Reviewer's report

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

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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
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.
3) Was albuminuria used in the diagnostic criteria?
4) The reference cited for recruitment details (#22) has many more participants. How were the final 30 for this study selected?
5) How was normal kidney function in endemic controls defined? Specifically what cut-offs for serum creatinine and albuminuria were used?
6) How was normal kidney function in group 3 controls defined since serum creatinine was not obtained?
7) Add sample size to table 1
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.
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 artifactually 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.

- 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?

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.

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.

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

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

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

- 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 CKDu terminology will be helpful in ultimately determining the etiology of this disease.

Level of interest: An article of importance in its field

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