PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	Angiogenic Factors during Pregnancy in Asian Women with Elevated Blood Pressure in Early Pregnancy and the Risk of Preeclampsia: a longitudinal cohort study
AUTHORS	Zhu, Jing; Zhang, Jun; Ng, Mor Jack; Chern, Bernard; Yeo, George S. H.; Tan, Kok Hian

VERSION 1 – REVIEW

REVIEWER	Shigeki Matsubara Jichi Medical University, Japan
REVIEW RETURNED	13-Jun-2019

GENERAL COMMENTS	To authors, The theme is worthy. I have some advice. 1. Abstract: “developing hypertensive disorders” should be hypertensive disorders in pregnancy”. Or if your targets were preeclampsia, this should be replaced by preeclampsia. 2. Abstract: last sentence: the issue of endothelium (vascular) vs. placenta cannot be deduced from this study, especially when we consider the “two-step theory” of PE, and thus I ask you to rephrase this sentence with some appropriate sentences. I wrote this advice also later. 3. Page 5: line 45 “made” should be “produced”. 4. Page 6; line 6: If you wish to state “Asian”, please state the meaning that “there may be some racial difference”. In other words, this suggests that “preceding articles regarding this theme have already been reported but it was targeted non-Asian population. Anigogenetic-related factors are reported to show racial differences and thus we studied here this issue on “Asian” population”: one will interpret the context as above. Please state if preceding article exits and also the context of mentioning “Asian”. 5. Discussion first paragraph: Please use past tense (not present tense) because the study has already finished. You used present tense also to the sentences that should be written in past tense elsewhere. Please reconfirm the whole manuscript. 6. Discussion second paragraph: “Our results suggest an imbalanced angiogenic environment in early pregnancy along with chronic inflammation may injure maternal vascular function”: this study did not show cause-effect relationship between angiogenetic-factor-imbalance and endothelial dysfunction; i.e., egg-chicken relationship. Please soften the expression. You only showed that hypertensive women were more likely to show this imbalance and this imbalance tended to continue during pregnancy: that is all. You cannot deduce any conclusion as to cause-effect relationship. You only observed this “fact”. 7. Page 18: line 34: You too much simplify the story; your point is that “sFlt1 from endothelium and PlGF from the placenta”. Things

	are not so simple. You had better at least to add “as far as the data of PlGF here indicated are concerned” “When looking at this solely from the viewpoint of PlGF level during pregnancy here observed” (example). Rather, to extinguish readers’ misunderstanding, you had better soften the expression. You need not forcefully “tell some interesting story”. State things definitely based on the present findings. 8. Conclusion: As I stated above, please soften the expression. To state this pathophysiology, much more data is definitely/undoubtedly needed. Your findings themselves are worthy and do not “jump” to the pathophysiology. This holds true all through the manuscript. Please recheck if you state “too much” also elsewhere. The knack of the paper writing is that “conclusion should be modest”. If the Editor may consider that you can say it more straightforwardly, editors will advise you to do so. Once again, do not state too much. 9. Please definitely state whether this was the first-time observation. Or for the first observation on “Asian” women?

REVIEWER	Jan Danser Erasmus MC, Rotterdam, The Netherlands
REVIEW RETURNED	26-Jun-2019

GENERAL COMMENTS	This is a prospective cohort study involving 923 women to compare angiogenic markers throughout pregnancy in women divided by blood pressures in early pregnancy. The main finding of this study is that women with preexisting hypertension display elevated sFlt-1 levels between weeks 28-32. However, the novelty of this observation is limited, and thus the question is what the actual goal of this study was other than confirming existing knowledge. 1. The authors found that women with high blood pressure (Hypertension stage 1 & 2) are at increased risk of developing preeclampsia (PE). It is common knowledge that women with preexisting hypertension (if hypertension is diagnosed < 20 weeks) have higher risk of developing PE. What is the additional value of this finding? 2. The authors have performed logistic regression for blood pressure to predict the onset of PE. Given the topic of the study, it would be more interesting to determine whether the sFlt-1 levels in early pregnancy (e.g., measured at 11-14 weeks) can predict the onset of PE (and for example how this compares with the predictive value of elevated blood pressure). Why hasn't this been done? A problem might be that actually between 11-14 weeks, the sFlt-1 levels were not significantly elevated (P=0.08). 3. In the group of women with preexisting hypertension, 12 women developed PE. This means that 63 women did not develop PE, despite having ‘higher sFlt-1 levels’ (?) in early pregnancy. Where there any differences in sFlt-1 levels between the PE group in comparison with the women that did not develop PE? And what happened with these markers throughout pregnancy in the ones that did not develop PE in comparison with the ones that did develop PE? The same might be asked about the PE women in the other groups. The Discussion contains remarks about "chronic inflammation that may injure maternal vascular function", and "preexisting

	endothelial dysfunction playing a critical role in the development of PE" etc. None of this has been investigated here.

REVIEWER	Ali Khashan University College Cork, Ireland
REVIEW RETURNED	24-Aug-2019

GENERAL COMMENTS	This study was performed to examine the dynamic changes of angiogenic and anti-angiogenic factors throughout pregnancy in Asian women with elevated blood pressure in early gestation and the risk of pre-eclampsia. I have few comments that the authors may wish to consider: In the statistical analysis section, the authors describe statistical tests including ANCOVA, linear regression and logistic regression. The outcome measure for each analysis should be stated clearly. What is the advantage of using ANCOVA instead of a linear regression model? Were the variables in the ANCOVA analysis treated as predictors of the outcome measure or there was more into the ANCOVA analysis than this? The results of these analyses should be reported in terms of effect size and 95% CIs. For example, in Table 3, the mean difference and 95% CIs should be reported with the p-values if necessary. In Table 4, the crude ORs should also be reported with 95% CIs. Where are the geometric means reported? Was the log-transformation successful in dealing with the deviations from the normal distributions? Were the assumptions of the statistical analyses evaluated for any violations? The Discussion should address the study limitations including the limitations of the study design as an observational study, the potential residual confounding and selection bias etc.

VERSION 1 – AUTHOR RESPONSE

Reviewer 1:

C1. Abstract: “developing hypertensive disorders” should be hypertensive disorders in pregnancy”. Or if your targets were preeclampsia, this should be replaced by preeclampsia.
R1. Thank you. We have used preeclampsia instead of “hypertensive disorders” in the abstract and throughout the manuscript.

C2. Abstract: last sentence: the issue of endothelium (vascular) vs. placenta cannot be deduced from this study, especially when we consider the “two-step theory” of PE, and thus I ask you to rephrase this sentence with some appropriate sentences. I wrote this advice also later.
R2. Thank you for the suggestion. We have deleted related sentence in the Abstract (Page 3, line 13-14).

C3. Page 5: line 45 “made” should be “produced”.
R3. Thank you. We have used “produced” instead of “made” (Page 5, line 17).

C4. Page 6; line 6: If you wish to state “Asian”, please state the meaning that “there may be some racial difference”. In other words, this suggests that “preceding articles regarding this theme have already been reported but it was targeted non-Asian population. Angiogenetic-related factors are reported to show racial differences and thus we studied here this issue on “Asian” population”: one

will interpret the context as above. Please state if preceding article exits and also the context of mentioning “Asian”.
R4. Thank you. We have stated (Page 5, line 21) as follows: “Besides, evidence suggests that there might be some racial differences in maternal angiogenic and anti-angiogenic factors.” References have also been added.

C5. Discussion first paragraph: Please use past tense (not present tense) because the study has already finished. You used present tense also to the sentences that should be written in past tense elsewhere. Please reconfirm the whole manuscript.
R5. Thank you for the suggestion. We have checked carefully throughout the text.

C6. Discussion second paragraph: “Our results suggest an imbalanced angiogenic environment in early pregnancy along with chronic inflammation may injure maternal vascular function”: this study did not show cause-effect relationship between angiogenetic-factor-imbalance and endothelial dysfunction; i.e., egg-chicken relationship. Please soften the expression. You only showed that hypertensive women were more likely to show this imbalance and this imbalance tended to continue during pregnancy: that is all. You cannot deduce any conclusion as to cause-effect relationship. You only observed this “fact”.
R6. Thank you. We have rewritten the sentence in the second paragraph of Discussion (Page 18, line 17-20) as follows: “Our results showed that hypertensive women in early pregnancy might have an imbalanced angiogenic factors level and such imbalanced angiogenic environment tended to continue during pregnancy, which might be associated with the increased risks of preeclampsia.”

C7. Page 18: line 34: You too much simplify the story; your point is that “sFlt1 from endothelium and PlGF from the placenta”. Things are not so simple. You had better at least to add “as far as the data of PlGF here indicated are concerned” “When looking at this solely from the viewpoint of PlGF level during pregnancy here observed” (example). Rather, to extinguish readers’ misunderstanding, you had better soften the expression. You need not forcefully “tell some interesting story”. State things definitely based on the present findings.
R7. The point is well taken. We have changed the sentence to (Page 19, line15): “Thus, our findings seem to suggest that the placental implantation and development might not be impaired in these women.”

C8. Conclusion: As I stated above, please soften the expression. To state this pathophysiology, much more data is definitely/undoubtedly needed. Your findings themselves are worthy and do not “jump” to the pathophysiology. This holds true all through the manuscript. Please recheck if you state “too much” also elsewhere. The knack of the paper writing is that “conclusion should be modest”. If the Editor may consider that you can say it more straightforwardly, editors will advise you to do so. Once again, do not state too much.
R8. Thank you. We have deleted the sentence “suggesting that it is the vascular dysfunction, but not the placenta, that plays a critical role in the pathogenesis of preeclampsia” in Conclusion (Page 21, line 2-4) and rewritten it as follows: “Our findings suggest that the imbalanced angiogenic factors levels throughout gestation might play a crucial role in developing preeclampsia in women with preexisting elevated blood pressure.”. We also checked the manuscript whether we had overstated in other places.

C9. Please definitely state whether this was the first-time observation. Or for the first observation on “Asian” women?
R9. As far as we know, the study was the first-time observation and we have stated it (Page 20, line 7-9) as follows: “To our best knowledge, this was the first prospective cohort study that illustrated the dynamic changes of angiogenic and anti-angiogenic factors throughout pregnancy in women with different blood pressure status in early pregnancy.”

Reviewer 2:

C1. The authors found that women with high blood pressure (Hypertension stage 1 & 2) are at increased risk of developing preeclampsia (PE). It is common knowledge that women with preexisting hypertension (if hypertension is diagnosed < 20 weeks) have higher risk of developing PE. What is the additional value of this finding?
R1. Indeed, it is well established that women with preexisting hypertension have increased risks of preeclampsia but the underlying mechanisms and how the circulating sFlt-1 and PlGF levels change in preexisting hypertensive women throughout pregnancy are still unclear. Our study aimed to illustrate the dynamic changes of angiogenic and anti-angiogenic factors throughout gestation in women with high blood pressure in early pregnancy. We felt that this information may help us to sort out whether preeclampsia in these women is likely to be attributable to vascular or placental problem.

C2. The authors have performed logistic regression for blood pressure to predict the onset of PE. Given the topic of the study, it would be more interesting to determine whether the sFlt-1 levels in early pregnancy (e.g., measured at 11-14 weeks) can predict the onset of PE (and for example how this compares with the predictive value of elevated blood pressure). Why hasn't this been done? A problem might be that actually between 11-14 weeks, the sFlt-1 levels were not significantly elevated (P=0.08).
R2. Thank you. A number of studies have consistently shown that sFlt-1 level in early pregnancy alone is not a good predictor for preeclampsia (Schneuer FJ, et al. Pregnancy Hypertens. 2013;3(4):215-21; Diguisto C, et al. J Matern Fetal Neonatal Med. 2017;30 (13):1514-1519). This was confirmed in our analysis (results not shown). The purpose of this study, instead, is to illustrate the angiogenic and anti-angiogenic factors levels throughout pregnancy in women with various blood pressure status in early pregnancy. Higher levels of sFlt-1/PlGF ratio were observed during pregnancy in preexisting hypertensive women, although the sFlt-1 level itself was not significantly elevated at 11-14 weeks. This finding suggests that blood pressure levels in early pregnancy are associated with circulating angiogenic and anti-angiogenic factors levels.

C3. In the group of women with preexisting hypertension, 12 women developed PE. This means that 63 women did not develop PE, despite having ‘higher sFlt-1 levels’ (?) in early pregnancy. Where there any differences in sFlt-1 levels between the PE group in comparison with the women that did not develop PE? And what happened with these markers throughout pregnancy in the ones that did not develop PE in comparison with the ones that did develop PE? The same might be asked about the PE women in the other groups.
R3. We compared angiogenic factors between PE and non-PE in normal BP, elevated BP and hypertension groups, respectively. Please see the Supplementary Table 2. A trend of higher levels of sFlt-1/PlGF ratio throughout gestation was observed in preexisting hypertensive women who developed PE later than those who did not. Unfortunately, because the number of PE cases in each group was small, we did not have sufficient statistical power to draw a conclusion.

C4. The Discussion contains remarks about "chronic inflammation that may injure maternal vascular function", and "preexisting endothelial dysfunction playing a critical role in the development of PE" etc. None of this has been investigated here.
R4. Thank you. We have deleted related sentences in the second paragraph of Discussion (Page 18, line 20-22) and rewritten it as follows: “Our results showed that hypertensive women in early pregnancy might have an imbalanced angiogenic factors levels and such imbalanced angiogenic environment tended to continue during pregnancy, which might be associated with the increased risks of preeclampsia.” We also deleted related sentences in the Conclusion (Page 21, line 4-6) and rewritten it as follows: “Our findings suggest that the imbalanced angiogenic factors levels throughout

gestation might play a crucial role in developing preeclampsia in women with preexisting elevated blood pressure."

Reviewer 3:

C1. In the statistical analysis section, the authors describe statistical tests including ANCOVA, linear regression and logistic regression. The outcome measure for each analysis should be stated clearly. What is the advantage of using ANCOVA instead of a linear regression model? Were the variables in the ANCOVA analysis treated as predictors of the outcome measure or there was more into the ANCOVA analysis than this?
R1. We have stated each statistical analysis for the outcome in the Statistical analysis section. We used ANCOVA analysis to identify the variance of angiogenic factors levels between groups (normal BP vs. elevated BP; normal BP vs. Hypertension) and we adjusted for maternal race, smoking during pregnancy, body mass index (BMI) and gestational age at blood collection as covariant. Linear regression model was used to identify the effects of maternal blood pressure at 11-14weeks on angiogenic factors levels (Supplementary Table 1).

C2. The results of these analyses should be reported in terms of effect size and 95% CIs. For example, in Table 3, the mean difference and 95% CIs should be reported with the p-values if necessary. In Table 4, the crude ORs should also be reported with 95% CIs. Where are the geometric means reported?
R2. We have added 95%CIs to the crude ORs in Table 4. ANCOVA analysis was performed after logarithmic transformation of angiogenic factors. To demonstrate the results clearly, we provided the Supplementary Table 3. Results calculated based on the logarithm-transformed values were presented in the Supplementary Table 3, including mean difference and 95% CIs. Geometric means and 95% CIs were calculated by taking the exponent of the logarithm transformed mean and they were presented in Table 3.

C3. Was the log-transformation successful in dealing with the deviations from the normal distributions? Were the assumptions of the statistical analyses evaluated for any violations?
R3. We assessed the normality of continuous variables by the Kolmogorov-Smirnov test and angiogenic factors values were not normally distributed. After logarithmic transformation, the skewness of logarithm-transformed values was within the range of ±1. All the statistical analyses were applied when the assumptions were satisfied.

C4. The Discussion should address the study limitations including the limitations of the study design as an observational study, the potential residual confounding and selection bias etc.
R4. Thank you. We have stated as follows (Page 20, line17-18): "As it was an observational study, the potential residual confounding and selection bias might have some impacts on our results."

VERSION 2 – REVIEW

REVIEWER	Shigeki Matsubara Jichi Medical University, Japan
REVIEW RETURNED	19-Sep-2019

GENERAL COMMENTS	To authors, The authors faithfully reacted to my suggestions and incorporated all my advice into this version, which greatly improved the manuscript quality. Hypertensive women at the first trimester of

	pregnancy already showed higher sFLT1/PlGF level, which continued/exaggerated through pregnancy, making a stark contrast to those of counterparts. Occurrence of later PE increased according to the higher level of this ratio measured at the first trimester. Although how the present data is useful to the daily obstetric practice, thus, its clinical utility waits for further consideration/study, this data may contribute to better understanding this issue. I have very small suggestions. Please check them if you may have chance. 1. Page 4, line 2: “This study was based on a well-performed perspective cohort”: Is this “prospective”? Please confirm it. 2. Page 6; line2: The objective “was”: Please write all these in paste tense because the study has been finished. To editors, I read authors’ response not only to me but also other reviewers. As for me, the authors incorporated all my advice into this version. Importantly, they did soften the expression, not jumping to the conclusion or mentioning pathophysiology (that was not demonstrated in this study). I agree with their edition. Only trivial typological errors should be edited. I consider that this revised one is suitable to your journal.

REVIEWER	Jan Danser Erasmus MC, Rotterdam, The Netherlands
REVIEW RETURNED	30-Oct-2019

GENERAL COMMENTS	No further comments.

REVIEWER	Ali Khashan University College Cork
REVIEW RETURNED	23-Oct-2019

GENERAL COMMENTS	I would like to thank the authors for addressing my comments.

VERSION 2 – AUTHOR RESPONSE

Reviewer 1:

C1. Page 4, line 2: “This study was based on a well-performed perspective cohort”: Is this “prospective”? Please confirm it.

R1. Thank you. We have corrected it as “prospective” (Page 4, line 2).

C2. Page 6; line2: The objective “was”: Please write all these in paste tense because the study has been finished.

R2. Thank you. We have used “was” instead of “is” (Page 6, line 1). We also checked carefully throughout the text.

Reviewer: 2

No further comments.

Reviewer: 3

I would like to thank the authors for addressing my comments.