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Original Article

Booster Vaccination in Infancy Reduces the Incidence of Occult HBV Infection in Maternal HBsAg-positive Children

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Abstract

Background and Aims: Occult HBV infection (OBI) in children has proven to be associated with their immune response to hepatitis B vaccine (HepB). This study aimed to investigate the effect of a booster HepB on OBI, which is rarely investigated. ***Methods:*** This study enrolled 236 maternal HBsAg-positive children who were followed up annually until 8 years of age and were hepatitis B surface antigen (HBsAg) negative. Of those 100 received a booster HepB between 1 and 3 years of age (booster group), and 136 were never boosted (non-booster group). Serial follow-up data of children and baseline data of their mothers were collected and between-group differences were analyzed. ***Results:*** The incidence of OBI varied dynamically during follow-up, with 37.14% (78/210), 19.09% (42/220), 20.85% (44/211), 31.61% (61/193), 8.65% (18/208) and 12.71% (30/236) at 7 months, 1, 2, 3, 4, and 8 years of age. At 8 years of age, the negative conversion rate of HBV DNA in the booster group was significantly higher than that in non-booster group [57.89% (11/19) vs. 30.51% (18/59), p=0.032]. For children without OBI at 7 months old, the incidence of OBI in booster group was significantly lower than that in non-booster group [25.64% (10/39) vs. 67.74% (63/93), p<0.001]. ***Conclusions:*** The incidence of OBI in maternal HBsAg-positive children was high, serum HBV DNA in children with OBI was intermittently positive at low levels, and a booster HepB in infancy reduced the incidence of OBI in children with HBsAg-positive mothers.

Keywords: Hepatitis B virus; Mother-to-child transmission; Hepatitis B vaccine; Occult hepatitis B virus infection; Boost vaccination.

Abbreviations: ACIP, Advisory Committee on Immunization Practices; Anti-HBs, hepatitis B surface antibody; CI, confidence interval; HBeAg, hepatitis B e antigen; HBIG, hepatitis B immunoglobulin; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; HepB, hepatitis B vaccine; LOD, limit of detection; MTCT, mother-to-child transmission; OBI, occult hepatitis B virus infection; OR, odds ratio; PVST, post-vaccination serologic testing.

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Introduction

Mother-to-child transmission (MTCT) of hepatitis B virus (HBV) is one of the main transmission routes of hepatitis B. In recent years, with the improvement of immunization strategy including antiviral treatment for pregnant women with high levels of serum HBV DNA, combined immunization measures for newborns with hepatitis B vaccine (HepB) and hepatitis B immunoglobulin (HBIG), as well as post-vaccination serological testing (PVST), the MTCT of HBV has been greatly reduced.[1–3] According to the PVST results of infants at 7–12 months of age, the immunoprophylaxis success of infants born to mothers with chronic HBV infection were defined as hepatitis B surface antigen (HBsAg) negative and hepatitis B surface antibody (anti-HBs) positive (≥10 mIU/mL). However, with the application of high-sensitivity HBV DNA detection technology, many studies reported that some children born to HBsAg-positive mothers were positive for serum HBV DNA despite successful immunoprophylaxis, that is, occult HBV infection (OBI) after immunoprophylaxis.[4–12]

OBI is defined as the presence of HBV DNA in the liver or serum of people who test negative for HBsAg with currently available assays.[13] The clinical implications of OBI are mainly focused on HBV transmission and disease progression. (a) A donor with OBI can transmit HBV to the recipient by blood transfusion or liver transplantation.[14,15] (b) Individuals with OBI may experience HBV reactivation in an immunosuppressed state and lead to acute hepatitis.[16,17] (c) Patients with chronic liver disease may develop cirrhosis and even hepatocellular carcinoma (HCC) under the procancer mechanism of OBI.[18,19] Currently, OBI has been categorized as a diagnostic type of chronic HBV infection.[20] Therefore, after prevention MTCT of HBV, the children with OBI are in fact immunoprophylaxis failures, which may pose a huge threat to the prevention and management of HBV infection, and need

to be controlled by an optimal strategy in order to achieve the goal of eliminating viral hepatitis by 2030.

The incidence of OBI in maternal HBsAg-positive children after immunoprophylaxis varies greatly in different studies, ranging from 3.1% to 64.0%,[4–12] which may result from different inclusion criteria for pregnant women and their neonates, the HBV immunization procedures of children, the prevalence of HBV in different regions, sample size, as well as the sensitivity and specificity of the detection methods that were used. It is worth noting that 8.3% (12/144) of children with maternal antiviral therapy developed OBI at 12 months old, and no difference was noticed compared with those without maternal antiviral therapy.[10] As suggested by some studies, poor response to HepB, that is, a low level of anti-HBs after immunization is a risk factor for the occurrence of OBI in infancy.[7,10,12] Similarly, our previous study revealed that after increasing the dosage of HepB from 10 μg to 20 μg/dose, the anti-HBs levels of children with a high maternal viral load were significantly improved and the incidence of OBI in the children was significantly decreased.[9] An increased dosage of HepB, booster vaccination also can significantly increase the anti-HBs level.[21] Therefore, whether a booster HepB would reduce the incidence of OBI in children born to mothers with chronic HBV infection is worth exploring.

Based on a prospective follow-up cohort of chronic HBV infected mothers and their children, this retrospective longitudinal study aimed to further explore the risk factors of OBI occurrence in the children after immunoprophylaxis and whether the incidence of OBI can be reduced with a booster dose of HepB in infancy, as well as to provide a theoretical basis for making an optimal strategy to prevent MTCT of HBV.

Methods

Serological and virological assays

HBV DNA load (the limit of detection, LOD: 1.18 $\log_{10}$IU/mL) was quantitated by an Abbott real-time HBV DNA assay (Abbott Molecular, Des Plaines, IL, USA) and an Abbott m2000 system. Serum HBsAg (LOD: 0.05 IU/mL), anti-HBs, and hepatitis B e antigen (HBeAg) were measured by an Abbott i2000 chemiluminescent microparticle immunoassay (Abbott Diagnostic, Chicago, IL, USA). Subjects were considered anti-HBs positive if the level ≥10 mIU/mL and considered HBeAg positive if the S/CO values >1.

Statistical analysis

Geometric mean concentration (GMC) and associated 95% confidence intervals (CIs) were calculated for anti-HBs levels. Categorical variables were expressed as % (m/n) and examined by χ^2/Fisher's exact test. Non-normally distributed data were expressed as median (range) and compared by Mann-Whitney U-test. All *p*-values were two-tailed, and *p*-values <0.05 were considered significant. The statistical analysis was performed with SPSS software V.25.0 (IBM Corp., Armonk, NY, USA) and graphs were plotted using Graphpad Prism 8.0 (GraphPad Software, San Diego, CA, USA).

Results

Enrollment process and related definitions

The children included in this study were from a previously established prospective cohort of maternal HBsAg-positive children.[22] The inclusion criteria were (1) a mother who was HBsAg positive and without antiviral treatment during pregnancy; (2) full term pregnancy; (3) APGAR score of 7 at 1 min; (4) a birth weight ≥2.0 kg; (5) normal body temperature; and (6) a normal jaundice index. The exclusion criteria were (2) congenital malformations; (2) acute neonatal infections; (3) developmental disorders; and (4) family history of neurological disorders, coagulation disorders, immune dysfunction, or allergy to vaccine components. All the children received three doses of recombinant yeast-derived HepB (10 μg/0.5 mL; Dalian Hissen Biopharm Inc., Dalian, China) at birth, 1 month, and 6 months, combined with 100 IU or 200 IU HBIG (Hualan Biological Engineering Inc., Xinxiang, China) within 12 h after birth. The children were followed up annually, and a booster HepB was recommended if their anti-HBs was <10 mIU/mL during follow-up.[23] Other than that, there were no other interventions.

A total of 241 children from Chuzhou city of Jiangsu province were followed up in 2019 at 8 years of age. Five of the 241 were HBsAg positive. Three of the five were immunoprophylaxis failures and two had vaccine-breakthrough infections and were excluded. The remaining 236 children who were HBsAg negative at 8 years of age were included, and their follow-up data at 7 months, 1, 2, 3, 4, and 8 years of age were collected.

As shown in Figure 1, 136 of the 236 children had never received a booster HepB (non-booster group) and 100 had received a booster HepB between 1 and 3 years of age (booster group), of which 44 were boosted when their anti-HBs was <10 mIU/mL, and 56 had a booster HepB at the request of their guardians when the were anti-HBs positive. In non-booster group, 123, 126, 121, 106, and 113 of the 136 children had follow-up data at 7 months, 1, 2, 3, and 4 years of age. In the booster group, 87, 94, 90, 87, and 95 of the 100 children had follow-up data at 7 months, 1, 2, 3, and 4 years of age.

OBI was defined as HBsAg negative, anti-HBs-positive or negative, and serum HBV DNA positive, which is consistent with the international statement on OBI and Chinese hepatitis B guidelines.[13,20] Infants with anti-HBs levels of 10–99.99, 100–999.99, and ≥1,000 mIU/mL at their first PVST were defined as low, medium, and high responses to HepB, respectively.

Comparison of the baseline data of children in non-booster and booster groups

A total of 236 children were included in this retrospective longitudinal study, 136 of whom never had booster HepB (non-booster group) and 100 had a booster HepB between 1 and 3 years of age (booster group). As shown in Table 1, none of the differences of any of the baseline characteristics in the two groups of children at 7 months old or the mothers' prenatal baseline data were significant.

Dynamics of anti-HBs GMCs in children

As shown in Figure 2A, the anti-HBs positivity rate of children in the non-booster group decreased with age, and was 100.0% (123/123), 99.21% (125/126), 72.73% (88/121), 67.92% (72/106), 72.57% (82/113) and 77.21% (105/136) at 7 months, 1, 2, 3, 4, and 8 years of age, respectively. The low, medium, and high response rates to HepB at the age of 7 months were 12.20% (15/123), 65.04% (80/123), and 22.76% (28/123), respectively.

To objectively present the effect of a booster HepB on anti-HBs levels, only data from children who had been boosted at that time were included at each follow-up point at and after 2 years of age. Specifically, 16, 58, 95, and 100 children were included at 2, 3, 4, and 8 years of age, respectively. As shown in Figure 2B, The anti-HBs positivity rate of children in booster group was consistently higher than 90%,

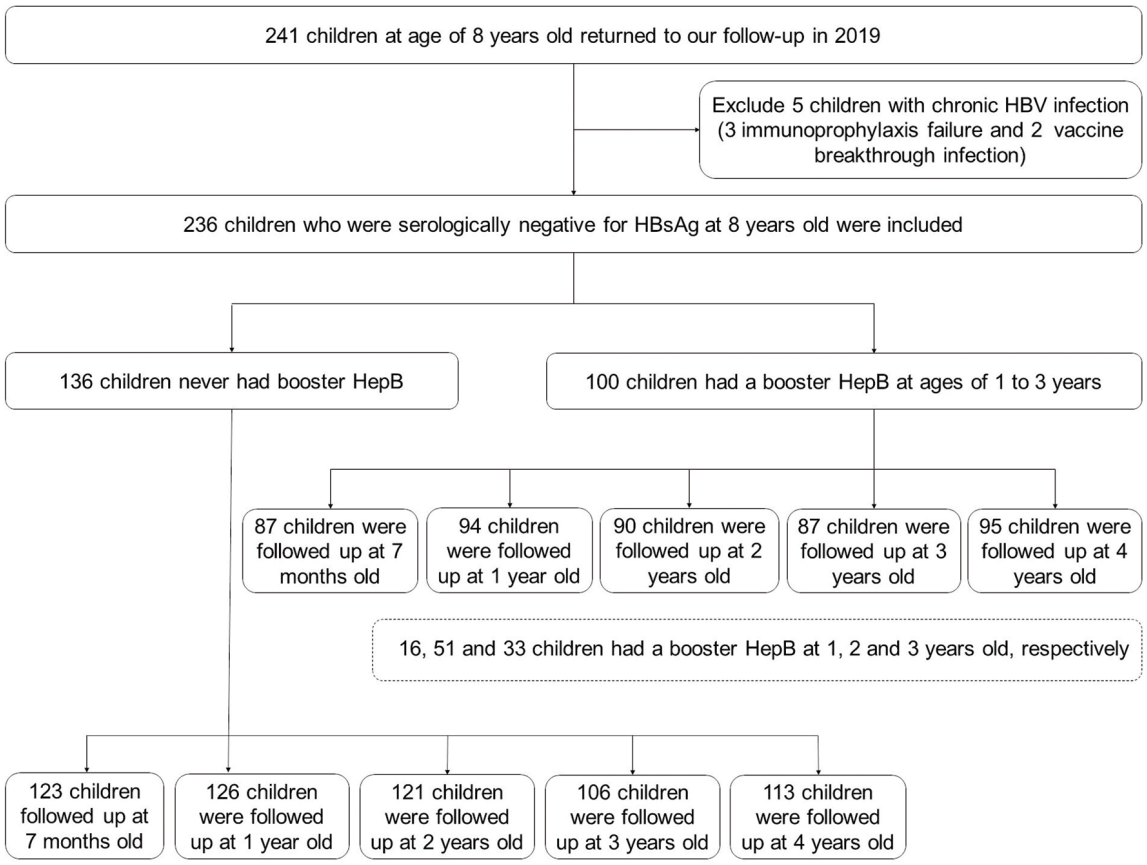

Fig. 1. Enrollment flowchart. HepB, hepatitis B vaccine; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus.

with 96.55% (84/87), 96.81% (91/94), 100.00% (16/16), 96.55% (56/58), 92.63% (88/95), and 92.00% (92/100) at 7 months, 1, 2, 3, 4, and 8 years of age, respectively. The anti-HBs positivity rates in the booster group were significantly higher than those in the non-booster group at and after 2 years of age ($p<0.001$). The low, medium, and high response rates to HepB at 7 months of age were 11.49% (10/87), 47.13% (41/87), and 37.93% (33/87), respectively, which were similar to those in the non-booster group ($p=0.138$).

As shown in Table 1, there were no significant differences

Table 1. Baseline data of children and their mothers in the non-booster and booster groups

	Children in non-booster group (*n*=136)	Children in booster group (*n*=100)	*p*
Mothers			
Age in years	24.21 (18.73–39.81)	24.4 (18.96–42.6)	0.625
HBsAg titer as $\log_{10}$IU/mL	3.55 (1.14–4.97)	3.69 (1.56–4.89)	0.714
HBeAg positive rate[a], *n* (%)	43 (31.85)[a]	33 (35.00)	0.612
HBV DNA level as $\log_{10}$IU/mL	3.09 (1.18–8.97)	3.22 (1.19–9.00)	0.723
ALT in U/L	22.00 (3.00–40.00)	22.00 (9.00–40.00)	0.377
Children			
Male: female	75:61	55:45	0.982
Vaginal: cesarean delivery	22:114	20:80	0.448
Breast:[b] formula fed	51:85	34:66	0.580
HBIG dose, 100 IU: 200 IU	75:61	46:49	0.314
Anti-HBs GMC in mIU/mL at 7 months of age	499.67 (396.79–629.22)	452.17 (325.19–628.72)	0.723

[a]One mother's HBeAg was not available; [b]Breast-feeding included mixed feeding; *p*-values were calculated by Mann-Whitney U-test and χ^2 test. Anti-HBs, hepatitis B surface antibody; HBeAg, hepatitis B e antigen; HBIG, hepatitis B immunoglobulin; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus.

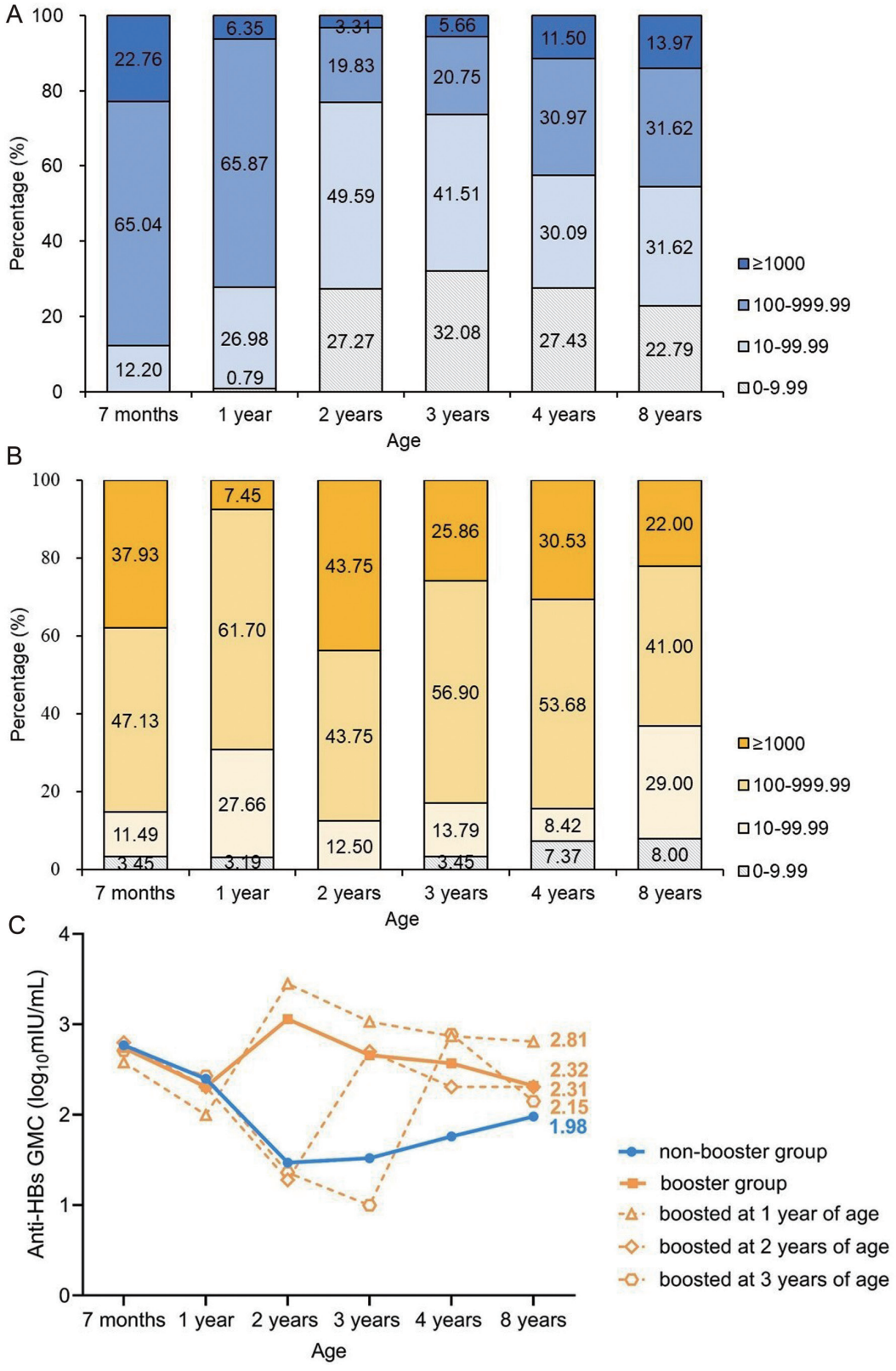

Fig. 2. Dynamic changes of anti-HBs composition ratios and anti-HBs GMCs in children. (A) Dynamics of the anti-HBs composition ratio in the (A) non-booster group and (B) booster group. (C) Dynamics of anti-HBs GMCs in the non-booster group (blue solid line), booster group (yellow solid line), and in the three groups of children boosted at different times (yellow dashed line). anti-HBs, hepatitis B surface antibody.

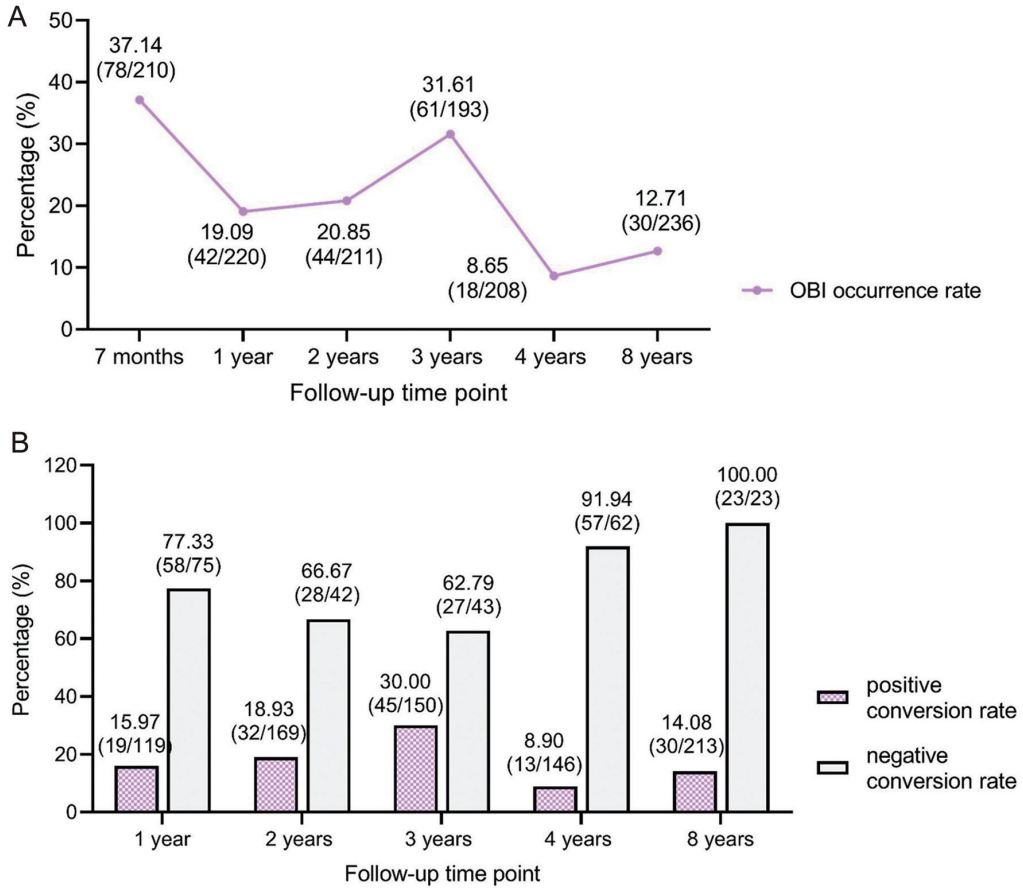

Fig. 3. Dynamic changes of the incidence of OBI in children at each follow-up time point. (A) Overall HBV DNA positivity rate at each follow-up. (B) Dynamics of negative and positive HBV DNA transition rates in children. HBV DNA positive conversion rate. Children who were HBV DNA negative at the last follow-up were found to be HBV DNA positive shown in purple. HBV DNA-negative conversion rate (children who were HBV DNA-positive at the last follow-up were found to be HBV DNA negative) shown in gray. anti-HBs, hepatitis B surface antibody; HBV, hepatitis B virus; OBI, occult hepatitis B virus infection.

between the anti-HBs GMCs of the booster and non-booster groups at 7 months of age [552.72, 95% CI: (383.47–796.67) mIU/mL vs. 586.56, 95% CI (430.45–799.29) mIU/mL, p=0.635], and at 1 year of age [205.25, 95% CI: (144.85–290.84) mIU/mL vs. 248.32, 95% CI: (187.64–328.62) mIU/mL, p=0.562]. However, the GMCs of anti-HBs in booster group were significantly higher than those in non-booster group at and after 2 years of age [8 years old: 207.42, 95% CI: (117.92–364.87) mIU/mL vs. 96.48, 95% CI: (53.57–173.73) mIU/mL, p<0.001]; (Fig. 2C). The results suggest that a booster dose of HepB can significantly increase the GMCs of anti-HBs in children, and thus may enhance the long-term immune barrier against HBV infection. Interestingly, the anti-HBs GMCs of the non-booster group dropped sharply to the lowest level at 2 years of age and then increased slightly after 3 years of age. That might be the result of close contact with their HBsAg-positive mothers, which put them at high risk of exposure to HBV. In addition, 27.27% of those children lost protective levels of anti-HBs at 2 years of age (Fig. 2A). The occurrence of natural booster in children may have thus resulted in a slight increase of the anti-HBs level after 3 years of age.

Dynamics of the incidence of OBI in children after immunoprophylaxis

All the enrolled 236 children had four to six return visits during follow-up. Only 70 of the 236 children (29.66%) tested negative for serum HBV DNA every time. The other 166 children (70.34%) were positive for serum HBV DNA at least once during follow-up. Specifically, 89, 52, 21, three, and one of the children were HBV DNA positive one, two, three, four, and five times, respectively. As shown in Figure 3A, the incidence of OBI in children varied dynamically during follow-up, found in 37.14% (78/210), 19.09% (42/220), 20.85% (44/211), 31.61% (61/193), 8.65% (18/208), and 12.71% (30/236) at 7 months, 1, 2, 3, 4, and 8 years of age, respectively.

Further, the dynamics on the OBI occurrence of children in conjunction with the dynamics of negative and positive HBV DNA transitions in children were analyzed. At each follow-up visit, 8.90–30.00% of children who were HBV DNA negative at the last follow-up were found to be HBV DNA positive, and 62.79–100.00% of the children who were HBV DNA positive at the last follow-up had become HBV DNA negative (Fig. 3B). The results indicated a high incidence of OBI in children born to HBsAg-positive mothers. In addition, alternating low positive or negative serum HBV DNA levels in children with OBI indicated that a single test may not be able to fully determine the status of OBI in children. Therefore, children born to HBsAg-positive mothers should be followed up, and additional monitoring of serum HBV DNA is necessary.

Table 2. Univariate and multivariate analysis of risk factors associated with the occurrence of OBI in children at 7 months of age

Variable	Univariate		Multivariate	
	OR (95% CI)	*p*	Adjusted OR (95% CI)	*p*
Mothers				
Age, per 1 year increase	0.97 (0.92–1.03)	0.308		
HBsAg titer as $\log_{10}$IU/mL	1.29 (0.9–1.84)	0.161		
HBeAg positive rate (%)	1.33 (0.73–2.45)	0.353		
HBV DNA level >6 $\log_{10}$IU/mL (%)	1.06 (0.95–1.19)	0.308		
ALT level, per 1 U/L increase	1.02 (0.98–1.06)	0.432		
Children				
Male vs. female	1.12 (0.64–1.97)	0.689		
Cesarean vs. vaginal birth	0.35 (0.17–0.72)	0.004	0.32 (0.15–0.67)	0.002
Breast[a] vs. formula fed	0.88 (0.5–1.57)	0.670		
HBIG dose[b], 200 IU vs. 100 IU	0.61 (0.34–1.08)	0.090	0.58 (0.32–1.05)	0.073
Birth weight, per 1 kg increase	1.46 (0.77–2.77)	0.251		
Anti-HBs GMC in mIU/mL at 7 months of age[c]	1.17 (0.62–2.2)	0.629		

[a]Breast-feeding included mixed feeding; [b]Lack of HBIG dose data for one child with OBI and three children without OBI at 7 months of age. Anti-HBs, hepatitis B surface antibody; CI, confidence interval; HBeAg, hepatitis B e antigen; HBIG, hepatitis B immunoglobulin; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; OR, odds ratio.

Risk factors for the occurrence of OBI in children at 7 months of age

A total of 210 of the 236 children returned for follow-up at 7 months of age, of whom 78 (37.14%, 78/210) had OBI. According to the baseline data of the two groups, univariate and multivariate analyses were conducted for the occurrence of OBI in children at 7 months of age. The results showed that the baseline age, HBsAg titer, HBeAg status, HBV DNA load, and alanine aminotransferase (ALT) level of the mothers and the baseline sex, feeding method, HBIG dose, birth weight, and anti-HBs level of the children had no significant effect on the occurrence of OBI at 7 months of age. The mode of delivery (cesarian or vaginal) was a risk factor [odds ratio (OR) 0.32, 95% CI: (0.15–0.67), p=0.002]. However, 37.18% of children (29/78) with OBI at 7 months of age had no detectable HBV DNA at any subsequent follow-up, and 55.30% (73/132) of children who were HBV DNA negative at 7 months of age had detectable HBV DNA at subsequent follow-ups (Table 2). Considering that HBV DNA status in children was dynamic, we further analyzed the effect of the type of delivery in 49 children who were consistently positive for HBV DNA except at 7 months of age and in 59 who were consistently negative for HBV DNA except at 7 months of age. The results showed that the delivery mode had no significant effect on the occurrence of OBI in children at 7 months old, 71.43% (35/49) delivered by cesarean section vs. 84.75% in those with vaginal birth (50/59), p=0.092].

Effect of booster HepB on outcome of HBV infection in 7-month-old children with OBI

Thirty-one of the 78 children with OBI at 7 months of age had a booster HepB between 1 and 3 years of age. The other 47 were never boosted with HepB. Seventy-six, 72, 65, 66, and 78 children returned for follow-up at 1, 2, 3, 4, and 8 years of age, respectively. At those visits, 76, 65, 42, 38, and 47, respectively had not been given a booster and 7, 23, 28, and 31, respectively had received a booster. The incidence of OBI in non-boosted children was 22.37% (17/76), 26.15% (17/65), 23.81% (10/42), 2.63% (1/38) and 34.04% (16/47) at 1, 2, 3, 4, and 8 years of age, respectively. The incidence in boosted children was 14.29% (1/7), 26.09% (6/23), 17.86% (5/28) and 9.68% (3/31) at 2, 3, 4, and 8 years of age, respectively (Table 3). At 8 years of age, the incidence of OBI was significantly lower in children with a HepB booster than that in those without a booster [9.68% (3/31) vs. 34.04% (16/47), p=0.014].

Table 3. Comparison of the incidence of OBI between children with OBI at 7 months of age with and without a booster HepB during follow-up

Age, years	Children without HepB booster		Children with a HepB booster		*p*
	No. of children followed up	Incidence of OBI, % (n/m)	No. of children followed up	Incidence of OBI, % (n/m)	
1	76	22.37 (17/76)	0	–	–
2	65	26.15 (17/65)	7	14.29 (1/7)	0.818
3	42	23.81 (10/42)	23	26.09 (6/23)	0.838
4	38	2.63 (1/38)	28	17.86 (5/28)	0.076
8	47	34.04 (16/47)	31	9.68 (3/31)	0.014

HepB, hepatitis B vaccine; OBI, occult hepatitis B virus infection.

Table 4. Comparison of the incidence of OBI in children without OBI at 7 months of age with and without a booster HepB during follow-up

Age, years	Children without HepB booster		Children with a HepB booster		*p*
	No. of children followed up	Incidence of OBI, % (n/m)	No. of children followed up	Incidence of OBI, % (n/m)	
1	120	15.83 (18/120)	0	–	–
2	113	19.47 (22/113)	4	0.00 (0/4)	1.000
3	80	33.75 (27/80)	27	51.85 (14/27)	0.094
4	62	6.45 (4/62)	54	3.70 (2/54)	0.684
8	76	10.53 (8/76)	56	3.57 (2/56)	0.246

HepB, hepatitis B vaccine; OBI, occult hepatitis B virus infection.

We investigated the difference of HBV DNA-negative conversion rates in children with a booster HepB and in those without a booster. Of the 31 children with a booster, 12 had received it after an OBI was detected, therefore those 12 children and the 47 children without a HepB booster were included in a non-booster group (a total of 59 children). The other 19 of the 31 children were included in a booster group. At 8 years of age, 29 (37.18%) of the 78 children with an OBI at 7 months of age were never found to be HBV DNA positive after 7 months of age, and thus might have achieved HBV DNA clearance. Of the 29 children, 18 were in the non-booster group and 11 children in the booster group. The rate of HBV DNA-negative conversion was significantly higher in the booster than that in the non-booster group [57.89% (11/19) vs. 30.51% (18/59), p=0.032], suggesting that a HepB booster promoted serum HBV DNA-negative conversion in children with OBI. Put another way, among children with OBI at 7 months old, the risk of re-occurrence of OBI was reduced in children with booster HepB, with an absolute risk reduction of 27.38%, a relative risk reduction of 39.40%, and a number needed to treat of 2.54.

Effect of booster HepB on outcome of HBV infection in 7-month-old children without OBI

At 7 months of age, 132 children were negative for HBV DNA, of whom 56 had a booster HepB between 1 and 3 years of age, and 76 never been boosted with HepB. There were 120, 117, 107, 116, and 132 children with return visits at 1, 2, 3, 4, and 8 years of age, respectively. Of those, 120, 113, 80, 62, and 76, respectively, had not received a booster and 4, 27, 54, and 56, respectively, had received a booster. The incidence of OBI in non-boosted children was 15.83% (19/120), 19.47% (22/113), 33.75% (27/80), 6.45% (4/62), and 10.53% (8/76) at 1, 2, 3, 4, and 8 years of age, respectively. The incidence in boosted children was 0.0% (0/4), 51.85% (14/27), 3.70% (2/54) and 3.57% (2/56) at 2, 3, 4, and 8 years old, respectively (Table 4).

We also determined the difference of the HBV DNA-positive conversion rate between children with a HepB booster and those without a booster. Of the 56 children with a HepB booster, 17 had received it after an OBI had been detected Those 17 children and the 76 without a HepB booster were included in non-booster group of 93 children. The other 39 of the 56 children were included in a booster group. At 8 years of age, 73 (55.30%) of the 132 children without OBI at 7 months of age tested positive for HBV DNA at least once during follow-up. Ten were in the booster and 63 were in the non-booster groups. The rate of HBV DNA positive conversion was significantly lower in the booster group than that in the non-booster group [25.64% (10/39) vs. 67.74% (63/93), p<0.001], suggesting that a HepB booster reduced the incidence of OBI in children after immunoprophylaxis, with an absolute recurrence rate of 42.10%, relative recurrence rate of 62.15%, and number needed to treat of 1.61.

Discussion

In recent years, more and more studies have shown that a certain proportion of infants develop OBI after MTCT of HBV despite successful immunoprophylaxis.[4–12] Children with OBI not only risk of HBsAg reactivation, but also may be a potential source of HBV transmission, and pose a major challenge in controlling HBV infection and achieving the goal of eliminating viral hepatitis at 2030. In this study, the high 37.14% incidence of OBI in 78 of 210 infants born to mothers with chronic HBV infection at their first follow-up visit at the age of 7 months, was similar to the prevalence of OBI in children reported in other studies, such as 28.0% in children 10–128 months of age in Iran,[4] 42.0% in children 6–72 months of ager in India,[5] and 36.4% in children 3–36 months in China.[7] The detection of serum HBV DNA and the occurrence of OBI in maternal HBsAg-positive children after immunoprophylaxis was worthy of careful consideration. Our previous study showed that the occurrence of OBI in children after immunoprophylaxis was characterized by intermittent viremia,[6] and similar results were found in this study. The immune system of children is not fully developed until the age of 5 years, and viremia of OBI may change dynamically with the fluctuation of immune status *in vivo*. After 5 years of age, and especially at 8 years of age, the immune system of children is basically stable, and the status of OBI thereafter deserves to be further explored. Our findings showed that the prevalence of OBI in children was 19.09% (42/220), 20.85% (44/211), 31.61% (61/193), 8.65% (18/208) and 12.71% (30/236) at 1, 2, 3, 4, and 8 years of age, respectively. In this study, only 29.66% (70/236) of the children were HBV DNA negative at each follow-up visit; that is, only about 30% of the children never developed OBI. The results indicate that the incidence of OBI after immunoprophylaxis was higher in children born to HBsAg-positive mothers, and that children with OBI had intermittent low levels of positive serum HBV DNA. Thus, a single test may not be accurately determine the occurrence of OBI in children, suggesting that children born to HBsAg-positive mothers should be followed up and that additional monitoring of serum HBV DNA is necessary.

After full immunization of HepB, the anti-HBs levels in children gradually decline over time.[24] When their anti-HBs are <10 mIU/mL, which is the protective antibody level, whether a booster dose of vaccine is needed is based on their maternal HBsAg status. Children of HBsAg negative mothers are not recommended for booster vaccination, as immune memory persists when anti-HBs disappears.[25] However, some maternal HBsAg-positive children may lose immune memory and

become HBsAg positive when they become adolescents.[21,26] Therefore, for children born to HBsAg-positive mothers, the Advisory Committee on Immunization Practices (ACIP) and the Centers for Disease Control and Prevention of the USA recommend booster vaccination with a single dose of HepB vaccine if the anti-HBs concentration is <10 mIU/mL.[27] In this study, 100 children received one dose of booster HepB between 1 and 3 years of age, and the other 136 never had a HepB booster. Compared with children without a HepB booster, children with a booster in infancy had significantly higher rates of anti-HBs positivity [92.00% (92/100) vs. 77.21% (105/136, p=0.002)] and anti-HBs GMCs (207.42 mIU/mL 95% CI: (117.92–364.87) vs. 96.48 mIU/mL, 95% CVI: (53.57–173.73), p<0.001] at 8 years of age. Consistent with other studies,[24,28,29] booster vaccination had a significant long-term effectiveness on enhancing anti-HBs positivity rate and level.

Interestingly, our results showed that for children with OBI at 7 months of age, the incidence of OBI at 8 years of age was significantly lower in those with a booster HepB than that in those without a HepB booster [9.68% (3/31) vs. 34.04% (16/47), p=0.014]. In addition, 37.18% (29/78) of all children who had OBI at 7 months of age were consistently negative for HBV DNA at follow-up, suggesting that approximately 40% of children with OBI at the first PVST achieved clearance of HBV DNA with the help of anti-HBs as their immune system developed. Notably, the rate of HBV DNA-negative conversion was significantly higher in children with a HepB booster than that in children without a HepB booster [57.89% (11/19) vs. 30.51% (18/59), p=0.032], which suggests that a HepB booster facilitated the achievement of HBV DNA-negative conversion in children with OBI. Moreover, for children who were HBV DNA negative at 7 months of age, the incidence of OBI was lower in children with a HepB booster than that in children without a booster at most follow-up time points, although the difference may not have been significant because of the small sample size. Furthermore, the cumulative incidence of OBI at follow-up was significantly lower in children with a HepB booster than that in children without a booster [25.64% (10/39) vs. 67.74% (63/93), p<0.001], suggesting that a booster in infancy significantly reduced the incidence of OBI. A long-term follow-up study showed that a HepB booster vaccination at 10–14 years of age reduced the risk of OBI at 23–28 years of age.[26] The evidence suggest that a booster reduces the risk of OBI occurrence in children, but further validation with an expanded sample is needed.

Several studies have explored the risks of OBI in immunized maternal HBsAg-positive infants, but no consistent conclusions have been reached because differences in sample enrollment, selection of factors, and analysis methods.[5] Our results show that maternal age, HBsAg titer, HBeAg status, and HBV DNA load, child sex, mode of delivery, feeding pattern, initial antibody level, HBIG dose, and birth weight, were not significantly associated with the occurrence of OBI in children. Only a HepB booster in infancy (1–3 years) significantly reduced the occurrence of OBI in children at 8 years of age. There were some study limitations. Firstly, the number of cases was limited because of the difficulty of obtaining long-term follow-up data. A study with a large sample is needed for further confirmation. Secondly, further clarification is needed on the relevant indications or optimal timing of a booster HepB for children.

In conclusion, we found a high incidence of OBI in children born to HBsAg-positive mothers, and serum HBV DNA in children with OBI was intermittently positive at low levels. Further, we demonstrated that a booster HepB in infancy reduced the incidence of OBI in maternal HBsAg-positive children, which provides evidence for improving the current immunization procedures for this high-risk population.

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Conflict of interest

The authors have no conflict of interests related to this publication.

Author contributions

Obtained funding (JL), designed the study (JL, JW), joint first authors (YL, LL), performed the study (YL, LL, YS, ML, FD), collected the serum samples (XZ, ZD, LZ, JJ, HZ), analyzed data and drafted the manuscript (YL, LL), contributed to the interpretation of the results and critical revision of the manuscript for important intellectual content (JL, JW), read and approved the final manuscript (All authors), study guarantors (JL, JW).

Ethical statement

Use of the cohort data was approved by the Bioethics Committees of Peking University. We confirm that we have the necessary consents from all individuals involved in the study.

Data sharing statement

The datasets used during the current study are available from the corresponding author on reasonable request.

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