Author's response to reviews

Title: Chronic Kidney Disease of uncertain aetiology; prevalence and causative factors in a developing country

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Author's response to reviews:

The comments made by reviewers have been addressed as follows

Reviewer 1:
1. A lot of literature has been already published in the past on the topic of this peculiar chronic renal disease in Sri Lanka. Thus in the background of the manuscript the Authors should briefly mention the state-of-the art about prevalence and etiology of the disease.
A paragraph has been added to the background section on work published already.

2. Criteria for case definition of chronic kidney disease of uncertain etiology (CKDu) as well as the paragraph about how this population prevalence study was conducted are now reported in the Background Section. They should be more appropriate for Methods and should be moved in this section of the manuscript.
Criteria for case definition have been moved to the methodology section.

3. To grade CKDu, beside albuminuria they have considered estimated GFR (eGFR) using the 4-variable MDRD. They may be aware of the new CKD-EPI equation to estimate GFR, that apparently provides better performance than MDRD, especially for CKD stage 1 and 2 and eventually a more suitable classification of screened population. Thus, the Authors should analyze their data according to the CKD-EPI formula. Moreover, the term “grade” for CKD should be changed into “stage”.

4. Body weight was measured using a calibrated weighing scale to the nearest
0.1 kg. More information about weight measurement are required, including whether the procedure was performed with or without garments.

This information has been added under methods in subsection population prevalence study line 15.

5. A key element of the study is to dissect possible etiologies for CKDu. To this purpose they have planned to analyze cadmium, arsenic, and lead in urine, blood, hair, and nails. It is unclear, however, why this analysis was not performed in all 733 individuals diagnosed with CKDu in the three districts in the endemic area. For example, as for urine analysis, the evaluation was performed only in randomly selected 495 cases. Does this shortcoming contribute to less robust conclusion?

In order to ensure accuracy of results all heavy metal assays were done outside Sri Lanka in a reputed laboratory. This increased the cost of the study a great deal as samples had to be shipped and also because the cost of analytical tests were quite high. Hence a decision was made to take a random sample of the CKDu cases for heavy metal analysis.

6. Results are intriguing, reporting in page 9 higher prevalence of CKDu in females than males, with the latter accounting for higher rate of progression to CKD stage 3-

4. This is an interesting observation that needs to be adequately addressed in the Discussion session, by providing a suitable explanation for this discrepancy, and comparing the findings with those previously published in the literature.

A possible explanation for this discrepancy is provided in the discussion section –paragraph one, lines 3-7

7. On the basis of their findings the Author hypothesize that chronic exposure to low levels of cadmium as well as low serum selenium, a metal that protect the kidney from oxidative stress, may be causative factors of CKDu in Sri Lanka. However, by ROC analysis they should define the threshold of urinary cadmium concentration and serum selenium levels that anticipate higher risk CKDu in the study population.

ROC analysis has been done for cadmium and selenium as well as for lead and arsenic (figure 3). The thresholds of urinary cadmium and serum selenium and the respective sensitivity and specificity levels are given in the Results section in subsection Arsenic, cadmium and lead and other elements in urine, lines 5-10 and in subsection Serum aluminum, chromium, selenium and strontium lines 7-8.

Reviewer 2:
1. Change study objectives to text rather than bullet points
This has been done in paragraph 6 of the background section.

2. Move inclusion criteria to methods
Inclusion criteria have been moved to the first paragraph of the methods section.

3. Better explain (in the introduction) the focus on environmental exposures. Second paragraph of the background section explains environmental exposures.

4. The definition of CKD that was selected requires albuminuria. Why were people with low GFR but no albuminuria considered to be free of CKD? For venepuncture people have to travel a long distance to health facilities with physicians. For logistic reasons as well as to contain the costs, creatinine levels were done only in people who had raised ACR in two visits. People with low GFR but no albuminuria were excluded due to this design of the study. This is mentioned under the limitations section of the paper.

5. The definition of CKD lumps together “grade 1” (albuminuria only; less clinically relevant) with “grade 4” (albuminuria+very low GFR; very severe). A sensitivity analysis would be helpful, focusing on those with more severe disease such as grade 3 and 4. We now have provided more results divided into the different grades which should be enough to see if there are large differences between grades 1&2 and 3&4.

6. The authors speculate that raised serum strontium levels are due to tubular dysfunction. Alternatively, might it be due to strontium in the food/water? We did not analyse strontium in the food and water. This is now mentioned in the discussion paragraph 7.

7. Table 1 should include more information on blood pressure (measured values) and its treatment. Information on baseline history of vascular disease would also be useful. Information on blood pressure, treatment, baseline history of vascular disease has been added to the Background section paragraph 6 lines 6-11.

8. Table 2: I did not understand how the variable "agriculture type" was defined for non-farmers. Agriculture type has been categorized to those who cultivate paddy and those who cultivate vegetables and other crops in chenas (slash and burn cultivation).

9. Tables 5 and 6 are hard to read, and might be better replaced by box plots. Tables 5 and 6 are replaced with box plots (figures 3 and 4).

10. There are a lot of tables and figures. Perhaps some more of them could be moved to e-appendices -- especially for the analyses that did not demonstrate an association between the putative exposure and CKD. Current version has 5 tables and 5 figures. Another table has been moved to the e appendix.
The major comment concerns interpretation: it seems clear that the patients ingest more heavy metals than is advisable. However, the link between heavy metal ingestion/body burden and CKD in the present study is less clear. This is especially important because other potential causes have not been completely ruled out. For example, what about traditional/herbal medicines, over-the-counter analgesic use, or other contaminants of the food supply (other than heavy metals)? Infections (either incompletely resolved acute infections, or chronic infections such as tuberculosis) are another possibility, as are previously undescribed hereditary kidney disease.

Discussion and conclusion sections have been rewritten to make the interpretation clear. Only a few CKDu cases gave a history of long term use of herbal medicines (0.4%) or analgesics (0.6%). Both these therefore are unlikely to play an important role in CKDu. Other contaminants of food such as mycotoxins have been investigated in previous studies and were not positive. Hereditary kidney disease is unlikely to be the underlying cause as kidney disease has not been reported as a health issue in populations who have lived in these regions for decades. People with history of kidney diseases including chronic pyelonephritis, hydronephrosis, renal calculi, nephrotic syndrome, etc were exclusion criteria in the case definition. Ongoing studies nearing completion on past infection with leptospirosis show that it is not a significant risk factor of CKDu.

Additional comments

Could authors test if there is a dose-effect relationship between CKDu stage and level of heavy metal or cumulative exposure risk/protective factor after adjustment on age and sex?

Dose effect relationship between heavy metals and CKDu stages has been added.

Could authors explain the significant association between female and higher risk of CKDu while male is a classical risk factor of CKD and the lower risk associated with paddy agriculture?

A possible explanation is provided in the discussion. The lower risk associated with paddy cultivation and high risk associated with chena cultivation may be related to the type of pesticides used. Heavy pesticide use is a common feature in vegetable cultivations. The present study has not looked into the exact type of pesticides used in the two categories and contamination of vegetables and rice with pesticide residues.