Author's response to reviews

Title: Relationship between arsenic skin lesion and the age of natural menopause

Authors:

Fakir Md Yunus (dryunus155@gmail.com)
Musarrat Jabeen Rahman (jabeen@icddrb.org)
Md. Zahidul Alam (mzalam@uta.edu)
Samar Kumar Hore (samar@icddrb.org)
Mahfuzar Rahman (mrahman1@uchicago.edu)

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Author's response to reviews: see over
Dear Sir,

Many thanks for your feedback on the manuscript ‘Relationship between arsenic skin lesion and the age of natural menopause’. We have responded in the following pages point-by-point to the concerns of both reviewer’s comments and incorporated their valuable suggestion on the manuscript. We have enclosed the revised version of our manuscript for your convenience as well. Therefore, we are looking forward to publish this original research article in your reputed journal soon.

Finally, we appreciate your time and hard work on our manuscript.

Your sincerely-

Corresponding author on behalf of all authors

Fakir Md. Yunus

Fakir Md. Yunus MBBS. MPH
Senior Research Associate
BRAC Research and Evaluation Division.
BRAC Centre, 75 Mohakhali, Dhaka-1212
Bangladesh.
dryunus155@gmail.com
yunus.fm@brac.net
Authors’ response to Reviewer #1 [Response review comments (in bold)]

ABSTRACT, Under the section results, the last sentence is not useful. The p value can be added to the previous sentence.

We changed the text as you suggested (page 2). We also deleted the last sentence as suggested.

Under the section conclusions: I suggest the following: The study showed a statistically significant association between chronic exposure to arsenic and age of menopause. Heavily exposed women experienced menopause two years earlier than those with lower or no exposure.

We have re-written the text in the conclusion as follows, “The study showed a statistically significant association between chronic exposure to arsenic and age of menopause. Heavily exposed women experienced menopause two years earlier than those with lower or no exposure.”

BACKGROUND: Page 3 line 4 should be 10/ug/L.

We have changed the text as followed “10/ug/L”

Page 3 line 3 from the bottom resulting instead of results.

We have corrected the text resulting instead of results.

Page 4 please do not use delays onset or delays menopause but use “delayed”

We have corrected the text as “delayed”

Page 4 next to the last sentence I would say “women are more at risk” not “will likely”

We have re-written the text as suggested by reviewer#2 “women would likely to experience of cardiovascular diseases, neurological diseases, osteoporosis and psychiatric diseases earlier in their lives [17].”

Page 4 last sentence: delayed. Menopause menopause may increase the risk (not “referred”)

We have re-written the last sentence as suggested “may increase the risk”

METHODS
Please explain briefly what was the random clinical trial. Were these women in treatment? For what? For arsenic intoxication?

We have added as follows, “This clinical trial aims to determine the supplementation of vitamin E, selenium or their combination that could have beneficial effect on arsenic induced skin cancer. It is a double blind, randomized, placebo controlled clinical trial conducted among the arsenicosis patients in Bangladesh.”

Also what was the “ongoing cohort study” It is not clear why you used two different populations if both of them were exposed to As. (“from the same tube”).
We selected arsenic skin lesion participants from Laksham area because clinical trial was conducting in that area which enrolled only arsenicosis patients. We assumed that arsenic skin lesion participants were more likely to be found in that area. Participants without arsenic skin lesion were selected from ongoing cohort study which enrolled participants arsenic contaminated area (Araihazar). Although in this study, we collected participants from two different areas but socio-demographic characteristics were almost similar as these two study sites were selected from two typical rural villages and located side by side in the eastern part of Bangladesh.

Page 6 last line and p. 7 first line: not clear” exists among the respondents

We have re-written the text suggested by reviewer #2 as follows ‘Several studies suggest that a number of factors may affect natural menopausal age such as smoking, educational attainment, income, marital status, employment, parity, contraceptives use, family history of premature menopause, cancer and history of chemotherapy or radiotherapy and body mass index [25-33]. Among these, a few factors such as smoking, parity, marital status, income, use of contraceptive methods, body mass index and family history of premature menopause prevailed among the respondents.’

Page 7 second paragraph “reviewed retrospectively” not clear. Also last sentence “during that time of menopause”

We meant, “reviewed retrospectively” by looking at those variables (smoking history and Body mass index) at the time of their menopause. We have changed that text as follows: “at the time of menopause” and deleted the text “during that time of menopause”

STATISTICAL METHODS
p. 7 “adjusting for potential confounding variables

We have corrected the text as suggested “potential confounding variables” instead of “potential factors.”

RESULTS
Table 1 is not useful. All characteristics should be shown separately for the two groups. Add the data of table 1 to table 2 and eliminate table 1.

We agree with the reviewer’s comment and we have added the data of table 1 to table 2 and eliminate table 1. And we have also re-written the result section.

Page 8 fifth line from the bottom:“were not significantly different. NOT “not associated”.

We have changed the text “were not significantly different”.

Page 9, before table 5 please repeat the variables for which you adjusted.

We have added the text as follows “contraceptive use, urinary arsenic level, body mass index and family history of premature menopause.”

Please do not say possible potential factors, but potential confounding factors.
We have changed the text to “potential confounders”. We deleted “possible potential factors”

When reporting urinary arsenic levels, please always write the units used (ug/g (creatinine)?)

We have reported µg/g creatinine instead of µg/L.

CONCLUSION
Line 3 “in women having an increased risk”

We have changed as “in women having an increased risk”.

Line 6 not “less” but shorter

We have corrected the text “shorter” instead of “less”
Authors’ response to Reviewer # 2 [Response review comments (in bold)]

Abstract:
1. The last line of the ‘Methods’ section of the abstract should be written as, ‘Multivariable linear regression was used to estimate the relationship between arsenic skin lesion and age of natural menopause in women’.

Response: We have changed text as suggested ‘Multivariable linear regression was used to estimate the relationship between arsenic skin lesion and age of natural menopause in women’

2. The first sentence of the ‘Conclusion’ section should be written as, ‘This study suggests a relationship between chronic arsenic exposure (as indicated by presence of arsenic skin lesion) and age of natural menopause.

Response: We changed the text as suggested by reviewer#1 ‘The study showed a statistically significant association between chronic exposure to arsenic and age of menopause. Heavily exposed women experienced menopause two years earlier than those with lower or no exposure.’

Background:
3. The second sentence should be written as, ‘Bangladesh retained WHO’s previous Guideline value for arsenic concentration in drinking water as a national standard which suggests 50µg/L or lesser amount as safe [2], although the current WHO guideline value suggests it as 10 µg/L or less [3].

Response: We have changed the text accordingly, ‘Bangladesh retained WHO’s previous Guideline value for arsenic concentration in drinking water as a national standard which suggests 50µg/L or lesser amount as safe [2], although the current WHO guideline value suggests it as 10 µg/L or less [3].’

4. On the page 3, paragraph 2, line4, along with the references 11,12,13, the following reference should be added: Milton AH, Rahman B, Hasan Z, Kulsum U, Dear K, Rakibuddin M, Ali A. Chronic arsenic exposure and adverse pregnancy outcomes in Bangladesh. Epidemiology. 16 (1) 2005, pp 82-86.

We have included the suggested references.

5. On the page 4, line 1, the sentence should be written as, ‘In relation to this, another study suggested that arsenic causes delay in onset of menarche [16].

Response: We have changed the text as suggested by reviewer #1 ‘In relation to this, another study suggested that arsenic causes delayed menarche [16].’

6. On the page 4, paragraph 2, Line 3-9, it should be written as, ‘Considering both human and animal studies, this study hypothesized that chronic arsenic exposure characterized by presence of arsenic skin lesion could have a relationship with the natural menopausal age. This may mean that arsenic associated with early or delayed menopause - in either way, this could be an undesirable outcome. If menopause occurs early, there will be a reduction in reproductive period, that means women would likely to experience of cardiovascular diseases, neurological diseases, osteoporosis and
psychiatric diseases earlier in their lives [17]. On the other hand, delayed menopause referred to higher risk of breast and ovarian cancers due to their prolonged exposure to oestrogen [18].

**Response:** we have re-written the text as “Considering both human and animal studies, this study hypothesized that chronic arsenic exposure characterized by presence of arsenic skin lesion could have a relationship with the natural menopausal age. This may mean that arsenic associated with early or delayed menopause - in either way, this could be an undesirable outcome. If menopause occurs early, there will be a reduction in reproductive period, that means women would likely to experience of cardiovascular diseases, neurological diseases, osteoporosis and psychiatric diseases earlier in their lives [17].”

And re-written as suggested by reviewer#1 “On the other hand, delayed menopause may increase the risk of breast and ovarian cancers due to their prolonged exposure to oestrogen [18].”

**Methods (or METHODS?):**

**Study area and population:**

7. On the page 4, paragraph 3, 2nd sentence of this section should write as, ‘Participants with arsenic skin lesion were selected from Laksam upzilla as this area is highly contaminated to arsenic [19].

We have changed as suggested “Participants with arsenic skin lesion were selected from Laksam upzilla as this area is highly contaminated to arsenic [19].”

8. On the page 4, paragraph 4, the first sentence should write as; ‘Participants with arsenic skin lesion were selected randomly using the database from an ongoing randomized clinical trial conducted in Laksam, Comilla.’

We included the line ‘an ongoing randomized clinical trial’

9. On the page 4, paragraph 4, the line 5 should write as, ‘That cohort enrolled apparently healthy participants (both male and female) with a history of drinking water for at least three years from the same tube-well within the age limit of 18-65 years.’

We changed the word ‘three’ instead of 3

10. The study recruited apparently healthy participants (both male and female) from both the study area? What do you mean by ‘apparently healthy participants’ and what is the significance of mentioning this in the ‘study area and population sub-section’? Do you mean that unhealthy individuals were excluded from the study? In that case, you need to be specific. It is preferred to mention as ‘inclusion and exclusion criteria for the study participants’.

For better understanding, we dropped ‘apparently healthy participants’ from the text.

11. On the page 5, 2nd paragraph, the first sentence should write as, ‘Considering 80% power, 5% level of significance, unexposed/exposed ratio of 1, percent of unexposed with outcome 30 and percent of exposed with outcome 50 using the “OpenEpi” version 2.2 software (Developer: Emory University, Rollins School of Public Health) the sample size was calculated [20].”
We have rewritten the text as suggested.

12. The paper mentioned that, 'The first step was to identify and discard potential outliers. Using face validity, some outliers appeared on both sides of the bell curve.' This is not accepted. The authors need to mention why they discarded the outliers. Is it due to data entry error or whether these outliers are not biologically plausible? If inclusion of outliers affects the distribution of the variable, this needs to be dealt with appropriate statistical technique.

We have re-written the text as follows 'Using the range of eligibility from 35 to 51 years yielded a total of 187 participants for further analysis out of total interviewed 210 participants.'

13. On the page 5, 2nd paragraph, lines 11-12, the sentence should be written as, 'Using the range of 35 - 51 years, the rest were discarded.'

We have changed the text to “the rest were discarded.”

Data collecting procedure:

14. On the page 5, 3rd paragraph, lines 15-17 should be written as, ‘Detail menopausal history and socio-demographic information was collected using close-ended structured questionnaires consisting of easy to understand questions with appropriate response options.’

We have re-written the text as followed “Detail menopausal history and socio-demographic information was collected using close-ended structured questionnaires consisting of easy to understand questions with appropriate response options.”

15. On the page 5, 3rd paragraph, lines 25-27 should be written as, ‘Moreover, interviewers were kept blinded to the objectives of the study and in addition to that, enough time was allocated for the interviewers to conduct each interview’.

We have changed the text as followed “Moreover, interviewers were kept blinded to the objectives of the study”

Exposure assessment:

16. The paper has determined arsenic exposure as the presence or absence of arsenic skin lesions which has been used as a measure of exposure in the past by some researchers. However, I believe that arsenic concentration of their drinking water sources is also available for this study population as they have been recruited from a well-designed RCT and a Cohort study. Therefore, it would be worthwhile to explore the association between chronic arsenic exposure determined by drinking water arsenic concentration and age of natural menopause in addition to the arsenic skin lesions.

We agree with the reviewer’s response that it would be worthwhile to explore such association. We did not have any data on participant’s tube-well arsenic concentration. However, we did Pearson’s correlation test between urinary arsenic level and age of menopause which we found there is no statistical significant correlation (p =0.639) between groups.
Outcome assessment:
17. On the page 6, 3rd paragraph, the first sentence should be written as, ‘Natural menopause was determined according to the World Health Organization (WHO) definition “Permanent cessation of menstruation resulting from the loss of ovarian follicular activity” which corresponds to a single point in time- the final menstrual period (FMP) [24].’

We have changed as followed ‘Natural menopause was determined according to the World Health Organization (WHO) definition “Permanent cessation of menstruation resulting from the loss of ovarian follicular activity” which corresponds to a single point in time- the final menstrual period (FMP) [24].’

18. The outcome variable was measured based on self-reported age of natural menopause. Hence, there is a risk of recall bias through differential recall of natural age of menopause among women with arsenic lesions. This should be mentioned as a limitation in the study and how it may affect the study finding. Although you have mentioned it on page 6, paragraph 1, line 9, this does not ensure accuracy of self-reporting.

We agree with the reviewer’s on recall bias. We have rewritten the text “One potential limitation of the study is using the self-reported data on the age of menopause and family history of premature menopause, therefore, there is a risk of recall bias through differential misclassification of natural age of menopause among women with arsenic lesions. To avoid differential misclassification of outcomes, we blinded all study staff that includes interviewers and participants assessing for both exposures to outcome and outcome to exposures status. Alone with this, we conducted training among the interviewers in order to adhere to the question, and answer the format strictly, with the same degree of questioning on objective measures (hard data) for both with or without arsenic skin lesion participants. However, we assume that any fluctuation of recalling menopausal age and the family history of premature menopause is likely to affect equally in participants with or without arsenic skin lesion”.

Covariate and other variables:
19. On the page 6, 4th paragraph, the first and second sentences should be written as, ‘Several studies suggest that a number of factors may affect natural menopausal age such as smoking, educational attainment, income, marital status, employment, parity, contraceptives use, family history of premature menopause, cancer and history of chemotherapy or radiotherapy and body mass index [25-33]. Among these, a few factors such as smoking, parity, marital status, income, use of contraceptive methods, body mass index and family history of premature menopause prevailed among the respondents.’

We have changed as suggested ‘Several studies suggest that a number of factors may affect natural menopausal age such as smoking, educational attainment, income, marital status, employment, parity, contraceptives use, family history of premature menopause, cancer and history of chemotherapy or radiotherapy and body mass index [25-33]. Among these, a few factors such as smoking, parity, marital status, income, use of contraceptive methods, body mass index and family history of premature menopause prevailed among the respondents.’

20. On the page 7, lines 3-4 should be written as, ‘Socio-demographic information such as number of children, education, marital status, monthly household income was collected.’
We have changed the word ‘was’ instead of ‘were’

21. The paper mentions that they asked the participants whether their mother or sister had experienced premature menopause. I wonder how accurate it would be to obtain information on menopausal history of family members from the study participants! This needs to be mentioned as a limitation of the study in the discussion section.

We agree with the reviewer’s comments on this. As suggested by the reviewer, we have mentioned it in the limitation of the study in the discussion section.

Statistical analysis:

22. Overall, appropriate statistical analyses have been done. However, some of the analyses need further explanation.

We have rewritten the result section and explained more on the analysis.

23. Please replace the words ‘multivariate linear regression’ by ‘multivariable linear regression’ throughout the manuscript.

We have replaced the as suggested “multivariate linear regression” by ‘multivariable linear regression’ throughout the manuscript.

24. Please replace the words ‘dependent variable’ by ‘outcome variable’ throughout the manuscript.

We have replaced as suggested.

25. The authors need to mention whether the outcome variable ‘age of natural menopause’ was normally distributed or not. If the outcome variable was not normally distributed, then appropriate statistical measure needs to be taken.

The outcome variable ‘age of natural menopause’ was normally distributed. We included the text in the first paragraph under result section as follows “Mean age (±SD) of menarche, menopause and reproductive age was 14.00±1.36, 44.95±3.35 and 30.94±3.51 respectively. The outcome variable ‘age of menopause’ was normally distributed.”

26. On the page 8, lines 1-3, the authors mentioned that, ‘Although participant’s age difference was statistically significant (p <.001) between two groups, we did not include participant’s age in the final model because age of menopause was our variable of interest.’ This is not appropriate as participant’s age is an explanatory variable in this linear regression analysis whereas the age of natural menopause is the outcome variable. Therefore, you need to include participant’s age as an explanatory variable in the regression analysis.

We agree with the reviewer concern that ‘age’ should be included as an explanatory variable in the regression analysis. However, in this study, we collected participant’s both ‘current age’ and their ‘menopausal age’. This ‘current age’ should not have any relation with their previously occurred ‘menopausal age’. Therefore could not be an explanatory variable. On the other hand, we think that ‘age’ should not be considered as ‘explanatory/independent variable’ because ‘age’ is the ‘dependent/outcome variable’ in this study. Therefore, we understand that ‘age’ should not include as both dependent and independent variable in the regression analysis. For better understanding, we dropped the previously written text and included the text as “In the
Ethical review

27. The paragraph should be written as, ‘The study protocol and recruitment procedure (access to the databases) was approved by the Ethical Review Board of the James P Grant School of Public Health, BRAC University as per the existing rules.’

We have changed the text ‘The study protocol and recruitment procedure (access to the databases) was approved by the Ethical Review Board of the James P Grant School of Public Health, BRAC University as per the existing rules.’

Results

28. The first sentence should be written as, ‘Participants background information is presented in Table 1. Mean age (+ SD) of the respondents was 48.0 (+ 3.6) years. Mean age (+ SD) of menarche, menopause and reproductive period was 14.00 + 1.36, 44.95 + 3.35 and 30.94 + 3.51, respectively.

We have re-written the result section and changed the text as suggested.

29. Please report the monthly household income in US$ along with Bangladeshi taka.

We reported US$ along with BDT

30. On the page 9, first and second sentences should be written as, ‘Continuous variables such as body mass index (BMI) was found significantly lower (p=.03) among women with arsenic skin lesion (18.93 + 3.03) in comparison to women without arsenic skin lesion (19.94 + 3.48). However, urinary arsenic levels were found significantly higher (p= 0.001) among individuals with arsenic skin lesion (328.01 + 284.17) in comparison to individuals without arsenic skin lesion (156 + 193.00).

We changed the text as followed “Continuous variables such as body mass index (BMI) was found significantly lower (p=.03) among women with arsenic skin lesion (18.93 + 3.03) in comparison to women without arsenic skin lesion (19.94 + 3.48). However, urinary arsenic levels were found significantly higher (p= 0.001) among individuals with arsenic skin lesion (328.01 + 284.17) in comparison to individuals without arsenic skin lesion (156 + 193.00).”

31. On the page 9, 2nd paragraph, first and second sentences should be written as, ‘Mean difference of age of menarche, age of menopause, and reproductive age of the participants are presented in Table 4. Age of menarche was similar (p=0.06) between two groups, however the menopausal age (p <0.001) and reproductive period (p <0.001) were different between the groups.’

We have changed the text as followed “Age of menarche was similar (p=0.06) between two groups, however the menopausal age (p <0.001) and reproductive period (p <0.001) were different between the groups.”

32. On the page 9, lines 11-13 should be written as, ‘Approximately, 1.9 years decrease in reproductive period (p= 0.001) occurred among participants with arsenic skin lesion (29.99 + 3.31 years) compared to participants without arsenic skin lesion (31.91 + 3.48 years).’
We have changed the text as followed “Approximately, 1.9 years decrease in reproductive period (p=0.001) occurred among participants with arsenic skin lesion (29.99 ± 3.31 years) compared to participants without arsenic skin lesion (31.91 ± 3.48 years)”

33. On the page 9, lines 13 - 14 should be written as,’ Multivariable linear regression model was undertaken to adjust for all the potential confounders presented in Table 5’.

We have changed the text as suggested.

34. Table 2 describes the study population adequately. Therefore, Table 1 should be removed. Additionally, Table 4 should be merged with the proposed Table 1.

We agree with the reviewer’s comment and we have added the data of table 1 to table 2 and eliminate table 1. Additionally, we have merged Table 4 with the proposed Table 1.

We have also re-written the results section.

35. Please prepare all the ‘Tables’ according to the journal’s recommended format. All the numbers with decimal point should be presented in a uniform manner.

We have changed the ‘Tables’ format according to the journal’s recommended format. We have corrected the numbers with decimal point in the uniform manner.

36. Table 3, regarding the variable ‘smoking’ one cell contains a ‘zero’ value. Did you consider that in your analysis? Fisher’s exact test may be an appropriate test for this. The reported ‘p’ value is ‘1’ which is very unlikely. It is better to report the ‘p’ value as <0.001.’

We agree with the reviewer’s assessment of the analysis. Yes, we considered Fisher’s exact test for the variable ‘smoking’. We agree about reviewer’s concern about the ‘p’ value is ‘1’, however, our Fisher’s exact test gives us ‘p’ value is ‘1.000’.

37. Table 3 also reports about ‘number of children’. The authors need to report whether this number of children was normally distributed or not?

The number of children was normally distributed. We added the text as follows ‘Participant’s number of children was normally distributed and mean (±SD) was 4.74±1.79.’

38. At the bottom of Tables 1-3, the authors do not need to mention ‘*p = <0.05’ as they have reported the actual ‘p’ values in the tables.

We cut the text ‘*p = <0.05’ at the end of each table

39. Table 4 should be merged with the proposed Table 1 as I have mentioned earlier.

We have merged Table 4 with the proposed Table 1 and re-written the results section.
40. Table 5, the first row about the ‘constant’ should be removed from the table. At the bottom of the Table, please replace the words ‘Dependent Variable’ by ‘Outcome variable’ as I have mentioned earlier.

We have deleted the ‘constant’ from the regression model and replaced the words ‘Dependent Variable’ by ‘Outcome variable’

Discussion

41. On the page 13, second sentence should be written as, ‘An overall 3.5 months of delay was observed on the menarcheal age (p= 0.06) among the participants.’

We changed the text as suggested ‘An overall 3.5 months of delay was observed on the menarcheal age (p= 0.06) among the participants.’

42. On the page 14, lines 3 - 4 should be written as, ‘Although we did not have reliable data on their arsenical skin lesion at the time of their menarche, however in…….’

We changed the text as suggested ‘Although we did not have reliable data on their arsenical skin lesion at the time of their menarche, however in…….’

43. On the page 14, lines 8 – 9 should be written as, ‘Secondly, our finding revealed that chronic arsenic exposure seems to have a relationship with the menopausal age.’

We changed the text as suggested ‘Secondly, our finding revealed that chronic arsenic exposure seems to have a relationship with the menopausal age.’

44. On the page 14, lines 11 – 14 should be written as, ‘Mean reproductive period was 1.9 years lower among the participants with arsenic skin lesions compared to the participants without arsenic skin lesion. The finding was also similar in the multivariable linear regression analysis.’

We have changed the text as followed ‘Mean reproductive period was 1.9 years lower among the participants with arsenic skin lesions compared to the participants without arsenic skin lesion. The finding was also similar in the multivariable linear regression analysis’

45. On the page 14, lines 14 - 19 should be written as, ‘This result suggests that chronic exposure to arsenic, as marked by the presence of skin lesions is associated with early menopause, that means holding all other potential confounders constant, women with arsenic skin lesion would experience 2.1 years early menopause than the women without arsenic skin lesion. Therefore, these women are at higher risk of developing non-communicable diseases including cardiovascular diseases, neurological diseases, osteoporosis and psychiatric diseases earlier in their lives compared to the women without arsenic skin lesion [17].’

We have changed the text as suggested.

46. On the page 15, lines 2 - 4 should be written as, ‘Arsenic also may cross placental barriers and exposure to this toxicant may occur when the foetus is still within the mother’s womb, although its effects may visibly appear much later in life [35, 37].’

We have changed the as suggested.

47. On the page 15, line 5 should be written as, ‘In this study, all the participants with or
without arsenic skin lesion have high urinary arsenic……'.

We have changed as suggested.

48. On the page 15, line 13 should be written as,’… and this long-term arsenic exposure is more important to consider for certain health conditions than recent exposure.’

We have changed as suggested.

49. On the page 15, lines 17 - 19 should be written as, ’Another positive issue was the adjustment for relevant early menopausal risk factors among the study women such as number of children, family history of premature menopause, use of contraceptives, urinary arsenic level and BMI.’

We have changed as suggested.

50. On the page 15, lines 19 - 20 should be written as, ‘As participants (with or without arsenic skin lesion) were selected randomly, selection bias was less likely to occur.’

We have changed the text as suggested.

51. On the page 15, lines 20 – 22 the authors have written,’ It would be desirable to have menopausal data with or without arsenic skin lesion participants over time. In the absence of any reliable data on menopause in the past, it had to rely on individual’s recall.’ These sentences do not make any sense as menopause does not occur over time. Furthermore, the authors may follow my previous comments on potential recall bias and write about this recall bias as a limitation here.

We agree with the reviewer concern on this and we have deleted those lines and rewritten the limitation focusing on the recall bias.

52. On the page 15, lines 26 - 27 should be written as, ‘As this is a cross-sectional study, temporality between arsenic exposure and menopause cannot be claimed.’

We have changed the text as suggested.

53. Finally, the manuscript should be prepared according to the recommended format of the journal.

We have changed the manuscript format as per recommended by the journal.

Level of interest:An article of importance in its field

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

Statistical review:Yes, and I have assessed the statistics in my report.

Declaration of competing interests:

'I declare that I have no competing interests'