Author's response to reviews

Title: Survey of Human Papillomavirus Types and Maternal-fetal Transmission in Pregnant Women

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Author's response to reviews: see over
Sepehr Tabrizi  
Section Editor  
*BMC Infectious Diseases*  

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Dear Dr. Tabrizi,

We have significantly revised our manuscript and it is now entitled “Survey of Human Papillomavirus Types and Their Vertical Transmission in Pregnant Women”. We would like to resubmit our revised manuscript for publication as a *Research article* in *BMC Infectious Diseases*.

Please find below our point-by-point responses to the comments of the reviewers. Additions to our manuscript have been highlighted in yellow, for ease of review.

This paper has not been published elsewhere, nor is it under consideration by another journal. All authors have read and approved the content, and to its resubmission to *BMC Infectious Diseases*. The authors have no ethical or legal conflicts to declare.

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We look forward to hearing from you at your earliest convenience.

Yours sincerely,

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Reviewer 1

We thank the reviewer for their helpful comments and suggestions. We have revised and corrected every section of the manuscript as suggested by the reviewer. We have also had our revised manuscript edited by a native-speaking English scientist, so as to include the most appropriate language to describe our findings. We hope that you will now consider our manuscript suitable for publication.
Reviewer 2

We are thankful to the reviewer for their comments and suggestions. We have made many revisions to our manuscript as indicated by the yellow highlighting. We have also had our revised manuscript edited by a native-speaking English scientist, so as to include the most appropriate language to describe our findings. We hope that you will now consider our manuscript suitable for publication.

1. In the introduction, please indicate the significance of neonatal HPV infections -what is the prevalence of HPV-related disease in neonates (either globally or in China/regionally)? What is the likely outcome of a neonatal infection - morbidity/mortality?

Response
The revised introduction now includes discussion of the significance of neonatal HPV infections, and includes the prevalence of HPV-related diseases in neonates and the outcome of infection (lines 42–45, lines 51-58).

2. It is stated in the Materials and Methods that the women were included in a follow-up study after delivery -please detail what was studied. Were samples collected at this time for HPV testing, and if so, was it these results or the 24-26 week sample that were used for comparison with neonatal HPV results? If both, then please provide a figure comparing the results obtained at these two different time-points and discuss. If not, then why weren't maternal samples collected at the time of birth -discuss.

Response
The revised manuscript now includes a description of what was studied and the collection times of samples. PENG et al. [32] (PENG Ping, WENG Xiayun, GU Zhiyuan, et al. Detection of the Asymptomatic Infection by Human Papillomavirus in Pregnant Women and Neonates. Chinese Journal of Obstetrics and Gynecology 2000, 35:523-525.) reported using PCR for the detection HPV in cervical and vaginal secretions, and in peripheral venous blood samples. They found no significant difference between genotypes detected during pregnancy, and at birth. Thus, maternal samples were not collected at birth.
3. Page 8, Line 10: Please describe or reference the PCR primers used for HPV detection/typing in the Materials and Methods.

Response
The HPV GenoArray Test Kit is based on “Flow-through Hybridization” technology for the rapid detection of up to 21 HPV genotypes. Unfortunately, the primers were part of a kit and their sequence is not disclosed by the manufacturer, therefore we are unable to include this information in our manuscript. Page 7 (lines152-165).

4. Page 8: Please provide details of the line-probe assay used to perform reverse-hybridisation, including name, manufacturer and number of genotypes detected. Also, two different HPV genotyping technologies were listed (gene-chip and line-probe assay) but it is unclear which was used how; the methods begin by describing the gene-chip assay but finish up with the line-probe assay; please clarify. Also clarify in the discussion (third paragraph) as this only mentions the gene-chip test.

Response
The methods section now includes a description of the line-probe assay (lines 153–156).

5. The suggestion that samples were experimentally contaminated is of concern and should be addressed more thoroughly -if this is a serious concern, I would like to see a review of the rate of laboratory contamination of samples in the particular facility (with the same staff) where testing was carried out. Questions which should be addressed include: were negative controls used during testing and what is the rate of false positives? If possible, it would be best to be able to rule out laboratory contamination as a factor in discordant results. If not possible, then the rate of false positives should be recorded.

Response
The current study was performed with strict measures enforced to limit the possibility of contamination. The suggestion of contaminated samples was only an attempt to explain the results obtained (lines 153–154).
6. Given the high rate of non-genotype concordance between mother and neonate, it would be useful to comment on the rate of HPV positivity in neonates born to HPV-negative mothers. As it does not appear that these children were tested, some reference to the literature should be attempted. Please also do some testing (or comment on) the prevalence of HPV on equipment/surfaces/staff involved in childbirth and postnatal care (again, possibly from a review of relevant literature on the prevalence of HPV on equipment/staff in maternity wards).

**Response**

Our laboratory is in very good condition and our quality control is very strict. Contamination is a theoretical possibility, not a fact.

7. The assertion of this study that the results make a case for vaccination of pregnant women and newborns has not been well discussed. Vaccination of newborns with a prophylactic vaccine is clearly pointless and the safety of such has not yet been evaluated (and is not mentioned in this article -please discuss). Vaccination of pregnant women may also occur too late to prevent transmission, but regardless, no mention has been made of any literature covering this topic. Vaccination of pregnant women may well be important in preventing vertical transmission, and studies such as these will potentially make a case for such; however the authors have failed to impress upon me the significance and potential impact of vaccinating pregnant women. Please include some literature and discuss further.

**Response**

We sought to investigate types of HPV that were vertically transmitted. We do not yet have a vaccination guide for HPV during pregnancy.

8. Why weren't skin swabs, vaginal swabs and anal swabs taken from mothers, as these are also potential sources of transmission? Discuss.

**Response**

We did not include swabs from these locations because the HPV detection rate was previously shown to be very low and unreliable for these.

9. Please explain why only 233 infants (of 422 HPV-positive women) were tested.
Response
The data collection section of our revised manuscript includes a statement that the discordant mother-infant pairs are due to the mother refusing examination and sampling of the infant (lines 111–116).

10. Statistics: when citing mean +/-, please indicate what the +/-indicates: is this standard deviation/error, two standard deviations/error, or range?
Response
We have revised the manuscript to include the standard deviations associated with our data.

11. Page 9 (Results, third sentence): this data is repeated two sentences later. Please remove this sentence.
Response
These redundant sentences have been removed in our revised manuscript.

12. Page 11, second sentence of Discussion: should read “Our results revealed that type-specific HPV DISCORDANCE...” (not concordance) "...was high......"
Response
The discussion has been revised to include this suggestion. Page 10 second sentence of Discussion (lines 217–218).
It now reads as follows:
“Our findings revealed that type-specific HPV discordance was high, suggesting other infection routes besides the maternal-neonate route.”

13. Page 11, line 9: do the authors mean HPV DNA transmission or HPV (virus) transmission? Please be clear, as the presence of DNA does not necessarily indicate infection.
Response
The prevalence of HPV in women (76.2%) was determined by abnormal cervical cytology (lines 218–221).
14. Which genotypes were most commonly discordant? Discuss.

**Response**
Discordant is one of the few.

15. There was no mention of the possibility of previous HPV infections which had been transmitted to the foetus prior to birth, but cleared in the mother - which would contribute to some of the apparent discordance.

**Response**
Quality control is very strict in our laboratory.

16. Page 12, third paragraph, first line: the GSK prophylactic vaccine Cervarix is bivalent, not quadrivalent.

**Response**
This has been corrected in the revised manuscript (lines 270).

17. References contain some typographic errors - please revise and correct.

**Response**
The references have been revised and corrected.

18. In several places, including the abstract, "HPV-DNA concordance" is listed as a result. This is a strange phrase and should perhaps be clarified.

**Response**
We have corrected this phrase in the revised manuscript.

19. Rather than focussing on vaccination of newborns (which is going to occur too late to afford any protection against exposure at birth), based on the high rate of non-concordance and the authors' apparent conclusion that up to 50% of neonatal infections occur after birth and perhaps independently of the mother, I would suggest
more attention be paid to preventing post-partum exposure from, for example, hospital staff/equipment. At present there appears to be only one line suggesting this course of action, but without further consideration or discussion.

Response
Currently, no recommendations have been introduced for the vaccination of infants and pregnant women.

20. The results are obviously only based on detection of HPV DNA (which does not necessarily mean infection), can the authors cite any research on rates of neonatal infection with HPV compared with rates of detection of HPV DNA?

Response
This has been corrected in the revised manuscript (lines 200–206).

21. I am not sure how the safety of an HPV vaccine during pregnancy relates to the results of this study, which was presumably carried out in unvaccinated women. The authors may wish to clarify or place this paragraph AFTER discussion of the merits of vaccinating pregnant women.

Response
We have revised our manuscript to address this point. Our discussion now reads (lines 270–277):
“We sought to investigate types of HPV vertical transmission, pre-pregnancy to vaccination related HPV vaccine. We do not yet have a vaccination guide for HPV inoculation in pregnancy.”

**Response**

We thank the reviewer for providing us with this reference. We have incorporated it into our revised manuscript.