Author’s response to reviews

Title: Biomarker Screening for Antenatal Depression in Women Who Underwent Caesarean Section: A Matched Observational Study with Plasma Lipidomics

Authors:
Zhuoxi Wu (WUSISSI527@163.com)
Peng Zhao (66949366@qq.com)
Zhonghong Long (FANNLZH@163.com)
Jie Li (824679901@qq.com)
Guiying Yang (472151685@qq.com)
Qingling Zhang (2805604446@qq.com)
Guangyou Duan (dgy1986anesthesia@126.com)
Hong Li (lh78553@163.com)

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Author’s response to reviews:

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Dear editor

I along with my co-authors would like to re-submit the manuscript entitled “Biomarkers Screening for Antenatal Depression in Caesarean Women: A Matched Observational Study with Plasma lipidomics” to BMC Psychiatry. The previous manuscript ID is BPSY-D-18-00982R1.

Thank you for the opportunity to respond to the reviewer/editorial comments and queries. The manuscript has been carefully rechecked, and appropriate changes have been made in accordance with the reviewer/editorial comments and queries. And we have sought for a professional language editing company to improve the English usage throughout the manuscript.
QUESTION 1
Chutima Roomruangwong (Reviewer 1): Introduction: The authors did not provide sufficient rationale why they need to study in female subjects who were the "elective" caesarean cases. The literatures that they had reviewed only demonstrated the relationship of antenatal depression with poor obstetrics outcome, which finally leads to caesarean section. Thus, the elective caesarean case in this study might not be explained by the effects of antenatal depression.
Response: Sincerely thanks for the reviewer’s thoughtful comments and suggestions regarding the manuscript, and it’s our pleasure to make a response. As we know, cesarean section is one of the most commonly used ways for maternal delivery. According to the WHO survey, the global cesarean section rate was 27.3% in 2008[PMID: 20071021]. The recent data showed that the cesarean section rate in China reached 34.9% in 2014[PMID: 28030701], and the US in 2014 was 32.2% [PMID: 26114874]. It is estimated that tens of millions of women will give birth through cesarean section every year. Caesarean sections in women are often correlated with different psychological and socioeconomic conditions and involve different clinical complications compared to vaginal delivery. And previous studies have reported that the incidence of perinatal depression in cesarean section is even higher than that in women who have had vaginal deliveries [PMID: 15339755, 28112056, 21489126, 21877916]. As we know, depression disorders are associated with more severe postoperative pain, worse postoperative recovery and long-term postpartum outcomes. In addition, women with untreated antenatal depression (AD) have more than seven times risk of postpartum depression than women without AD [PMID: 27959754]. Therefore, preoperative identification of AD can be helpful for potential treatment to improve the maternal outcomes in women who have undergone cesarean section. Additionally, it is important to consider AD before a caesarean section. We have rewritten these statements in the revision manuscript. (Page6-7 , lines80-99)

QUESTION 2
Methods: What is ASA I –II ? The authors should provide the full name before abbreviations. Either "DSM-5" or "DSM-V" should be consistently use along the manuscript.
Response: We have provided the full abbreviations for ASA I - II and unify the "DSM-5" and "DSM-V" as "DSM-V" in the revision manuscript. (Page 10, lines 146; Page 7, lines 106; Page11, lines 164, 169, 172, 177; Page 12, lines 180, 182)

QUESTION 3
Discussion: (1)The potential mechanisms for this biomarker that related to AD should be more extensively review and (2)the authors should mention about the usefulness of this biomarker as a predictor of antenatal depression when compare to a standard screening method (e.g. EPDS).
Response: Thanks for the reviewer’s insightful comments. We have added a paragraph about the review of biomarkers for antenatal depression in the discussion. However, regarding the identified CS and PC in the current study, we didn’t find other previous direct evidence to explore their effects in AD according to our literature review, although some physiological trends in the depressive state have been discovered. Therefore, at present it’s difficult to make a broader review regarding the effects of these identified biomarkers in AD. Based on the current finding, we think it is meaningful to further explore the potential mechanisms for this biomarker that related to AD. We have revised these statements and
(1) DISCUSSION: Some studies have found that increase in blood concentration of 25-hydroxyvitamin D (25(OH)D [PMID: 29649128] and urine average 8-isoprostane[PMID: 30723278], were significantly associated with AD. Another study has showed that women with untreated AD had higher TNFα levels[PMID: 29862416]. Osborne S, et al. have reported that women with depression during pregnancy had raised inflammatory biomarkers (IL-6, IL-10, t TNFα and vascular endothelial growth factor) and cortisol levels[PMID: 30033161]. In addition, Bodnar LM, et al. [PMID: 22152590] indicated that some nutritional biomarkers (Essential Fatty Acids, Micronutrients, Carotenoids) may be a modifiable risk factor for antenatal depression. Although at present, some physiological trends in the depressive state have been discovered through previous studies, studies investigating potential markers for AD are still lacking. Regarding the identified CS and PC in the current study, any other previous supporting evidence regarding the biomarkers identified in the current study, are not available according to our literature review, although some physiological trends in the depressive state have been discovered. Therefore, currently it is difficult to present a comprehensive review on the effects of these identified biomarkers for AD. Based on the current findings, we think it is important to further explore the potential mechanisms of these biomarker related to AD.

(2) At present, the EPDS-10 is a widely used instrument for screening perinatal depression. But as we know, clinical diagnosis of depression depends on psychiatric interviewing by professionally trained experts. Accurate diagnosis of depression is difficult and time consuming for busy non-psychiatric clinicians, especially for obstetricians and anaesthetists prior to performing a caesarean section. Therefore, it may be helpful to diagnose AD using a similar process to that used for the prediction of gestational trophoblastic diseases (GTD) in routine clinical practices. As we know, obstetricians may be able to identify the possibility of developing GTD based on serum hCG levels with a simple and objective blood test. In a similar way, based on the current study, non-psychiatric doctors might simply and objectively predict AD according to the plasma levels of CS. Compared to the EPDS-10, this method may be more convenient and applicable for clinicians who have not undergone psychiatric diagnostic training. However, the sensitivity and specificity of the current biomarkers used for the prediction of AD in the clinical practice needs further investigation in the future.

QUESTION 4
Since the gestational age in both groups were about 38 weeks (very near term), the usefulness of this biomarker profile might not be as a predictor for AD or for management plan in pregnant women.
Response: Thanks for the reviewer’s insightful comments. In the study only women patients with full-term pregnancy were recruited at one day before caesarean section, thus whether or not the current finding can also be applicable to the other trimesters of pregnancy remains unclear. We have added it as a limitation in the revision manuscript. (Page29 , lines 421-424). Previous studies have demonstrated that antenatal depression is a neglected component of care for women in the third trimester of pregnancy[PMID: 25455248 , 24020729]. And in the third-trimester the possibility of AD is often higher than other trimesters due to more maternal sleep problems, worry about caesarean delivery and child's health[PMID: 26342890]. In addition, after admitting to the hospital caesarean women will undergo a series of preoperative examinations. We think to perform
blood screening for AD at this time might be optimized, and based on the test results immediate management plan and medical intervention for improve maternal outcome could be scheduled in advance. Therefore, we chose this time period before cesarean as the time point of assessment in the current study.

TEXT: Second, in the study only women patients with full-term pregnancy were recruited at one day before caesarean section, thus whether or not the current finding can also be applicable to the other trimesters of pregnancy remains unclear.

QUESTION 5
It is will be more valuable and very helpful if the authors could demonstrate that the biomarker could predict postpartum blues/ postpartum depression.
Response: We agree with the reviewer that it is also valuable and very helpful if the biomarker could predict postpartum blues/ postpartum depression. However, based on the results of this study, postpartum blues/postpartum depression cannot be directly predicted. But according to previous studies, it is clear that early screening and diagnosis of depression in the prenatal stage can be helpful to identify the risk of PD. One study have reported that women who have untreated AD have a risk of PD that is more than seven times that of women without AD[PMID: 27959754]. Further research is needed later to explore the association between the identified biomarker in the study and the risk of PD.

QUESTION 6
Potential treatment in the future based on the recent findings should be discussed.
Response: We have revised the discussion part according to the reviewer’s suggestion.

TEXT: Clinical diagnosis of depression depends on psychiatric interviewing by professionally trained experts. Accurate diagnosis of depression is difficult and time consuming for busy non-psychiatric clinicians, especially for obstetricians and anaesthetists prior to performing a caesarean section. But, based on the current study, non-psychiatric doctors might simply and objectively predict AD according to the plasma levels of CS. Compared to the EPDS-10, this method may be more convenient and applicable for clinicians who have not undergone psychiatric diagnostic training. However, the sensitivity and specificity of the current biomarkers used for the prediction of AD in the clinical practice needs further investigation in the future.

Reviewer 2 (Reviewer 2): PEER REVIEWER ASSESSMENTS:

OBJECTIVE - Full research articles: is there a clear objective that addresses a testable research question(s) (brief or other article types: is there a clear objective)?
Yes - there is a clear objective

DESIGN - Is the current approach (including controls and analysis protocols) appropriate for the objective?
Yes - the approach is appropriate

EXECUTION - Are the experiments and analyses performed with technical rigor to allow confidence in the results?
No - there are minor issues
STATISTICS - Is the use of statistics in the manuscript appropriate?  
No - there are issues with the statistics in the study

INTERPRETATION - Is the current interpretation/discussion of the results reasonable and not overstated?  
No - there are minor issues

OVERALL MANUSCRIPT POTENTIAL - Is the current version of this work technically sound? If not, can revisions be made to make the work technically sound?  
Probably - with minor revisions

PEER REVIEWER COMMENTS:

GENERAL COMMENTS: Potentially innovative contribution to furthering understanding of biomarkers in antenatal depression. Literature review seems thorough. A bit better description of sampling and potential limitations besides the sample sizes should be added to the manuscript. In addition some issues about statistical analyses need to be addressed.  
Response: Sincerely thanks for the reviewer’s thoughtful comments and suggestions regarding the manuscript, and it’s our pleasure to make a response. We have revised the manuscript according to the reviewer’s suggestion.

REQUESTED REVISIONS:

QUESTION 1  
Authors need to describe better the process of enrollment and reasons for exclusions given that hundreds were screened and only 33 were enrolled with AD and then matched. The flow chart clarifies the criteria used to screen and match but not how many women at each stage were excluded and for what reasons. Matching criteria are also slim.  
Response: Thanks for the reviewer’s suggestion, which can make us better explain this part of article. To better describe the screening and matching process, we have rewritten this part and remade the flowchart (Figure 1) in the revised manuscript.(Page 11-12, lines 173-188; Page 17, lines 261-264;)

TEXT:
2.3.3 Screening and matching  
Screening and matching were conducted according to a one-way process (Figure 1). After one eligible positive subject was enrolled in the AD group, one NAD subject was selected, matched to the subject with AD in terms of age ±1 year and BMI ±1 kg/m2. In subjects with an EPDS-10 ≥ 10 or a positive answer to the 10th item, the DSM-V diagnosis was performed by a psychiatric expert, subsequent to which the eligible positive subject was allocated to the AD group. In subjects with an EPDS-10 < 10 and with a negative answer to the 10th item, the DSM-V diagnosis was performed only if the subject met the matched criteria (age ±1 year and BMI ±1 kg/m2) compared to the previously included subjects in the AD group. If both the EPDS and DSM-V had negative answers, the subjects were allocated to the Non-AD group. Cases with unsuccessful matches were considered invalid and were not used for matching in subsequent cases. The inclusion of subjects was terminated after the number of eligible matched subjects in both the AD group and the NAD group reached 33. Peripheral blood samples were collected only from the patients who were finally included in the study.
3.1 General results  
A total of 484 pregnant women were screened in the study; 246 cases who presented with complications were excluded, and 172 cases were excluded because their age and BMI did not match
the criteria for inclusion in the AD group, according to the study design.

QUESTION 2
In the table the percentages under quality of marriage also are confusing. At the very least the discussion should address other factors that might account for different lipid profiles that could confound the findings.
Response: Thanks for the reviewer’s suggestion, which can make us better explain this part of article. We have added the related descriptions regarding this part of results and added new table 4 in the revision manuscript. (Page 16, lines 251-256; Page 22, lines 319-323; Page 26, lines 381-386)

TEXT:
2.7 Statistical analysis
Then, an exploratory multiple regression analysis using the entire model was performed to investigate whether the main identified biomarker was affected by the clinical characteristics; age, BMI and EPDS; educational level (9 to 12 years = 1, over 12 years = 2), marital relationship (Bad = 1, General = 2, Good = 3) and sleep quality (Very poor – poor = 1, General = 2, Good -very good = 3) were regarded as independent variables of the main identified biomarker.

3.3 Identification biomarker for antenatal depression
To investigate whether CS is affected by clinical characteristics of the pregnant women, we performed an additional exploratory multiple regression analysis using the entire model. The results showed that age (P=0.055, approximate statistical significance), marital relationship (P=0.027), and EPDS (P < 0.001) significantly influenced plasma CS levels (Table 4).

4. Discussion
Via additional multiple regression analyses, we found that marital relationship and EPDS also have an effect on the plasma levels of CS. Age [PMID: 22882967] and marital relationships [PMID: 26650969] have been demonstrated to be risk factors for AD. Combined with the results that CS plasma levels were positively correlated with AD in this study, these analyses further suggest that plasma CS levels could be affected by high risk factors for AD and this might help us better understand the effects of CS in AD

QUESTION 3
Also, the reasons in the intro for need for biomarkers (e.g. inadequate clinical opinion) should be modified somewhat as patient-reported screening instruments have shown to be valid and reliable.
Response: Thanks for the reviewer’s thoughtful comments and suggestions. We have revised this part in the introduction of revision manuscript. (Page 7-8, lines 100-117)

TEXT: Due to the lack of a laboratory diagnostic strategy [PMID: 10814765], dentification of AD often needs comprehensive clinical interviewing by a psychiatry expert [PMID: 26180865] through a subjective judgment based on the clinical symptoms, behaviour, and psychological characteristics, etc. At present, the 10-item Edinburgh postpartum depression (EPDS-10) scales [PMID: 3651732, 25932866, 20974776] are commonly used in AD screening and the diagnostic criteria mainly depends on the American Psychiatric Association’s Diagnostic and Statistical Manual of Mental Disorders, fifth edition(DSM-V) [PMID: 29395259]. However, it is difficult for a non-psychiatric doctor such as an obstetrician and an anaesthesiologist to accurately diagnose depression without the necessary long term professional training [PMID: 17968628, 11354234]. A credible meta-analysis [PMID: 19640579]
showed that the rate of false positives would surpass that of true positives by about 50% in primary care practices if the diagnosis of depression were performed by general practitioners (non-psychiatrists). One study indicated that diagnosis of AD is difficult because the physiological signs of pregnancy overlap with the symptoms of AD [PMID: 17535161]. In addition, not all antenatal-depressed individuals voluntarily and willingly manifest emotional symptoms. Furthermore, in clinical practice, obstetricians and anaesthesiologists often pay little attention to AD before a caesarean delivery. Therefore, it is necessary to find an objective, fast and practical indicator to help clinicians identify or predict AD in their daily clinical practices.

QUESTION 4
The reverse association of biomarker to self-reported depression needs to be considered. What percent of patients with the identified 'biomarker' are not depressed? What is the predicted sensitivity and specificity of such use of biomarkers?
Response: Thanks for the reviewer’s comments. In our study, the sensitivity and specificity of CS have been calculated from the cut-off values according to the Yoden index (Page21, lines301-304). However the sensitivity and specificity of direct usage of the current cut-off values in prediction of AD in clinical practice needs further study in the future. We have added calculations for false positive and false negative rates, and the results are shown in the figure below.

<table>
<thead>
<tr>
<th></th>
<th>EPDS (+)</th>
<th>EPDS (-)</th>
<th>Sum</th>
</tr>
</thead>
<tbody>
<tr>
<td>CS (+)</td>
<td>34</td>
<td>11</td>
<td>45</td>
</tr>
<tr>
<td>CS (-)</td>
<td>0</td>
<td>21</td>
<td>21</td>
</tr>
<tr>
<td>Sum</td>
<td>34</td>
<td>32</td>
<td>66</td>
</tr>
</tbody>
</table>

Sensitivity =100%
Specificity =65.625%
False negative =0
false positive =34.375%
Accuracy =83.33%

QUESTION 5
Can the authors also analyze the strength of the association depending on the level of self-reported depression (e.g. very high scores on the EPDS vs lower) or suggest that as other steps to study (given the limited sample size in this study).
Response: Thanks for the reviewer’s suggestion. We agree with the reviewer that it would be meaningful if such analysis could be done. However, our study was designed as a one-to-one match research between the two groups. The cases in Non-AD group with unsuccessful match according to age and BMI were excluded and did not perform the DSM-V diagnosis. Therefore, although these patients were screened using EPDS, the final diagnosis was not performed by the psychologist. This will make significant bias and it is infeasible to perform such analysis in the current study.

QUESTION 6
Authors also need to describe how they selected variables for the logistic regression.
Response: Thanks for the reviewer’s suggestion. In order to observe which differential lipid has the greatest effect on AD, we performed a Conditional Logistic regression analysis. The total number of included cases in this study was 66. Because ten times of subjects compared to number of included
independent variables are needed based on the sample size principle for logistic regression analysis, 6 factors could be selected in the current regression model. Thus we chose the top six identified biomarkers with the maximum area under the curve in the previous ROC analysis as independent variables. (Page16, lines246-250)

TEXT:
2.7 Statistical analysis
Conditional Logistic regression analysis for antenatal depression was also performed. The total number of included cases in this study was 66. Because ten times of subjects compared to number of included independent variables are needed based on the sample size principle for logistic regression analysis, 6 factors could be selected in the current regression model. Thus we chose the top six identified biomarkers with the maximum area under the curve in the previous ROC analysis as independent variables.

QUESTION 7
Further, p-value comparisons for 35 variables should be Bonferonni corrected as there is room for Type 2 error.
Response: Thanks for the reviewer’s suggestion. We have added Bonferonni corrected into table 2. As we know, Bonferonni correction is an applicable but very strict method of correcting the P value. It reduces the chance of making a type I error while reducing type II errors. The current study is designed as an exploratory study for finding of potential biomarkers of AD. Thus we have adjusted P value using the Benjamini–Hochberg procedure with the critical false discovery rate (FDR) set to 0.05, and we didn’t use Bonferonni correction. We have added the correction P values in the Table 2 and also added the related description in the revised manuscript. (Page 15, lines 230-234).

TEXT: Then, we adjusted the P-value of the T-test using the Benjamini–Hochberg procedure with the critical false discovery rate (FDR) set at 0.05. The reference standard for screening is VIP > 1 and an FDR < 0.05. Bonferroni correction with P values multiplied 35 times are also presented in table 2.

QUESTION 8
ADDITIONAL REQUESTS/SUGGESTIONS:
Manuscript could use some English language editing.
Response: Sincerely thanks for the reviewer’s suggestions regarding the manuscript, and we have sought for a professional language editing company to improve the English usage throughout the manuscript.

QUESTION 9
Also, some general references to the development of biomarkers are used to support the specific link of biomarkers to depression and after checking some of these references did not specifically support the points being made in the text (eg, 24-27) and some others.
Response: Thanks for the reviewer’s comments regarding the references, we have revised corresponding descriptions in the revised manuscript. (Page 8, lines 118-126)

TEXT: A ‘biomarker’ is defined as a specific biomolecule that is objectively measured and evaluated as an indicator of normal biological and pathogenic processes or pharmacological responses to a therapeutic intervention [PMID: 11240971]. The National Institute of Mental Health (NIMH) Research Domain Criteria (RDoC) has set the discovery of biomarkers as a priority for clinical research [PMID:
26877115]. Objective biomarkers that can be measured externally have been demonstrated to be good predictors of the personalized diagnosis and treatment of depression [PMID: 11240971, 20978388]. For this purpose, metabolomics is currently a viable and being widely used method for finding biomarkers for diseases including depression[PMID: 18843269].

We thank you and the reviewers again for your thoughtful suggestions and insights. We hope that the revised manuscript is now suitable for publication in your journal.

I look forward to your reply.

Sincerely,
Hong Li, Director, Department of Anesthesiology, Xinqiao Hospital, Third Military Medical University, Chongqing,400037, China.
Telephone: (+86)13608380123; Email: lh78553@163.com.