PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	Serum hepcidin and iron status parameters in pregnant women and the association with adverse maternal and foetal outcome: a study protocol for a prospective cohort study
AUTHORS	Amstad Bencaiova, Gabriela; Vogt, Deborah; Hoesli, Irene

VERSION 1 – REVIEW

REVIEWER	Olof Stephansson Karolinska Institutet, Sweden
REVIEW RETURNED	24-Jun-2019

GENERAL COMMENTS	¬¬¬Review of manuscript BMJ_Open_2019-032280 Thank you for the opportunity to review this study protocol. This is a study protocol for a prospective Swiss observational study of obese and non-obese controls on the association between serum hepcidin and adverse maternal and neonatal outcomes. General comments 1. With a sample size of less than 200 pregnant women the study has the power to analyse the primary endpoint hepcidin. However, many of the secondary outcomes are rare and thus it is not likely that they will be able to be studied. I would recommend the authors to revise secondary outcomes of the study or increase the number of study participants. Specific comments 1. In Methods and analysis, the primary endpoint is not the same as the primary objective. Please rephrase. 2. It is not clear how all the variables for the study will be collected. From the medical record system? Who will retrieve the information? 3. Do all women deliver in the same unit? Do all women attend the same antenatal care?

REVIEWER	Dr. Manisha Nair University of Oxford, UK
REVIEW RETURNED	29-Jul-2019

GENERAL COMMENTS	This will be an interesting study and I will look forward to reading the results. A few comments - Methods: Primary objective - states the primary endpoint, not objective - please correct this. Study design - This is not clear. Can the author please provide the research questions? If the author is interested in simply looking at the difference between Hepcidin level between obese and non-obese women at 11-14 weeks of gestation, then the study is simply a cross-sectional study with two groups, obese and non-obese. However, the author presents a large number of secondary

	objectives, some of which in my opinion are important - example change in Hepcidin during pregnancy and its association with blood iron markers. Since the author is following up a cohort of women, the design becomes more like a prospective cohort study with two population groups - obese and non-obese. The authors are assessing multiple exposures in each group and will be following up the cohort until delivery to assess outcomes. Inclusion/ exclusion criteria - will parity be a criterion - will you recruit both primi and multi? If multiparous women are included - any thoughts on history of previous pregnancy complications? Sample size calculations are based on the primary objective of finding a statistically significant difference in Hepcidin level between obese and non-obese pregnant women at 11-14 weeks. Can the author comment on whether the expected difference of 4.0 ng/ml is a clinically significant difference as well? I am worried about the secondary analyses. The author is proposing to analyse 15 different maternal outcomes and 12 neonatal outcomes with just 169 expected participants divided into 84 in each of two groups - obese and non-obese. This could result in bias due to multiple testing and the author is likely to find significant results just by chance. Further, I believe that the incidence of the proposed outcomes is low in the study setting, which will further create problems with study power. Thus, the author needs to explain how they will address both plausible Type-I and Type-II errors? Discussion - in the last paragraph on page 23, the author writes, 'We expect that the usefulness of hepcidin in the early detection of women with an increased risk of developing maternal and foetal complications could be confirmed by our prospective cohort study'. Why is this mentioned as a prospective study in the discussion, but not in the study design section? The claims about clinical and public health impacts from this study should be balanced by discussing the limitations related to the secondary objectives. There is no doubt that the proposed secondary objectives are important, but this study is unlikely to be able to measure these with rigour since it is nor powered/ designed to do so. It can lead to hypothesis generation, but is not likely to impact clinical and public health pathways/ policies. Please include a STROBE checklist as per journal requirement. A few typos in the discussion section - not many.

VERSION 1 – AUTHOR RESPONSE

Reviewer 1:

1) With a sample size of less than 200 pregnant women the study has the power to analyse the primary endpoint hepcidin. However, many of the secondary outcomes are rare and thus it is not likely that they will be able to be studied. I would recommend the authors to revise secondary outcomes of the study or increase the number of study participants.

The study has been powered in order to show a difference in the primary endpoint (serum hepcidin in first trimester) between obese and non-obese women. We agree that secondary analyses should per se not be interpreted as confirmatory but rather as hypothesis generating. We would like to point out that we will investigate two composite event outcomes: a) adverse maternal outcome and b) adverse neonatal outcome. The first one includes either or several of the following outcomes: pre-eclampsia, gestational hypertension, GDM, cholestasis of pregnancy, peripartal haemorrhage, infections, anaemia and placenta abruption. Hence, although some of these outcomes are rare, we expect the number of the composite 34 (20% of women) to be sufficient for logistic regression analyses.

Accordingly, the second event outcome b) adverse neonatal outcome is the composite including either or several of preterm birth, IUGR, PPROM, macrosomia, Apgar score < 7 at 5, still birth and neonatal death. Here we expect a number of 34 events (20% of children) for the composite endpoint. We further account for the relatively large number of predictors by using a forward model selection approach. An alternative approach might be to use LASSO. These specifics will be determined in detail in the statistical report and analysis plan which will be finalised before data base closure.
As a side comment, we would like to mention that the sample size will be 169 at least, as sample size might be increased based on the blinded sample size re-estimation. We added and corrected this point in the Strengths and Limitations section as well as in Discussion.
2) In Methods and analysis, the primary endpoint is not the same as the primary objective. Please rephrase.
It was corrected.
3) It is not clear how all the variables for the study will be collected. From the medical record system? Who will retrieve the information?
We added this information in data collection section, page 15.
4) Do all women deliver in the same unit? Do all women attend the same antenatal care?
All women will deliver in the same unit and attend the same antenatal care. See Study setting, page 8.

Reviewer 2:
1) Primary objective - states the primary endpoint, not objective - please correct this.
It was corrected.
2) Study design - This is not clear. Can the author please provide the research questions? If the author is interested in simply looking at the difference between Hepcidin level between obese and non-obese women at 11-14 weeks of gestation, then the study is simply a cross-sectional study with two groups, obese and non-obese. However, the author presents a large number of secondary objectives, some of which in my opinion are important - example change in Hepcidin during pregnancy and its association with blood iron markers. Since the author is following up a cohort of women, the design becomes more like a prospective cohort study with two population groups - obese and non-obese. The authors are assessing multiple exposures in each group and will be following up the cohort until delivery to assess outcomes.
Thank you for pointing this out. Indeed, our primary analysis is the comparison of serum hepcidin levels at a fixed timepoint between obese and non-obese women. Considered in isolation, this is clearly a case-control situation. However, as the reviewer points out, women are followed until delivery and several outcomes (e.g. biomarkers) are measured repeatedly, as we are also interested in the time course of hepcidin and other biomarkers (secondary outcomes). Hence our study design is a prospective cohort study with a planned comparison of obese and nonobese women. We have adapted the study title accordingly.
3) Inclusion/ exclusion criteria - will parity be a criterion - will you recruit both primi and multi? If multiparous women are included - any thoughts on history of previous pregnancy complications?
We will recruit both primipara and multipara. To our knowledge, parity does not have an influence on hepcidin level.
4) Sample size calculations are based on the primary objective of finding a statistically significant difference in Hepcidin level between obese and non-obese pregnant women at 11-14 weeks. Can the author comment on whether the expected difference of 4.0 ng/ml is a clinically significant difference as well? I am worried about the secondary analyses. The author is proposing to analyse 15 different maternal outcomes and 12 neonatal outcomes with just 169 expected participants divided into 84 in each of two groups - obese and non-obese. This could result in bias due to multiple testing and the author is likely to find significant results just by chance. Further, I believe that the incidence of the proposed outcomes is low in the study setting, which will further create problems with study power. Thus, the author needs to explain how they will address both plausible Type-I and Type-II errors?

This is an important point, and we would like to refer to our answer to reviewer 1 who raised the same concerns. Since indeed certain events are rare, we will analyse the two composite outcomes adverse maternal and adverse neonatal outcome. Other secondary outcomes are continuous variables which should be available for each participant at each measurement time (e.g. serum ferritin). Here we plan to describe the time course (visualisation and summary statistics) and use linear mixed effects models, accounting for repeated measurements, in order to test for a difference between obese and non-obese women. We are aware that any p-values resulting from these tests should not be interpreted as confirmative (significant vs non-significant effect) but can nevertheless be useful in identifying hypotheses worth of further investigation. We have added a corresponding sentence to the manuscript (in the Strengths and Limitations section, Sample Size, Discussion). We added a clinically significance of hepcidin difference in Sample Size section, page 14.

5) Discussion - in the last paragraph on page 23, the author writes, 'We expect that the usefulness of hepcidin in the early detection of women with an increased risk of developing maternal and foetal complications could be confirmed by our prospective cohort study'. Why is this mentioned as a prospective study in the discussion, but not in the study design section?

It was added in study design section.

6) The claims about clinical and public health impacts from this study should be balanced by discussing the limitations related to the secondary objectives. There is no doubt that the proposed secondary objectives are important, but this study is unlikely to be able to measure these with rigour since it is nor powered/ designed to do so. It can lead to hypothesis generation, but is not likely to impact clinical and public health pathways/ policies.

We are grateful for this comment and following the suggestion of the reviewer we corrected and rewrote this part in the discussion.

7) Please include a STROBE checklist as per journal requirement.

A STROBE checklist is included.

8) A few typos in the discussion section - not many.

Typos in the discussion section were corrected.

VERSION 2 – REVIEW

REVIEWER	Olof Stephansson Karolinska Institutet, Sweden
REVIEW RETURNED	06-Sep-2019

GENERAL COMMENTS	The authors have addressed my comments in the revised manuscript. No additional questions.

REVIEWER	Manisha Nair University of Oxford, UK
REVIEW RETURNED	13-Sep-2019

GENERAL COMMENTS	The authors have considered my comments and have incorporated my suggestions. I have no further queries or concerns. I wish the authors the very best of luck with their study.