Author's response to reviews

Title: High-risk Human Papillomavirus Infection is associated with Premature Rupture of Membranes

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Author's response to reviews: see over
Dear Section Editors:

Please find our revised manuscript entitled: High-risk human papillomavirus infection is associated with premature rupture of membranes. All authors verify that this revised manuscript is original and is not currently under consideration for publication in other journals.

On next pages, we have described a point-by-point response to reviewer’s comments. Please check this manuscript carefully.

Best Regards

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REVIEWER 1

I appreciate your review. Your comments have been a great help to our study.

< MAJOR COMPULSORY REVISIONS >

Reviewer (Luis Gomez)'s comment 1

Premature rupture of membranes (PROM) is defined with no criteria of gestational age. PROM at term does not have the same clinical impact as PROM that occur preterm (PPROM), especially when PPROM occurs before 34 weeks. I think that this is the major limitation of this paper: it does not detail if HR-HPV is more prevalent in the cohort that experiences PPROM especially <34 weeks.

Authors’ response

Like reviewer’s comment, PROM at term does not have the same clinical impact as PPROM. But, because PROM at term also has several complications, such as increased maternal infections and neonatal intensive care unit admissions, we think that our study has enough meaning[1]. In addition, in our study, 43 pregnant women delivered at less than 34 weeks. Detailed analysis was performed for those.

<table>
<thead>
<tr>
<th>Maternal complications (%)</th>
<th>HR-HPV infection test</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative ($N = 39$)</td>
<td>Positive ($N = 4$)</td>
<td></td>
</tr>
<tr>
<td>PPROM</td>
<td>15.4</td>
<td>25.0</td>
</tr>
</tbody>
</table>

We thought that there was not a statistical significance because the number of cases is small. But, these values are similar to our results, 14.3% in HR-HPV-negative and 27.3% in HR-HPV positive. We were trying to compensate the effect of gestational age through by
multivariate logistic regression analysis (Table 3). After adjusting for gestational age at
delivery, HR-HPV infection showed a statistically significant association with PROM.
Therefore, large scaled studies should be needed on the association of HR-HPV infection and
PROM according to gestational age.

1. Dare MR, Middleton P, Crowther CA, Flenady VJ, Varatharaju B: Planned early
birth versus expectant management (waiting) for prelabour rupture of
membranes at term (37 weeks or more). Cochrane database of systematic reviews

Reviewer (Luis Gomez)’s comment 2

There is no mention to the role of known historical confounders for PPROM and spontaneous
preterm delivery, such as previous PPROM, tobacco use, other genital infections.

Authors’ response

We collected history of PROM and preterm birth. We inserted the tow about these historical
confounders in 2nd paragraph of Methods. After adjusting these factors, HR-HPV infection
showed a statistically significant association with PROM (Table 3). In addition, all
participants answered by “No.” when we asked about smoking status at enrollment. These
may not be the honest answers. It seems to be associated with Korean cultural characteristics
that pregnant women have been reluctant to talk about smoking. According to the study of
Jhun et al, the percentage of smoking revealed by self-reporting was even lower than that of
revealed by urinary cotinine measurement, 0.55% and 3.03%, relatively [2]. Based on this
study, the smoking did not have a significant impact on our results.

On the other hand, we did not collect and mention about other confounders for PPROM and
spontaneous preterm delivery. So, we inserted the sentence about the above into the 4th
paragraph of Discussion, as “Second, we lacked information regarding sexual behavior (i.e.,
number of sexual partners, use of condoms, and other active sexually transmitted infections)
and previous medical histories (i.e., immunocompromised, and HIV), which can act as or increase the risk factors for PPROM, and HR-HPV infection during pregnancy.”


Reviewer (Luis Gomez)’s comment 3

Why did the authors decide to test for the detection of HR-HPV at 6 weeks postpartum and not during the early prenatal care before delivery? Wouldn’t it better to test at the first prenatal visit? The virus has a slow incubation period, but how can the authors assure that HR-HPV detected one-and-a half month after delivery is responsible for adverse outcomes that took place antepartum?

Authors’ response

Like reviewer’s comment, we also worried about when to test would be the most accurate to expect the pregnancy outcome. After several considerations, for the following reasons, we decided to test for the detection of HR-HPV at 6 weeks postpartum. First, like reviewer’s comment, the virus has a slow incubation period and also a slow clearance time. According to the research of Brown et al., the median clearance time for high-risk HPV was 226 days [3]. This means that HR-HPV infected during pregnancy can affect the outcome of pregnancy and that to test HR-HPV at postpartum may reflect the effect of HR-HPV on pregnancy outcome. And the hospital where the study was conducted in is a tertiary hospital. There are more pregnant women that were requested for a variety of reasons than the mother who take a full antenatal care at tertiary hospital.
< MINOR ESSENTIAL REVISIONS >

Reviewer (Luis Gomez)’s comment 4

The authors do not specify where in the female genital tract samples were obtained; I assume it was in the cervix but this needs to be written.

Authors’ response

After your kind comment, we were aware that we skipped the mention. We are very sorry for that. So, in Methods, we inserted a sentence, “The samples were obtained in the cervix.”

Reviewer (Luis Gomez)’s comment 5

Gomez et al (reference 4) described the association of HPV with spontaneous preterm birth. Instead of studying the association with all spontaneous and indicated preterm birth, could the authors describe the association only with spontaneous preterm delivery? The authors also should include a cohort of spontaneous preterm delivery before 34 weeks.

Authors’ response

In our study, women with indicated preterm birth, such as preeclampsia and placenta previa, were 28 of 80 women who gave birth to a baby prematurely. We analyzed the rate of preterm birth (gestational age at delivery < 37 weeks or < 34 weeks) according to HR-HPV infection. There was no difference in both group, 14.2% vs. 16.3% (p=0.717) in women with less than 37 weeks and 10.0% vs. 15.2% (p=0.438) in women with less than 34 weeks, relatively.
Reviewer (Luis Gomez)’s comment 6

Hermonat et al (reference 3) reported the association of HPV with first trimester losses. The authors should report also the association of HR-HPV with miscarriages.

Authors’ response

The entire participant delivered at more than 24 gestational weeks except two women who delivered at 21 weeks and 23 weeks. From this finding, it is unreasonable to infer the association HR-HPV infection and PROM.

And, we cited these studies (reference 3 and 4) to give examples about the effect of HPV on pregnancy outcome and to confirm that there was no similar research on Korean women. We hope that it would be a sufficient answer to the reviewer’s comment.

Reviewer (Luis Gomez)’s comment 7

Did the authors also test the placenta for HR-HPV? It would have been interesting to assess the correlation with positive HR-HPV in the genital tract.

Authors’ response

Unfortunately, we did not give enough consideration to that part. Thank you for providing the interesting idea. If we have a chance, we will proceed with the study.

< DISCRETIONARY REVISIONS >

Reviewer (Luis Gomez)’s comment 8

The Discussion section is too large and scattered; it should be more focused and oriented to the main findings.
Authors’ response

We acknowledge the reviewer’s comments. So, we deleted the vain repetitions and tried to focus on the main findings.
REVIEWER 2

I appreciate your review. Your comments have been a great help to our study.

< MAJOR COMPULSORY REVISIONS >

Reviewer (Jeroen Vanderhoeven)’s comment 1

A statistician should verify the use of multivariate logistic regression in this study. After controlling for 6 variables, there appear to be insufficient number of events per variable which may affect the validity of statistical inference. (IE, PROM occurred in a total of 12 HPV positive subjects).

Authors’ response

With respect for the opinions of the reviewer, we consult a statistician to verify the usefulness of multivariate logistic regression. Although the number of PROM is a few relatively, he certifies that the current results have statistically sufficient significance for the following reasons: A model of multivariate logistic regression analysis was used to evaluate the principal variables’ relationship with HPV infection after adjustment for the covariates that had statistical value of p < 0.15 in the univariate analysis or the covariates that are interesting on the basis of their hypothesized relationship of PROM. Odds ratios of each variable were not significantly different from both before and after adjustment. In order to verify the above the Table 3 was replaced. And the total number of enrolled women is enough for multivariate logistic regression (44 women in HR-HPV positive and 51 in PROM). According to the research of Peduzzi et al., events per variable values of 10 or greater are enough for logistic regression analysis [4]. Lastly, if the number of PROM is insufficient, 95% confidence interval of HR-HPV infection would have been even appropriate. We hope that it is a sufficient answer to the reviewer’s comment.

< MINOR ESSENTIAL REVISIONS >

Reviewer (Jeroen Vanderhoeven)’s comment 2

In Methods: Define parameters for cross sectional study. Use specific date ranges. Did all patients at KUMC participate in HPV testing? What was the loss to follow up at the 6wk postpartum visit?

Authors’ response

Please forgive that we have missed important points, such as the definition of parameters and date ranges for enrollment. In Methods, we defined parameters and date ranges for enrollment exactly, as “We conducted a cross-sectional study of 311 women who gave a birth at Korea University Medical Center from February 2010 to January 2011.” and “We collected the basic characteristics, such as age, parity, number of abortions, body weight and height of pregnant women, gestational age at delivery, Apgar score and birth weight of each neonate, and delivery mode by medical chart review. Body mass index (BMI) is defined as the individual’s body weight (kilogram) divided by the square of their height (meter).” And all participants were taken HR-HPV test at KUMC.

< DISCRETIONARY REVISIONS >

Reviewer (Jeroen Vanderhoeven)’s comment 3

In Methods: list which subtypes HPV are noted to be "high risk" by the Hybrid Capture II diagnostic system.
Authors’ response

According to reviewer’s comment, we described “high-risk” HPV subtypes by Hybrid Capture II system in 2nd paragraph of Methods, as “We used the Hybrid Capture II system (Digene Diagnostics Inc., Gaithersburg, MD, USA) for detection of HR-HPV infection (HPVs 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68) at six weeks postpartum.”.

Reviewer (Jeroen Vanderhoeven)’s comment 4

In Results: Known risk factors for HPV infection include immunocompromised, HIV, and smoking status. Inclusion of this data would benefit this study; exclusion should be cited as an additional limitation. While it may be that HPV infection is associated with increased risk of PROM via a common modality of tobacco use. The authors stimulate interesting discussion by highlighting novel research implicating HPV-mediated increased MMP activity as a possible mechanism for their finding.

Authors’ response

All participants answered by “No.” when we asked about smoking status at enrollment. These may not be the honest answers. It seems to be associated with Korean cultural characteristics that pregnant women have been reluctant to talk about smoking. According to study of Jhun et al, the percentage of smoking revealed by self-reporting was even lower than that of revealed by urinary cotinine measurement, 0.55% and 3.03%, relatively [2]. Based on this study, the smoking did not have a significant impact on our results.

We did not collect and mention about known risk factors for HPV infection. So, we inserted the sentence about the above as an additional limitation in 4th paragraph of Discussion, as “we lacked information regarding sexual behaviors (i.e., number of sexual partners, use of condoms, and other active sexually transmitted infections) and previous medical and
environmental histories (i.e., previous preterm premature rupture of membrane (PPROM), immunocompromised, and HIV)” And, we acknowledge the reviewer’s comments. So, we deleted the vain repetitions and tried to focus on the main findings.