Author's response to reviews

Title: Simultaneous Exposure to Multiple Heavy Metals and Glyphosate May Contribute to Sri Lankan Agricultural Nephropathy

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Version: 4 Date: 16 May 2015

Author's response to reviews: see over
16th May 2015.

Dear Editor,

Thank you very much for sending reviewer’s comments. Herewith we submit the revised manuscript. We have accepted all suggestions made by reviewers and amendments were done accordingly. Below we elaborate all the changes. Further, we have added reference 2, 4, 33 and 47.

Thanking you,

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Reviewer's report

Title: Simultaneous Exposure to Multiple Heavy Metals and Glyphosate May Contribute to Sri Lankan Agricultural Nephropathy

Version: 3 Date: 22 April 2015

Reviewer: Virginia M Weaver

Reviewer's report:

This article addresses an important public health concern that is a major health burden in Sri Lanka and other affected communities. Identification of the etiology for this type of kidney disease is essential. Small study size is a major limitation for this manuscript, however the concern regarding toxicant exposures is valid, particularly in relation to multiple exposures.

- Major Compulsory Revisions

1) Note sample size in abstract
done

2) In Methods (Line 126), briefly state diagnostic criteria (i.e., no diabetes mellitus, history of snakebite, or kidney disease of known etiology; glycosylated hemoglobin level # 6.5%; blood pressure #160/100 mmHg untreated or #140/90 mmHg on up to two antihypertensive agents) with the Ministry of Health reference as well as the reference for additional recruitment details.

The diagnostic criteria was added

3) Was albuminuria used in the diagnostic criteria?

No, only creatinine and Ultrasound evidence were positive criteria and others mentioned above are negative criteria.

“Ultrasound evidence of contracted kidneys and serum creatinine> 1.3 mg/dL was used to diagnose CKD”

4) The reference cited for recruitment details (#22) has many more participants.

How were the final 30 for this study selected?

Ten Cases were selected from the patients presented to Padavi-siripura hospital clinic in one day. No sampling was done.

Reference (#22) was cited only for the diagnostic criteria.

5) How was normal kidney function in endemic controls defined? Specifically what cut-offs for serum creatinine and albuminuria were used?
A sentence inserted in the revised manuscript.

“They had negative albuminuria (albumin creatinine ratio less than 30 mg/g) and serum creatinine less than 1.3 mg/dL”.

6) How was normal kidney function in group 3 controls defined since serum creatinine was not obtained?

By obtaining a history of not having kidney disease or any other chronic disease.

7) Add sample size to table 1: done.

8) For trimmed means, how did the authors remove the highest and lowest 5% when only 10 samples were present? Median values may be preferred for such small data sets. If trimmed means are used, this term should be used in abstract so reader knows arithmetic means were not used.

We have removed the trimmed mean and used the median values.

9) In Table 2, trimmed means for all the urine chemical levels increase in patients when urine creatinine adjustment is performed. However, just the opposite is true for almost all values in the controls. As the authors note in line 151, patients with renal impairment may have low urinary creatinine. That low excretion could contribute to the higher urine creatinine adjusted values reported. This could result in artifactualy increased urine toxicant levels. Recently, the challenges of adjusting for urine concentration when measuring urine biomarker levels has been a topic of discussion among researchers using biomarkers. This is particularly challenging when levels are measured in patients with CKD because not only could urine creatinine be lower, urine toxicant levels could be as well. As the authors note in their discussion, “Once the renal functions are compromised, SAN patients lose their ability to excrete the heavy metals resulting in their accumulation in the body tissues over the time and reduced excretion in urine.” Thus, the analysis in Table 3 should also be shown both with and without urine creatinine adjustment. It may be that the two control group comparisons may be the most informative if their kidney function is similar.

We have added the unadjusted creatinine values to the table 3 in addition to the adjusted creatinine values.

- Minor Essential Revisions

1) I appreciate the paragraph in the discussion on dehydration since this has been a risk factor studied in the outbreak in Central America. Can the authors comment on the occupations of their study participants and the potential for dehydration?

This we have added the occupation of the participants to the table one. In the last para of the discussion, we have dwelt on the potential for dehydration among people living in the endemic area.

2) Line 234 – Bruce Fowler and colleagues have published studies describing mixed exposures to various heavy metal combinations that the authors may wish to read. I am
not sure any of those publications fit the exact criteria of chronic low-level exposures, however.

Thank you for the information that we were unaware before. We have commented on his studies in the discussion.

3) Use of the term bio-accumulate is a bit tricky in this manuscript. Metals such as lead and cadmium, which have very long half-lives in the body, are considered to bio-accumulate. And this can occur for many chemicals in CKD where they are not excreted. However, in controls, elevated levels of chemicals with short half-lives are more likely to reflect increased exposure rather than bioaccumulation.

We agree that the term bio-accumulate is tricky and we have removed it.

4) If article needs to be shortened, could eliminate paragraph 1 of intro

Other reviewer has commented on that paragraph hence, we have made the changes accordingly.

5) Minor editing noted below and in subsequent instances not noted:

Line 84 – replace sight with site done
Line 96 - Comma after hypertension done
Line 103 – should be hypokalemia done
Line 108 – toxicants is the preferred term for chemicals whereas toxins is preferred for biological done

- Discretionary Revisions

I recommend that the authors use the term Chronic Kidney Disease of unknown etiology (CKDu) rather than Sri Lankan Agricultural Nephropathy (SAN). Chronic kidney disease very similar to that in Sri Lanka has been reported in a number of other locations globally. Therefore, use of the more widely accepted CKD terminology will be helpful in ultimately determining the etiology of this disease.

Thank you for the comment. In Central America CKDu is named as Mesoamerican nephropathy to draw attention to the epidemic. In our case control study (22) we have shown all potential risk factors are associated with agriculture and would like to name the disease as such.

Level of interest: An article of importance in its field

Quality of written English: Needs some language corrections before being published

Language corrections were done.

Statistical review: No, the manuscript does not need to be seen by a statistician.

Declaration of competing interests: I declare that I have no competing interests.
Reviewer's report 2

Title: Simultaneous Exposure to Multiple Heavy Metals and Glyphosate May Contribute to Sri Lankan Agricultural Nephropathy

Version: 3 Date: 30 April 2015

Reviewer: Keith Levine

Reviewer's report:

Major Compulsory Revisions

Sri Lanka's emerging chronic kidney disease epidemic is a public health crisis and is of significant interest to the Sri Lankan and international scientific communities. The authors of this manuscript are to be commended for drawing further attention to this serious public health issue. Reports of the association between chronic, low-level exposure to heavy metals and other persistent environmental chemicals and non-communicable disease genesis and progression are becoming more frequent in the literature. As a result, biosample data from a broad panel of metals and a frequently used pesticide (glyophosate) are of potential interest.

Thank you for the comments.

The primary limitation of this study is the limited sample size for the case and control groups. The authors acknowledge this in the Discussion section of the paper, but it should be stated in the abstract section and emphasized elsewhere in the paper.

We have mentioned the number in each group in the abstract. We also have mentioned this in the methods where we have explained why the non-parametric tests were used.

Even though the sample size was limited, some interesting data were obtained that may warrant further study. Additional information about the study participants would be of interest if data are available (for example, stage of CKD, BMI, other risk factors, dietary recall prior to spot urine sample, etc.). It would also be helpful to summarize criteria for assignment to case/control groups in table 1 (i.e. biochemical marker levels, medical criteria, etc.).

Additional available information is provided in table-1, such as history of kidney disease, stage of CKD and occupation.

From data tables 2 and 3, it was noted that no values were flagged as less than detection or quantitation limit. It would be of interest to compare collected data with these method detection/quantitation limits to put reported data into context.

We accept this limitation and one reason again may be small sample size. We have added a comment in the discussion.
Also in relation to glyphosate the detection limit in ELISA is lower than HPLC. “ELISA method has lower detection limit of glyphosate (0.6 µg/L) compared to HPLC method (050 µg/L)”.

For this broad panel of analytes, however, it seems unlikely that measurable values would be obtained at high confidence for all samples. Additional information on sample preparation, quality control, and analysis procedures may be helpful for both ICP-MS and kit-based assays, as appropriate.

Additional information is provided under “accuracy precision and detection limits”

**Minor Essential Revisions**

There are editorial changes throughout the manuscript that should be made prior to publication. For example, ‘manufacturer's’ on line 148, potentially repeated text on line 179, and so on.

Done ('manufacturer's' )

Repeated text, is removed

It may be of benefit for the authors to review the manuscript looking for these minor items.

The manuscript would also benefit from an expansion of the background about heavy metals (lines 69 - 80) with additional references.

**Background is expanded with additional references.**

**Discretionary Revisions**

The authors discuss similarities with other international CKD epidemics and mention other confounding factors (i.e. chronic dehydration). It would add value to this manuscript if these discussions could be expanded with additional references added.

We have discussed the mechanism of dehydration and its effect in dry zone of Sri Lanka.

**Level of interest:** An article whose findings are important to those with closely related research interests

**Quality of written English:** Needs some language corrections before being published

Language corrections were done.

**Statistical review:** Yes, but I do not feel adequately qualified to assess the statistics.

**Declaration of competing interests:**

I declare that I have no competing interests.