Reviewer's report

Title: Effect of maternal manganese exposure on birth weight: a birth cohort study

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Reviewer: maryse F bouchard

Reviewer's report:

Major Compulsory Revisions

There are many problems with the way the data are interpreted in this manuscript; both data from the literature, and their own data. I think it needs to be extensively rewritten. The authors must acknowledge that their observation of a relation between blood Mn and birth weight is very far from proving causation. They need to rewrite the Discussion to reframe their arguments without assuming such causative relation. I believe that caution in interpreting these findings is especially important because the association could be gravely biased. A major weakness of this study is the lack of data on iron stores. It is well known that Mn and Fe share uptake mechanisms, and individuals with low iron stores have increase Mn uptake and retention. Early studies reported that workers with anemia had higher Mn levels (e.g. Mena et al. Neurology. 1969; 19:1000). Therefore, women with low iron stores could have higher blood Mn levels, and the lower birth weight could actually be due -- entirely or in part -- to iron deficiency. The potential role of iron should be acknowledged by the authors.

Also the authors' argumentation rest on the assumption that high Mn levels during pregnancy = high exposure levels. This is clearly an over simplification, and may even be false. Indeed, population studies have demonstrated that Mn levels in pregnant women are much higher that non-pregnant women, especially towards the end of pregnancy. Non pregnant women have levels around 8-10 ug/L, whereas levels increase during pregnancy to culminate around 20-40 ug/L at birth. Levels are even higher in cord blood. These high levels are observed in women with no known source of overexposure to Mn. The most logical explanation is that it reflects high physiological needs during gestation. This background should appear in the Introduction of the article. Review the important work from Takser et al. on this topic (Manganese levels during pregnancy and at birth: relation to environmental factors and smoking in a Southwest Quebec population. Environ Res. 2004; 95:119).

Based on these considerations, the title should be changed to remove the term 'exposure'. Clearly, the term 'effect' is also completely inappropriate as well, since it suggests causation.

I addition, the references are often inadequate. However, the statistical analyses are appropriate, although I have a few suggestions (see below)
INTRODUCTION

I would urge the authors to review their manuscript to ensure proper use of references. It is important that they be pertinent and actually address the topic of the statement authors are making. In the 1st paragraph of the introduction, I found several problems with references. For instance, the 1st sentence reads Manganese (Mn) is one of the essential nutrients for humans and animals, and is intensively needed during gestation and early infancy [1].

The reference 1 is from Dorman et al. 2005. Well, this animal study showed that in utero exposure to inhaled Mn was associated only to limited increase in fetal Mn levels, limited to the liver and not to blood, lung, brain, or skull cap. This experiment does not address the Mn physiological needs during growth at all.

Again in the 1st paragraph of the introduction, the sentence “Mn deficiency may result in poor bone formation, birth defects, and increased susceptibility to seizures [4-6].” References 5 is a review about Mn transport in the brain in the context of high exposure levels causing neurotoxic effects. It does not address Mn deficiency at all.

2nd paragraph, 1st sentence: I would refrain from using the term ‘exposure’ in this context. The presence of manganese in the system is not properly referred to as exposure because Mn is an essential nutrient. For instance, we don’t talk about iron exposure, or calcium exposure.

2nd paragraph, last sentence “Excessive concentrations of Mn are potentially harmful to the foetus due to the underdeveloped ability to eliminate Mn [9,10].” This statement is not clearly supported by the literature Actually, the reference no. 1 (Dorman et al. 2005) supports the contrary, since this an animal study showed increase in Mn levels limited to the liver in fetus exposed in utero exposure to airborne particulates of Mn (there was no increase in fetal blood, lung, brain, or skull cap Mn). Very high Mn levels are found in pregnant women and in cord blood of individuals with no known source of overexposure to Mn, so these high levels probably indicate high physiological needs during gestation. The reference 9 and 10 provided to support this statement are, again, inappropriate.

3rd paragraph, 1st sentence, should read “ One of the reported maternal and developmental toxic effects in studies(…)”

3rd paragraph, 3rd sentence, “However, these studies were conducted in a population with a high level of environmental exposure,”. Firstly, the studies cited in the previous sentence were conducted in 2 separate groups, one in the U.S. and one in Iran, so it is not a single population. Secondly, no unusual sources of exposure are mentioned in paper on the Iran study, so it is unclear why they authors think this population had high levels of exposure.

Last sentence of Data Analysis: “ Statistical significance was determined using a p value of < 0.05, but extended to <0.1, when active interpretation is needed.” I am not sure what active interpretation means.
RESULTS

2nd paragraph. This sentence makes no sense; it need to be rewritten. Also, the R2 from model 3 is not really different from that of Model 2 (0.1693 and 0.1631, respectively).

You should justify choosing the 3000g cut-off for birth weight?

Figure 1 shows OR for birth weight below 3000g per group of blood Mn levels. Table 1 (mislabelled Table 3) presents different groups for blood Mn. You should use the same groups throughout the paper and justify how the grouping of values was done.

The confidence intervals for the lowest and highest blood Mn are extremely wide, so much that it is not very informative. It must be that there were very few observations. It would be more informative to present a table (not a figure) and add the number of ‘cases’ of birth weight below 3000 g (or another value well justified) per blood Mn group.

Table 1 is mislabelled Table 3.

DISCUSSION

1st paragraph: sentence “This study focuses on the relationship between deficiency or high level exposure to Mn in utero and infants’birth weight.” This is a misleading statement. You observed that the lowest and the highest levels of blood Mn during pregnancy were association with lower birth weight, but this is clearly no proof that Mn caused these changes in birth weight. I don’t think you can conclude anything about Mn deficiency.

2nd paragraph. “This study provides epidemiologic evidence for adverse effects of both deficiency and excess of Mn during pregnancy from a cohort based on the general population.” This statement is extremely overreaching. Your study provide no evidence of effect at all; you merely show an association, which could be completely biased, for instance by iron levels.

3rd paragraph. “The results of the present study confirm that a high level of blood Mn is associated with low birth weight in full-term infants.” Low-birth weight is defined as below 2500g, and you did not show associations of Mn levels with odds for the prevalence of such birth weights. Please consider using another term than ‘low birth weight’ elsewhere in the manuscript when you refer to your results. Again, the term “confirm” is way too strong and definitive in light of this study.

5th paragraph. “Nonetheless, the results of these human studies suggest that abnormal Mn exposure during early life may affect foetal growth and result in low birth weight.” Your study did not examine ‘abnormal’ exposures to Mn. You have no data on exposures. You only have blood Mn levels, which has not been proven to reflect exposures in this particular group, i.e. pregnant women.

CONCLUSION:
Again, high Mn levels is not synonymous with high exposure.

Minor Essential Revisions

Table 3: The term ‘unknown’ should be changed for ‘missing’.

There are too many digits for p-values.

Level of interest: An article of limited interest

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 have no competing interest