Author’s response to reviews

Title: Is the staple diet eaten in Medawachchiya, Sri Lanka, a predisposing factor in the development of chronic kidney disease of unknown etiology? - A comparison based on urinary beta2-microglobulin measurements

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Author’s response to reviews: see over
E A R I E Siriwardhana  
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26/05/2014  

Editor,  
BMC Nephrology,  

Dear Sir,  

Title: Is the staple diet eaten in Medawachchiya, Sri Lanka, a predisposing factor in the development of chronic kidney disease of unknown etiology? – An experience from urinary β₂-microglobulin measurement  

I am much pleased to submit this revised manuscript titled “Is the staple diet eaten in Medawachchiya, Sri Lanka, a predisposing factor in the development of chronic kidney disease of unknown etiology? – A comparison based on urinary β₂-microglobulin measurements” to BMC Nephrology, on behalf of all the co-authors. We are much thankful to the editorial committee for extending the deadline for submitting the revised manuscript till 26th May 2014. We have answered all the queries raised by the reviewers and herewith submit the revised manuscript and the revised manuscript with track changes. The responses to the queries raised by reviewers are mentioned below. Please inform me on any additional procedures I should follow.  

I look forward for a favorable respond from you.  

Thanking You,  

Sincerely,  

Ms. E A R I E Siriwardhana
Responses to reviewers’ comments

Reviewer 1: Jennifer Hoponick Hoponick Redmon

MINOR ESSENTIAL REVISIONS

1. Major parts of the discussion are written similar to a results section (e.g. page 13 and 14). These paragraphs should be moved to the results section instead and a more comprehensive discussion should take place in the discussion section that discusses the overall implications of the study results, data gaps, and future recommendations.

   As suggested by the reviewer, the contents of page 13 and 14 which consist of parts similar to the discussion section were moved into the results part. The discussion was made more comprehensive by adding overall implications of the study results, data gaps and future recommendations.

2. For the article to be used in risk assessment, amounts of food eaten should be added to the study if they were collected. It is especially difficult to estimate what amount of "accompaniments" the subjects may have eaten based on the data tables shown. Furthermore, detailing how the consumption bins were developed in greater detail would allow others to more easily use the study findings.

   The current study consisted of 24 hour dietary recalls that were performed on three occasions per individual and in that case only a qualitative data collection was done. The subjects of both groups in the dietary study were not inward patients and therefore doing a quantitative assessment of food intake was not feasible. The Sri Lankan staple diet is rice based mixed diet which generally includes a major portion of rice and small portions of few accompaniments. This constraint has been discussed under the limitations of the dietary study. This information was included in the manuscript.

3. The first sentence in the background section is written as a statement but is instead a hypothesis and should be modified in structure or changed to say that some studies suggest that metals may be a potential contributor to CKDu.

   The first sentence in the background section was modified.

4. "Normal" subjects should be defined in the abstract section as well and further clarification should be noted on p. 5 whether the subjects were deemed healthy or whether they just were tested to not have CKDu. If the latter is the case, perhaps
stating non-CKDu subjects is more apt than referring to them as "normal" or healthy. Furthermore, the authors should specify whether other CKD risk factors (and CKDu exclusion factors) were considered for the "normal" subjects such as snake bite.

The subject group referred to as normal subjects was selected subsequent to using all the indices and exclusion criteria used for CKDu patients. Further subjects with any other visible or diagnosed illnesses were excluded from the study. However as suggested by the reviewer, it is felt that the term “non-CKDu subjects” is more appropriate than “normal subjects” and the manuscript was revised accordingly.

5. ELISA should be spelled out as the first reference on p. 3.
Corrected.

6. In p. 3 methods section, language correction is needed in the last sentence where "frequency consumption of foods of animal origin" is written. Language correction also needed in abstract conclusion sentence where "CKDu affected from" is written. Modify last sentence of introduction. Small language corrections needed throughout. Can provide scanned hard copy markup if requested.

Suggested changes were made.

7. Add fish consumption frequency number in abstract results section

Fish consumption frequency numbers are added to the abstract section.

8. P. 11 notes that rice consumption is higher among "normal subjects" thus it could be interpreted that it is less likely to contribute to CKDu. However, it should be noted that 1) the overall rice consumption is very similar across CKDu and normal subjects, and 2) rice consumption could go down once someone is diagnosed with CKDu and trying to live healthier or has a decreased appetite, and this instead could explain for higher rice consumption among healthysubjects. Same general comment for accompaniments on P. 15 first paragraph.

- P. 11 also notes that since rice consumption frequency is almost equal there is no evidence to support heavy metals in rice contribute to CKDu. This section would be more complete if there is a discussion that perhaps other risk factors may play a role in the development of CKDu or work in concert with heavy metals to contribute to its incidence.

- P. 11 notes that a detailed food intake study needs to be conducted prior to "coming to a conclusion" that metals are not a CKDu contributor. The authors should also note that a multifactorial study of the issue is needed to more comprehensively and simultaneously consider all risk factors that may be associated with CKDu.
- The Jayatilake et al. study findings do not support the study results, and caution should be taken in reporting their overall conclusions. A better approach would be to interpret their results individually.
- In conclusion paragraph, last sentence, it should be noted that other risk factors could be at play for CKDu patients.

The manuscript was revised taking these valuable comments into account.

DISCRETIONARY REVISIONS
1. In sentence before conclusion section, it would be apt to note that associated metals testing would also be useful.

This part is added to the sentence

Reviewer 2: Keith Levine

Discretionary Revisions:

1. Recommend significantly expanding introduction (if not word limited) to include a more extensive discussion on the CKDu epidemic in Sri Lanka and provide relevant supporting references. For example, is the issue predominantly impacting a certain geographic region on the country, people of a certain occupation or socioeconomic status, and so on? This information would help put the study findings in context.

These details were included in the introduction in brief with appropriate references.

2. In the materials and methods section, a discussion of the 30 CKDu patients identified for the study is provided. Is additional, specific information available on the biochemical measurement levels for these cases and age sex matched controls? What was the threshold for inclusion or exclusion?
Threshold values of biochemical measurements were included in the manuscript.

3. What were the biochemical metric thresholds for different stages of CKDu?

Internationally accepted National Kidney Foundation, Kidney disease outcomes quality initiative classification was used for getting thresholds for different stages of CKDu
<table>
<thead>
<tr>
<th>Stages of CKD</th>
<th>Description</th>
<th>GFR (mL/min/1.73m²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Kidney damage with normal or increased GFR</td>
<td>≥ 90</td>
</tr>
<tr>
<td>2</td>
<td>Kidney damage with mild decreased GFR</td>
<td>60 – 89</td>
</tr>
<tr>
<td>3</td>
<td>Moderately decreased GFR</td>
<td>30 – 59</td>
</tr>
<tr>
<td>4</td>
<td>Severely decreased GFR</td>
<td>15 – 29</td>
</tr>
<tr>
<td>5</td>
<td>Kidney failure</td>
<td>&lt; 15</td>
</tr>
</tbody>
</table>

The CKDu patients were randomly selected from the patients diagnosed and confirmed to be having CKDu. The stages of the disease of CKDu of the subjects of the urinary β₂m study are given in the table 2.

Further the CKDu patients of the dietary study were in stage II (n=16), stage III (n=58), stage IV (n=24) and stage V (n=2) respectively and these information were included in the manuscript.

4. Are some of the cases and controls the same for the urinary study and the dietary study, or are these different groups entirely?

Some of the subjects for urinary study and dietary study were same.

5. In addition, are some of the cases and controls from the same family or residence?

None of the subjects from each group were from the same family, but from the same residential area.

6. How were the GFR data presented in Table 2 collected? Were these data collected as part of this investigation, or were they provided by medical professionals at the onset of the study?

GFR data were given by the consultant nephrologist (fourth author) who is conducting the renal clinic in the study region and the GFR values at the time recruitment were considered for this study.

7. Is the diet of the Medawachchiya region typical of the Sri Lankan diet? From the discussion, it seems that most of the food consumed in this region is harvested locally from home plots. Are foods from the region exported to other parts of the country?
The diet in Medawachchiya is typical to general Sri Lankan diet, but may vary in composition based on the region and most of the food consumed in this region is harvested locally as majority of them are farmers. The food from this region (especially rice) are exported to other parts of the country, but the food consumption frequency of the same local food items in other parts of the country is low as this supplies only a smaller portion of the requirement of the overall population.

8. Was information about food preparation practices collected as part of the dietary recall questionnaire? Are these practices fairly consistent across the region? Yes.

09. Was information collected about the water source(s) used for cooking?
10. Was information collected about the water source(s) used for drinking and the general volume of consumed water?

The subjects were recruited for an epidemiological study of the same issue in addition to the current study and the water sources and amounts used by the subjects for different purposes were assessed in detail during the said epidemiological study. This information will be analysed separately in detail for the extensive exploration of the epidemiological aspects of this issue.

11. Could BMI be predictive of CKDu onset and severity in this region? If appropriate, please consider expanding discussion on this (page 7).

BMI of the tested populations were calculated with the objective of finding any association (if present) with the disease. As the BMI of both groups were within the normal range for Asians this was not extensively discussed.

12. The authors designing the study to capture seasonal variations in diet. Does the seasonal diet in this region vary significantly?

The availability of certain food types vary among dry and wet seasons and it was felt best to capture any such variations.

Minor Editorial Comments:

1. Abstract (Background): Suggest changing ‘chronic exposure to heavy metal’ to ‘…heavy metals’.

   Revised

2. Recommend consistency throughout manuscript with use of β2-microglobulin acronym.
   Consistency of β2-microglobulin is checked and corrected
3. Abstract (Results): Suggest adding period to end of paragraph.

The study period was included at the end of the methods section of the abstract.

4. Suggest to capitalize “Study” in the “Dietary study” section header and to change “The Urinary” to “The urinary” in the first sentence of that section.

Revised

5. Suggest adding spacing between paragraphs throughout.
Spaces are added between the paragraphs

The correction is done

Reviewer 3: Ranil Gajanayaka

Reviewer's report:

1. Urinary Beta 2 Microglobulin (B2M) is highly unstable at room temperature especially in acidic environment. (Urine pH <6). Authors do not describe how they collect these urine samples, what was the urine pH and how they maintain the cold chain. Without fulfilling that condition, authors cannot say that healthy sample had normal B2M.

Upon collection of the sample pH was adjusted to >6, aliquoted and immediately transported to the laboratory in ice boxes and stored at -40°C till the analysis is done. The laboratory for storing and analysis was located about 30-50 km away from all the collection sites and cold chains were maintained during all the procedures. This information was added into the manuscript.

2. Urine B2M level increase with any protenuric CKD and the amount of B2M increase with the CKD stages. It could have been nice if it was compared with patients with CKD due to other etiologies.

We agree with this valuable comment which can add some important facts to the manuscript. However the current study lacks the data for comparing with CKD due to other etiologies as this was not considered in the initial study design.

3. Based on their dietary recall results, over 90 % of both groups had rice for 3 meals. They haven’t looked into the source of water they drank. I don’t think they could use the word "dietary pattern" at the conclusion. So the conclusion may need to be changed according to the research questions.
Suggested change in the conclusion was done.

Reviewer 4: Shanthi mendis

Reviewer's report:

1. Is the question posed by the authors well defined?

The authors say that the current study intended to compare the variation in urinary β2-microglobulin excretion of CKDu patients and normal subjects inhabited in a high CKDu endemic area and to compare the dietary patterns of CKDu patients and normal subjects from the same endemic area. The research question that they are trying to address through this comparison is not clearly defined. What do the investigators mean by `an experience from urinary β2-microglobulin measurement`?

As stated under methodology section, analysis of urinary β2-microglobulin was followed by the dietary study further clarifications were made within the manuscript and changes in the title objectives were done.

2. Are the methods appropriate and well described?

Without a clear research question it is not possible to comment on whether or not the methods are appropriate. Methods are described. However if the intention was to investigate the role of heavy metals in the causation of CKDu a 24 hour heavy metals should have been analyzed. No analysis of heavy metals in the food has been done.

Role of heavy metals in the development of CKDu was not explored during the current study and the focus of the study was to compare the variation in urinary β2m excretion of CKDu patients and non-CKDu subjects, inhabiting in a CKDu endemic area, and to compare the dietary patterns of CKDu patients and non-CKDu subjects from the same area.

3. Are the data sound?

The CKDu group has subjects with moderate and severe kidney disease. Their diet intake will be influenced by a) loss of appetite b) need to reduce consumption of proteins as per medical advice. Further, if heavy metals are playing a role, longterm intake will determine the accumulation of metals in the body which cannot be assessed by a 24 hour dietary recall. Both these would interfere with the data and the conclusions that have been made.

We agree with this valuable comment made by the reviewer and the possible dietary changes associated with the disease itself is a practical limitation of this study, which was unraveled and discussed in the manuscript. However authors did not intend to explore the effects of heavy metals during the current study, hence we believe that a
dietary recall would be sufficient to study the effects of general food consumption. Further the entire manuscript including the abstract was extensively revised to clarify the possible misinterpretations.

4. Does the manuscript adhere to the relevant standards for reporting and data deposition?
   Yes

5. Are the discussion and conclusions well balanced and adequately supported by the data?
   The discussion and conclusions are not adequately supported by the data. The CKDu group has subjects with moderate and severe kidney disease. Their diet intake will be influenced by Loss of appetite as well as the need to reduce consumption of proteins as per medical advice. These factors would interfere with the data and the conclusions that have been made. The role played by heavy metals is discussed but heavy metals have not been analysed in the food. A 24 dietary recall of food types and quantities consumed will not give the answer to the question whether heavy metals are causative factors in the causation of CKDu; if indeed this is the question that is been asked by the investigators.

   As mentioned above the current study did not intend to study heavy metals as a causative factor. Further discussion and conclusions are revised with the comments.

6. Are limitations of the work clearly stated?
   Some of the limitations are stated.

   Limitations were revised.

7. Do the authors clearly acknowledge any work upon which they are building, both published and unpublished?
   Yes

8. Do the title and abstract accurately convey what has been found?
   No

   Title and abstract are revised.