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Original Article

Vaginal Delivery and Breastfeeding Benefit Infant Immune Response to Hepatitis B Vaccine: A Prospective Cohort Study

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Abstract

Background and Aims: Natural vaginal delivery and breastfeeding favor the development of a strong immune system in infants, and the immune response of infants to vaccines is closely related to their immune system. This large prospective cohort study aimed to explore the effects of delivery and feeding mode on infant's immune response to hepatitis B vaccine (HepB). ***Methods:*** A total of 1,254 infants who completed the whole course of HepB immunization and whose parents were both HBsAg negative were enrolled from infants born in Jinchang City during 2018–2019 by cluster sampling method. ***Results:*** Twenty (1.59%) of the 1,254 infants were nonresponders to HepB. Among the other 1,234 infants, 10.05% (124/1,234), 81.69% (1,008/1,234) and 8.27% (102/1,234) of infants had low, medium, and high responses to HepB, respectively. Logistic regression analysis showed that cesarean section (OR: 8.58, 95% CI: 3.11–23.65, $p<0.001$) and birth weight <3.18 kg (OR: 5.58, 95% CI: 1.89–16.51, $p=0.002$) were independent risk factors for infant nonresponse to HepB, and cesarean section (OR: 7.63, 95% CI: 4.64–12.56, $p<0.001$), formula feeding (OR: 4.91, 95% CI: 1.47–16.45, $p=0.001$), maternal anti-HBs negativity (OR: 27.2, 95% CI: 10.67–69.35, $p<0.001$), paternal non-response history of HepB (OR: 7.86, 95% CI: 2.22–27.82, $p=0.014$) and birth weight <3.22 kg (OR: 4.00, 95% CI: 2.43–6.59, $p<0.001$) were independent risk factors for infant low response to HepB. In cases where birth weight and genetic factors are unmodifiable and maternal anti-HBs effects are controversial, it makes sense to enhance infant response by changing delivery and feeding patterns. ***Conclusions:*** Natural vaginal delivery and breastfeeding are beneficial to the infant's immune response to HepB.

Keywords: Hepatitis B vaccine; Immune response; Anti-HBs; Vaginal delivery; Breastfeeding.

Abbreviations: Anti-HBs, hepatitis B surface antibody; CI, confidence interval; GMC, geometric mean concentration; HBV, hepatitis B virus; HBsAg, hepatitis B surface antigen; HepB, hepatitis B vaccine; MTCT, mother-to-child transmission; OR, odds ratio; PVST, post-vaccination serological testing.

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Introduction

Hepatitis B virus (HBV) infection is a major public health problem with an estimated 296 million people worldwide living with chronic HBV infection in 2019.[1] Due to the persistence of replication-active closed circular DNA (cccDNA) in hepatocytes, spontaneous or therapeutic eradication of chronic HBV infection is rarely achieved.[2] Thus, universal vaccination of hepatitis B vaccine (HepB) is considered to be the most effective means to reduce the harm of HBV.[3–6] Nevertheless, despite receiving the standard primary HepB vaccination, approximately 3–10% of healthy infants have no response or a low response to the vaccine respectively, which places them at high risk of HBV infection.[7–11] More important, it has been well established that people infected with HBV in infancy or early childhood have an almost 80–90% risk of developing chronic HBV infection. Therefore, it is necessary to investigate the occurrence rate and related risk factors of nonresponse and low response to HepB in infants, so as to find a solution to improve the level of immune response to HepB.

As with most vaccines, the protection induced by the HepB is mediated through a complex interaction between innate, humoral and cellular immunity. There is growing evidence suggesting that the gut microbiome, which changes dynamically early in life, plays an important role in the development of the infant's immune system.[12–15] Combining the fact that there are individual and geographical differences in the effectiveness of vaccines, and that these are also true in the polymorphism of the gut microbiome, researchers have begun to explore the possible impact of the gut microbiome on vaccine efficacy.[16–23] Recently, it was reported that the prevaccination gut microbiome composition differed between infants response to rotavirus vaccine and those not responsive to the vaccine.[21] Two studies by the same author on HepB, Bacillus Calmette Guerin, oral polio vaccine, and tetanus toxoid vaccine showed that high abundance of stool Actinobacteria, such as *Bifidobacterium*, was associated with an increased immune response to the vaccine and immune memory re-

sponse 2 years after vaccination.[22,23] The above evidence suggests that favorable early development of the infant gut microbiome is highly correlated with the effectiveness of vaccine immunization, including HepB, suggesting the possibility of improving infant response to HepB by modifying gut microbiome.

The composition of an infant's gut microbiome in early life is heavily influenced by the mode of delivery and feeding. Many studies have shown that both natural vaginal delivery and breastfeeding result in a higher diversity of gut microbiome and a higher relative abundance of *Bifidobacterium* species, which are overall closer to the mature gut microbiome of the mother.[24–29] Interestingly, a study found that infants born by cesarean section developed a gut microbiome similar to that of infants born by natural vaginal delivery after they orally took diluted feces from their mothers.[29] That suggested that natural vaginal delivery could facilitate mother-to-child transmission of microorganisms, which was important for the development of intestinal microbiota in the early life of infants. All the evidence indicated that the indirect effect of natural vaginal delivery and breastfeeding on infant's immune response to vaccines is great of concern. Some studies have demonstrated that natural delivery and breastfeeding improved infant immune response to vaccines such as oral polio vaccine and tetanus toxoid vaccine,[30,31] but there is a lack of such studies related to HepB. In this large prospective cohort study, we investigated the impact of delivery and feeding patterns on the immune response to HepB in infants born to mothers without HBV infection. Other potential risk factors that might lead to nonresponse or a low response to HepB were also analyzed with a view to addressing the cause of poor response to HepB and finding a feasible way to improve infant's immune response.

Methods

Subjects

In this study, 1,254 infants were enrolled from those born in 2018–2019 in Jinchang City by a cluster sampling method. The inclusion criteria were (1) completion of the whole course of immunization with three doses of HepB following the 0-, 1-, and 6-month schedule; (2) both parents were both HBsAg negative; (3) gestational age of ≥37 weeks; (4) healthy (no jaundice); (5) birth weight ≥2 kg and (6) returned for post-vaccination serologic testing (PVST) at 7 months of age.

According to the results of PVST at 7 months of age, infants who were HBsAg negative and those with anti-HBs <10 mIU/mL were regarded as nonresponsive to HepB, those who were HBsAg-negative and anti-HBs levels of 10–99.99, 100–999.99, and ≥1,000 mIU/mL were defined as having low, medium, and high immune responses to HepB, respectively. All infants with nonresponse and low response to HepB were boosted with two doses of recombinant CHO HepB (20 μg/1.0 mL; North China Pharmaceutical Jintan Biotechnology Co., Ltd., Hebei, China), and the PVST was performed 8 days after the first dose and 1 month after the second dose. A flowchart of enrolment and follow-up is shown in Figure 1.

Serological and virological assays

The level of serum anti-HBs was detected with a MAGLUMI 2000 Plus Automatic Chemiluminescent Microparticle Immunoassay (CMIA) Analyzer (New Industries Biological Engineering Co., Ltd., Shenzhen, China). Anti-HBs of ≥10 mIU/mL was considered positive. Infants with anti-HBs <10 mIU/mL were also tested for HBsAg and HBV DNA. HBsAg was determined with an RT-6100 ELISA Microplate Reader (Rayto Life and Analytical Sciences Co., Ltd., Shenzhen, China). HBV DNA was quantitated with an NP968 Nucleic Acid Extractor (Tianlong Technology Co., Ltd., Xian, China). HBsAg <0.05 IU/mL and HBV DNA <1.18 $\log_{10}$IU/mL were considered as negative.

Statistical analysis

Anti-HBs levels were reported as geometric mean concentrations (GMCs) with 95% confidence intervals (CIs). Non-normal variables were reported as medians (range) and compared by Mann-Whitney U-tests. Categorical variables were compared by chi-squared (χ^2), or Fisher's exact tests when the expected count in one cell was less than 5. All *p*-values were two-tailed and those <0.05 were considered significant. Odds ratios (ORs) and 95% CIs of each factor were derived by logistic regression analysis. The birth-weight cutoff for infants with no response or a low response to HepB was estimated with receiver operating characteristic (ROC) curves. The statistical analysis was performed with SPSS V.24.0 (IBM Corp., Armonk, NY, USA); graphs were plotted by Graphpad Prism software V.8.0 (Dotmatics, Boston, MA, USA).

Results

First PVST in infants at 7 months of age

All 1,254 infants tested negative for HBsAg, and 20 (1.59%, 20/1,254) of the 1,254 were negative for anti-HBs and nonresponsive to HepB; the other 1,234 (98.41%, 1,234/1,254) were positive for anti-HBs, and the infants with low, medium, and high responses to HepB were 10.05% (124/1,234), 81.69% (1,008/1,234) and 8.27% (102/1,234), respectively. In addition, the anti-HBs GMC for low, medium and high responders were 82.97 (78.61–87.57) mIU/mL, 521.36 (504.69–538.57) mIU/mL, and 1,826.48 (1,666.05–2,002.37) mIU/mL, respectively.

Booster immunization in infants with no response and low response to HepB vaccine

All 20 nonresponders and 124 low responders were boosted with two doses of 20 μg CHO HepB, of which 17 nonresponders and 106 low responders returned for PVST 8 days after the first dose and 1 month after the second dose.

As shown in Figure 2, anti-HBs levels increased significantly with booster doses in both nonresponders and low responders ($p<0.001$). For nonresponders, 29.41% (5/17) remained anti-HBs-negative even though anti-HBs GMC increased from 6.10 (5.15–7.23) mIU/mL to 39.6 (19.69–79.65) mIU/mL after the first booster HepB. After the second booster HepB, the anti-HBs positivity rate reached 100.00% (17/17) and the anti-HBs GMC increased to 263.63 (157.80–440.35) mIU/mL. For low responders, 7.55% (8/106) still had low anti-HBs levels after the first booster HepB, but all infants had anti-HBs ≥100 mIU/mL after the second booster HepB. In addition, the anti-HBs GMC of the low responders increased from 82.97 (78.61–87.57) mIU/mL to 471.63 (408.04–545) mIU/mL after the first HepB booster and then to 833.49 (754.4–920.87) mIU/mL after the second HepB booster.

Comparison of baseline characteristics in infants at 7 months of age with different anti-HBs profiles

According to anti-HBs levels of infants at 7 months of age, infants were divided into four groups, nonresponders, low responders, medium responders, and high responders. The comparison of baseline data in the four groups was shown in Table 1. There were no significant differences in infant

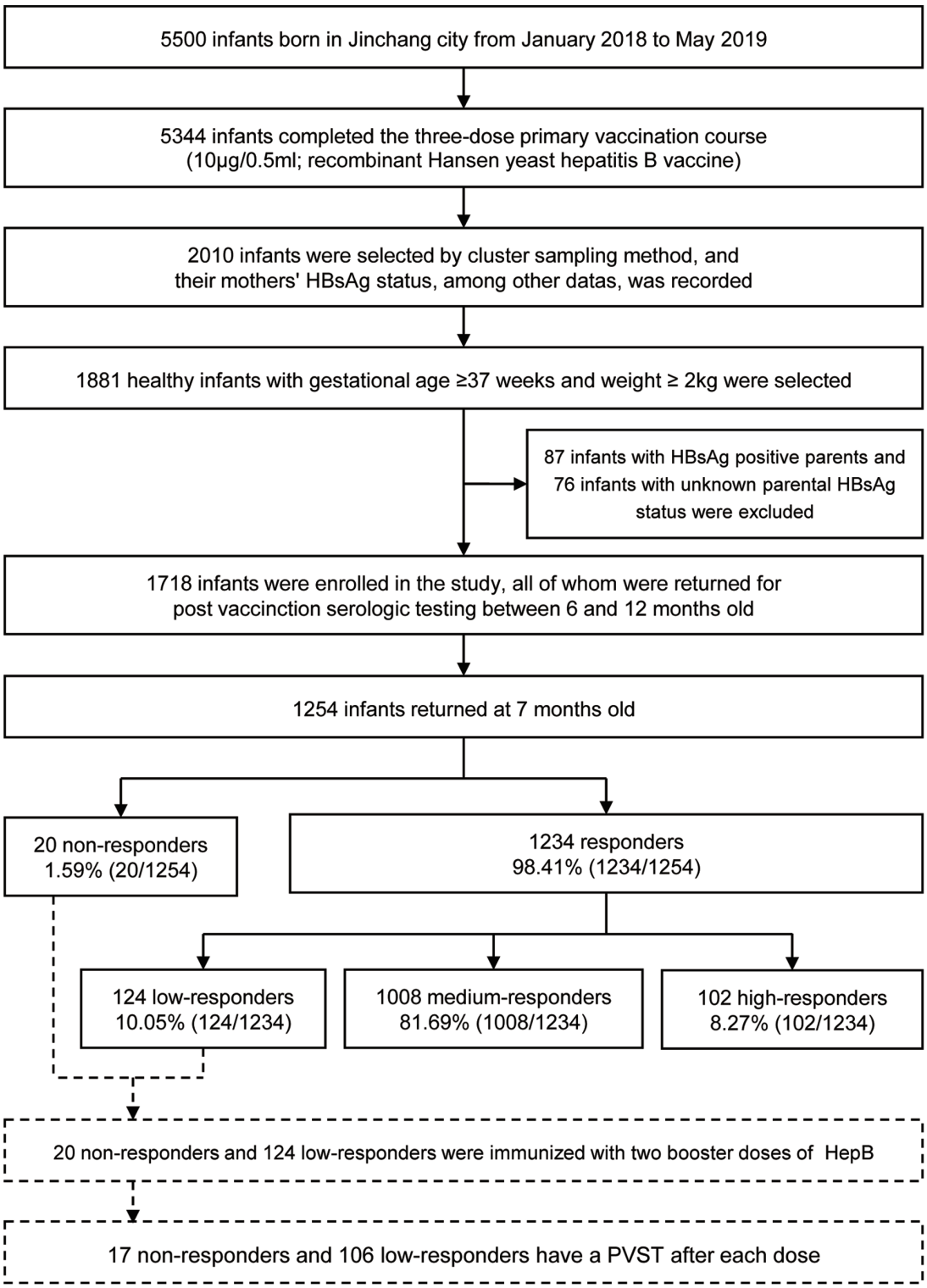

Fig. 1. The enrolment flowchart of infants. HBsAg, hepatitis B surface antigen; HepB, hepatitis B vaccine.

gender. Other baseline data, including delivery mode, feeding pattern, maternal anti-HBs negativity, parental history of nonresponse to HepB, parental immune diseases, and birth weight differed significantly among the four groups. The proportions of cesarean section, formula feeding, maternal anti-HBs negativity, parental nonresponse and parental immune diseases were significantly higher in nonresponders and low responders than that in the medium and high responders.

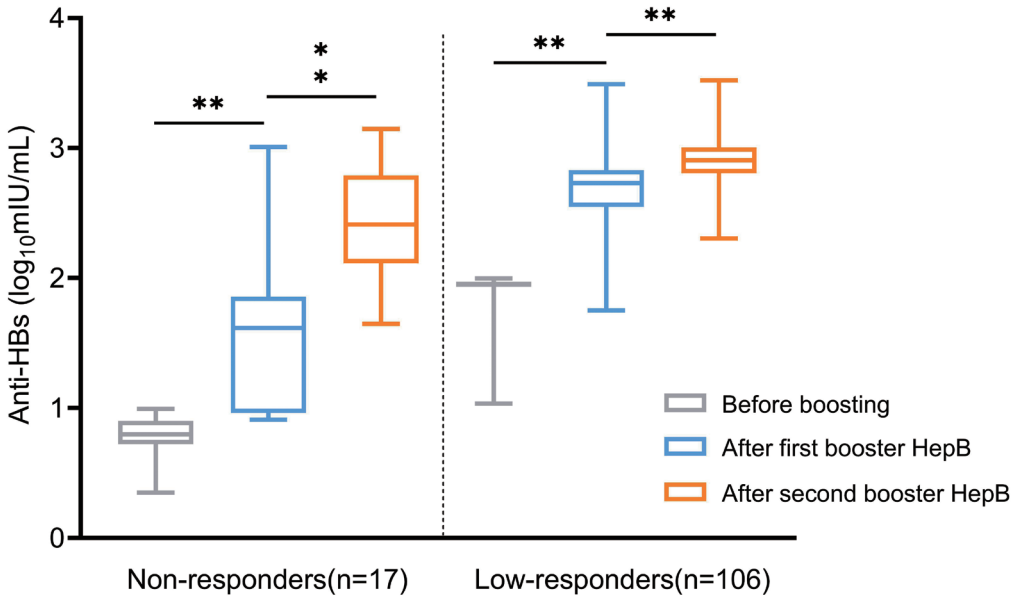

Fig. 2. Immune response to the first booster HepB and the second booster HepB in nonresponders and low responders. **p<0.01. anti-HBs, hepatitis B surface antibody; HepB, hepatitis B vaccine.

Moreover, the birth weight of infants was significantly lower in nonresponders than in low, medium, and high responders.

Risk factors of nonresponse to HepB vaccine

The nonresponse rate among the enrolled infants was 1.59% (20/1,254). The results of univariate and multivariate analysis of risk factors associated with nonresponse are shown in Table 2. Three possible risk factors of nonresponse were identified by univariate analyses. Among them, cesarean section (OR: 6.99, 95% CI: 2.82–17.31, p<0.001), formula feeding (OR: 7.51, 95% CI: 1.62–34.78, p=0.010) and maternal anti-HBs negativity (OR: 7.27, 95% CI: 2.33–22.72, p=0.001) were positively associated with nonresponse; Birth weight (OR: 0.16, 95% CI: 0.04–0.62, p=0.008) was negatively associated with nonresponse after primary immunization. To clarify the specific impact of birth weight on the risk of nonresponse, we obtained a cutoff value of 3.18 kg for birth weight associated with infant nonresponse by ROC analysis (AUC: 0.693, p=0.002). A birth weight of <3.18 kg was also significant in the univariate analysis (OR: 3.95, 95% CI: 1.43–10.92, p=0.008). The results of multivariate regression analysis showed that cesarean section (OR: 8.58, 95% CI: 3.11–23.65, p<0.001) and a birth weight of <3.18 kg (OR: 5.58, 95% CI: 1.89–16.51, p=0.002) were independent risk factors for infant nonresponse to HepB. Feeding pattern (p=0.289) and maternal anti-HBs negativity (p=0.360) were excluded from the multivariate analysis.

Risk factors of low response

As there were no significant differences of the factors in medium and high responders, both the groups were combined in the following analyses. Of the 1,234 responders, the rates of low and medium-high response were 10.05% (124/1,234) and 89.95% (1,110/1,234), respectively. The results of univariate and multivariate analysis of risk factors of low response are shown in Table 3. In the univariate analysis, four factors including cesarean section (OR: 6.12, 95% CI: 4.14–9.04, p<0.001), formula feeding (OR: 7.59, 95% CI: 2.94–19.6, p<0.001), maternal anti-HBs negativity (OR: 42.55, 95% CI: 19.71–91.85, p<0.001), maternal history of nonresponse (OR: 11.42, 95% CI: 3.43–38.00, p<0.001), and paternal history of nonresponse history (OR: 7.65, 95% CI: 2.96–19.76, p<0.001), were identified as positively associated with a low response to HepB. Birth weight (OR: 0.30, 95% CI: 0.17–0.51, p<0.001) was identified as negatively associated with a low response. To clarify the specific impact of birth weight on the risk of a low response to HepB, we obtained a cutoff value of 3.22 kg for birth weight associated with a low response by ROC analysis (AUC: 0.615, p<0.001). A birth weight of <3.22 kg was also significant in the univariate analysis (OR: 2.22, 95% CI: 1.59–3.29, p<0.001). Notably, the proportion of parents with immune disease in low responders was 6.45% (8/124) compared with 0.00% (0/1,110) in medium/high responders (p<0.001). Therefore, parental immune disease might also be also a risk factor for a low response to HepB in infants. That it was not included among the risk factors might be due to the small sample size of the infants with parental immune disease in this study.

To exclude bias, multivariate analysis was performed after excluding eight infants with parental immune disease. All factors that had significant results in the univariate analysis remained significant in the multivariate analysis. Cesarean section (OR: 7.63, 95% CI: 4.64–12.56, p<0.001), formula feeding (OR: 4.91, 95% CI: 1.47–16.45, p=0.001), maternal anti-HBs negativity (OR: 27.20, 95% CI: 10.67–69.35, p<0.001), paternal nonresponse history (OR:2.27, 95% CI: 2.22–27.82, p=0.014), and birth weight <3.22 kg (OR: 4.00, 95% CI: 2.43–6.59, p<0.001) were independent risk factors for infant low response. Maternal history of nonresponse (p=0.297) were excluded from the multivariate analysis. Risk factors of nonresponse and low response to HepB in infants are shown in Figure 3.

Immune response to HepB in infants with different delivery and feeding patterns

To further explore the effect of delivery and feeding pattern on infant's immune response to the HepB, anti-HBs composition ratios and anti-HBs GMCs of infants in the vaginal de-

Table 1. Baseline characteristics of infants at 7 months of age, and with different anti-HBs profiles

Characteristic	Nonresponder	Low responder	Medium responder	High responder	*p*
Case	20	124	1,008	102	
Sex, *n* (%)					0.316
Male	7 (35.00)	66 (53.23)	513 (50.89)	46 (45.10)	
Female	13 (65.00)	58 (46.77)	495 (49.11)	56 (54.90)	
Delivery mode, *n* (%)					<0.001
Vaginal	8 (40.00)	62 (50)	872 (86.51)	82 (80.39)	
Cesarean	12 (60.00)	62 (50)	136 (13.49)	20 (19.61)	
Feeding pattern, *n* (%)					<0.001
Breast[a]	18 (90.00)	116 (93.55)	1,002 (99.4)	98 (96.08)	
Formula	2 (10.00)	8 (6.45)	6 (0.6)	4 (3.92)	
Maternal anti-HBs negativity, *n* (%)	4 (20.00)	32 (25.81)	7 (0.69)	2 (1.96)	<0.001
Maternal nonresponsive history, *n* (%)	1 (5.00)	6 (4.84)	4 (0.4)	1 (0.98)	0.001
Paternal nonresponsive history, *n* (%)	1 (5.00)	8 (6.45)	9 (0.89)	1 (0.98)	0.003
Parental immune diseases, *n* (%)	1 (5.00)	8 (6.45)	0 (0.00)	0 (0.00)	<0.001
Birth weight in kg	3.01 (2.51–4.21)	3.10 (2.05–4.11)	3.25 (2.41–4.20)	3.25 (2.43–4.06)	<0.001
Anti-HBs GMC in mIU/mL[b]	6.10 (5.15–7.23)	82.97 (78.61–87.57)	521.36 (504.69–538.57)	1,826.48 (1,666.05–2,002.37)	<0.001

[a]Breast feeding included mixed feeding. [b] Mean (95% confidence interval). *p*-values were calculated by Mann-Whitney U-test and χ^2 test. anti-HBs, hepatitis B surface antibody.

livery, cesarean delivery, breastfeeding and formula feeding groups were analyzed (Fig. 4A). Of the 1,254 infants enrolled, 1,024 were vaginally and 230 by cesarean section. The proportions of nonresponders [0.78% (8/1,024) vs. 5.22% (12/230), $p<0.001$] and low responders [6.05% (62/1,024) vs. 26.96% (62/230), $p<0.001$] were significantly lower in infants born by vaginal delivery than in those born by cesarean section. The anti-HBs GMC was significantly higher than in those born by cesarean section [494.54 (469.03–521.31) mIU/mL vs. 290.07 (241.82–347.94) mIU/mL, $p<0.001$]. Among all infants, 1,234 were breastfed and 20 were formula fed. Breast fed infants had lower rates of both nonresponse [1.46% (18/1,234) vs. 10.00% (2/20), $p<0.001$] and low response [9.40% (116/1,234) vs. 40.00% (8/20), $p<0.001$] than formula-fed infants, and had higher anti-HBs GMC than formula-fed infants [454.57 (430.23–480.29) mIU/mL vs. 192.8 (90.51–410.68) mIU/mL, $p=0.012$].

We divided the infants into four groups by the mode of delivery and feeding (Fig. 4B), and calculated anti-HBs composition ratios and anti-HBs GMCs for each group separately. In the four groups of infants born vaginally and breastfed, born vaginally and formula fed, born by cesarean section and breastfed, and born by cesarean section and formula fed, the nonresponse rates were 0.79% (8/1,013), 0.00% (0/11), 4.52% (10/221) and 22.22% (2/9), respectively; The low response rates were 5.73% (58/1,013), 36.36% (4/11), 26.24% (58/221) and 44.44% (4/9), respectively; anti-HBs GMCs were 496.82 (471.19–523.84), 321.07 (155.67–662.06), 302.48 (252.64–362.24) and 103.37 (22.42–476.65) mIU/mL, respectively. The results further suggest that both vaginal delivery and breastfeeding were good for immune response to HepB in infants.

Discussion

HepB is one of the safest and most effective vaccines. However, it is well established that a few infants are not responsive or have low responses to HepB after the standard schedule of vaccination at 0, 1, and 6 months of age, which puts them at high risk of HBV infection. The anti-HBs induced by the HepB is mediated by a complex interaction between innate, humoral and cellular immunity. An increasing number of studies suggest that the early gut microbiome is highly correlated with the development of the infant immune system and may therefore alter the effectiveness of vaccines, including the hepatitis B vaccine in infants.[12–23] Some studies showed that natural vaginal delivery and breastfeeding resulted better early development of the infant's gut microbiome.[24–29] This large prospective cohort study was designed to explore the potential factors associated with no response and a low response to HepB in infants born to HBsAg-negative mothers. Our study excluded in advance the effects of geography, the vaccine itself, parental HBV infection status and infant health status on vaccine efficacy. All infants included were born to HBsAg-negative parents in the same city, had normal birth weight, full gestational age, healthy, and received three doses of the same HepB according to standard procedures. Finally, our study was the first to demonstrate that vaginal delivery and breastfeeding were beneficial to infant's immune response to HepB.

Consistent with previous studies,[7–11] the rates of nonresponse and low response were 1.59% (20/1,254) and 10.05% (124/1,234), respectively. Infant sex, delivery mode, feeding pattern, maternal anti-HBs negativity, parental history of nonresponse to HepB, parental immune diseases and birth weight were included in this study of nonresponse and low response to HepB in infants. Among them, only infant gender had no effect on the immune response

Table 2. Univariate and multivariate logistic regression analyses of risk factors related to nonresponse to HepB in infants

Variable	Univariate		Multivariate	
	OR (95% CI)	*p*	OR (95% CI)	*p*
Male vs. female	0.53 (0.21–1.32)	0.172	–	–
Cesarean vs. vaginal birth	6.99 (2.82–17.31)	<0.001	8.58 (3.11–23.65)	<0.001
Formula vs. breast[a] feeding	7.51 (1.62–34.78)	0.010	2.50 (0.46–13.64)	0.289
Maternal anti-HBs negativity	7.27 (2.33–22.72)	0.001	1.83 (0.50–6.68)	0.360
Maternal nonresponse history	5.84 (0.72–47.51)	0.099	–	–
Paternal nonresponse history	3.55 (0.45–27.96)	0.229	–	–
Parental immune diseases	8.07 (0.96–67.71)	0.054	–	–
Birth weight (per 1 kg increase)	0.16 (0.04–0.62)	0.008	–	–
Birth weight ≤3.18 kg	3.95 (1.43–10.92)	0.008	5.58 (1.89–16.51)	0.002

[a]Breast feeding included mixed feeding. CI, confidence interval; HepB, hepatitis B vaccine; OR, odds ratio.

to HepB. Cesarean section (OR: 8.58, 95% CI: 3.11–23.65, $p<0.001$) and a birth weight of <3.18 kg (OR: 5.58, 95% CI: 1.89–16.51, $p=0.002$) were identified as independent risk factors for nonresponse. Cesarean section (OR: 7.63, 95% CI: 4.64–12.56, $p<0.001$), formula feeding (OR:4.91, 95% CI: 1.47–16.45, $p=0.001$), maternal anti-HBs negativity (OR: 27.2, 95% CI:10.67–69.35, $p<0.001$), paternal HepB nonresponse history OR: 7.86, 95% CI: 2.22–27.82, $p=0.014$), and a birth weight of <3.22 kg (OR: 4.00, 95% CI: 2.43–6.59, $p<0.001$) were identified as risk factors of a low response in infants. The risk factors of nonresponse differed from those of a low response, probably because of the small sample size of nonresponders.

Cesarean section was found to be an independent risk factor both for infant nonresponse and low response to HepB. Further analysis revealed that the proportions of nonresponders and low responders among infants delivered by natural was significantly lower than that of infants delivered by cesarean section, and that the anti-HBs GMC of infants born naturally was also significantly higher than that of infants born by cesarean section. It has been reported that infants delivered by cesarean section do not acquire microbial colonization, which is necessary for the development of the immune system and immune regulation, from the birth canal.[24,32,33] Our preconjecture that vaginal delivery might increase infant response to HepB based on the effect of delivery mode on intestinal bacteria and immune system development was confirmed.

Although the proportion of formula-fed infants was higher in both nonresponders and low responders than in medium and high responders. Formula feeding was a risk factor for low response, not lack of response. The lack of a significant difference might have been a consequence of the small sample of nonresponders. Extensive evidence has shown that breastfeeding not only provides many direct health benefits to infants, but also plays a positive role in their response to multiple vaccines.[22,23,31] Consistent with cesarean section, formula feeding has been proved to hinder the formation of infant's intestinal microbiota.[34] Furthermore, many studies have shown that, compared with formula feeding, breastfeeding promotes the development of thymus which plays a central role in the establishment of T-cell mediated immunity.[35–37]

As in our preconjecture, different delivery and feeding pattern are seen to affect the immune response capacity of infants by altering the gut microbiome. In order to confirm that delivery and feeding pattern could individually influence the infant's immune response to HepB, we further divided the infants into four groups by delivery and feeding pattern and calculated the anti-HBs composition ratios and anti-HBs GMCs for each group separately. The results showed that there were significant differences in anti-HBs levels between infants fed differently, both in vaginal and cesarean births. Likewise, there were significant differences in immune response to HepB between infants delivered by different modes in both breastfed and formula-fed infants. In addition, we found that infants born by cesarean sec-

Table 3. Univariate and multivariate logistic regression analysis of factors associated with low response in infants

Variable	Univariate		Multivariate	
	OR (95% CI)	*p*	OR (95% CI)	*p*
Male vs. female	1.12 (0.77–1.63)	0.545	–	–
Cesarean vs. vaginal birth	6.12 (4.14–9.04)	<0.001	7.63 (4.64–12.56)	<0.001
Formula vs. breast[a] feeding	7.59 (2.94–19.60)	<0.001	4.91 (1.47–16.45)	0.001
Maternal anti-HBs negativity	42.55 (19.71–91.85)	<0.001	27.2 (10.67–69.35)	<0.001
Maternal nonresponse history	11.42 (3.43–38.00)	<0.001	2.93 (0.39–22.20)	0.297
Paternal nonresponse history	7.65 (2.96–19.76)	<0.001	7.86 (2.22–27.82)	0.014
Birth weight, per 1 kg increase	0.30 (0.17–0.51)	<0.001	–	–
Birth weight ≤ 3.22 kg	2.22 (1.50–3.29)	<0.001	4.00 (2.43–6.59)	<0.001

[a]Breast feeding included mixed feeding. HepB, hepatitis B vaccine; OR, odds ratio.

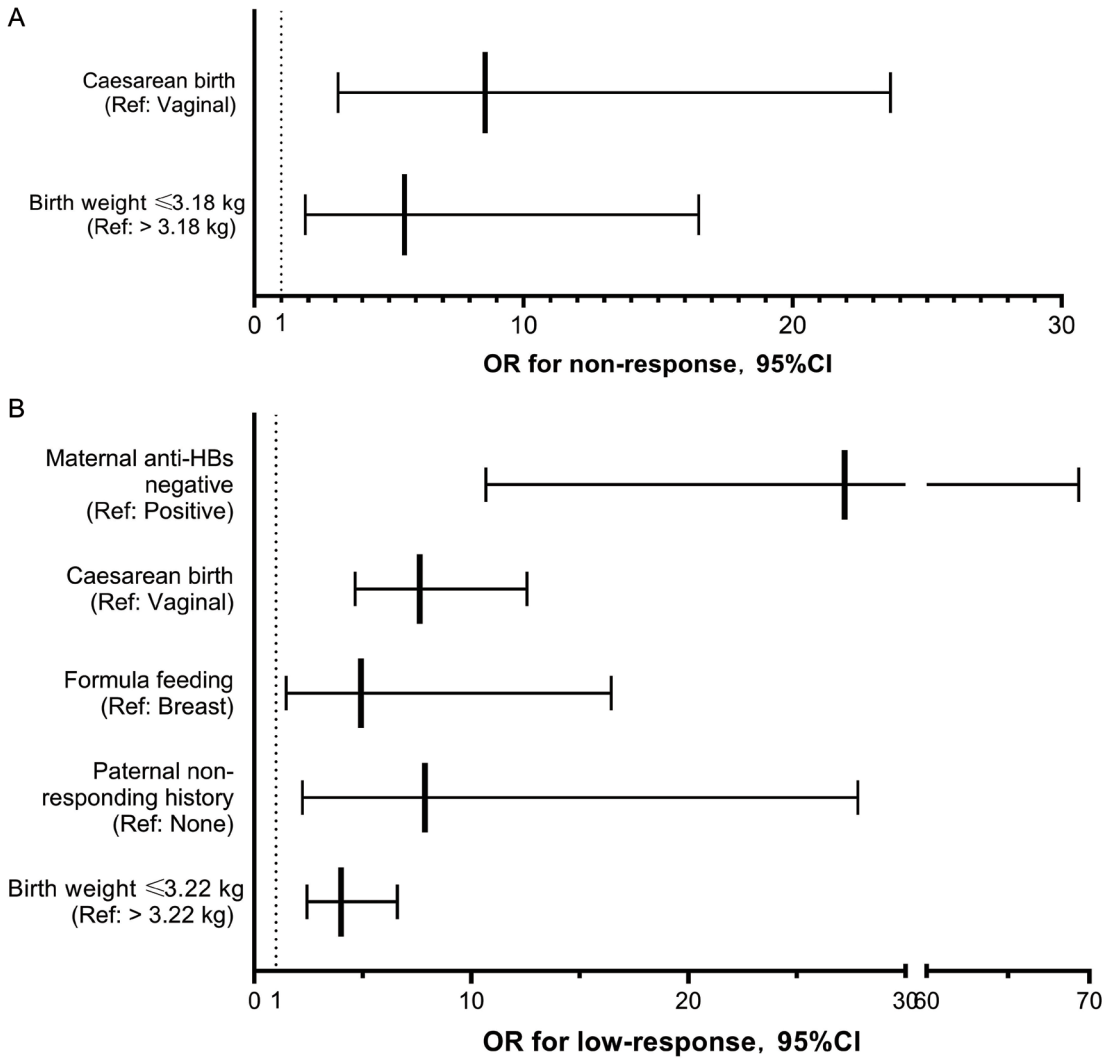

Fig. 3. Risk factors for nonresponse (A) and low response (B) to HepB in infants. CI, confidence interval; OR, odds ratio.

tion and formula fed had high HepB nonresponse and low response rates of 22.22% and 44.44% respectively, while infants born vaginally and breastfed had a significantly lower nonresponse and a low response rates of 0.79% and 5.73%, respectively.

For the effect of birth weight on the immune response to HepB, previous studies were based on premature or low birth-weight infants, and poor vaccine response in infants with birth weight <2 kg has been observed with common vaccines such as diphtheria, tetanus and hepatitis B.[38,39] In this study, normal-weight infants delivered at term were selected as subjects and it was found that lower weight may also lead to poorer immune responses even in infants weighing >2 kg. Specifically, ROC analysis and logistic regression identified birth weight <3.18 kg and birth weight <3.22 kg as independent risk factors for nonresponse and low response to HepB in infants, respectively. Notably, we also found the effect of parental nonresponse history and parental immune diseases on infant low response. The proportion of infants born to parents with immune diseases was 6.45% (8/124) in the low responders and 0.00% (0/1,110, $p<0.001$) in the medium/high responders. Besides, parental nonresponse history was found to be an independent risk factor of low response to HepB. The results suggest that the HepB response in infants was significantly influenced by immune-related genetic factors. Whether maternal anti-HBs levels affect infant response is inconclusive,[40–42] and in our study, infants with maternal anti-HBs negativity had a higher risk of a low response. Nevertheless, maternal nonresponse was shown to be associated with a low infant response in both the univariate and multivariate analysis when maternal anti-HBs negativity was not introduced. Therefore, further studies are needed to determine whether the effect of maternal anti-HBs negativity on the low response in infant is primarily due to genetic factors of poor response to the vaccine or to placental transmission of anti-HBs.

This study also analyzed the immune response to the first booster dose and the second vaccination in nonresponders and low responders. The results showed that for infants who did not respond to the primary immunization, two doses of booster HepB should be given to ensure the production of protective anti-HBs. In addition, for infants with a low response to primary immunization, one booster dose of vaccine was sufficient to significantly raise the anti-HBs level to a high

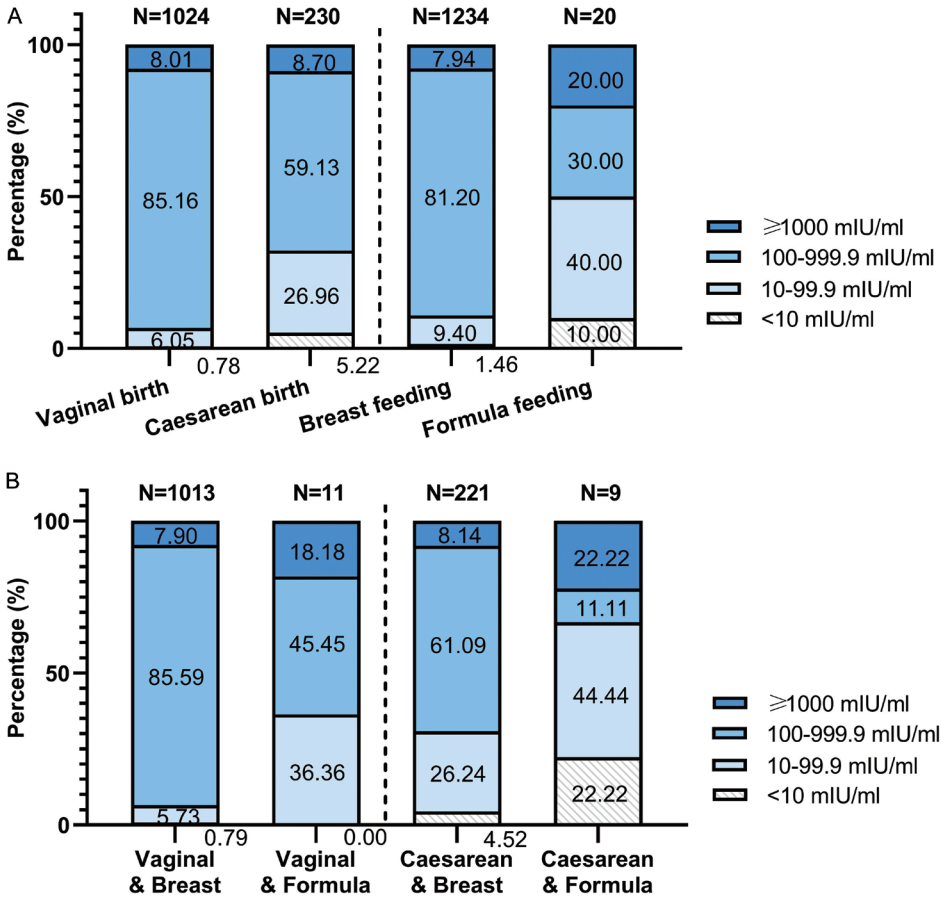

Fig. 4. Composition ratio of and GMC of anti-HBs in infants with different delivery and feeding patterns. GMC, geometric mean concentration; anti-HBs, hepatitis B surface antibody.

enough level.

The study included some limitations. First, the sample sizes of low-response infants and formula-fed infants were relatively small. Second, analysis of some factors, such as parental HepB nonresponse history, were based on parental reports, which could result in potential recall bias.

In conclusion, natural vaginal delivery and breastfeeding are beneficial to infants in immune response to HepB. Although delivery mode, feeding pattern, maternal anti-HBs status, birth weight, and genetic factors are all associated with infant's immune response to HepB, neither birth weight nor genetic factors can be modified, but the effect of maternal anti-HBs status needs further confirmation. It is of interest to enhance the infant immune response through delivery and feeding pattern. It has been reported that the prevalence of cesarean section has recently increased worldwide.[43] Cesarean section rate in China is estimated as 47.6% in 2010–2011 by the WHO.[44] According to a recent report from China, only 79.6% infants are breastfed, and only 20.8% infants are exclusively breastfed before 6 months of age.[45] Therefore, in order to improve the effectiveness of HepB and further control the spread of HBV, there is an urgent need to promote vaginal delivery and breastfeeding.

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Conflict of interest

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

Author contributions

Designed the study (JL, XZ), joint first authors (HL, LL), performed the study (HL, LL, YL, ML, YS, FD), obtained funding and collected the serum samples (HL), analyzed data and drafted the manuscript (LL), contributed to the interpretation of the results and critical revision of the manuscript for important intellectual content (JL, XZ), read and approved the final manuscript (All authors), and served as study guarantors (JL, XZ).

Ethical statement

The cohort data involved in the study was approved by the Bioethics Committees of Jinchan city. We confirm that we have all necessary consents from any 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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