-40 (Reference)	15	38	1.00
0-10	5	18	0.90 (95% CI = 0.24 - 3.42)
10-20	6	24	1.50 (95% CI = 0.47 - 4.82)
20-30	8	48	0.63 (95% CI = 0.22 - 1.86)
40-50	40	26	0.16 (95% CI = 0.06 - 0.45)
50-60	32	18	0.14 (95% CI = 0.05 - 0.41)
60+	19	8	0.11 (95% CI = 0.03 - 0.36)
30 + (Reference)	106	90	1.00
0-29	19	90	5.58 (95% CI = 3.16 - 9.86)

Arsenic is known to have higher affinity to keratin. Hence comparatively high concentration of arsenic has been observed in high keratin containing tissues such as hair and nails (Table 5). Concentration of arsenic in hair of humans with no known exposure to arsenic, generally ranges 0.02-0.2 mg/kg (Valentine et al.1979; Narang et al.1987; Wang et al.1994 and Kurttio et al.1998). Hair samples of CKDu patients contained high amount of arsenic compared to that of individuals in the control. It further substantiates the chronic arsenic poisoning of CKDu patients in the study area.

Table 5: Arsenic content in hair samples of CKDu patients and individuals of the control group

Hair samples	Arsenic -mg/kg
Mean-Normal (Muzumdar 2000)	0.46
Control group	0.22 \pm 0.01 to 4.58 \pm 0.98
CKDu patients	1.27 \pm 0.22 to 7.03 \pm 1.06

04. Discussion and conclusion

Dermatological features supported by toxic levels of arsenic in urine and hair are confirmatory for chronic arsenic poisoning. (Muzumdar 2000) There is a wide variation in the incidence of chronic arsenic poisoning in an affected population. In a study done at west Bengal has shown that even not all members of an affected family show clinical symptoms. (Muzumdar 2000) Dermal manifestation such as hyper pigmentation and hyperkeratosis are diagnostic of

chronic arsenic poisoning. However dermal manifestations depend on age, gender, ethnicity, skin colour and type of arsenic that is ingested.

Findings of the present study reveal that 48% of the CKDu patients and 17.4% of the subjects in the control group have fulfilled the requirements to be diagnosed CAT. Percentage CKDu patients who fulfill the requirements for CAT were greater than that among the control group. Calculation of ODDS ratios shows that the individuals who excrete more than 35µg/L of arsenic in urine are 5.58 times more likely to develop CKDu. Observable CAT symptoms among individuals of the control group indicate that being inhabitants of the same area and having to consume contaminated water and food majority of the population has already bio-accumulated arsenic, nevertheless to a lesser extent than those who have been diagnosed as CKDu patients. This also implies that these individuals are of high risk in acquiring CKDu in near future.

CKDu represents an emerging new type of nephropathy, caused potentially by chronic arsenic poisoning with the contribution of heavy metals, pesticide residues, heat stress and chronic repeated dehydration (Personal communication Daniel Brooks, Boston University, USA 2012), poor quality of drinking water and genetic variations (Specially AS3MT gene). (Fig 03)

It is appropriate therefore to subject the deceased patients to complete medico-legal autopsy including histopathological and toxicological investigations. Ideally, as this is not a deliberate self poisoning clinical subjects are supposed to get a medico-legal referral. But it has not taken place in the clinical setup yet.

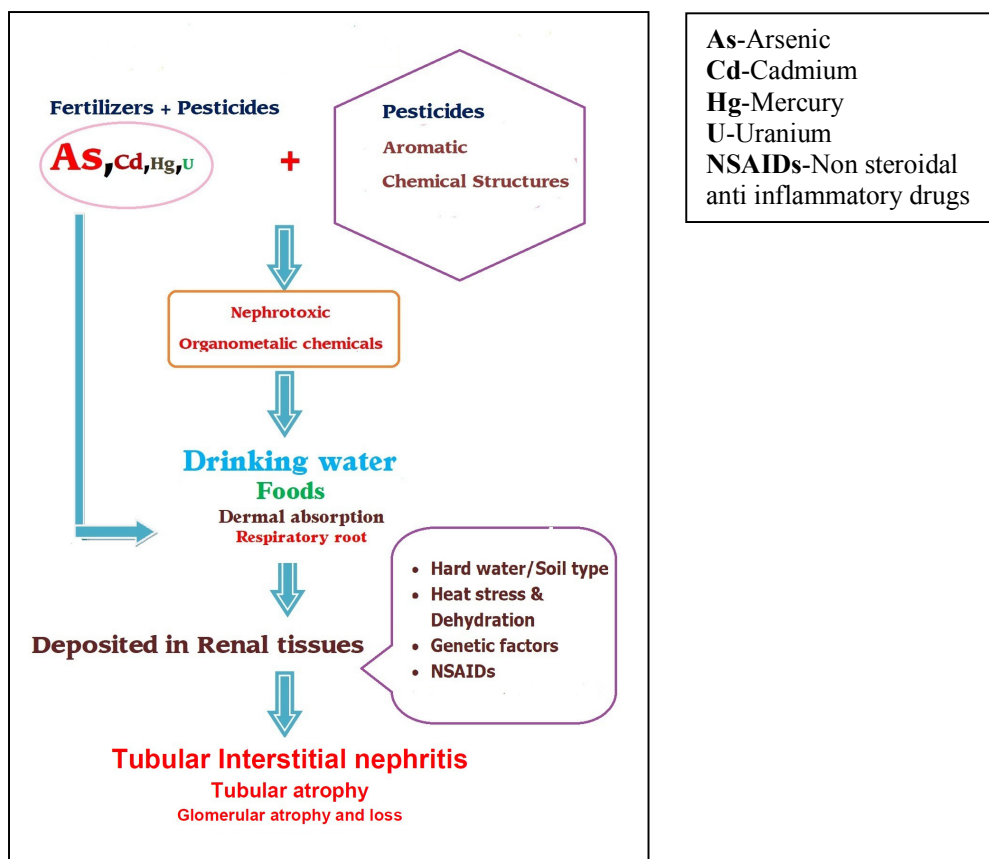


Fig. 03-Possible mechanism to explain the occurrence of CKDu in Sri Lanka

Continuation of this research to explore the possible contamination of arsenic among the farmers with CKDu suggested that the arsenic is not present naturally in the soils of the study area and thus investigations have been

carried out to test the agrochemicals, namely, pesticides, herbicides and inorganic fertilizer for arsenic. The agrochemicals were tested in an accredited laboratory in Malaysia for arsenic and the results have confirmed the presence of arsenic in agrochemicals. (Jayasumana et al.2011) Since no reported work is available as to the presence of arsenic in the bedrocks below Sri Lankan land mass, pesticides and fertilizers that are excessively used in paddy farming should be attributed as the most likely sources of arsenic in CKDu patients in the study area.

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06.References

- Apel, M., and M. Stoepler. (1983). Speciation of arsenic in urine of occupationally non exposed persons. paper presented at international Conference on Heavy Metals in the Environment, September, in Heidelberg, Germany.
- Athuraliya, T.N., D.T. Abeysekera, P.H. Amerasinghe, P.V. Kumarasiri, and V. Dissanayake. (2009). Prevalence of chronic kidney disease in two tertiary care hospitals: high proportion of cases with uncertain aetiology. *Ceylon Med Journal* 54(1):23-5.
- Bandara, J.M., D.M. Senevirathna, D.M. Dasanayake, J.M. Bandara, D.T. Abeysekera, and K.H. Rajapaksha. (2008). Chronic renal failure among farm families in cascade irrigation systems in Sri Lanka associated with elevated dietary cadmium levels in rice and freshwater fish (Tilapia). *Environ Geochem Health* 30(5):465-78.
- Bhattacharya, P., S.H. Frisbie, E. Smith, R. Naidu, G. Jacks, and B. Sarkar. (2002). *Handbook of Heavy Metals in the Environment: A Global Perspective*. New York: Marcel Dekker.
- Buchet, J.P., and P. Hoet. (1998). Consistency among biomarkers for the assessment of exposure to inorganic arsenic: a literature review. Third International Conference on As Exposure and Health Effects, July 12-15, in San Diego, CA, USA.
- Chandrajith, R., C.B. Dissanayake, T. Ariyaratna, H.M.J.K.M. Herath, and J.P. Padmasiri. (2011). Dose dependent Na and Ca in fluoride rich drinking water-another major cause of chronic renal failure in tropical arid regions. *Science of the Total Environment* 409: 671-5.
- Chandrajith, R., S. Nanayakkara, K. Itai, T.N.C. Athuraliya, C.B. Dissanayake, and T. Abeysekera. (2010) Chronic kidney disease of uncertain etiology in Sri Lanka: geographic distribution and environmental implications. *Environ Geochem. Health* DOI10.1007/s10653-0109339-1.
- Chronic Kidney Disease of Unknown etiology, Circular No 01-10/2009, Ministry of Health, Sri Lanka.
- Dalal, P.K., A.K. Gangopadhyay, and A. Roychowdhury. (2008). Identification of indicators of arsenic induced nephrotoxicity in humans. *The internet Journal of Toxicology*:5(2).
- Foncin, J.F., and K.H. El Hachimi. (1986). Neurofibrillar degeneration in Alzheimer's disease: A discussion with a contribution to aluminium pathology in man. *Senile dementias early detection* 191-201.
- Haw, T., W. Sue-Sun, and L. Gwo-Chen. (2004). Heavy Metal Content of Rice and Shellfish in Taiwan, *Journal of Food and Drug Analysis* 12(2):167-74.
- Henke, Kevin. (2009). *Arsenic*. Chichester, West Sussex: John Wiley & Sons Ltd.
- Herath, K.R.P.K., O.A. Illeperuma, H.A. Dharmagunawardhane, and K.J. Haller. (2005). Environmental health risk for the chronic renal failure in Sri Lanka. paper presented at the 31st congress on Science and Technology, October, in Suranaree University of Technology, Thailand.
- Hindmarsh, J.T. (1998). Hair arsenic as index of toxicity. Third International Conference on As Exposure and Health Effects, July 12-15, in San Diego, CA, USA.
- Jayasumana, M.A.C.S. (2010). Chronic kidney disease of unknown etiology in north central province, Sri Lanka: a meta analysis-Paper presented at the Annual research symposium, Rajarata university of Sri Lanka, September 20, In Anuradhapura, Sri Lanka.

Jayasumana, M.A.C.S., P.Paranagama, and M.Amarasinghe. (2011). Chronic kidney disease of unknown etiology and arsenic in ground water in Sri Lanka. paper presented in workshop on challenges in groundwater management in Sri Lanka, March 15, in Colombo, Sri Lanka.

Jayasumana, M.A.C.S., P.Paranagama, M.Amarasinghe, D.V.K. Wijekon and S.I.Fonseka. (2011). Presence of Arsenic in pesticides used in Sri Lanka. Paper presented at Water professionals' day symposium, University of Peradeniya, October 01, in Peradeniya, Sri Lanka.

Jayasumana, M.A.C.S. (2012). Histopathological features of Sri Lankan Agricultural nephropathy. Presentation done at the first international workshop on Mesoamerican Endemic Nephropathy, November 28-30, San Jose, Costa Rica.

Kamel, E.G., and O.El-Minshawy. (2010). Environmental Factors Incriminated in the Development of end stage renal disease in El-Minia Governorate, Upper Egypt *Int J Nephrol Urol* 2(3): 431-7.

Kavanagh, P., M.E. Farago, I. Thronton, W. Goessler, D. Kuehnelt, C.Schlagenhaufen, and K.J. Irgolic. (1998). Urinary arsenic species in Devon and Cornwall residents, UK. *Analyst* 123: 27-9.

Kurtio, P., H.E., E.Komulainen, E.Hakala, H. Kahelin, and J. Pekkanen. (1998). Urinary excretion of arsenic species after exposure to arsenic present in drinking water. *Arch. Environ, Contam. Toxicol* 34:297-305.

Meharg, A.A., and A.Raab. (2010). Getting to the bottom of arsenic standards and guidelines, *Environ. Sci. Technol* 44, 4395-99.

Muzumder, D.N. (2000). *Diagnosis and treatment of chronic arsenic poisoning*, Institute of post graduate medical education and research, Calcutta, India.

Narang, A.P.S., L.S. Chawls, S.B.Khurana. (1987). Levels of arsenic in Indian opium eaters. *Drug Alcohol Depend.* 20: 149-53.

Orantes, C.M., R.Herrera, M.Almaguer, E.G.Brizuella and C.HE.Hernandez, (2011). Chronic kidney disease and associated risk factors in the Bajo Lempa region of El Salvador: Nefrolempa study, 2009, *MEDICC Review* 13(4):14-21

Patel, H.V., and K.Kalia. (2010). Sub-chronic arsenic exposure aggravates nephrotoxicity in experimental diabetic rats, *Indian Journal of Experimental Biology* 48:762-8.

Prasad, G.V.R., and N.F.Rossi. (1995). Arsenic intoxication associated with tubule interstitial nephritis. *Am J Kidney Diseases* 26(2):373-6.

Trepka, M.J.J., C.Schulz, C.Krause, M.Popescu, M.Wjst, and H.E.Wichmann. (1996) Arsenic burden among children in industrial areas of eastern Germany. *Sci. Total Environ* 180: 95-105.

Valentine, J.L., H.K.Kang, and G.Spivey. (1979). Arsenic levels in human blood, urine, and hair in response to exposure via drinking water. *Environ. Res.* 20: 24-32.

Wang, T.S., and H.Huang. (1994). Active oxygen species are involved in the induction of micronuclei by arsenite in XRS-5 cells. *Mutagenesis* 9: 253-7.

Wanigasuriya, K.P., R.J.Peiris-John, R.Wickremasinghe, A.Hittarage. (2007). Chronic renal failure in North Central province of Sri Lanka: an environmentally induced disease. *Trans R Soc Trop Med Hyg* 101(10):1013-17.

WHO Guidelines for drinking water quality. (1996). *Health criteria and other supporting information*. Geneva:940-94.