Reviewer's report

Title: Reproductive factors and its association with intima media thickness and carotid plaques in a cross sectional study of postmenopausal women enrolled in the population-based KORA F4 study

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Reviewer: Samar El Khoudary

Reviewer's report:

Using data from the population-based KORA F4 study of 843 postmenopausal women (35-81 years old); the authors evaluated the cross-sectional associations between several reproductive parameters and early measures of atherosclerosis (intima-media thickness and plaque of the common carotid artery). The authors reported independent associations between younger age at menarche and ever use of hormone therapy with presence of carotid plaque. In order to ensure clarity of presenting these results the following changes are recommended.

Major Compulsory Revisions

1. The wide age range of women included in this analysis may impact the results. The study included postmenopausal women between 35-81 years old. According to the results' section, first paragraph “the youngest women were included in the analysis based on their postmenopausal status after bilateral oophorectomy”. It is well known that surgical menopause women differ from natural menopause women in several aspects related to the menopausal transition (as the authors acknowledged in reference 17: Oophorectomy before natural menopause increases IMT independent of age and time since menopause). In addition, the young age of part of the study population indicates the possibility of premature menopause or the existence of other conditions that lead to bilateral oophorectomy in such young age. It would be of interest to know the distribution of type of menopause in this study. I am wondering why the authors did not create a menopausal status variable and adjust for it in their analysis. In order to insure that the results were not biased by including young postmenopausal women, it would be of great interest to reanalyze the data excluding women younger than 50 years old (with surgical menopause) and check if the results are the same. Please provide a table showing how women younger than 50 years old differs from those older than 50 years old in relation to study outcomes and main independent variables.

2. The description of the study population as well as the timeline of the reproductive factors was not clear. In specific, it was not clear if the reproductive factors were collected as part of KORA S4 or KORA F4. If the data was collected at both visits, please clarify which time point was used and why? What was the level of agreement between the two time points? Also did all women include in this analysis came from KORA F4? The method section gave the impression that
some of the data were derived from the baseline visit as well. This needs to be clearly stated.

3. Methods’ section, page 5, first paragraph, the authors specified that the sample size of KORA S4 was 6640 subjects. Then the authors stated in the second paragraph of the method section, page 5 “of the 4261 participants in S4, 3080 took part in the F4”. Please clarify which number is the correct one for the sample size of KORA S4. In the same section, the authors indicated that 943 subjects were included. According to the exclusion criteria which were listed in first paragraph of page 6, the final study population sample should be 715 and not 843. This needs to be clarified. Please show how those excluded differ from included and discuss the ramifications of the exclusion criteria on the results.

4. Data collection: “a fasting venous blood sample was obtained from all study participants”. Please clarify what was the purpose of these blood specimens, and how the related data were used in the current paper. If the participants had lipid profile, insulin resistance index, inflammatory markers, it would be very important to assess if the detected associations are independent of these potential covariates.

5. Page 7, 2nd paragraph, the authors stated the following: “Women were classified postmenopausal at the absence of menstrual bleeding for 12 consecutive months, if they had bilateral oophorectomy (either alone or in combination with hysterectomy) and had hysterectomy without bilateral oophorectomy and were above 50 years (without reported menopause before hysterectomy)”. What about those who were younger than 50 years old with hysterectomy with/out bilateral oophorectomy? How those were classified and what was the distribution of the different categories of post menopause in this study (natural post menopause, hysterectomy with/out bilateral oophorectomy, bilateral oophorectomy).

6. Please clarify how absence of menstrual bleeding for 12 consecutive months was verified. Was that self-reported or based on menstrual bleeding data?

7. Statistical analyses, page 9. Since the study included both continuous and dichotomous outcomes, I assume that the authors meant to say “PROC GENMOD” generalized linear models were used rather than “PROC GLM” general linear models were used. This needs to be corrected.

8. Table 1 should be presented for the total population as well as by menopausal status category. P value should be provided. It was not clear why the authors chose to present differences of study covariates by plaque but not by IMT categories. Also why IMT was not listed in the table? What was the mean IMT of the current study population? Please include summary statistics of both outcomes for the full cohort as well as by menopausal status. Also, please provide “n” along with “%”. Please clarify what the superscript “2” stand for in Table 1?. Although the variable “menarche” is a continuous variable, the symbol “%” was listed after it to indicate that it is a categorical variable. Please correct.
9. Table 2, were lipids, insulin resistance and/or inflammatory markers available? If yes, please provide models that are additionally adjusted for these potential covariates. I assume that what was presented in this table were the estimated means of IMT from the linear regression analysis. Please add unit of IMT to the table and indicate that estimated means of IMT was presented. The presented means (estimates) of IMT are higher than what was previously reported. This could be an indication of old age. What was the proportion of participants who were older than 60 years in the current study? The authors should discuss the compatibility of IMT level of their study population as compared to other study populations. Please discuss the clinical implication of the reported effect size (differences of IMT level between groups) in light of other clinical studies.

10. Given the emerging concept of “timing hypothesis”, did the authors tested possible interactions between current use of HT and time since menopause as well as age at menopause?

11. Did the study collected information about CVD events? were the study population healthy women with no reported CVD events? If these data are not available then this should be discussed as one important limitation of the study.

12. The following statement was not clear “however, it could explain why the association between ever use HRT and IMT loses its statistical significance in the multivariable adjust model in this study”. Please clarify.

13. The authors stated that IMT is a less reliable marker for early atherosclerosis than CP and that is why they were not be able to report significant associations between reproductive parameters and IMT. This explanation is not convincing given that IMT is widely used in clinical studies as an early marker of atherosclerosis or vascular remodeling. As the authors acknowledged in reference # 2, IMT predict CVD in the general population based on a recently published meta-analysis.

14. The final conclusion in the abstract as well as in the manuscript “in general, more gender based studies on cardiovascular risk factors in population based samples are necessary to better” is not directly related to the reported finding. Please provide a more clinically related conclusion.

Minor Essential Revisions

1. Some of the statements made by the authors were too strong and should be modified. For example, in the introduction section, the author stated that “the menopausal transition is associated with CVD”. It would be better to say “the menopausal transition has been shown to be associated with CVD” instead. Additionally the reference which was used assessed association between metabolic syndrome and menopausal status (cross-sectional) and not specifically CVD.

2. Table 2: was generalized linear model used for both table 2 and 3 or general linear model for table 2 and logistic regression for table 3. Please clarify in the
method section and under each table.

3. Table 2 and 3, please remove the symbol “%” for evaluated independent variables as this is not related to the tables contents.

Discretionary Revisions

1. Please state what “KORA” is stand for.

**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

**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