HBsAg-positive and HBsAg-negative hepatitis B virus infection among mother-teenager pairs 13 years after neonatal hepatitis B vaccination

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Abbreviations:

- HBsAg: Hepatitis B surface antigen
- HBV: Hepatitis B virus
- HBI: Hepatitis B virus infection
- hHBI: Hepatitis B virus infection, HBsAg-positive
- nHBI: Hepatitis B virus infection, HBsAg-negative
- OBI: Occult hepatitis B virus infection
- IU: International unit
- anti-HBc: Antibody to hepatitis B core antigen
- anti-HBs: Antibody to hepatitis B surface antigen
- ALT: Alanine aminotransferase
- PCR: Polymerase chain reaction
Abstract

Aim

It is unclear whether a mother of negative hepatitis B surface antigen (HBsAg) but positive hepatitis B virus (HBV) is at potential risk for mother-to-child transmission of HBV. This study using a paired mother-teenager population aims to assess whether maternal HBsAg-negative HBV infection (hnHBI) is a significant source of child HBV infection (HBI).

Methods

A follow-up study with blood collection has been conducted on the 93 mother-teenager pairs from the initial 135 pregnant woman-newborn pairs 13 years after neonatal HBV vaccination. Serological and viral markers of HBV has been tested, and phylogenetic analysis of HBV isolates has been done.

Results

The HBI prevalence is 1.9% (1 hnHBI /53) for teenagers of non-HBI mothers, compared with 16.7% (1 hnHBI /6) for those of hnHBI mothers and 2.9% [1 HBsAg-positive HBV infection (hpHBI) /34] for those of hpHBI mothers. Similar viral sequences have been found in one pair of which both mother and teenager have had hnHBI. In comparison with the hpHBI cases, those hnHBI have a lower level of HBV viral load and a higher proportion of genotype-C strains, which are accompanied by differentiated mutations (Q129R, K141E and Y161N) of the “a” determinant of the HBV surface gene.

Conclusion

Our findings suggest that mother-to-teenager transmission of hnHBI can occur among those under neonatal HBV vaccination program.
Keywords: hepatitis B virus infection, vaccination, teenager, phylogenetic analysis, mother-to-child transmission
Introduction

Infection with the hepatitis B virus (HBV) accounts for a significant portion of morbidity and mortality worldwide (15). With the introduction of a safe and effective HBV vaccination for neonates, the prevalence of chronic carriers, which are identified as being hepatitis B surface antigen (HBsAg)-positive, has markedly dropped to 1%~2% among the vaccinees (17, 37).

The HBV vaccination protocols for neonates vary according to their mothers’ HBV status (37). Since it has been established that the combined three 10 μg-dose HBV vaccines plus hepatitis B immune globulin (HBIG) would provide better protection than the three 5 μg- or 10 μg-dose HBV vaccines alone (16), the Chinese government has introduced a compulsory neonatal HBV vaccination program in 1992 (17): for babies born to HBsAg-positive mothers, three 10 μg-dose HBV vaccines plus a dose of 200 IU HBIG will be provided; whereas for those born to HBsAg-negative mothers, only three 5μg-dose vaccines will be used.

Determining HBsAg status has been routinely undertaken for the mothers during prenatal visit or before delivery through serological methods, which target the major “a” determinant of HBsAg. However, current available commercial assays could not recognize the following scenarios, i.e., the early window period of acute HBV infection (HBI), occult hepatitis B virus infection (OBI) [defined as the presence of HBV DNA in the liver (with or without detectable HBV DNA in the serum) combined with a negative HBsAg] with an HBV load below 200 IU/mL (24, 26), and false OBI due to a modified HBsAg (caused by the “a” determinant mutations) (7-8, 13, 38). In current practice, differentiation among the above scenarios is unlikely unless follow up studies are performed. Therefore, nearly all serology-based studies have treated such HBsAg-negative
HBI (hnHBI) cases as OBIs (2). The substantial impacts of hnHBI occur in a variety of clinical settings (1, 3, 6, 9, 11, 22, 25, 28, 32, 35), including the reactivation or transmission of HBV, the progression of liver diseases and the development of hepatocellular carcinoma, etc.

Mother-to-child transmission of HBsAg-positive HBI (hpHBI) but not hnHBI have been well documented (16, 20). Scientific evidence suggests that HBV DNA, rather than HBsAg, is the determinant of this transmission (10). However, the inability to identify hnHBI routinely has resulted in that an hnHBI pregnant woman would be treated as a non-HBI case, and her newborn baby would be vaccinated with only the three 5 μg-dose HBV vaccines. Contrasted with the hpHBI, the prevalence of hnHBI was much higher among the vaccinees or even those with high-level antibodies against HBsAg (anti-HBs) (5, 21, 34). Recent publications reported that the prevalence of hnHBI was 10.9% for vaccinees aged 1-13 years in Taiwan, China (21), 20.0% for those under 15 years of age in Singapore (5), and 3.25% for those aged 19-20 years in Qidong, China (34). One study reported a 28% prevalence of hnHBI among children born to hpHBI mothers despite prophylaxis with HBV vaccines and HBIG (29). Among teenagers who had a history of hpHBI but who were no longer tested positive to HBsAg, only 24% responded to HBV vaccines marked by positive anti-HBs (30). Therefore, it would be hypothesized that hnHBI in the vaccinees may originate mainly from their mothers.

In this study, we use a paired mother-teenager population to ascertain whether maternal hnHBI is a significant source of hnHBI for their child by analyzing the occurrence of hnHBI, determining the phylogenetic relationship between concurrent isolates, and assessing risk of child hnHBI attributable to maternal hnHBI.
Materials and Methods

Participants

From October 7th, 1996 to May 17th, 1997, 135 pregnant women-newborn pairs were enrolled in a follow-up vaccination program in Deqing County, Zhejiang Province, China (33). Of the 135 pregnant women, 100 were categorized as non-HBI and 35 had hpHBI; further, 16 out of the 35 hpHBI were also HBV e antigen-positive. At the 0-, 1-, and 6-months since birth, the newborns received HBV vaccinations with each a 5-μg dose of yeast-derived recombinant hepatitis B vaccine (Shenzhen Kangtai Biological Products Co., Ltd., Shenzhen, China). The infants’ anti-HBs levels were quantified at the ages of both 7 and 12 months. All HBV indexes were determined by using radioimmunoassay-based commercial kits (Shanghai Kehua Bio-engineering Co., Ltd., Shanghai, China) (33).

Follow up and data collection

From July to August 2010, a repeat study has been conducted on the 135 initial pregnant women-newborn pairs, who are now mother-teenager pairs. Informed consent was obtained from the teenagers’ parents or participating mothers prior to specimen collections. Demographic data on the teenagers and mothers by using a structured questionnaire and 5-mL blood were collected. Data on the administration of HBV booster vaccines for the teenagers were obtained from their vaccination records at the Center for Disease Prevention and Control, Deqing County, Zhejiang Province, China.
Serological and virological testing

All serum specimens were aliquoted into two separate sterile tubes. The first tube of serum was used for the alanine aminotransferase (ALT) assay by using commercial kits based on the method of lactate dehydrogenase by ultraviolet radiation (Shanghai Kehua Bio-engineering Co., Ltd., Shanghai, China); the second tube was used for the detection of HBsAg, anti-HBs and antibodies against hepatitis B core antigen (anti-HBc) by using commercial kits for an electrochemiluminescence immunoassay (Elecsys, Roche Diagnostics, Inc.). To avoid potential false-negative results by a single test, the HBsAg was further tested by enzyme-linked immunosorbent assay (ELISA)-based HBsAg kits (Beijing Wantai Biological Pharmacy Enterprise Co., Ltd., Beijing, China).

Viral DNA extractions from 100 μL of serum were performed in parallel for both the first and second tube. The levels of HBV DNA load were measured by using Premix Ex Taq™ (Perfect Real Time) kits [TaKaRa Biotechnology (Dalian) Co. Ltd., Dalian, China] and primer sets, described elsewhere (35). Positive results in both tubes indicated a positive HBV viral load. All tests were performed strictly according to the manufacturer’s instructions.

Nested polymerase chain reaction (PCR) for HBV surface gene, sequencing and phylogenetic analysis

The second tube of serum (200 μL) was also used for performing a nested PCR on the HBV surface gene (35). The DNA extraction was performed using QIAamp DNA Blood Mini kits (Qiagen, Hilden, Germany) according to the manufacturer’s instructions. Briefly, a 544-nt segment of the HBV surface gene, nt 223-766 relative to the HBV prototype (GenBank access no...
AB033557), was amplified using primers S1 (5'-CCTGCTGGTGGCTCCAGTTC-3') and S2 (5'-ATACCCAAAGACAAAAGAAAA-3') for the first round of PCR, and primers S3 (5'-GCCGGGTTTTCTTGTGAC-3') and S4 (5'-GGGACTCAAGATGTTGTACAG-3') for the second round. The PCR cycling conditions for both rounds consisted of denaturing for 40 seconds at 94, annealing for 40 seconds at 55, and extension for 40 seconds at 72 with 35 and 25 cycles for the first and second round, respectively.

The PCR products for the HBV surface gene were purified and further sequenced on an ABI Prism 3130X automatic genetic analyzer (Applied Biosystems Life Technologies Corporation). The viral sequences were aligned using Lasergene (version 7.10; DNASTAR Inc.). Genetic distances between pairs of virus isolates were calculated using the Tamura-Nei method. A phylogenetic tree was constructed using the maximum likelihood method and evaluated using the bootstrap test with 500 replications in MEGA software (version 5.05; available at: http://www.megasoftware.net/). Prototype HBV strains of all A-I genotypes were used as references in the analysis; their GenBank accession numbers are listed as follow: Genotype A – AY373432, DQ315784; Genotype B – BX97850, AY800391, AY206390, AY206391; Genotype C – AB112063, AB033557; Genotype D – EU939680, X65259; Genotype E – AB032431, AB091256; Genotype F – AB036905, AB116654; Genotype G – AB056515, AB064313; Genotype H – AB059661, AB375161; and Genotype I – AF241408, AF241409. The serotypes of all identified HBV strains were determined by their amino acid sequences as previously reported (19).
Positive HBV DNA was defined as being positive for either HBV viral load or the HBV surface gene for the HBsAg-positive participant, or being positive for both HBV viral load and the HBV surface gene for the HBsAg-negative. The current HBI (in 2010) was defined as being either hpHBI or hnHBI. The prenatal HBI (in 1996/1997) was defined as being HBsAg-positive only.

For teenagers without current HBI, a protective immunity to HBV was defined as an anti-HBs level ≥10.0 IU/L (20). Initial vaccine response was defined as an anti-HBs level ≥10.0 IU/L for the infants at 7 or 12 months.

The genotype-specific surface gene mutants of HBV in this study were compared with archived HBV strains (95 genotype-B and 48 genotype-C) that had been isolated from hpHBI from the general population in the same county (36).

Statistical analysis

The means, medians or proportions are presented as descriptive statistics. In the bivariate analyses, the student’s t-test, the Mann-Whitney test, and Pearson χ² test or the Fisher’s exact test were used to compare the means, medians and proportions, respectively. All P values were 2-sided. The results were considered statistically significant when P < 0.05. All analyses were performed using SAS 8.02 for Windows (SAS Institute, Cary, NC).

Ethical approval was granted by the Human Subjects Committee Review Board of the School of Public Health, Fudan University.
Results

Characterization of the follow up and HBI cases identified in the mother-teenager pairs

There were 16 mother-teenager pairs who refused to participate, 17 pairs who could not be contacted, and 8 mothers and 1 teenager alone who refused to provide blood samples. The remaining 93 pairs (68.9%) who had completed the questionnaire data and provided blood samples were included for further analysis (Figure 1). The follow-up rate, 77.1%, for the 35 HBsAg-positive pregnant women diagnosed in 1996/1997, was not significantly different from that of 66.0% (66/100) for HBsAg-negative pregnant women ($\chi^2=1.05, P=0.22$) (Figure 1).

The HBI status of the 93 pairs above was determined again in 2010 with the more sensitive assays, i.e., PCR, sequencing methods and new HBsAg assays. Forty HBI mothers (34 $\text{hpHBI}$ and 6 $\text{hnHBI}$) and three HBI teenagers (1 $\text{hpHBI}$ and 2 $\text{hnHBI}$) were identified (Figure 1). For the mothers, their HBI status exhibited a 75.3% concordance between in 1996/1997 and in 2010 ($\text{McNemar } \chi^2=7.3, P=0.01$); and all the six $\text{hnHBI}$ mothers classified in 2010 were also HBsAg-negative in 1996/1997 (Figure 1).

Feature comparison of the teenagers and the mothers by mothers' HBI status

In comparison with non-HBI mothers, mothers' age was slightly higher and proportion of mothers' being anti-HBs-positive was higher among HBI mothers; but the other features of the mothers (education level, occupation, annual family income, abnormal ALT level and anti-HBc status) and of the teenagers (age, sex ratio, delivery and feeding methods, infantile growth and development, history of diseases, dental treatment, injures, surgical operation, transfusion,
toothbrush sharing, passive smoking, initial response to and boost with HBV vaccine, and abnormal ALT level) were similar (Table 1).

**Relationship of HBI status among the 93 mother-teenager pairs**

For all teenagers, the prevalence of HBI (1 hpHBI plus 2 hnHBI), or of being anti-HBs-positive or anti-HBc-positive were 3.2%, 60.2% and 4.3%, respectively (Table 2).

Four anti-HBc-positive cases were identified exclusively among teenagers whose mothers were hpHBI in both 1996/1997 and 2010, together with a detectable HBV DNA in 2010 (Figure 1), but only one of the teenagers developed hpHBI. And the difference in proportions of being anti-HBc-positive was statistically significant in teenagers of mothers with different HBI status based on the results of either 1996/1997 or 2010 (Table 2, Figure 1).

The proportion of HBI was 16.7% (1/6), 2.9% (1/34) and 1.9% (1/53) respectively in teenagers of the mothers with hnHBI, hpHBI or non-HBI. However, the difference in proportions of HBI was not statistically significant in teenagers of mothers with different HBI status based on the results of either 1996/1997 or 2010 (Table 2, Figure 1).

**Viral and phylogenetic analysis of HBsAg-negative and HBsAg-positive HBV strains**

Of the 93 mother-teenager pairs, 29 mothers and 2 teenagers were identified as being HBV DNA-positive with the median HBV DNA load of 3.47×10^2 IU/ mL. The hpHBI cases exhibited a slightly higher but non-significant median HBV DNA level, 3.92×10^2 IU/ mL (23 cases, range:
< 5.0 IU/ mL ~ 1.91×10^9 IU/ mL), as compared with \( \text{hnHBI} \) cases, 2.47×10^2 IU/ mL (8 cases, range: 4.33×10^1 IU/ mL ~ 6.25×10^5 IU/ mL).

A total of 31 HBV strains, 29 from mothers (6 from \( \text{hnHBI} \) and 23 from \( \text{hpHBI} \)) and two from teenagers (both from \( \text{hnHBI} \)), were identified, and the overall ratio of genotype-B to genotype-C was 16/15 (Figure 2). The proportion of genotype-C was significantly higher for cases with \( \text{hnHBI} \) (7/8) than for those with \( \text{hpHBI} \) (8/23, \( P=0.02 \), Fisher’s exact test). All genotype-B and genotype-C HBV strains identified in this study had the serotypes of adw and adr respectively.

In comparison with the archived HBV strains that were isolated from individuals with \( \text{hpHBI} \) in the same region, the overall mutation rate per strain was consistent between \( \text{hnHBI} \) cases in this study and archived \( \text{hpHBI} \) cases (7.04 per 1000 and 7.19 per 1000, respectively). In the \( \text{hpHBI} \) cases from this study, the observed mutations of the 100\textsuperscript{th}, 120\textsuperscript{th}, 126\textsuperscript{th}, 156\textsuperscript{th}, 158\textsuperscript{th} and 164\textsuperscript{th} amino acid position for genotype-B strains and those of the 120\textsuperscript{th} and 141\textsuperscript{st} amino acid position for genotype-C strains were exactly the same as those archived. Neither of the specific mutants above nor those with high frequencies, such as the 133\textsuperscript{rd} and 161\textsuperscript{st} position, from the \( \text{hpHBI} \) cases were identified in the \( \text{hnHBI} \) cases in this study. The two alleles (Y161N and K141E) identified in the \( \text{hnHBI} \) cases in this study showed different mutation profiles compared with those archived (Y161F/S and K141G). In addition, the Q129R allele identified in two \( \text{hnHBI} \) cases (one teenager and one mother) was not observed in those archived.
There was one mother-teenager pair both infected with \textsubscript{hn}HBI and identified with HBV strains (MO-78 and TE-78 in Figure 2). Further sequence analysis showed the virus from this mother and her child are closely related with the only difference at the site of Q129R (Table 3).

**Discussion**

Our results show that one mother-teenager pair is both infected with \textsubscript{hn}HBI and their viral sequences are highly similar, which suggests that mother-teenager transmission of \textsubscript{hn}HBI has occurred among 13-year-old teenagers who have received neonatal HBV vaccination.

In this study, 3.2% of the 93 teenagers had infected with either \textsubscript{hn}HBI or \textsubscript{hp}HBI even though all of them have received HBV vaccination since birth and 81% of them have received at least one booster dose before the age of 13 years old. Further, this prevalence was markedly higher among teenagers of \textsubscript{hn}HBI mothers, 16.7% (1/6), than that among those of either \textsubscript{hp}HBI or non-HBI mothers, 2.9% (1/34) and 1.9% (1/53) respectively; but it was not significant. Thus, it remains inconclusive whether maternal \textsubscript{hn}HBI could play a major role in its transmission to child.

The mechanism of transmission of \textsubscript{hp}HBI has been well understood. Early-life HBV infection from mother-to-child, which is mainly determined by mother's HBV DNA level, can occur during the prenatal (through the placenta), perinatal or postnatal stages of life (4, 27, 31). Furthermore, natural and chronic-carrier infection of HBV can still occur over time, even for those who have received neonatal HBV vaccinations (20) or been living in highly endemic areas (12, 18, 23). Though \textsubscript{hn}HBI has been established in a variety of population including those with neonatal HBV vaccination (5, 21, 34), source of this infection remains largely unknown. In this
study, we did observe the transmission of maternal \textsubscript{hn}HBI to her child (one case), which would imply a possibly similar role of maternal \textsubscript{hn}HBI as that of maternal \textsubscript{hp}HBI. But other sources (father, siblings, friends, etc.) rather than the mother remain possible, as indicated by the ME-102 and TE-102 pair in this study.

In agreement with previous studies (34-35), we found more genotype-C HBV strains and a lower viral load in \textsubscript{hn}HBI cases than in \textsubscript{hp}HBI cases. The site-specific amino acid analysis identified three mutations (Q129R, K141E and Y161N) that were unique to \textsubscript{hn}HBI cases. The Q129R or K141E mutant, which has been suggested as a diagnostic-escape strain (13), had established its infection among the vaccinees. Interestingly, both \textsubscript{hn}HBI teenagers identified had received an extra booster dose of HBV vaccine in the past 13 years and had a detectable level of anti-HBs (Table 3).

Our findings raise the questions whether maternal \textsubscript{hn}HBI can play a main role in its transmission to their child under current HBV vaccination program. In comparison with the prevalence of \textsubscript{hp}HBI in the pregnant women [approximately 3% ~4% (Tao, personal communication)] and in the general population (7.2%) (17), the prevalence of \textsubscript{hn}HBI among the pregnant women is not insignificant or even higher (14), as indicated also by our results that the \textsubscript{hn}HBI prevalence was 10.2% (6/59) among the tested mothers. And the inability to routinely identify \textsubscript{hn}HBI in prenatal women means that they would be treated as non-HBV cases and their newborns will receive the less effective HBV vaccination protocol (16). Thus, mother-to-child transmission of \textsubscript{hn}HBI needs further investigation.
In conclusion, mother-to-teenager transmission of \textsubscript{hn}HBI occurs among those under neonatal HBV vaccination program. Due to this small study, the exact role of maternal \textsubscript{hn}HBI remains unknown. A large cohort of pregnant women with a longer follow-up period for both mothers and children would be ideal for investigating mother-to-child transmission of \textsubscript{hn}HBI and the effectiveness of current HBV vaccinations against both \textsubscript{hp}HBI and \textsubscript{hn}HBI.

Acknowledgements

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References


Figures

Figure 1 Flow chart for study design and results of hepatitis B virus related markers

VL: viral load; SG: hepatitis B virus surface gene

Figure 2 Phylogenetic tree of the 31 hepatitis B virus (HBV) strains isolated from a paired mother-teenager population

Each hepatitis B virus (HBV) strain is presented with a name connected by a hyphen, “-“. Those beginning with the letters from A to I denote genotypes A-I of the reference HBV and are further connected by hyphens with their corresponding GenBank accession numbers. Those beginning with the letters “MO” and “TE” denote our HBV strains isolated from the mothers and teenagers in this study. And those letters are further connected by the hyphens with one to three digits that represent the paired mother-teenager numbers. The dotted and hollow circles denote HBsAg-positive and HBsAg-negative HBV strains, respectively. The symbols of # at the right of our strain names indicate that the strains were isolated from a mother-teenager pair. The GenBank access numbers are KC117267-KC117297 for our 31 strains.
### Table 1 Features of the teenagers and the mothers by the mothers' HBI status

<table>
<thead>
<tr>
<th>Features</th>
<th>HBI mothers (n=40)</th>
<th>Non-HBI mothers (n=53)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No</td>
<td>Prop%</td>
<td>No</td>
</tr>
<tr>
<td>Mothers</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age in years (mean±SD)</td>
<td>38.5±2.4</td>
<td>39.9±3.9</td>
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</tr>
<tr>
<td>Education: junior middle school or above</td>
<td>31</td>
<td>77.5</td>
<td>39</td>
</tr>
<tr>
<td>Occupation: peasants</td>
<td>22</td>
<td>55.0</td>
<td>28</td>
</tr>
<tr>
<td>Annual family income ≥ 50,000 yuan</td>
<td>26</td>
<td>65.0</td>
<td>32</td>
</tr>
<tr>
<td>ALT ≥ 40 U/L</td>
<td>2</td>
<td>5.0</td>
<td>6</td>
</tr>
<tr>
<td>anti-HBe-positive</td>
<td>32</td>
<td>80.0</td>
<td>41</td>
</tr>
<tr>
<td>anti-HBs-positive</td>
<td>8</td>
<td>20.0</td>
<td>30</td>
</tr>
<tr>
<td>Teenagers</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age in years (mean±SD)</td>
<td>13.7±0.1</td>
<td>13.7±0.1</td>
<td>0.72</td>
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<tr>
<td>Sex (male)</td>
<td>22</td>
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<td>31</td>
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<tr>
<td>Vaginal delivery: yes</td>
<td>34</td>
<td>85.0</td>
<td>40</td>
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<tr>
<td>Full-term delivery: yes</td>
<td>40</td>
<td>100.0</td>
<td>51</td>
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<tr>
<td>Birth weight in kilograms (mean±SD)</td>
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<td>3.35±0.37</td>
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<tr>
<td>Only breastfeeding up to six months: yes</td>
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<td>Normal infantile growth and development: yes</td>
<td>33</td>
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</tr>
<tr>
<td>Dental treatment: yes</td>
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<td>23</td>
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<tr>
<td>History of diseases: yes</td>
<td>4</td>
<td>2</td>
<td>0.40*</td>
</tr>
<tr>
<td>History of injury: yes</td>
<td>36</td>
<td>49</td>
<td>0.72*</td>
</tr>
<tr>
<td>Sharing of toothbrush: yes</td>
<td>3</td>
<td>1</td>
<td>0.31*</td>
</tr>
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<td>Transfusion history: yes</td>
<td>2</td>
<td>0</td>
<td>0.18*</td>
</tr>
<tr>
<td>History of surgical operation: yes</td>
<td>4</td>
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<td>0.16*</td>
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<td>Initial HBV vaccine response: yes</td>
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<td>46</td>
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<tr>
<td>History of booster: yes</td>
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<td>ALT ≥ 40 U/L</td>
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<td>4</td>
<td>0.46</td>
</tr>
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</table>

* Fisher’s exact test

HBI: hepatitis B virus infection; Prop: proportion; ALT: alanine aminotransferase; anti-HBs: antibody to hepatitis B surface antigen; anti-HBc: antibody to hepatitis B core antigen; SD: standard error.
Table 2 Relationship of HBI status among the 93 mother-teenager pairs

<table>
<thead>
<tr>
<th>No.</th>
<th>Mother’s HBI status (Total no)</th>
<th>Teenagers’ HBI status (no, prop.)</th>
<th>Teenagers’ anti-HBc+ (no, prop.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>HBI</td>
<td>Non-HBI</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Anti-HBs +</td>
<td>Anti-HBs -</td>
</tr>
<tr>
<td>①</td>
<td>HBI (40)</td>
<td>2</td>
<td>26</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5.0%</td>
<td>65.0%</td>
</tr>
<tr>
<td>②</td>
<td>hHBI, HBV DNA+ (6)</td>
<td>1*</td>
<td>2</td>
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<td></td>
<td>16.7%</td>
<td>33.3%</td>
</tr>
<tr>
<td>③</td>
<td>hHBI, HBV DNA+ (25)</td>
<td>1#</td>
<td>18</td>
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<tr>
<td></td>
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<td>4.0%</td>
<td>72.0%</td>
</tr>
<tr>
<td>④</td>
<td>hHBI, HBV DNA- (9)</td>
<td>0</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.0%</td>
<td>66.7%</td>
</tr>
<tr>
<td>⑤</td>
<td>Non-HBI (53)</td>
<td>1*</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.9%</td>
<td>56.6%</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>3</td>
<td>56</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3.2%</td>
<td>60.2%</td>
</tr>
</tbody>
</table>

Note: *hHBI, #hHBI. HBI: hepatitis B virus infection; HBV: hepatitis B virus; anti-HBs: antibody to hepatitis B surface antigen; anti-HBc: antibody to hepatitis B core antigen.

Statistical significance was determined by Fisher’s exact test.

For teenagers’ HBI status: overall $P=0.46$ (① and ⑤), $P=0.14$ (②, ③+④ and ⑤), $P=0.28$ (②, ③, ④ and ⑤), $P=0.63$ (②+③, ④ and ⑤), $P=1.00$ (②+⑤, ③+④).
For teenagers' anti-HBe status: overall $P=0.04$ (① and ③), $P=0.05$ (②, ③+④ and ⑤), $P=0.02$ (②, ③, ④ and ⑤), $P=0.02$ (②+③, ④ and ⑤), $P=0.02$ (②+⑤, ③+④).
Table 3 Population characteristics of the eight mother-teenagers pairs with either HBI teenagers or HBsAg-negative HBI mothers

<table>
<thead>
<tr>
<th>M-T pairs</th>
<th>Sex (T)</th>
<th>Age (M/T)</th>
<th>ALT, U/L (M/T)</th>
<th>Casr (M/T)</th>
<th>HBsAg /HbsAg (M)</th>
<th>HbsAg /anti-Hbs (M)</th>
<th>HbsAg /anti-Hbs (T)</th>
<th>Booster age (Ye, T)</th>
<th>Anti-HBs, IU/ML (M/T)</th>
<th>HBV DNA, IU/ML (M/T)</th>
<th>HBV-SG (M/T)</th>
<th>Genotype/ Serotype/Mutants (M/T)</th>
</tr>
</thead>
<tbody>
<tr>
<td>73 G</td>
<td>38.8/13.7</td>
<td>21/13</td>
<td>-</td>
<td>-</td>
<td>-/+</td>
<td>+(-/-)</td>
<td>+/-</td>
<td>no</td>
<td>ND/ND</td>
<td>&lt;5/5.58×10^2</td>
<td>ND/ND</td>
<td>B/adr/wt, ND</td>
</tr>
<tr>
<td>78 B</td>
<td>41.0/13.8</td>
<td>14/15</td>
<td>-</td>
<td>-</td>
<td>-/+</td>
<td>+(-/-)</td>
<td>+/-</td>
<td>5.75</td>
<td>1003.5/38.8/372.0</td>
<td>1.42×10^7/7.83×10^6</td>
<td>ND/ND</td>
<td>C/adr/wt, C/adr/Q129R</td>
</tr>
<tr>
<td>102 G</td>
<td>37.8/13.9</td>
<td>45/11</td>
<td>-</td>
<td>-</td>
<td>-/+</td>
<td>+(-/-)</td>
<td>+/-</td>
<td>5.92</td>
<td>59.7/10/9.4/2.1</td>
<td>&lt;5/5.58×10^2</td>
<td>ND/x</td>
<td>ND, C/adr/K141E</td>
</tr>
<tr>
<td>118 B</td>
<td>39.2/13.6</td>
<td>6</td>
<td>-</td>
<td>-</td>
<td>+/-</td>
<td>+(-/-)</td>
<td>+/-</td>
<td>6.17</td>
<td>ND/31.1/42.3</td>
<td>3.02×10^5/5</td>
<td>ND/ND</td>
<td>B/adr/F111N, ND</td>
</tr>
<tr>
<td>36 G</td>
<td>37.9/13.7</td>
<td>7</td>
<td>-</td>
<td>-</td>
<td>+/-</td>
<td>+(-/-)</td>
<td>+/-</td>
<td>10.92</td>
<td>ND/ND/6.2</td>
<td>6.25×10^5/5</td>
<td>ND/ND</td>
<td>C/adr/wt, ND</td>
</tr>
<tr>
<td>38 B</td>
<td>37.7/13.8</td>
<td>20</td>
<td>-</td>
<td>-</td>
<td>+/-</td>
<td>+(-/-)</td>
<td>+/-</td>
<td>No</td>
<td>ND/31.4/48.9/2.0</td>
<td>3.02×10^5/5</td>
<td>ND/ND</td>
<td>C/adr/wt, ND</td>
</tr>
<tr>
<td>39 B</td>
<td>37.2/13.8</td>
<td>8</td>
<td>-</td>
<td>-</td>
<td>+/-</td>
<td>+(-/-)</td>
<td>+/-</td>
<td>4.92</td>
<td>688.9/5136.9/21.1</td>
<td>1.08×10^5/5</td>
<td>ND/ND</td>
<td>C/adr/A129R, ND</td>
</tr>
<tr>
<td>42 B</td>
<td>38.4/13.6</td>
<td>11</td>
<td>-</td>
<td>-</td>
<td>+/-</td>
<td>+(-/-)</td>
<td>+/-</td>
<td>4.75</td>
<td>ND/920.6/328</td>
<td>1.92×10^7/5</td>
<td>ND/ND</td>
<td>C/adr/wt, ND</td>
</tr>
</tbody>
</table>

Note: The results were from 1996-1997. HBI: hepatitis B virus infection; HBV: hepatitis B virus; anti-HBs: antibody to hepatitis B surface antigen; anti-HBc: antibody to hepatitis B core antigen; M: mother; T: teenager; G: girl; B: boy; Mo: month; Ye: year; ND: not detectable; wt: wild type; ALT: alanine aminotransferase; HBV-SG: HBV surface gene.
HBI transmission after neonatal HBV vaccination
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HBI transmission after neonatal HBV vaccination