RD/DOC/GC/06

Investigation and Evaluation of Chronic Kidney Disease of Uncertain Aetiology in Sri Lanka

Final Report

World Health Organization

RD/DOC/GC/06

Project: Investigation and Evaluation of Chronic Kidney Disease of Uncertain Aetiology in Sri Lanka

Section 1
Information regarding Project/ Project Personnel

Grant Number	**57445**
Title of the Project	Investigation and Evaluation of Chronic Kidney Disease of Uncertain Aetiology in Sri Lanka
Principal Investigators	**Ministry of Health** Dr Nihal Jayathilake – Secretary Health Dr Palitha Mahipala – Director General - Health **World Health Organization** Dr Shanthi Mendis – WHO - Geneva Dr Firdosi Rustom Mehta – WHO - Country Representative Dr Lanka Jayasuriya Dissanayake Dr Navaratnasingam Janakan
Co-investigators	Details attached below
Institute(s) where research was being carried out	Ministry of Health (MoH) in collaboration with World Health Organization (WHO)
Date of award	22 November 2010
Date of completion of Project	31 December 2012
Total allocation of funds (Rs)	70,000,000
Total spent (Rs)	Financial report attached
Number of Research Students employed	Hospital registry - 02 (pre-intern doctors) Literature repository - 02 (pre-intern doctors)
Post graduate degree completed with dates	Nil
Number of Technical Assistants and/ or labourers employed and period of service	45 Field Assistants worked for population prevalence study, environmental studies and analytical studies for a period of two years Two coordinators were appointed for coordination of field activities and administrative & secretarial work for the clinical trial for a period of one year Undergraduates of Sociology at Universities of Colombo and Rajarata took part at data collection for socio-economic and productivity impact study
Publications/Communications arising from the project during the reporting period	A paper entitled `Chronic kidney disease of uncertain aetiology (CKDu) in Sri Lanka; prevalence and causative factors` based on the content of Subproject I is under consideration for publication in a peer reviewed international medical journal

Background

An apparently new form of chronic kidney disease which cannot be attributed to diabetes mellitus, hypertension, primary glomerular nephritis or other known etiologies has emerged in the North Central Region of Sri Lanka. This Chronic Kidney Disease of uncertain etiology (CKDu) is slowly progressive, probably starting in the second decade of life, and asymptomatic until very advanced. The North Central Region includes North Central Province and parts of Eastern, North Western and Uva Provinces. In these areas, CKDu has become a major public health problem causing devastating social economic and health impacts.

The total number of affected individuals with CKDu is unknown; however hospital statistics suggest that in excess of 8,000 people are currently undergoing treatment for this condition. The financial cost for the management of these patients is considerable. Prevention would be the most cost-effective and affordable approach. However, currently it is not an option as the cause of the disease is unknown.

To resolve this public health issue, the Ministry of Health in collaboration with the World Health Organization conducted multisectoral and multidisciplinary research effort built on existing evidence. This coordinated series of research activities from varying disciplines was designed to generate conclusive evidence regarding the aetiology within a specified time period to make prevention an option.

A comprehensive project proposal was prepared and prioritized sub-projects listed in table below were commenced using funds from the World Health Organization (WHO). With the progress of the research there were study requirements that emerged as priority research activities which were also included in the research effort. The total budget for the comprehensive multidisciplinary research project was Rs. 100 million, of which Rs. 30 million was borne by the WHO. As this was of national importance, the National Science Foundation agreed to support this project, and submitted a research proposal to the Department of National Planning to secure the balance funds (Rs. 70 million) needed for the successful completion of the project.

Table - List of multi-disciplinary subprojects and the names of co-investigators involved

No.	Sub-project	Co-investigators
I.	Prevalence and causative factors for CKDu in Sri Lanka (Comprised of population prevalence, analytical and environmental studies, establishment of hospital based CKD registry and literature repository for CKD)	Dr Paba Palihawadana Dr Thilak Abeysekera Dr Meriyanthi Gunethilake Prof. J. M. R. S. Bandara Prof. Janitha Liyanage Prof. Rezvi Sheriff Prof. Oliver Ileperuma Prof. Ravindra Fernando Dr Samitha Ginige Dr S Mathu Dr Pathmalal Manage Dr L Rajapakse Dr P Bandara Dr U Karunaratne Dr D. L. Waidyarathne Dr R Alagiyawanna Dr A. V. Ranasinghe Dr A Bandaranayake Dr N Seneviratne Dr N. M. Perera Dr A Kulasinghe Dr H Karunathilake Dr A. M. C. D. Abeysinghe Dr H. T. Wimalasena Mr. Dilip Hensman Mr. Sumudu Hewawasam Mr. Ruwan Bandara Mr. S Sumanaweera
II.	Double blind randomized clinical trial to examine the renal effects of an Angiotensin Converting Enzyme Inhibitor (Enalapril) in adults with CKDu	Dr S. Mathu Prof. Rezvi Sheriff Prof. Saroj Jayasinghe Dr Thilak Abeysekera
III.	Socioeconomic and productivity impact of CKDu	Dr Chandani Liyanage Prof. Ramani Jayathilake Prof. Amala de Silva Prof. Saroj Jayasinghe Dr Anuradhani Kasthuriratne
IV.	Nephrotoxic herbal remedies used in Sri Lanka	Dr P. Hemachandra Dr P. Hewagamage Dr K. K. D. S. Ranaweera Dr D. N. Ethugala

Operational structure

An operational platform for the implementation of this research project was established with the formulation of three committees at different levels i.e. National steering Committee, Management Committee & Scientific Committee, with clearly defined roles and responsibilities (Annexure I). In addition to these committees, there was a panel of International Experts providing technical inputs for the conduct of research. They also helped in ensuring quality and ethical standards of research through peer review of protocols, reports and publications.

Sections 2 and 3

The sections that follow present the following components for different studies within the subprojects:

- Scientific scope and objectives
- Materials and methods
- Results/outputs
- Discussion and conclusions.

Sub-project I: Prevalence and causative factors for CKDu in Sri Lanka

Comprised of the following:
a) Establishment of hospital based CKD registry;
b) Establishment of a literature repository for CKD;
c) Population prevalence, analytical and environmental studies

a) Establishment of a Hospital-based CKD Registry and literature repository

Scientific scope and objectives

In order to monitor the trends in the disease burden attributable to chronic kidney disease (CKD) in general and chronic kidney disease of uncertain aetiology (CKDu) in specific, efforts were taken to establish a computerized database. As an initial step, hospital-based registries of patients attending renal clinics were established in selected hospitals in the North Central Province namely TH Anuradhapura, DGH Polonnaruwa, BH Medirigriya and BH Madawachchiya. For each patient, the database includes information on socio-demographics, lifestyle factors, environmental factors, information on co-morbidities and primary diagnosis that led to CKD, anthropometric measures and laboratory investigations.

This registry was established to

- identify CKDu cases for studies commissioned under the National Research Programme for CKDu, establishment of a National Registry
- characterize the CKD and CKDu cased with regard to socio-demographics, lifestyle and environmental factors and treatment modalities)

Materials and Methods (CKD registry)

Prior approval for data collection was obtained from the Provincial and Regional Directors of Health Services. Permission to get access to hospital records was obtained from the Directors/ Medical Superintendents of the selected hospitals. The study involved no additional risks to patients and the objectives of the registry were explained to them before collecting data.

Appropriate administrative, technical, procedural, and physical safeguards have been established to protect the confidentiality of the data and to prevent unauthorized access to it. Data from the registry will be provided to researchers those who conduct legitimate research and the researchers should not use the data for purposes that are not related to research. The data will not

be published or disclosed to any person unless the data have been aggregated (i.e. combined into groupings of data such that the data are no longer specific to any individual). A copy of any aggregation of data intended for publication should be submitted to the Scientific Committee, National Research Programme for CKDu and relevant health authorities for review, and written approval should be obtained prior to publication.

The hospital-based registries have been established only in selected hospitals. The data collection sheet used for the registry is given in the appendix. The database currently contains information on approximately 2,000 CKD patients and it has been operational since 2009. Two pre-intern medical officers under the supervision of Epidemiology Unit, Ministry of Health involved in the data collection. Data collection was mainly by interviewing the patients during clinic sessions and by reviewing the clinic records. If necessary, clarification on clinical records was obtained from the medical officers serving at the clinics. Basic socio-demographics and clinical data of CKD patients attending renal clinics at TH Anuradhapura, DGH Polonnaruwa, BH Medirigiriya & BH Medawachchiya entered into a database.

Statistical Analysis

The relevant categorical variables were dichotomized and bivariate analysis (preliminary analysis) was done. For all these variables, odds ratios (OR) and 95% confidence intervals (CI) were calculated to identify risk factors for CKDu. The software package SPSS 10 was used for data entry, analysis and editing. To identify the factors that were independently associated with CKDu, multiple logistic regression analysis was done.

Results/outputs

As per the preliminary analysis, out of the 1997 included in the registry 775 (39%) could be identified as CKDu. Others were due to hypertension 798 cases (40%), hypertension & diabetes 118 cases (6%), diabetes 90 (5%), snakebite 121 (6%), obstructive uropathy 32 (2%), glomerulonephritis 30 (2%), polycystic kidney disease 10 (1%) and other known causes 18 cases (1%).

Bivariate analysis found that compared to CKD of known aetiology patients, CKDu patients were more likely to be males (OR = 2.4, 95% CI: 1.9, 3.0), aged ≤50 years (OR = 1.6, 95% CI: 1.3, 2.0), residents of Anuradhapura district (OR = 1.26, 95% CI: 1.04, 1.5), less educated (OR = 1.3, 95% CI: 1.04, 1.7), past or current smokers (OR = 1.9, 95% CI: 1.6, 2.3), regular consumers of alcohol (OR = 1.9, 95% CI: 1.6, 2.4), consumers of illicit liquor (OR = 1.9, 95% CI: 1.6, 2.3) and exposed to agrochemicals (OR = 1.9, 95% CI: 1.6, 2.4).

Aetiology (known vs. unknown) was not related to ethnicity (OR = 1.1, 95% CI: 0.5, 2.2), income (OR = 1.1, 95% CI: 0.9, 1.4), occupation (OR = 0.9, 95% CI: 0.7, 1.2) and source of drinking water (OR = 1.3, 95% CI: 0.98, 1.6).

Discussion/ Conclusions

As the classification of aetiology of CKD was based on secondary data, it was not possible to confirm the sequence of events especially that of hypertension i.e. whether hypertension was primary (led to CKD) or secondary (due to CKD) diagnosis. Therefore, all the patients with

hypertension were classified as "CKD cases due to hypertension". Considering these facts, it can be stated that the proportion of CKDu cases derived from the registries was an underestimate. Even for other known causes of CKD, secondary data analysis would not suffice to attribute CKD totally to that particular cause/s.

Future activities

Long term aims of this project are:

- To determine the disease burden attributable to CKD in terms of prevalence and incidence of CKD/ CKDu along with geographic and temporal trends
- To investigate relationships among primary diagnosis, socio-demographics, lifestyle and environmental factors, treatment modalities etc
- To support geographic mapping of CKD/ CKDu cases
- To identify new areas for special renal studies and stimulate and facilitate investigator-initiated research (e.g. descriptive, analytic and hypothesis generating studies)

The registry is intended to be a truly national registry. Steps will be taken to expand this activity in a stepwise manner to all renal centres/ clinics in both public and private hospitals in Sri Lanka. In addition to the objectives listed earlier, it can also be used to evaluate renal replacement therapy programmes e.g. outcomes and factors influencing renal replacement therapy, information on patients waiting for renal transplant etc. There are plans to introduce electronic data capture via the internet in the near future. Periodic reports containing descriptive and analytic epidemiologic data on CKD patients will be published in future and this registry is expected to serve as a resource to the academic and clinical medicine communities.

Annexure II: Data collection sheet used for hospital-based registry

b) <u>Establishment of a literature repository for CKD</u>

There are several studies that have investigated the prevalence, aetiology, clinical and histopathological features of CKD and CKDu. Collating these data (published/ unpublished/ grey literature related to CKD/ CKDu into a repository can contribute to the national research effort by facilitating knowledge sharing between researchers and dissemination of results of studies to other stakeholders and policy makers. A request was made to researchers, academics and all other stakeholders to share copies of relevant research work especially papers published in peer-reviewed journals during the period 2000 - 2012. The literature/ data collected were compiled under different topics.

c) Population prevalence, analytical and environmental studies

(The content of this section has been submitted for publication in a peer reviewed Medical Science Journal)

Scientific scope and objectives

The prevalence of CKDu is not known. Studies conducted up to now have either been hospital-based rather than population- based or have not used specific diagnostic criteria (1-13). A population prevalence study was conducted during 2010-2012. CKDu is endemic in the North Central Region of Sri Lanka. The role of known nephrotoxins arsenic (As), cadmium (Cd) and lead (Pb) pesticides (14-16) and other metals and pesticides that could influence the natural history of kidney disease (aluminium, copper, chromium, sodium, potassium, calcium, magnesium, copper, zinc, selenium, titanium and strontium) (17), in the pathogenesis of CKDu have not been hitherto investigated comprehensively.

This study was therefore undertaken

- To confirm or refute previously reported wide ranges in the prevalence of CKDu
- To identify the risk factors associated with CKDu
- To compare and contrast CKDu cases and controls in relation to exposure to heavy metals/ metalloids and pesticides through analysis of biological samples (urine, hair, nails, blood and tissue)
- To estimate quantitatively the potential nephrotoxins in a wide range of environmental samples including water, soil, weedicides and fertilizers obtained from endemic and non-endemic areas

Materials and Methods

Case definition of CKDu

Participants who had persistent albuminuria, i.e. albumin-creatinine Ratio (ACR) ≥30mg/g in initial urine sample and at a repeat visit were considered to have CKDu if they satisfied all the following criteria:

- No past history of ureteric calculi, glomerulonephritis, pyelonephritis or snake bite
- Not on treatment for diabetes.
- Normal HbA1c (<6.5%)
- If on treatment for raised blood pressure - BP <140/90 mmHg if not on treatment for blood pressure BP <160/100 mmHg

CKDu was graded as follow:

- Grade 1: Persistent albuminuria, (i.e. ACR ≥30mg/g in initial and repeat urine sample) and eGFR (using 4-variable MDRD (Modification of Diet in Renal Disease) equation (51)) >90ml/min/1.73m^2
- Grade 2: Persistent albuminuria and eGFR 60 - 89 ml/min/1.73m^2
- Grade 3: Persistent albuminuria and eGFR 30-59 ml/min/1.73m^2
- Grade 4: Persistent albuminuria and eGFR <30ml/min/1.73m^2

Population prevalence study

Ethical approval for the study was obtained from the Ethical Review Committee of the Sri Lanka Medical Association. Six divisional secretariat areas (administrative divisions) were selected randomly from the three districts. Twenty two villages (Grama Niladari areas) were selected randomly from the six divisions. Using the electoral lists of these villages, 100 households were randomly selected for the study. Males and females between 15-70 years (n=6698), were invited to participate. The response rate was 74 %. All participants gave written consent to join the study. Early morning urine samples of the respondents were tested. Those with ACR ≥30mg/g were invited for further biochemical studies, repeat urine ACR, HbA_1C and serum creatinine. 97% of people with ACR ≥30mg/g responded. Those who did not satisfy the CKDu definition (people with diabetes and/or hypertension and/or history of snake bite or glomerulonephritis or pyelonephritis or ureteric calculi) were excluded (n=310) in calculating the prevalence rates of CKDu.

At the first visit, trained interviewers used a survey questionnaire to gather information on age, sex, marital status, education, occupation , smoking, alcohol consumption, current residence, duration of residence in study area, source of drinking water, storage containers used for drinking water, exposure to agrochemicals, history of snake bite, glomerulonephritis, pyelonephritis and renal calculi. Height was measured to the nearest 0.1 centimeter. Weight measurements were taken using a calibrated weighing scale to the nearest 0.1 kg. A medical officer verified the medical information gathered and measured the blood pressure using a mercury sphygmomanometer after 15 minutes rest. The average of two readings taken 5 minutes apart was used.

Analytical studies

<u>As, Cd and Pb in urine, blood, hair and nails</u>

Cd, As and Pb were analyzed in urine in a randomly selected subset of CKDu cases (n= 495) and randomly selected matched controls from the endemic area as well as from a non-endemic area (n= 250).

Sodium, potassium, calcium, magnesium, copper, zinc, and titanium were analyzed in the urine in a subset of people with CKDu (n=148). Their serum was also analyzed for selenium, aluminium, strontium, and chromium. Hair and nail samples were analyzed for Cd, As and Pb in a subset of CKDu cases (n= 80) and controls from the study area (n= 48).

Urine of people with CKDu (n=57) and people from the non endemic area (n=39) were analyzed for pesticide residues (2,4-D, 2,4,5-T, 2,4,5-trichlorophenol, isopropoxyphenol, pentachlorophenol, 3,5,6-trichloropyridinol, p-nitrophenol, 1-naphthol, 2-naphthol, glyphosate, AMPA).

<u>As, Cd and Pb in water, food, tobacco, pasture, weeds, soil, fertilizer, weedicides and pesticides</u>

Arsenic, Cd, Pb were analyzed in water, food, tobacco, pasture, weeds, soil and agrochemicals obtained from both the endemic area and a non endemic area.

Water samples taken from 234 different sources were analyzed for As, Cd and Pb. They comprised 99 drinking water sources of CKDu cases(from ground wells, tube wells and natural

springs), 123 other sources of water (from ground wells, tube wells, irrigation canals, reservoirs, natural springs) from the endemic area and 12 from the non-endemic area.

Rice, pulses, vegetables including leafy vegetables, coconut, yams and roots (e.g. kohila, lotus), fresh water fish, tobacco, betel leaf, pasture and weeds were analyzed for As, Cd and Pb. Samples were obtained from endemic (n= 119) and non-endemic areas (n=32).

Soil, phosphate fertilizer, pesticides and weedicides were analyzed for As, Cd and Pb. Soil samples were obtained from paddy fields, other types of cultivations, and reservoirs in the endemic area (n=88) and non endemic area (n=41) .

As, Cd and Pb in bone, kidney and liver (post mortem tissue)

Postmortem specimens (kidney cortex, liver and bone) were obtained from 26 CKDu patients and 16 accident victims aged between 40 - 60 years from the North Central Province. Written consent was obtained from the next of kin/ a close relative for obtaining postmortem tissues (kidney cortex, liver and bone) from the deceased who satisfied the selection criteria stated earlier. Powder free gloves were used during the procedure and a plastic knife was used for soft tissue handling and dissection. A piece of kidney was cut from middle third of left kidney (1cm x 1 cm x 1 cm, weighing more than 1 gm). A piece of liver was taken from the large lobe of the liver just beneath the lower surface, measuring 1cm x 1 cm x 1 cm and weighing more than 1 gm. Bone samples were obtained from anterior end of 2nd rib without cartilage. Tissues were stored at -20^{0}C until quantitative analyses was done at an international reference laboratory (University of Antwerp).

Specimen handling and analysis

Samples were collected in uncontaminated collection vials and stored frozen (-20°C) until transfer to the laboratory. All analyses were performed in a contract laboratory (Laboratory of Pathophysiology of the University of Antwerp, Belgium) which has a Trace Element External Quality Control Scheme.

Measurements of arsenic (As), cadmium (Cd), lead (Pb) and other elements in urine, water, vegetables, agrochemicals, soil, bone and soft tissue was performed by Inductively Coupled Plasma Mass Spectrometry.

Serum analyses were performed by electrothermal atomic absorption spectrometry. Limits of detection for Al, Sr, Cr, Se were 0.1 µg/l, 0.5 µg/l, 0.01 µg/l and 1 µg/L respectively.

Determination of pesticide residues in urine of people with CKDu

Urine was shipped on dry ice and were stored at -18 C until analysis. Analysis used validated liquid chromatography with tandem mass spectrometry (LC-MS/MS), Gas Chromatography-Mass Spectroscopy (GCMS) and Gas Chromatography with tandem mass spectrometry (GC-MS/MS) methods.

Statistical analysis

Normality of data distribution was assessed with histograms. All metals data had skewed distributions. After removal of a small number of outliers log-transformations were used to

normalize the data in order to conduct statistical analyses. The mean, median, minimum and maximum are reported on original data.

Chi square test was used to determine the differences in prevalence of CKDu in relation to age and sex. T-test of log-transformed values was used to test differences in quantitative variables. Results were also confirmed by non-parametric Wilcoxon rank-sum test.

To adjust for the potential effect of dehydration, all the urine measures are defined as microgram of metals per gram of creatinine.

A multiple logistic regression model was fitted for each of the two CKDu definitions. The models incorporated characteristics of interest including age, sex, education, smoking, illicit alcohol, occupation, type of agriculture, years of agriculture, drinking water source, drinking water from paddy, exposure to fertilizer, exposure to weedicides/pesticides, water container type, using protection against agrochemicals, and months living in the district. All were entered as categorical variables except months living in the district. These data analyses were performed using Stata 11 and p-values of less than 0.05 were considered statistically significant.

Results/outputs

Population prevalence study

The overall prevalence of CKDu was 15.3% with a higher prevalence in females (16.8%) than males (13.3%) ($p < 0.05$). More severe grades of CKDu were seen more frequently in males (grade 3: males vs. females =19.9 vs. 5.3%, grade 4: males vs. females = 16.1 vs. 3.8%). In both sexes prevalence was higher with increasing age ($p < 0.05$).

Being male reduced the risk of CKDu (odds ratio 0.745, 95% CI 0.562-0.988. $p < 0.05$), and being over the mean age of 39 years increased the risk of CKDu (odds ratio 1.926, 95% CI 1.561-2.376, $p < 0.001$). When separate logistic regressions were run for each potential exposure, only occupation type (being a farmer increased odds by 19.5%) and type of agriculture (paddy cultivation compared to cultivation of vegetables and other crops decreased odds by 26.8%) were significant. Other potential exposures that were analyzed and had no statistical significance included education, smoking, illicit alcohol consumption, years of agriculture, drinking water source and type of water storage container.

As, Cd, Pb and other elements in urine

Cadmium excretion in urine was significantly higher in healthy subjects living in the endemic area (mean 0.646, median 0.18, min 0.005, max 5.13 µg/g) compared to those living in the control area (mean 0.345, median 0.265, min 0.005 , max 2.079 µg/g) ($p < 0.001$).

In subjects with CKDu, urine Cd excretion was significantly higher (mean 1.039, median 0.695, min 0.005, max 8.93 µg/g) compared to healthy subjects in the endemic area (mean 0.646, median 0.18, min 0.005, max 5.13 µg/g) ($p < 0.05$). These results indicate a higher exposure of people in the endemic area to Cd.

Arsenic excretion in urine was significantly higher in healthy subjects living in the endemic area (mean 92.443, median 36.99, min 0.2, max 966.29 µg/g) compared to those living in the control area (mean 56.572, median 42.025, min 5.38, max 350.28 µg/g) ($p < 0.001$).

However, urine As excretion in CKDu subjects was significantly lower (mean 45.447, median 26.3, min 0.4, max 616.6 µg/g) compared with urine As excretion in normal subjects in the endemic area (mean 92.443, median 36.99, min 0.2, max 966.29 µg/g) ($p < 0.01$).

There was no significant difference in urine Pb excretion between CKDu subjects (mean 1.153, median 0.95, min 0.04, max 8.53 µg/g) and healthy subjects in the endemic area (mean 1.024, median 0.84, min 0.1, max 2.25 µg/g).

As shown in the table below urine concentrations of sodium, potassium, calcium, magnesium, copper, zinc, and titanium in CKDu cases were within normal limits.

Table: Concentration of metals in urine and serum of subjects with CKDu

Metals in urine n=107, (mg/g creatinine)				
	Mean	Median	Minimum	Maximum
Sodium	4105.50	3544.00	425.00	17458.00
Potassium	917.94	800.00	243.00	2469.00
Calcium	80.45	67.00	4.00	368.00
Magnesium	79.89	80.00	2.00	169.00
Copper	13.34	11.00	3.70	91.10
Zinc	229.99	235.99	31.00	510.00
Titanium	0.26	0.24	0.03	0.88
Serum concentration (ug/l), n=171				
	Mean	Median	Minimum	Maximum
Strontium	83.17	82.00	29.00	198.00
Aluminum	4.13	3.00	1.00	12.00
Selenium	88.27	84.5	50.0	121.8
Chromium	0.118	0.06	0.01	1.15

Serum aluminium, chromium, selenium and strontitium in people with CKDu

Serum aluminium and chromium levels were within normal limits. Serum selenium levels in subjects with CKDu ranged from 50.0 - 121.8 ug/l (reference range 54-163 ug/l). Serum strontium levels were above normal limits (mean 83.17 ug/l, SD 32.15 ug/l, normal range 14-84 ug/l).

Cd and As in hair and nails

A significantly higher Cd concentration was seen in the nails of CKDu cases (mean 0.017, median 0.007, min 0.001, max 0.347 ug/g) compared to controls (mean 0.009, median 0.001, min 0.001, max 0.091 ug/g) ($p < 0.05$).

As levels in hair were significantly higher in CKDu cases (mean 0.144, median 0.139, min 0.00, max 0.452 ug/g), compared to healthy subjects from the endemic area (mean 0.125, median 0.103, min 0.006, max 1.214 ug/g) ($P < 0.05$). There was no significant difference in the As concentration in nails in CKDu subjects compared to controls.

As, Cd and Pb in bone, kidney and liver (post mortem tissue)

The concentration of As, Cd and Pb (ug/l) in kidney, liver and bone were as follows:

- Cadmium: Kidney - mean 885.54, median 152.62, range 19.28-7458.54; Liver - mean 165.39, median 117.19, range 22.22-1471.41; Bone - mean 8.68, median 4.87, range 0.82-70.66
- Arsenic: Kidney - mean 4.04, median 2.34, range0.0384-14.16; Liver - mean 5.38, median 2.42, range 0.13-26.16; Bone-mean 6.47, median 4.34, range 0.47-28.84
- Lead: Kidney - mean 0.89, median 0.6, range 0.01-2.54; Liver - mean 4.56, median 3.93, range 0.98-13.33; Bone - mean 64.04, median 47.13, range 2.11-233.92

The mean cadmium and lead contents in bone were higher than the currently reported levels in healthy subjects.

As, Cd, Pb and uranium in water

As, Cd and Pb contents of water were sampled from 234 different sources in the study area and the control area. Cd and Pb levels in drinking water sources used by CKDu subjects (n= 99) were within normal limits (<3 ug/l and < 10 ug /l respectively) (18). As was borderline or raised in four samples (9.9 ug/l, 10.2 ug/l, 10.5 ug/l, 13.4 ug/l), analysis was repeated in 32 samples taken from the four sources. Repeat results for As, were within currently stipulated normal limits (less than <10 ug/l) (18).

In water samples analysed from other sources, As levels were 22.2 ug/l and 9.8 ug/l in two samples taken from a canal and a reservoir, Cd was 3.46 ug/l in one sample from a reservoir and Pb was 12.3 ug/l in one sample from a reservoir in the endemic area. All other samples from wells, tube wells, irrigation canals, pipe-borne water, reservoirs, and natural springs including those taken from the non-endemic area had normal As, Cd and Pb levels.

As, Cd and Pb in food, tobacco, betel leaves, pasture and weeds

Content of As, Cd and Pb were analysed in food, tobacco, pasture and weeds from endemic areas. The Cd levels in rice in both endemic and non endemic areas were less than the Codex Alimentarius Commission allowable limit (19-21). The maximum concentration of Cd in vegetables in the endemic area was 0.322 mg/kg and in the non endemic area 0.063 mg/kg. Cd in some vegetables such as lotus root was high. High concentration of Cd was also seen in tobacco. Cadmium levels in lotus and tobacco were higher in endemic than in non endemic areas (Lotus: mean 0.413 vs. 0.023, median 0.066 vs. 0.023, max 1.50 vs. 0.03 and tobacco: mean 0.351 vs. 0.316, median 0.351 vs. 0.316, max 0.44 vs. 0.351 endemic vs. non endemic respectively). The maximum concentration of Cd in fish (0.06 ug/g) also exceeded the European maximum limit of 0.05 mg/Kg stipulated for certain types of fish (21). In the non endemic area the maximum concentration of Cd in fish was 0.033mg/Kg. The maximum level of Pb permitted by the Commission of European Communities in vegetables is 0.10 mg/kg (21). The maximum concentration of Pb in vegetables in the endemic area was 0.476 mg/kg.

As, Cd and Pb in soil and agrochemicals in the endemic and non-endemic areas

The concentrations (range) of As, Cd and Pb in soil, weedicides, pesticides and fertilizers in the endemic and non endemic areas are shown in the table below. In the endemic area mean and median of Cd in surface soil (n= 94) (excluding samples from reservoirs), was 1.16 and 0.41 ug/g respectively. In the non endemic mean and median of Cd in surface soil (n = 45) (excluding samples from reservoirs), was 0.49 and 0.41 ug/g respectively.

Table: Concentration (range) of arsenic, Cd and Pb in soil, phosphate fertilizer, pesticides and weedicides

Source	Field	As µg/g	Cd µg/g	Pb µg/g
		Minimum, maximum		
Endemic area	Paddy cultivation (n = 45)	0.00, 0.85	0.16, 0.56	5.03, 34.54
	Chena (shifting) cultivation (n = 20)	0.00, 0.22	0.17, 1.27	8.25, 28.33
	Vegetable plot (home) (n = 23)	0.00, 0.46	0.16, 70.00	6.69, 41.02
	Crop land (n = 6)	0.00, 0.01	0.17, 1.47	9.98, 32.1
	Reservoir (n = 6)	0.17, 0.43	0.15, 1.36	7.11, 33.49
	Phosphate fertilizer (n = 13)	0.00, 0.19	0.01, 30.79	0.17, 823.41
	Weedicides and /pesticides (n = 26)	0.01, 94.93	0.05, 9.34	0.83, 930.81
Non-endemic area	Paddy cultivation (n = 21)	0.01, 0.99	0.01, 1.61	0.02, 39.95
	Chena (shifting) cultivation (n = 10)	0.09, 1.57	0.34, 0.93	5.42, 26.1
	Vegetable plot (home) (n = 10)	0.08, 0.53	0.29,0.84	5.57, 32.87
	Crop land (n = 4)	0.09, 0.18	0.24, 0.33	3.15, 12.77
	Phosphate fertilizer (n = 5)	0.00, 1.22	0.01, 1.28	0.09, 98.52
	Weedicides and pesticides (n = 8)	0.01, 13.15	0.05, 2.0	1.01, 56.39

Pesticide residues in urine

Pesticide residues were detected in the urine in people with CKDu as well as people from the control area. A high percentage of urine samples tested had detectable levels of certain pesticide residues. In the urine of people with CKDu, the frequency of detection of 2,4-D, 3,5,6-trichloropyridinol, p-nitrophenol, 1-naphthol, 2-naphthol, glyphosate, aminomethyl phosphonic acid (AMPA) were 33%, 70%, 58%, 100%, 100%, 65%, 28% respectively). The proportions of CKDu subjects with levels above reference values for different pesticide residues were: 2,4-D, (3.5%) Pentachlorophenol (1.7%), Chlorpyrifos (10.5%), Parathion (0%), Carbaryl (10.5%), Naphthalene (10.5%) and Glyphosate (3.5%).

Discussion

The overall prevalence of CKDu (15.3%), was higher than reported previously (2% to 8.5%) (3, 5, 13). Older age, being female, being a farmer and being engaged in non-paddy cultivation increased the odds of CKDu. In previous studies a family history of chronic kidney disease, taking ayurvedic treatment, and history of snake bite were identified as significant predictors for CKDu (3, 5, 6). In the present study, family history was positive in one fifth of those with CKDu and a history of snake bite was one of the exclusion criteria.

Previous studies have reported divergent information on the role of Cd in the causation of CKDu in Sri Lanka. Some have reported high levels of Cd in water from the endemic region (7, 8, 12), while others have refuted Cd as a causative factor (13). Our findings were different. We found significantly higher urine Cd excretion in healthy people in the endemic area compared to those living in a non-endemic area. People with CKDu excreted significantly higher levels of Cd compared to healthy people both in the endemic and non-endemic areas. Cadmium is nephrotoxic and urine Cd excretion is considered to be a reliable indicator of cumulative long term exposure to cadmium (14). The mean concentration of Cd in urine in people with CKDu was higher than levels demonstrated to cause oxidative stress damage to the kidney in recent studies (22-27). These findings support the contention that chronic exposure to low levels of Cd may be playing a role in the causation of CKDu in Sri Lanka.

The concentration of arsenic in urine in people with CKDu was above levels known to cause oxidative injury to the kidney (27). In people with CKDu, and in healthy people from the endemic area, concentrations of As in urine and in fingernails were higher than those reported in people living in unexposed or low exposure environments (28, 29). Total As level in urine is associated with chronic kidney disease in a dose-response relationship especially when the level is greater than 20.74 ug/g (30). Co-exposure to As is likely to aggravate the effect of Cd on the kidney making the changes more pronounced than exposure to Cd alone (31, 32).

Selenium has been shown to protect the kidney from oxidative stress (33). A selenium concentration of 80-95 ug/l is needed to maximize activity of the antioxidant enzyme glutathione peroxidase and selenoproteins in plasma (34, 35). Serum selenium was below 80 ug/l in 38% of people with CKDu. Low selenium levels may have been a contributory factor increasing the susceptibility of the kidneys to oxidative damage caused by heavy metals and metalloids. It is not clear why the serum strontium levels in people with CKDu were raised. The association of raised serum strontium levels with raised serum Cd levels has been reported previously (36).

Cd levels have previously been reported to be high in water sources in the domestic environment of people with CKDu and 10-20 fold the maximum stipulated level in reservoirs in the endemic area (8). Our results did not show this to be the case. On the contrary, Cd levels of all water samples analysed were within normal limits except in one sample from a reservoir which had a borderline Cd level (3.45 ug/l).

Drinking water is a major pathway for entry of inorganic As into the human body. The WHO guideline for As in drinking water is 10 µg/l (6). The U.S. Environmental Protection Authority has suggested that the concentration of As in drinking water should be no more than 5 µg/l (37).

Levels of Cd and Pb in vegetables and Cd in fresh water fish from the endemic area are above the maximum levels stipulated by certain Authorities (38). Since the Cd content of certain food items in the endemic area is above stipulated levels, the total weekly intake of Cd in people living in the endemic area could exceed these safe limits with detrimental effects on renal function particularly in vulnerable people (39, 40).

Reported mean dietary exposure to inorganic As in the US and various European and Asian countries range from 0.1 to 3.0 µg/kg bw per day (39). Recently the PTWI for As (0.015 mg/kg body weight per week) was withdrawn and environmental authorities are in the process of collecting more data for exposure assessment (40). The current recommendation is that every effort be made to keep concentrations of As as low as reasonably possible.

Previous studies have reported high Cd values in fertilizer (mean 47 ug/g) (8). The maximum Cd, Pb and As values in phosphate fertilizer from the endemic area in the present study were 30.8 ug/g, 823.4 ug/g and 0.19 ug/g respectively. The maximum acceptable level for As, Cd and Pb in phosphate fertilizer product at 1% of the nutrient level is 2, 4 and 20 parts per million (41). The mean Cd concentration of soil from the endemic area was 0.4 ug/g and is higher than the levels reported in agricultural soils in certain developed countries (42, 43).

The concentration of Cd , As and Pb in the soil and their impact on body burden and excretion is known to be influenced by many environmental factors such as the pH of soil, ability of soil to preserve and supply soil fertilizer, buffering capacity, content of soil organic matter and water quality, among others (43-46). The hardness and high content of fluoride in water in the endemic area may also influence the dynamics of Cd in soil (10, 47).

One or more pesticides residues were above reference levels in 31.6% of people with CKDu. Residues are demonstrative of the extent of the environmental distribution of pesticides and certain pesticides are nephrotoxic (48, 49). Simultaneous exposure to nephrotoxic pesticides may be contributing to the progression of the disease in people with CKDu.

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