

To the Editors:

Authors response: Ingestion of dug well water from an area with high prevalence of chronic kidney disease of unknown aetiology (CKDu) and development of kidney and liver lesions in rats – Toxicological viewpoint

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Justification for the selection of water sources for the study

Questionnaire based study was performed in two Medical Officer of Health (MOH) areas in North Central Province (NCP) namely, Dimbulagala and Medirigiriya in order to select suitable water sources. Previous investigations carried out by health authorities using dipstick method revealed that high microalbuminuria and these areas were identified as CKDu prevalent. Other causative factors such as diabetes mellitus, high blood pressure etc. were excluded in these investigations. Each MOH area comprised of Public Health Inspectors areas of New Town Medirigiriya (NTM) and Bisobandaragama (BB) from Medirigiriya and Divuldamana (DD) from Dimbulagala. People were questioned in order to identify whether they have CKDu patients in their families identified by health authorities, their regular water source, depth of their wells and number of years of consumption of water and boiled and unboiled water and nature of consumed water. One hundred and thirty nine families were recruited to the study and according to the questionnaire based study, 23 families were identified as affected and of them, 9 families were from New Town Medirigiriya and 9 families from Bisobandaragama Medirigiriya whilst 5 families were from Divuldamana, Dimbulagala [1]. Wells were selected considering number of CKDu patients in the locality or close proximity to the locality in the selected villages [2]. According to questionnaire based study there was a significant association between number of years of consumption of water and development of CKDu [2]. Water from Colombo was used as a control from a low CKDu disease prevalence area based on scientific evidence. A previous study by Gunaratne *et al* among

131 patients (2.5:1 male to female 2.5:1) with a mean age of 47.8±13.7 years in the National Hospital of Colombo, Sri Lanka revealed that common causes for CKD are diabetic nephropathy (n=37, 30.6%), hypertension (n=16, 13.2%), glomerular nephritis (n=12, 9.9%) and obstructive uropathy (n=10, 8.3%). The aetiology could not be identified in 25.6% of CKD patients. In this study group 50% of the patients were from the Western Province of Sri Lanka. The leading cause of CKD in patients from the Western Province of Sri Lanka was diabetic nephropathy (n=26, 37.7%). But aetiology of CKD was unknown in majority of the patients (n=14, 27.4%) from other provinces [3]. Though this difference was not statistically significant the authors hypothesized that water from Colombo was a good control to represent low endemic areas for CKDu. Not all but some suspected causative factors such as fluoride, Fe, calcium, cadmium and arsenic were analyzed in selected wells from high disease prevalent areas as well as in Colombo and summarized in the table provided with the original manuscript (Table 1, page 23).

Confounding variables from rat feed

We gave the laboratory rats a standard feed formula which has been given to Wistar rats at the Medical Research Institute, Colombo for more than 25 years. It was prepared using locally available ingredients and the authors assumed that dietary variations were equally distributed among groups. Rat formula was not analysed for suspected causative factors due to the high costs involved. Analysis of feed samples may not be useful as some of the raw ingredients are from Sri Lanka and others are imported from India. Authors have no control over

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raw ingredients available in the market. As our main objective was to investigate water as a possible causative factor detailed investigation of feed was not considered.

Not analysing cumulative toxic effect from different modes of exposure

This is a case control animal experiment and not a toxicity study. In toxicity studies animals are subjected to same toxic agents using different routes such as oral, nasal, skin, eye to evaluate the toxic effects when exposed to the same toxin via different routes. OECD, WHO or any other suitable guideline are used to conduct toxicity studies according to international standards. In a case control animal experiment there is no such requirement to expose rats using different routes.

Pre-existing hyperglycemia in Wistar rats

Queries were raised whether rats been used for this experiment are free from hyperglycaemia. This is not the first instance that these rats had been used for experimental purposes. Rats originating from this colony at the Medical Research Institute, Colombo have been used for many hypoglycaemic/hyperglycemic research for more than 25 years and their baseline blood glucose level have been analyzed and published in local and international peer reviewed journals. Though we have not analyzed their baseline blood glucose levels, their creatinine, AST and ALT levels were analyzed and are given in the manuscript. They were within the normal range of Wistar rats.

Evaluating similar biomarkers in human beings

Evaluating similar biomarkers in humans can lead to many issues. Feasibility of that kind of large scale study is questionable and it is difficult to convince ethics committees without having any pre existing evidences of liver lesions in human beings to analyze liver markers in the population. It would have been better if we were able to study the kidney markers in the study population despite of high cost involved.

Regarding detailed analysis of water samples

Regarding this statement on water analysis "It should be noted that while a large number of parameters can be measured in assessing water quality, the article provides analysis of only five elements". No reputed laboratories which were able to do detailed investigations applied when quotations were called for water analysis. Only the Industrial Technology Institute was able to provide a quotation. Because of the costs involved only the highly suspected agents were investigated. Authors are unaware about a reputed laboratory which can analyze pesticide residues.

Not analysing the quantity of food and water

Food and water quantities given to Wistar rats were measured throughout the experimental period and as there was no significant difference observed in test groups and control group it was not incorporated to the study as an important finding.

The correspondence states "authors suggest that small animals tend to eliminate metabolites more rapidly than humans". This was based on reference number 19 by Graham *et al* in the original manuscript.

Histopathological lesions

To explain the statement about the main lesions observed in Wistar rats, it is necessary to read the page number 22 of the results section in the original manuscript. Tubular lesions were statistically significant in all three experimental groups whilst glomerular lesions were statistically significant only in rats that ingested water from Divuldamana. All the slides were interpreted by three pathologists; one is a qualified veterinary pathologist from Camdon, Australia and others were two medical pathologist from the Medical Research Institute. Though species specific variations were observed, clear peritubular and periglomerular lesions were observed in experimental groups. Not only in diabetes, even in advanced CKDu, in addition to tubular lesions, glomerular lesions can also be observed as a prominent feature. It was reported by Senevirathne *et al* that CKDu patients are identified with interstitial fibrosis, interstitial inflammation, glomerular sclerosis and tubular atrophy [4].

Statement about hepatocellular carcinoma, hepatitis and adenoma formation and the query about whether the detected toxic agent has a cocarcinogenic effect on Wistar rats is explained by reference number 18 in the original manuscript.

Statement of not observing a significant difference in creatinine and Cystatine C after eight months is explained by published data. According to Wyne *et al* serum creatinine was reported as a less sensitive markers for kidney disease and it does not warrant early diagnosis [5]. Cystatine C concentration is totally depends on GFR it is now not considered as a good marker for kidney disease[5].

Possible underlying infections in experimental animals are ruled out by regular microbiological monitoring carried out in the Wistar rat colony at MRI. [6]. Though there is a possibility that environmental toxins can make similar changes in experimental animals, it is not possible to examine more than one variable at a time.

We have thus provided evidence which reconfirms the conclusion drawn in the original manuscript. This experiment did not violate ethics of animal experimentation.

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