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Chronic kidney disease of unknown aetiology in Sri Lanka; a journey to unravel the mystery

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Introduction

Chronic kidney disease of unknown aetiology (CKDu) is a major health problem in the North Central Region of Sri Lanka. First reported in the early 1990s, the disease mainly affects vulnerable populations from agricultural communities and results in many psycho-social, economic and cultural issues. The disease burden is most pronounced in the North Central Province and has extended to the adjacent provinces over the last two decades.[1] Currently, there are over 20,000 patients under treatment with a rise in the need for dialysis and kidney transplantation. Chronic kidney disease (CKD) imposes a high economic burden on the country's health budget, 4.6% of the Annual Health Budget in 2010 being spent on managing CKD patients.[2]

Geographic CKDu "hot spots" have appeared in several other countries, including El Salvador, Guatemala, Nicaragua and India. It had been debated whether CKDu is a new disease entity since no association was found with conventional risk factors of CKD. In 2004, our research team embarked on a journey to unravel this mysterious disease. Hospital-based studies were carried out to identify the disease profile, progression and risk factors. Community-based studies helped in documenting the disease prevalence and further evaluation of the risk factors.[3] Based on our findings, we postulated that environmental toxins affecting vulnerable populations in a specific geographical terrain as the likely cause of CKDu.[4] Further studies evaluated microbial toxins such as ochratoxins, pesticides and heavy metals as potential causes of kidney damage. Our current studies are focused on cyanobacterial toxins and water quality of hardness and fluoride. The usefulness of novel urinary biomarkers in the early diagnosis of CKDu is another area of research carried out by our research team. This oration summarizes our research work on the subject of CKDu methodically and sequentially.

Epidemiology of CKDu

In the year 2006, a community-based study was carried out in Medawachchiya, Padaviya and Rajanganaya to determine the prevalence of CKD in the North Central Province (NCP) of Sri Lanka. Urinary microalbumin (MA) was evaluated in 425 women and 461 men.[3] Sulphosalicylic acid and the Light Dependent Resister microalbumin gel filtration method was used for initial screening for microalbuminuria (MA) and reconfirmed by the Micral strip test. The prevalence of MA was 6.3% in women and 8.6% in men.[3] The majority, 82% of men and 71% of women were involved in agriculture.

Athuraliya et al in 2003 reported a CKDu prevalence of 3.9% in Medawachchiya (n=4107) and 3.7% in Girandurukotte (n=233) using urinary albumin as the screening test.[5] The WHO study in 2011 reported prevalence figures of 15.6% in Anuradhapura, 20.6% in Medirigiriya and 22.9% in Giradurukotte.[6] The WHO study used urine albumin to creatinine ratio and serum creatinine as diagnostic tests. These data indicate how the disease prevalence has risen over the years. However, in addition to a real increase in the prevalence, the methodology used (UAlb Vs MA), and the area of sampling also had an impact on the difference in the prevalence figures.

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Disease profile

Our hospital-based study at Anuradhapura hospital revealed that CKDu had a male preponderance (F: M 1:1.3), patients were at a relatively younger age of 35-60 years (54.22±12.50) and the majority were farmers by occupation (M 85%, F 71.1%).[4] They were asymptomatic in early stages; oedema and hypertension being less common and appearing late. Urinary sediments were bland and there were low levels of proteinuria, mainly tubular proteins.[5] Histopathology of renal biopsies were reported as a chronic tubulo-interstitial disease.[7]

Socio-demographic factors were evaluated in a recent study carried out in a community-based study in the endemic region of Padaviya.[8] Of CKDu and controls, 60% and 53% were men and the mean age was 61±11 years and 46±8 years, respectively. They were full-time farmers (55% and 44% respectively) for most of their adult years (32±7). Family income was below LKR 10,000/month (USD 56) in 58% of CKDu patients and 20% of controls. With regards to the level of education of CKDu patients, 15% studied up to Ordinary Level, 6% up to Advanced Level, 68% received only primary education, while 11% patients never attended school. It was noted that 45% of CKDu patients do not attend followup clinics.

It was reported that 90% of CKDu patients had consumed water from dug wells and tube-wells for a period of over ten years. After being diagnosed with kidney disease, 72% of people changed their drinking water to reverse-osmosis (RO) water and pipe-borne water. Average water consumption per day was 3.0±0.54 L. Agrochemicals were used by 90% of farmers several times per year without wearing personal protective equipment.

These studies highlighted the socio-demographic patterns associated with CKDu patients in Sri Lanka. Additional studies are needed to identify whether changing certain practices, such as changing the water source and use of filtered water, could reduce the prevalence of CKDu.

Aetiology

To find out the risk factors of the disease, a case-control study was carried out in 2007, comparing 183 patients with CRF of unknown aetiology at the renal clinic, Teaching Hospital Anuradhapura with a control group from the general medical clinic of the same hospital.[4] Several risk factors were evaluated. Being a farmer (p<0.001), using pesticides (<0.001), drinking well water at home (p<0.001) and in the field (p=0.036), having a family member with renal dysfunction (p=0.001), having taken ayurvedic treatment in the past (p<0.001) and a history of snakebite (p<0.001) were risk factors of CRF of unknown aetiology. In a multivariate logistic regression analysis, significant predictors of CRF of unknown aetiology included, having a family member with a history of chronic renal dysfunction, a history of having taken ayurvedic treatment and having had a snake bite in the past. *(Current definition of CKDu excludes patients with a history of bite.)* Subjects with a family member with renal dysfunction were 4.5 times more likely to have CRF of unknown aetiology compared with those without such a family history.

Considering the regional clustering of disease, risk factor profile and family history we postulated that the disease is triggered by an environmental factor in those who are genetically predisposed.

Environment factors and CKDu

CKDu may reflect a complex interplay between the environment and lifestyle. NCP was the seat of Sri Lanka's ancient kingdoms and the centre of civilization and Buddhism. The province extends over 10,530 m^3 in the dry zone of the country and has a sophisticated irrigation system of water storage reservoirs which was a major achievement of the Anuradhapura Kingdom. Despite its unique history and culture, NCP has been a fertile ground for infectious diseases such as malaria and Japanese encephalitis in the past. CKD is a major health problem in the province at present. The climate of the region is semi-arid and communities and the entire echo system are adapted to this. In ancient villages people lived for many centuries whereas new settlements related to main irrigation schemes were created since the 1930s.

During the last 50 years, several noticeable changes took place in the lifestyle of people who live in this region. In the ancient villages, people depended on surface water from reservoirs for drinking and other domestic purposes. As the population grew, they started using dug wells (deep and shallow) to meet their requirements. The green revolution is an agricultural package introduced in mid-1960s to become self-sufficient in rice production. Under this scheme, fertilizer subsidy program was introduced in 1962. High yielding rice varieties were introduced and traditional methods of farming were replaced by the use of agrochemicals and technology.

After considering factors specific to Sri Lanka and having reviewed the world literature on the topic, the following risk factors were considered for further research studies.

a) Drinking water
b) Pesticides
c) Cadmium and arsenic as a result of heavy use of agrochemicals
d) Plant toxins such as ochratoxins (association with Balkan endemic nephropathy) and cyanobacterial toxins

a) Pesticides and CKDu

A descriptive cross-sectional study was carried out in 2006 to determine the potential association between chronic renal failure (CRF) and low-level organophosphate pesticide exposure.[9] Red cell (RBC) acetylcholinesterase (AchE) was used as a surrogate marker of exposure. RBC AchE levels in farmers exposed to pesticides were significantly lower than in unexposed controls (p<0.05). Among CRF patients, RBC AchE levels were lower in the exposed, than in the unexposed (p<0.05). In conclusion, this study suggests a possible association between CRF of unknown aetiology and organophosphate exposure. However, long term effects of low-level exposure to OPs needs further investigations.

Added evidence pointing to nephrotoxic pesticide exposure in CKDu was provided by Jayatilake et al in the CKDu National Research Project Team study.[6] They reported that 10.5% of CKDu patients had chlorpyriphos, carbaryl and naphthalene above the reference limits in urine samples. However, they did not report comparative results among the controls. The studies available thus far do not provide conclusive evidence on the relationship between the use of pesticides and indicate the need for a more robust research agenda.

b) Heavy metals and CKDu

Cadmium (Cd), lead (Pb) and uranium (U) exposure are potential risk factors for decreased renal function. Inorganic arsenic (As) has a well-known toxicity profile, but no firm evidence has been established in CKDu aetiology. Cd has been implicated as a causal factor contributing to CKDu, the source of contamination being triple superphosphate (TSP).[10] Levels measured in soils and rice samples by one of the research groups have raised concerns[10], but further studies have questioned the veracity of these results. Findings of two of our studies that examined the association of heavy metals and CKDu is highlighted below.

Study 1

Randomly selected 37 CKDu patients attending a renal clinic in an endemic area in the North Central Province and two control groups namely a farmer group (n=39) and a non-farmer group (n=40) from a non-endemic area were included in the study. Urine samples were analyzed for heavy metals and five biomarkers at the Harvard School of Public Health Trace Metals Analysis Laboratory (Boston, USA).[11]

Urinary cadmium level in CKDu patients was lower than the farmer control and non farmer control groups [Mean (SD) 0.68 (0.39), 1.32 (1.27), 0.54 (0.31), respectively]. However, the farmer control group had a significantly higher urinary cadmium level (p<0.001) than the other two groups. Urinary arsenic levels were similar in all three groups of subjects. Urinary manganese and mercury levels in CKDu patients and in the control farmer group were significantly higher than that in non-farmer controls. KIM-1 and beta 2 microglobulin (biomarker associated with Cd toxicity) were not correlated with urinary Cd levels. It was concluded that there was no significant association of urinary Cd, As and CKDu in the patients participated in the study.

Study 2

The objective of this investigation was to determine the concentration of a suite of heavy metals and trace elements in biological media (blood and hair) collected from CKDu patients (n=39) and healthy subjects (n=34) along with environmental media (water, rice, soil and fish) collected from Medawachchiya and Medirigiriya of the NCP.[12] Broad panel approach was employed to generate data for multivariate statistical analyses, intending to identify potential risk factors associated with CKDu. Samples were analyzed by inductively coupled plasma mass spectrometry (ICP-MS, Thermo X-Series 2) at the Research Triangle Institute International, North Carolina, USA.

Blood – Cd and Pb concentrations in blood exceeded mean US reference values from healthy nonsmokers[13] for 68.7 and 89.2% of the samples, respectively.

Hair – Hg levels in hair exceeded the US mean reference values for women of childbearing age[14] in 66.1% of the samples.

Rice – The maximum measured Cd rice concentration was below the Codex Alimentarius Commission reference level[15] and the maximum allowable concentration (MAC) for Chinese rice[16].

Fish – Observed elemental content in freshwater fish samples collected from CKDu endemic areas were similar to or lower than other recent literature reports.[6].

Soil – As, Cr, Cu, Fe, Hg, Mn, Ni, Pb, and Se concentrations exceeded mean background US soil concentrations. No study samples exceeded maximum background US soil concentrations.

This study identified that specific constituents may be present above levels of concern, but does not compare against specific kidney toxicity values or cumulative risk related to a multi-factorial disease process. However, given the importance of rice in the Sri Lankan diet, it is possible that chronic exposure to Cd below available reference levels could act as an environmental nephrotoxin, especially if other nephrotoxins or additional CKDu risk factors are present.

c) Drinking water and CKDu

In our community-based study[3] we found that approximately 90% of individuals consumed water from dug wells and 75% consumed water from wells situated in paddy fields during the working hours. The subjects who drank well water were approximately 2.5 times more likely to have microalbuminuria than those who consumed water from other sources. A low CKDu prevalence was noted in communities where the water source is either from surface water or natural springs. CKDu was not reported from Anuradhapura urban area which receives water from Tissawewa supplemented with water diverted from the Mahaweli river. Similarly, no cases have been reported among the few villages of Kebithigollawa DSD in the endemic area where domestic water supply is natural springs.[17]

In the previous study [Study 2][12], we analyzed drinking water samples (n=93) for heavy metals and other constituents. Arsenic, cadmium and mercury were within the accepted limits of the World Health Organization (WHO2011) International drinking water standards.[18] Fe, Mn, Na, and Pb exceeded applicable drinking water standards in some samples. Fluoride levels were significantly high in most samples. A large number of samples (n=73) had hard or very hard water, as calculated from calcium and magnesium levels.[12]

Our findings regarding heavy metals in drinking water are consistent with available results of other studies.[19] The endemic CKDu area is also known to overlap with a hydrogeochemical region that contains elevated ground F levels and hard water. Overall, data collected from drinking water samples reflected the unique hydro-geochemistry of the region, including elevated levels of several elements (e.g., F and Fe) and the prevalence of hard or very hard water. Chandrajith et al argued that the cytotoxicity effect of fluoride appears to be the effect of Na^{2+} and Ca^{+} ratio of the ingested water on the F^{-} metabolism.[20] Therefore, fluoride can be considered as a significant risk factor in the aetiology of CKDu. Literature survey revealed few reports where fluoride is related to kidney disease[21,22] indicating the need for more research studies.

d) Plant toxins and CKDu

Ochratoxin A: At the early stages of our research, we considered Ochratoxin A (OA) as a potential cause for CKDu as it was under consideration for Balkan endemic nephropathy at the time. OA is a mycotoxin which has mutagenic, oncogenic and nephrotoxic properties. We tested A total of 98 samples of rice, cereals and legumes from Medawachchiya, Padaviya and Rajanganaya were tested for OA by solid-phase direct competitive enzyme immunoassay technique using Myco Monitor Ochratoxin A ELISA assay kit (Helica Biosystems Inc., Fullerton, CA, USA)[23]. OA concentration in samples of rice and cereals from the endemic area varied from 0.3-3.2 μg/Kg but levels were below the statutory maximum limit recommended by the FAO/WHO. These findings were confirmed in another study which reported mycotoxin levels in urine samples were comparable in CKDu patients and non-affected relatives.[24]

Cyanobacterial toxins: Global occurrence of cyanobacterial blooms in aquatic systems has increased over recent decades. Microcystins (MCs) and Cylindrospermopsin (CYN) are the commonest cyanotoxins. These toxins are ingested via water and food items and lead to hepatic and renal toxicity. WHO established a guideline for MC-LR in drinking water as 1 μg/l and CYN in drinking water as 2 μg/l.[25] Cyanobacterial blooms are frequently seen in reservoirs in the endemic region and the growth is thought to be facilitated by high phosphate levels in the water.

Our most recent study identified cyanobacteria in 75% of freshwater bodies tested in endemic areas compared to 40% of freshwater bodies in non-endemic areas.[26] The toxin-producing cyanobacteria identified in the majority of water sources (dug wells and surface water) were *Microcytis aeruginosa, Cylindrospermopsis* sp. and *Anabaena* sp. The cell density of cyanobacteria in well water significantly correlated with cyanotoxins levels. The detected cyanotoxin levels in well water were Cylindrospermopsin (CYN) (1.0±0.01 to 5.3±0.02 μgL^{-1}) and Microcystin-LR (MC-LR) (1.0±0.01 to 7.6 ± 0.34 μgL^{-1})] indicating that humans are exposed to cyanotoxins. This was confirmed by detection of high concentration of CYN (1000±10 to 86×10^2 ± 260 μgL^{-1}) and MC-LR (200 ± 10 to 24×10^2± 120 μgL^{-1}) in urine samples from 248 CKDu patients in the North Central Province, compared to control samples (n=72) from Agunukolapallassa, a non-endemic area in the Southern Province.[27] Further research includes the development of rat model and cell culture studies to investigate the effects of cyanotoxins on kidneys.

Early diagnosis of CKDu

Disease onset of CKDu is often silent and patients present at late stages requiring renal replacement therapy. There are limitations in using conventional diagnostic tools such as serum creatinine and urinary albumin in the early diagnosis due to the tubular-interstitial nature of the CKDu. Therefore, a new interest is focused on novel biomarkers of kidney injury.

We found that albuminuria was detected in only 62.1% of CKDu patients (ACR > 30 mg/g creatinine). Only 32.4% had albumin-creatinine ratio (ACR) above 300 mg/g creatinine, the level which can be detected by the albustix method.[11]. CKDu patients had significantly elevated urinary levels of fibrinogen ($p<0.001$), clusterin ($p<0.001$), cystatin C ($p<0.001$) and beta-2 microglobulin (nine $p<0.001$) compared to the control groups. Kidney injury molecule-1(KIM 1) was of borderline significance (780.8 ng/mg creatinine $p=0.079$). The comparison of ROC of biomarker levels revealed that Fibrinogen and β2 microglobulin has the potential of being candidates for use as a screening tool for detection of CKDu.[11] Their usefulness needs to be further validated in patients with early CKDu.

The diagnosis of kidney injury in high prevalence areas of developing countries can be very challenging due to the lack of highly sensitive and specific techniques that can be applied in point-of-care settings. A new technique of ‘micro-urine nano-particle detection (μUNPD), using urine samples of CKDu patients from NCP of Sri Lanka was developed in collaboration with the Harvard Medical School to address this issue.[28] This technique utilizes an automated on-chip assay followed by detection with a handheld device for the read-out. This μUNPD technology allows the detection of trace amounts of molecular markers in urine. KIM-1 and Cystatin C were detected down to concentrations of 0.1 ng/ml and 20 ng/ml respectively, which meet the cut-off range required to identify patients with acute or chronic kidney injury. Thus, the μUNPD technology provides a point of care device that has potential for applications in diagnosing kidney injury with high sensitivity in resource-limited settings.

Conclusions

Today, we have a better understanding of the burden, disease profile, geographical distribution and multi-factorial nature of CKDu. We have been able to exclude the role of some factors such as arsenic in the aetiology of the disease. Our results did not find any evidence for cadmium as a causative factor for CKDu. The evidence thus far favors a connection between groundwater, either the quality or some other constituent in water, with CKDu. Fluoride and its interaction with the hardness of water is a concern and need further investigations. Human exposure to toxins produced by freshwater cyanobacteria in the endemic areas is a recent finding and may play a role in aetiology and/or progression of the disease.

Ethical approval

Ethical approval for all the studies presented above has been obtained from the ERC of Faculty of Medical Sciences, USJP.

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Conflicts of interest

None.

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