NOTICE: This document contains correspondence generated during peer review and subsequent revisions but before transmittal to production for composition and copyediting:

- Comments from the reviewers and editors (email to author requesting revisions)
- Response from the author (cover letter submitted with revised manuscript)*

**The corresponding author has opted to make this information publicly available.*

Personal or nonessential information may be redacted at the editor's discretion.

Questions about these materials may be directed to the *Obstetrics & Gynecology* editorial office: obgyn@greenjournal.org.

Date: 04/17/2023
To: "Mona Prasad" [redacted]
From: "The Green Journal" em@greenjournal.org
Subject: Your Submission ONG-23-441

RE: Manuscript Number ONG-23-441

Risk Factors for Perinatal Transmission of Hepatitis C Virus (HCV): A prospective observational study

Dear Dr. Prasad:

Thank you for sending us your work for consideration for publication in Obstetrics & Gynecology. Your manuscript has been reviewed by the Editorial Board and by special expert referees. The Editors would like to invite you to submit a revised version for further consideration.

If you wish to revise your manuscript, please read the following comments submitted by the reviewers and Editors. Each point raised requires a response, by either revising your manuscript or making a clear argument as to why no revision is needed in the cover letter.

To facilitate our review, we prefer that the cover letter you submit with your revised manuscript include each reviewer and Editor comment below, followed by your response. That is, a point-by-point response is required to each of the EDITOR COMMENTS (if applicable), REVIEWER COMMENTS, and STATISTICAL EDITOR COMMENTS (if applicable) below. The revised manuscript should indicate the position of all changes made. Please use the "track changes" feature in your document (do not use strikethrough or underline formatting). Upload the tracked-changes version when you submit your revised manuscript.

Your submission will be maintained in active status for 21 days from the date of this letter. If we have not heard from you by 05/08/2023, we will assume you wish to withdraw the manuscript from further consideration.

EDITOR COMMENTS:

Please note the following:

Thank you for your submission to the Green Journal. The manuscript underwent peer review and review by the Statistical Editor. Significant concerns were raised at the Editorial Board discussion about its validity and interpretability as the planned sample size was not reached and the high rate of individuals who were lost follow-up or do not have outcome assessment introduces bias. The Journal is unable to publish the manuscript in its current form given these concerns, but is willing to consider a condensed version of the results in the form of a Research Letter.

If you do accept the opportunity to revise as a Research Letter, please include Figure 2 and Table 2 as the Letter's two exhibits. You can use the Supplemental Materials to include the remainder figures, if desired, and a more detailed description of the methods. Please address all of the Statistical Editor's points and incorporate the Reviewers' comments if/when they are relevant to the more condensed version. The Discussion section should highlight the limitations of this data and how it potentially affects the interpretation of the estimates and the generalizability.

* Help us reduce the number of queries we add to your manuscript after it is revised by reading the Revision Checklist at https://journals.lww.com/greenjournal/Documents/RevisionChecklist_Authors.pdf and making the applicable edits to your manuscript.

* Figures 1-3: Please upload as figure file on Editorial Manager.

REVIEWER COMMENTS:

STATISTICAL EDITOR COMMENTS:
General: The most serious limitation to this analysis is the loss to follow-up, especially among the patients identified as viremic (actual = 314 out of 548) lines 158, 164. This limits both the precision and potentially the accuracy of the estimates of maternal-infant HCV transmission. Should include both estimates of transmission rates (lines 170-171 and

173), but with appropriate caveat re: missing data.

The Letter should include Fig 2 and Table 2. Remainder of Tables and Figures could be supplemental material, as desired.

For Table 2, it should include in footnote the variables included in the final model (I presume the first two variables listed.)
For Figure 2, was there a stats test for the difference in titer distributions shown by the box-plots? If so, should include in figure legend.

Again, related to the missing data re: viremic patients, it is reasonable to surmise that an increased viral load is associated with higher probability of maternal-infant transmission, but the estimation of a precise threshold from these data is fraught. Please do not make a generalized claim about what threshold is associated with transmission risk. Although 20/26 instances of perinatal transmission occurred with HCV RNA titer > 10^6 IU/mL, among 148 instances of such titers, only 20/128 resulted in transmission. Thus, sensitivity > specificity, but each imperfect and limited by the missing data. Might be informative to include in supplemental a Table similar to present Table 2, but only including the viremic patients.

Reviewer #1:
This is a prospective, multicenter observational study including pregnant women with antenatal HCV testing and available neonatal screening data, between 2012 and 2018 in the United States (45 hospitals, NICHD MFMU Network and HIV Network).
Primary outcome of the study is HCV perinatal transmission, assessed with viremia (HCV RNA) and antibody testing in the neonates at two time-points.

Positive antenatal HCV serology was identified in 1.1% of pregnant women. Only 63% of HCV-positive women enrolled in the study, and only 56% of the enrolled women (35.3% of the HCV-positive women) had data available to assess the primary outcome.

I agree with the authors that is an extremely relevant topic, for which generation of new data is substantial to guide future studies and recommendations.
A few observations to improve the manuscript:
- Only 35.3% of HCV-positive women were assessed; this is a limitation and needs to be addressed adequately.
- Patients were enrolled over a period of 7 year, starting in 2012, more than 10 years ago; this needs to be addressed.
- Vaginal bleeding as risk factor: were only those episodes requiring hospitalizations considered or all episodes of vaginal bleeding prior to delivery?
- Provide a more in-depth description of the 26 HCV-positive neonates and their mothers' pregnancy course and data (possibly a Table)

Reviewer #2:
1. Consider different term than "perinates" in line 37.

2. The authors report "no threshold of viremia necessary for transmission has been established (lines 45-46)." This does not appear to be accurate as several studies have proposed threshold of 10^log6. Please refer to these historic studies.

3. Would recommend including lines 168-169 sooner in the results section. Additionally, when authors report "the study population" in line 168 does this refer to n=432 (viremia patients only)? Please clarify

4. Overall, authors should simplify the first 4 paragraphs (lines 144-167). Figure 1 details most of this commentary.

5. Additionally, lines 157-163 should come after the entire study population is described. Recommend completely summarizing the final number of included patients before providing details regarding lab tests across pregnancy.

6. Were lower viral loads that resulted in perinatal transmission associated with vaginal bleeding? In the absence of bleeding would the cutoff be different? This would be helpful to know.

7. The authors contend that "in the absence of viremia transmission does not occur" in lines 197-198. However, one case of transmission had an "indeterminant" viremia. Authors also note " 25/26 infants were born to viremia participants (lines 71-72).

8. Additionally, was the lowest viral load associated with transmission "12,244 (line 203) or was the value "indeterminate (line 172).

9. It does not appear that this study was powered to comment on secondary factors influencing perinatal transmission like "internal monitoring, prolonged rupture, duration of membrane rupture >6hrs" (lines 208-210). I do not think the authors should confidently conclude that labor management does not have to be changed based on this study alone. Please adjust language.

10. Overall, the authors do not assess their results in the context of what has been found or shown in prior studies at any point in the discussion. I urge the authors to assess aspects of their results such as antepartum bleeding, viremia and labor management recommendations in the context of what other large studies have shown- even if they are studies from other countries.

11. In strengths and limitations authors allude to their 44% loss to follow up rate. This manuscript could be strengthened by providing some suggestions for improving follow up for future prospective studies.

12. Table 1 reveals that the majority of included patients were unemployed ~70% and ~85% had public insurance. These attributes suggest many potential barriers to follow up (e.g. socioeconomic burden of transportation, childcare, etc). We participants given incentives (financial or otherwise)? Might this have improved retention?

13. Before undertaking this study were patients with HCV queried about the follow up plan and its feasibility? Was their a patient advisory board or qualitative component that informed this study design?

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Sincerely,
Mark A. Clapp, MD, MPH

The Editors of Obstetrics & Gynecology

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June 5, 2023

Dear Editors,

We respectfully submit revisions to the previously reviewed manuscript: Risk Factors for Perinatal Transmission of Hepatitis C Virus (HCV): A prospective observational study, NCT01959321, for consideration for publication in *Obstetrics and Gynecology*. As you are aware, this multicenter study was Institutional Review Board approved by each participating center. All listed authors have made significant contributions to the design, data acquisition, analysis and/or manuscript development for the study. Permission has been received to include those in our acknowledgements. A portion of this work was presented as an oral plenary at the virtual Society for Maternal-Fetal Medicine Annual Meeting on February 3, 2022.

Below you will find a summary of the editorial comments and our responses. We hope you find them satisfactory, and that this manuscript is deemed acceptable for publication.

Sincerely,

Mona Prasad DO MPH for the *Eunice Kennedy Shriver* NICHD Maternal Fetal Medicine Units Network.

EDITOR COMMENTS:

Please note the following:

Thank you for your submission to the Green Journal. The manuscript underwent peer review and review by the Statistical Editor. Significant concerns were raised at the Editorial Board discussion about its validity and interpretability as the planned sample size was not reached and the high rate of individuals who were lost follow-up or do not have outcome assessment introduces bias. The Journal is unable to publish the manuscript in its current form given these concerns, but is willing to consider a condensed version of the results in the form of a Research Letter.

Response:

We acknowledge that the lost follow-up and sample size were not ideal in this undertaking. However, we do feel that this is the best data that exists regarding an inherently difficult population to study. Outside of a research setting, 6% of this population is followed appropriately when diagnosed with HCV.

At the time of design of this study, such information was not available to inform sample size and feasibility calculations, and we learned in the process of research the nuances of following this population. As we were able to complete 18 month follow up in 56% of participants, when compared to the background likelihood of follow-up, we assert that this represents the best available data regarding the management of this high-risk population. To that end, we request the manuscript is reconsidered for Original Rather than a Research Letter.

We have included the referenced 6% follow up in the discussion and elaborated on loss to follow up more distinctly in the discussion. We have included comments regarding improving study design in future trials as well, as suggested by Dr. Clapp. The feedback was well taken, and it is our hope that this response is satisfactory. The response to editorial comments that reference line numbers are line numbers referenced in the tracked changes version.

REVIEWER COMMENTS:

STATISTICAL EDITOR COMMENTS:
General: The most serious limitation to this analysis is the loss to follow-up, especially among the patients identified as viremic (actual = 314 out of 548) lines 158, 164. This limits both the precision and potentially the accuracy of the estimates of maternal-infant HCV transmission. Should include both estimates of transmission rates (lines 170-171 and 173), but with appropriate caveat re: missing data.

Response:

We will be addressing this more specifically in the discussion to reflect this caveat. Please see line 213-215.

The Letter should include Fig 2 and Table 2. Remainder of Tables and Figures could be supplemental material, as desired.

Response:

This comment is not applicable, as we are hopeful to publish as Original Research.

For Table 2, it should include in footnote the variables included in the final model (I presume the first two variables listed.)

Response:

The footnote is revised as follows: Adjusted model included HCV RNA titer and any antepartum bleeding. See Table 2.

For Figure 2, was there a stats test for the difference in titer distributions shown by the box-plots? If so, should include in figure legend.

Response:

We did not perform a test to compare titers between perinatal transmission status because this was not truly a continuous variable (i.e. lower limit of quantitation). We did compare titer comparisons as categorical (>10^6 IU/mL).

Again, related to the missing data re: viremic patients, it is reasonable to surmise that an increased viral load is associated with higher probability of maternal-infant transmission, but the estimation of a precise threshold from these data is fraught. Please do not make a generalized claim about what threshold is associated with transmission risk. Although 20/26 instances of perinatal transmission occurred with HCV RNA titer > 10^6 IU/mL, among 148 instances of such titers, only 20/128 resulted in transmission. Thus, sensitivity > specificity, but each imperfect and limited by the missing data. Might be informative to include in supplemental a Table similar to present Table 2, but only including the viremic patients.

Response:

We have updated the discussion to address these concerns. Due to constraints and the ask for another supplemental table, we have not pursued the table suggested above. Attempt to address this query is in lines 229-232.

Reviewer #1:
This is a prospective, multicenter observational study including pregnant women with antenatal HCV testing and available neonatal screening data, between 2012 and 2018 in the United States (45 hospitals, NICHD MFMU Network and HIV Network).
Primary outcome of the study is HCV perinatal transmission, assessed with viremia (HCV RNA) and antibody testing in the neonates at two time-points.

Positive antenatal HCV serology was identified in 1.1% of pregnant women. Only 63% of HCV-positive women enrolled in the study, and only 56% of the enrolled women (35.3% of the HCV-positive women) had data available to assess the primary outcome.

I agree with the authors that is an extremely relevant topic, for which generation of new data is substantial to guide future studies and recommendations.
A few observations to improve the manuscript:

- Only 35.3% of HCV-positive women were assessed; this is a limitation and needs to be addressed adequately.

Response:

We agree with this limitation and will address in the text as well. Only 35.3% were enrolled and able to be followed up. Patients initial blood testing was for screening only, and if screened positive, they were approached for enrollment. We experienced a high rate of loss at the enrollment stage due to refusal of

consent and unwillingness to commit to follow up. These were reasons for loss to follow up after enrollment as well. Please see lines 264-268 for attempt to clarify.

- Patients were enrolled over a period of 7 year, starting in 2012, more than 10 years ago; this needs to be addressed.

Response:

We agree that this requires more discussion and will address in text as well. It took significant time to enroll this cohort which was unexpected given the prevalence of HCV anticipated with the coexisting opioid epidemic. The MFMU is considered to be a generalizable population, but this cohort was not easily ascertained by this network, for reasons we do not fully understand. The length of time it took to identify and enroll our cohort exceeded our anticipated timeline significantly, which impacted our feasibility and acquisition of optimal sample size. Please see lines 262-265 to attempt to clarify.

- Vaginal bleeding as risk factor: were only those episodes requiring hospitalizations considered or all episodes of vaginal bleeding prior to delivery?

Response:

Any bleeding prior to delivery was captured as a risk factor. This is defined in methods.

- Provide a more in-depth description of the 26 HCV-positive neonates and their mothers' pregnancy course and data (possibly a Table)

Response:

We have included a descriptive table. If useful it can be included. If not useful, we can exclude it. Please see table 3.

Reviewer #2:
1. Consider different term than "perinates" in line 37.

Response:

We agree and will change to children.

2. The authors report "no threshold of viremia necessary for transmission has been established (lines 45-46)." This does not appear to be accurate as several studies have proposed threshold of 10^log6. Please refer to these historic studies.

Response:

We agree with the interpretation of the existing literature. Our intent at the outset was to identify a threshold characterized as a continuous rather than a categorical variable, which is why we reported it as such. This assertion will be revised. Please note change in line 45.

3. Would recommend including lines 168-169 sooner in the results section. Additionally, when authors report "the study population" in line 168 does this refer to n=432 (viremia patients only)? Please clarify

Response:

Regarding lines 144-169: We agree that this section can be made clearer. We will reformat. Please see reorganization lines 162-175.

4. Overall, authors should simplify the first 4 paragraphs (lines 144-167). Figure 1 details most of this commentary.

Response:

Regarding lines 144-169: We agree that this section can be made clearer. We will reformat. Please see reorganization lines 162-175.

5. Additionally, lines 157-163 should come after the entire study population is described. Recommend completely summarizing the final number of included patients before providing details regarding lab tests across pregnancy.

Response:

Regarding lines 144-167: We agree that this section can be made clearer. We will reformat. Please see reorganization lines 162-175.

6. Were lower viral loads that resulted in perinatal transmission associated with vaginal bleeding? In the absence of bleeding would the cutoff be different? This would be helpful to know.

Response:

Our statisticians did check for interactions and there was **no significant interaction between viral load and vaginal bleeding** (p=0.47) for MTCT

7. The authors contend that "in the absence of viremia transmission does not occur" in lines 197-198. However, one case of transmission had an "indeterminant" viremia. Authors also note " 25/26 infants were born to viremia participants (lines 71-72).

Response:

We agree that this could be clarified, and have changed the language to demonstrate this. Please see lines 216 for clarification.

8. Additionally, was the lowest viral load associated with transmission "12,244 (line 203) or was the value "indeterminate (line 172).

Response:

We agree that this could be clarified, and have changed the language to demonstrate this. Please see lines 203-204 for clarification.

9. It does not appear that this study was powered to comment on secondary factors influencing perinatal transmission like "internal monitoring, prolonged rupture, duration of membrane rupture >6hrs" (lines 208-210). I do not think the authors should confidently conclude that labor management does not have to be changed based on this study alone. Please adjust language.

Response:

We understand the concern and will adjust the language. Please see lines 239-243 for clarification.

10. Overall, the authors do not assess their results in the context of what has been found or shown in prior studies at any point in the discussion. I urge the authors to assess aspects of their results such as antepartum bleeding, viremia and labor management recommendations in the context of what other large studies have shown- even if they are studies from other countries.

Response:

We will update the discussion to make this context more clear. Please see line 239-240. Have added a reference as well.

11. In strengths and limitations authors allude to their 44% loss to follow up rate. This manuscript could be strengthened by providing some suggestions for improving follow up for future prospective studies.

Response:

We agree that this is one of the biggest lessons learned from this study. Improving follow up could be accomplished by seeking understanding of barriers, creating incentives that uniquely address the barriers, and a patient advisory board at the outset to help craft the work more specifically to this population. We have attempted to address throughout the discussion, lines 269-278.

12. Table 1 reveals that the majority of included patients were unemployed ~70% and ~85% had public insurance. These attributes suggest many potential barriers to follow up (e.g. socioeconomic burden of transportation, childcare, etc). We participants given incentives (financial or otherwise)? Might this have improved retention?

Response:

Patients were incentivized financially, and each site chose their incentives specifically. Will incorporate this information into the revision because this would be a place that could have been enhanced to improve retention. See lines 269-278.

13. Before undertaking this study were patients with HCV queried about the follow up plan and its feasibility? Was their a patient advisory board or qualitative component that informed this study design?

Response:

All patients were informed of the follow up plan at the outset of the study. We had clues that formal follow up would be difficult in this setting with our lack of ability to enroll people at the outset. Similarly, follow up was difficult with this population. We did not have a patient advisory board in this study design, but I will suggest it for future studies because you raise a very good point. A better understanding of barriers could help us address follow up. Please see lines 269-278.

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Sincerely,
Mark A. Clapp, MD, MPH

The Editors of Obstetrics & Gynecology