Author's response to reviews

Title: Dynamic changes in HBV surface marker titers and HBV DNA loads of infants born to HBsAg-positive mothers: can positivity for HBsAg or HBV DNA at birth be an indication for HBV intrauterine infection in newborns?

Authors:

Tianyan Chen (chentianyan@126.com)
Jing Wang (kidip@163.com)
Yuling Feng (fengyulingbest@sohu.com)
Zhi Yan (samsnally@gmail.com)
Tieying Zhang (zhangtieying36@126.com)
Minghui Liu (13509188790@163.co)
Yun Bai (shangluoyiyuan@163.com)
Hongxia Song (songccccp@yahoo.com.cn)
Hongli Liu (liuhonglili@sina.com)
Yuan Yang (xayangyuan@126.com)
Jingfeng Liu (sourmilk2003@163.com)
Yingli He (heyingli2000@163.com)
Yunru Chen (chenyunru_2002@126.com)
Shulin Zhang (zhangsl451206@vip.163.com)
Guihua Zhuang (zhuanggh@mail.xjtu.edu.cn)
Xiaofeng Liang (liangxf@hotmail.com)
Zongyin Liu (angelfly85@163.com)
Xiaguang Xu (xuzhuren123@163.com)
Wei Chen (chw62@163.com)
Yong Liu (liuy5599@mail.xjtu.edu.cn)
Yingren Zhao (zhaoyingren@mail.xjtu.edu.cn)

Version: 3 Date: 27 September 2013

Author's response to reviews: see over
Dear Dr. Philippa:
We appreciate your patient work. Our responding to each single comment from both reviewers was presented as follows.

Response to reviewer Dr. Selda Polat:
We thank Dr. Selda Polat for the thoughtful comments on our paper. Several important issues are raised. The responding was presented as follows.

Question 1: In methods, in data collection section; there has been written that the developmental status of the subjects were recorded. Does this mean physical growth (weight, length or head circumference??) or neurodevelopmental status? Is it asked roughly to the mothers or assessed with objective follow up tests? Did any part of this data used in any part of the research? If these data is not used, there is no need to mention.
Response: “Developmental status of the subjects” mentioned in the article means the weight, length, Apgar score, and congenital anomalies of infants at birth. All those data were collected from the medical records and assessed by the obstetricians.
Those data gave us the general information (presented in page 22 Table1) on the infants of HBV infected mothers, which was indeed not closely related to the topic of this article. Thanks for your advice, we will not display those data in the manuscript.

Question 2: It was mentioned 85.1% of the newborns were term and the rest were preterm but there is only 2 infants weighting 2000 and 2050 grams. Were the other 20 preterms all above 2000 grams? How were their birth weeks?
Response: We are sorry for not clearly describing the infants’ general conditions. Among the newborns of HBV infected mothers, 126 (85.1%) were full-term, and 20 (13.5%) were post-term, while 2 (1.4%) were preterm weighting 2000 and 2050 grams. And we found neither preterm nor postterm was related with the vertical transmission and none of the preterm or postterm infants has been infected with HBV. Please refer to table 1 for detail information. As you suggested, those data were deleted without sacrifice the integrity of this article.

Question 3: In statistical analysis there might be need to investigate if there was any differences between ‘cesarean section or vaginal route’ of births or being ‘preterm or term’ on being infected in utero or becoming chronic hepatitis.
Response: We have retrospected Pubmed from 1970 to 2013, according to published data [3-6], the role of cesarean section in preventing mother-to-child transmission of HBV and the relation between HBV infection of infants and preterm birth were uncertain, most previous studies showed HBV infection of infants was not influenced by mode of delivery (whether cesarean section or vaginal route) and gestational week (preterm, term or postterm). Our data displayed the same results as table 1. This will be further evidenced with a larger sample size in our subsequent work, so these data were not presented in this paper.
<table>
<thead>
<tr>
<th>Variables</th>
<th>Intrauterine Infection (n=9)</th>
<th>Non-Intrauterine Infection (n=139)</th>
<th>$\chi^2$</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mode of delivery</td>
<td>3 vs. 6</td>
<td>72 vs. 67</td>
<td>1.153</td>
<td>0.323</td>
</tr>
<tr>
<td>Cesarean section vs. Vaginal route</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gestational week</td>
<td>0 vs. 9 vs. 0</td>
<td>2 vs. 117 vs. 20</td>
<td>0.494</td>
<td>0.781</td>
</tr>
<tr>
<td>Preterm vs. term vs. postterm</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 1. The influence of delivery modes and gestation weeks on infants HBV infection.

Question 4: Is there any explanation for the decrease in anti-HBs levels in healthy subjects? It was mentioned that anti-HBs levels are maximum at the end of 1st month and higher than 7th month levels. As the third dose of immunization was given at 6th month and as the infants went on receiving HBs Ag by the infant-mother relation there had to be an effect of natural boosting also. Then why the anti-HBs levels were decreased by time?

On page 9, line 8: it was said the levels of anti-HBs were maximum at the end of 1st month, but on page lines 17-18 it was told the levels were maximum at the end of 7th month. Which one is true?

Response: The positive rate of anti-HBs in the infants maximize at the age of the 1st month, while the titer of anti-HBs was peaked at the age of 7th month (Fig1). We are sorry that the describing is not clear. We have rewritten these results in our revised manuscript.

![Fig1. The positivity rate (A) and the titer (B) of anti-HBs in the infants](image)

The effect of natural boosting can be observed in the infants born to HBsAg positive mothers[7-9], the anti-HBs titer in the children born to HBsAg-positive mothers was higher than that of those born to HBsAg-negative mothers, while the titer of anti-HBs in HB immunized infants was gradually decreased after the peak, no matter whether the infant born from HBV positive mother or negative mother [8, 10]. In our data, after 3 doses of HB immunization (0, 1, 6 mo), the anti-HBs titer of the infants was decreased at 12 mo as compared to 7 mo (438.1 ± 375.4 mIU/mL vs. 601.2 ± 393.4 mIU/mL, p=0.008), which was consistent with the reported data. However, the anti-HBs titer increased in 12 mo as compared to 7 mo in a small subgroup of those subjects (17.6%). But we do not know the reason for it. Moreover,
in order to minimize the risk of horizontal transmission in daily life, which may also minimize the possibility of natural boosting, all the mothers enrolled had been educated to change their life style before and after childbirth. To confirm the effect of natural boosting, longer and more thoughtfully observation on these infants is needed.

**Question 5:** On page 10, last 2 lines: it was mentioned that ‘24 infants remained HBV DNA and HBsAg positive during follow up’ what was the duration of this positivity? Was not the number of HBV DNA and HBsAg positive infants 9??

**Response:** We are so sorry for not clearly representing the detailed numbers on page 10 and we have reorganized the sentences as following (Fig1A, B):

“At birth, 24 newborns were positive for HBV DNA, 41 infants were positive for HBsAg at birth. 17 infants were double positive for HBV DNA and HBsAg. Although evidences exists that HBV DNA and HBsAg can be diagnostic indicators for HBV infection, the specificity and sensitivity of those two markers remains to be studied. Ours results shown that high levels of HBV DNA load at birth (more than $10^5$ IU/mL) detected in 5 infants was a robust predictive marker for HBV infection at 12 mo. Among those 19 infants with low level HBV DNA (less than $10^5$ IU/mL), 15 infants who were anti-HBs detected positive at 1 mo were negative for HBV DNA and HBsAg at the age of 12 months, 4 infants were persistently positive for HBV DNA and HBsAg and negative for anti-HBs (Figure.4A).

As shown in Figure 4, 4 infants with high levels of HBsAg titer (more than 250 IU/mL) at birth, persistently positive for HBV DNA and HBsAg, were diagnosed as HBV infection: 32 in 37 infants with low levels of HBsAg titer (from 0.05 IU/mL to 250 IU/mL) eliminated HBV virus at the age of 12 months, other 5 infants with persistently HBV DNA(+) and HBsAg(+), anti-HBs was negative.”

---

**Fig1A The Number of infants with positive HBV DNA**
**Question 6:** In section ‘Dynamic changes in HBV surface marker titers and HBV DNA loads in infants’ lines 8,9,10 the construction of the sentence is wrong. The concomitant use of hepatitis B vaccine and HBIG does not decrease the transition of the virus from mother to infant. What does it mean? Protection of the infant is another thing!!!

In section ‘Comparison of the dynamic changes in HBV surface marker titers and HBV DNA loads between infected and uninfected infants’ (page 10) it is very difficult to understand this section, the content and language should be corrected.

On page 11 line 1: ‘HBV DNA loading’ should be corrected. Load is a plural word but it is used as ‘loads’ whole the manuscript.

Table 2: the headings of the columns are not unique [(n (%)) or (% n)]???

There are many language mistakes, a native English speaker/writer should recheck the article.

**Response:** I am so sorry for the improper use of words and phrases in the article that makes you confused. As a non-native English speaker, I will try my best to improve the confluence of the article. To make an improvement, two native speakers majoring in virology and gynecology reviewed this version of manuscript. The wrong sentences and misused words were corrected as listed below:

No.1 in section ‘Dynamic changes in HBV surface marker titers and HBV DNA loads in infants’ lines 8,9,10 has been corrected as “Next, we investigated changes in the positive rates of HBV markers and HBV DNA over the first year of the infants’ lives. The rates of HBV DNA(+), HBsAg(+), HBeAg(+), and anti-HBc(+) in infants reduced gradually during the follow-up (χ²: 9.67, 592.01, 36.83, and 190.7, respectively; P = 0.022, <0.01, <0.01, and <0.01, respectively).”

No.2 the content and language in section ‘Comparison of the dynamic changes in
HBV surface marker titers and HBV DNA loads between infected and uninfected infants’ (page 10) has been corrected as:

“As shown before, 9 infants with continuous HBV DNA and HBsAg(+) were diagnosed HBV infection. Dynamic tendency in HBV DNA load, HBsAg, HBeAg, anti-HBc, and anti-HBs titers were significantly different between 9 infected infants and 139 uninfected infants (F = 2.13 × 10^{10}, P < 0.01; F = 87.78, P < 0.01; F = 2.59 × 10^{7}, P < 0.01; F = 6.73, P < 0.01; and F = 2.82, P = 0.047, respectively; Figure. 3). HBV DNA load, HBsAg and HBeAg titer of the 9 HBV intrauterine infected infants increase gradually, while 139 uninfected infants went opposite way. Nine infected infants presented anti-HBs(-) even under consecutive detection, whereas the titer in the 139 non-infected infants increased.”

No.3 “Loading” and “loads” have been corrected as “load” in the entire article.

No.4 the data in table 2 have shown in figure.1 of the revised manuscript.

Response to reviewer Dr. Seyed mohammad Mohammad jazayeri:
We thank Dr. Seyed mohammad Mohammad jazayeri for the precious comments. The responding was presented as follows.

**Question:** The authors claimed that HBs negativity at 1 month of age may contribute to the identification of HBV intrauterine infection in infants with low levels of HBV DNA loading and HBsAg titers at birth, however, if the mother is positive for anti-HBs (a rare case though), how can they distinguish between passive transfer of Ab from mother to infant and/or real Anti-HBs negativity?

**Response:** Anti-HBs, an IgG antibody, can transfer human placenta from mother to infant [11, 12]. If the mother is positive for anti-HBs, at present no methods available to distinguish passive transfer from real anti-HBs positivity, however, all the mothers enrolled in this study were negative for anti-HBs (0.06±0.21 mIU/mL, data not shown), and all the infants were negative for anti-HBs at birth (0.03±0.04 mIU/mL).


