RESEARCH PAPER

Effects of hepatitis B vaccine boosters on anti-HBs-negative children after primary immunization

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\textbf{ABSTRACT}
This study was aimed at evaluating the changes of hepatitis B surface antibody (anti-HBs) titer after booster vaccinations in 5–15-year-old children with negative antibodies (<10 mIU/mL). 225 subjects (mean age, 9.28 ± 2.95 years) included in the study consisted of 123 males and 102 females, with a complete hepatitis B vaccination during infancy. The participants were divided into 3 groups according to their pre-booster anti-HBs level: Group I, <0.1 mIU/mL; Group II, 0.1 to <1.0 mIU/mL; Group III, 1.0 to <10.0 mIU/mL. All the participants were administrated 3 doses of booster hepatitis B vaccination (0-1-6 month, 20 μg), and changes in the levels of antibodies were examined at 4 time-points (one month after the first and the third dose, one year and 5 years after the third dose). The seroprotective rate (defined as anti-HBs >10.0 mIU/mL) among 225 subjects at the 4 time-points were 93.8%, 100%, 83.6% and 73.4%, respectively (χ\textsuperscript{2} = 90.29, p < 0.05). The seroprotective rate (>10 mIU/mL) and anti-HBs geometric mean titer (GMT) in Group III were always higher than those in the other 2 groups (all p < 0.05). The immune effect of a 3-dose booster revaccination is good, and the booster-induced immune response was correlated with their pre-booster levels, and ≥1.0 mIU/mL ensuring a robust positive response, whereas titers below this value may indicate the need for a course of booster vaccination.

\textbf{Introduction}

Hepatitis B virus (HBV) infection is one of the leading causes of chronic hepatitis, liver cirrhosis, and hepatocellular carcinoma, and has a worldwide distribution with significant morbidity and mortality.\textsuperscript{1-3} Vaccination is the most efficient and safest method of conferring long-term protection against the virus.\textsuperscript{4} In 1992, the World Health Organization (WHO) endorsed the recommendation for the integration of HBV vaccination into national immunization,\textsuperscript{5} with a target for worldwide implementation by 1997. In 2008, universal childhood HBV vaccination programs were administered in 177 countries, leading to substantial reduction in the global burden and transmission of HBV-associated complications.\textsuperscript{6}

A national seroepidemiological survey in 2006 revealed a high HBV infection rate in China, with an HBsAg carrier rate of 7.2% in 1–59 y old individuals. Since 1992, universal infant vaccination planning began in China and 2005, the government required that all infant vaccinations be administered free of cost. After decades of hepatitis B mass vaccination, a national serosurvey in 2006 showed the prevalence of HBsAg in Chinese population aged 1–59 y had decreased to 7.2%, from 9.8% in 1992, and the number of HBV-infected children has declined by 80 million.\textsuperscript{7,8}

The long-term protection induced by 3 doses of hepatitis B vaccine is well-established. Previous studies have demonstrated that the majority of individuals elicit a strong anamnestic response upon exposure to HBV even 10–15 years after primary vaccination in infancy.\textsuperscript{9-11} However, a number of studies have indicated that the antibody titers decline with increasing age following immunization, leading a higher risk of infection.\textsuperscript{12,13} In addition, a small number of vaccination failures occur.\textsuperscript{14,15} Approximately 10–20% of neonatal HBV vaccines develop an anergy or a poor response, and 5% acquire HBV infections despite vaccination.\textsuperscript{16,17} HBV vaccine failure may be attributed to an immature immune system. Consequently, the necessity of booster vaccination raises concern. While our earlier study\textsuperscript{20} has observed that, the one year effect of a 3-dose revaccination in anti-HBs negative children was closely related to their pre-booster level of anti-HBs.

The follow-up periods of most previous studies were short. Hence, a longer follow-up is essential to assess the HBV revaccination efficacy. In the present study, which is a follow-up to our previous study,\textsuperscript{20} we examined protective immunity to HBV in 225 children whose anti-HBs levels were <10.0 mIU/mL more than 10 years after primary vaccination; we evaluated the efficacy of the 3-dose booster over a 5-year follow-up period.
Results
In all, total of 2022 children were investigated and 795 children were selected and enrolled. One month after the first dose, 9 subjects refused to blood sample collection. One month after the third dose, 21 subjects dropped out of the study. One year after vaccination, 206 subjects were unavailable to follow-up. Four years later, 225 subjects were remaining while the other 299 were lost to follow-up (Fig. 1). The 225 participants included 123 males and 102 females, with an average age of 9.28 ± 2.95 years. 41, 69, and 115 subjects were randomized into 3 groups, respectively. No statistically significant differences were observed in age or gender between the groups (Table 1).

Antibody titer distribution after the first and the third dose of booster vaccination
A total of 211 children showed protective antibodies after the first dose of hepatitis B vaccine, with a total positive seroconversion rate of 93.8%; the positive rate of group III was the highest. The difference among the groups was statistically significant (Fisher’s exact probability test, \( p < 0.001 \)). After the first dose, the GMTs of 225 subjects was 323.13 mIU/mL, and the difference among the groups was significant (\( F = 44.56, p < 0.001 \)).

A cohort of 225 subjects achieved satisfactory response after the third dose of the booster vaccine, with a total positive seroconversion rate of 100% and an anti-HBs GMT of 616.27 mIU/mL. The differences in anti-HBs GMT between the 3 groups were statistically significant (\( F = 16.95, p < 0.001 \)). Further multiple comparisons showed that the GMT in Group III was significantly higher than that of the other 2 groups (Table 2).

Antibody titer distribution at 1-year post booster vaccination
At one-year after the third booster dose, 188 children maintained protective antibody levels, with a total anti-HBs positive rate of 83.6%, and an anti-HBs GMT of 48.40 mIU/mL. Significant differences were observed in the positive rate and anti-HBs GMTs between the groups (\( \chi^2 = 23.27, p < 0.001, F = 13.64, p < 0.001 \)), and anti-HBs positive rate as well as GMT in Group III were the highest (Table 2).

Antibody titer distribution at 5-year post booster vaccination
At five-year after the third booster dose, 116 children maintained protective antibody levels, with a total anti-HBs positive
rate of 73.4%, and an anti-HBs GMT of 24.60 mIU/mL. Significant differences in the positive rate and anti-HBs GMT between groups ($\chi^2 = 27.75, p < 0.001, F = 32.80, p < 0.001$) were observed. In addition, anti-HBs positive rate and GMT in Group III were the highest (Table 2).

**Comparison of antibody titer at 4 time-points (one month after the first and the third dose, one year and 5 years after the third dose)**

One month after the first and third dose, one year and 5 years after the third dose, and anti-HBs positive rates were 93.8%, 100%, 83.6%, and 73.8%, respectively. The total anti-HBs positive rate increased by 6.2% after 2 additional doses, which decreased by 16.4% after one year followed by a further decrease by 9.8% after 5 years (all $p < 0.05$).

After stratified by groups, the anti-HBs positive rates increased to 100% in Group I ($p < 0.05$) and Group II ($p < 0.05$) at one month after the third dose. And the anti-HBs positive rates among the 3 groups declined at one year post the third dose, respectively ($p < 0.05$), then unchanged 4 years later.

Anti-HBs GMTs of group I and II at one month after the third dose were higher than those at one month after the first dose (all $p < 0.05$), whereas the anti-HBs GMTs of group III were similar. Subsequently, the anti-HBs GMTs decreased with time in the 3 groups (all $p < 0.05$) (Table 2, Fig. 2).

**Discussion**

In this study, total of 795 children with $\leq 10$ mIU/mL anti-HBs titer after finishing a primary HBV vaccination in infancy were divided into 3 groups: Group I, $< 0.1$ mIU/mL; Group II, 0.1 to $< 1.0$ mIU/mL; and Group III, 1.0 to $< 10.0$ mIU/mL, respectively. We then evaluated the change of anti-HBs antibodies level over 5-year period after a second serial 3-dose HBV vaccination. The result showed that the humoral immune response after receiving booster vaccines was observed to be significantly different among the groups. Anti-HBs titers in Group III were seen to be able to elicit a rapid and robust anamnestic response after the first dose, at the same time, the value of antibody titers in Group III were higher than those in both Group I and Group II. It demonstrates that Group III may continue to maintain the good immune memory based on earlier hepatitis B vaccination scheme in their infancy, which indicate that no second-serial 3-dose HBV vaccination may be need for children in Group III. However, for Group I, about 20.0% of children did not develop anamnestic response after receiving the first dose booster. So a second serial dose is needed to ensure sero-protection for these children. These results in our present study were consistent with previous studies. Federica Chiara et al. found that $\geq 2$ mIU/mL pre-booster antibody titers might be predictive of an anamnestic response to booster vaccination and $< 0.1$ mIU/mL, pre-booster levels were prone to predictive of failing to achieve the protective antibody level after a booster. Spradling et al. observed that a pre-booster level $\geq 1$ mIU/mL could potentially respond to a booster dose.22

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**Table 1. Age and sex distribution of study subjects.**

| Group | No. | Male | Female | $\chi^2$ | $P$ | Age | $F$ | $P$ value |
|-------|-----|------|--------|---------|-----|-----|-----|---------|
| I     | 41  | 19   | 22     | 1.776   | 0.411| 9.67 ± 2.81 | 0.257 | 0.774    |
| II    | 69  | 37   | 32     |         |      | 9.27 ± 2.92 |      |          |
| III   | 115 | 67   | 48     |         |      | 9.35 ± 3.03 |      |          |

$^a$ Anti-HBs level prior to immune booster: Group I $< 0.1$ mIU/mL; Group II 0.1 to $< 1.0$ mIU/mL; Group III 1.0 to $< 10.0$ mIU/mL.

**Table 2. Anti-HBs titer distribution at 4 time-points.**

| Group | No. | Antibody titers after booster vaccination (mIU/mL) | GMTs | $P$ value$^a$ |
|-------|-----|-------------------------------------------------|------|-------------|
|       |     | 10 (n (%)) | $\geq 10$<100 (n (%)) | $\geq 100$<1000 (n (%)) | $\geq 1000$ (n (%)) |      |
| 1 m after the dose-1 | I 41 | 8 (19.5) | 12 (29.3) | 14 (34.2) | 7 (17.1) | 81.96 | Total$^b$ |
| II 69 | 6 (6.7) | 21 (30.4) | 35 (50.7) | 7 (10.1) | 133.39 | III vs. I$^c$ |
| III 115 | 0 (0) | 2 (1.7) | 56 (48.7) | 57 (49.6) | 896.04 | III vs. II$^c$ |
| Total 225 | 14 (6.2) | 35 (15.6) | 105 (46.7) | 71 (31.6) | 348.62 |
| 1 m after the dose-3 | I 41 | 0 (0) | 7 (17.1) | 28 (68.3) | 6 (14.6) | 357.15 | Total$^b$ |
| II 69 | 0 (0) | 11 (15.9) | 43 (62.3) | 15 (21.7) | 356.51 | III vs. I$^c$ |
| III 115 | 0 (0) | 1 (0.9) | 64 (55.7) | 50 (43.5) | 1039.59 | III vs. II$^c$ |
| Total 225 | 0 (0) | 19 (8.4) | 135 (60.0) | 71 (31.6) | 571.20 |
| 1 y after the dose-3 | I 41 | 14 (34.2) | 23 (56.1) | 4 (9.8) | 0 (0) | 18.84 | Total$^b$ |
| II 69 | 17 (24.6) | 43 (62.3) | 9 (13.0) | 0 (0) | 25.04 | III vs. I$^c$ |
| III 115 | 6 (5.2) | 54 (47.0) | 52 (45.2) | 3 (2.6) | 100.06 | III vs. II$^c$ |
| Total 225 | 37 (16.4) | 120 (5.3) | 65 (28.9) | 3 (1.3) | 48.40 |
| 5 y after the dose-3 | I 41 | 19 (46.3) | 21 (51.2) | 1 (2.4) | 0 (0) | 9.69 | Total$^b$ |
| II 69 | 27 (39.1) | 39 (56.5) | 3 (4.4) | 0 (0) | 11.54 | III vs. I$^c$ |
| III 115 | 13 (11.3) | 68 (59.1) | 30 (26.1) | 4 (3.5) | 54.62 | III vs. II$^c$ |
| Total 225 | 59 (26.2) | 128 (56.9) | 34 (15.1) | 4 (1.8) | 24.60 |

$^a$ Based on One-way ANOVA test to compare the anti-HBs GMTs of all 3 groups, and LSD to further compare the anti-HBs between each of the 2 groups.

$^b$ $P < 0.001$.
Based on the study results, we suggest that the current threshold value of 10 mIU/mL may be inappropriate to consider to differentiate protected individuals from non-protected individuals. The exist of immunological memory is reflected via the ability to elicit an anamnestic response to a booster dose. In this study, the Group III whose pre-anti-HBV antibodies level falls below the threshold value (10 mIU/mL) can generate a significant immune response after booster vaccinations, which means the Group III have good immunological memory. Similarly with our study results, previous studies also demonstrated that <10 mIU/mL anti-HBV antibodies titer may not indicate the loss of protection from HBV; and the duration of immunological memory is considered to be longer than that of the circulating antibodies. Poorolajal and Hooshmand observed that <10 mIU/mL anti-HBs antibodies titer or an absence of anamnestic immune response could not be considered as an absence of immunity. This threshold value of <10 mIU/mL anti-HBs antibodies titer can continue to show the positive immune effect after a booster vaccination.

In the current study, the results also showed that the total anti-HBs positive rate after the 3-dose vaccine was 100%. Similarly, one study in central Taiwan displayed a success rate of 97.2% for developing seroprotection following an HBV booster vaccination of 3 doses in adolescents. In another Taiwanese study, the anti-HBs positive rate for subjects was higher than 99.2% after the 3 doses vaccination among young people around 20 y. These data indicated that the 3-dose revaccination benefits non-responders and low-responders with a sufficient conversion of anti-HBs.

Earlier report from our group observed that participants experienced significant declines in their levels of anti-HBs during the first year after the revaccination, especially among those having low pre-booster antibody titer (in Group I and Group II). This study, which is a further follow-up to this earlier study, showed during the next 4 y, the seroprotection levels showed a sustained but downward trend while the protective rates was invariable, with approximately 58.0% of the participants in Group I and Group II having protective levels of anti-HBs at 5 years after booster doses. A bivariate correlation between the anti-HBs level one month and 5 years post complete vaccination indicated that the higher the anti-HBs level at one month was greater than at 5 y (r = 0.706, p < 0.001). The significant decrease in the levels of anti-HBs in Group I and Group II during the 5-year follow-up period indicate that the duration of the 3 doses booster vaccination in these individuals would not be at a prolonged interval. On the other hand, previous studies repeated that the anti-HBs antibody titer in children with full vaccination in infancy gradually declined with age. According to a study conducted in Taiwan, its result showed that the seropositive rate of anti-HBs in among adolescents at 18 years after primary vaccination was low, only 33.6%, while in Saudi Arabia it was 38%. In fact, children in HBV prevalence areas with anti-HBs titers below the protective level or undetectable are at a high risk of HBV infection, and booster vaccination is needed. Thus, we suggest that the use of HBV vaccine booster doses should be based on the prevalence of the disease in the population, rather than on levels of anti-HBs.

There are some limitations in our study. First, we failed to collect the data on the immune response after the completion of the primary vaccination. Therefore, we could not determine how the current sero-negative results after the booster had related with the previous immunological response after the primary immunization course. Second, immunological memory is the main determinant of present seroprotection, however, some bias exists in judging protective effect of boosters via anti-HBs positive rate and titer level, such as we may mistakenly believed some subjects with waring antibody titer lost seroprotection despite immunological memory, and underestimated the effect of boosters. Third, since we grouped the participants based on school enrollment, some subjects were transferred to schools which are not involved in our study during the whole study period, though, the subjects who were lost to follow-up had similar demographic characteristics to those who completed the 5-year follow-up.

In conclusion, the 3-dose booster revaccination has a good medium-term immune effects among children with nonseroprotective anti-HBs levels. A booster dose might not need to be recommended for the children with a pre-booster anti-HBs titer of 1–9.9 mIU/mL, however, individuals with pre-booster anti-HBs titers <1.0 mIU/mL are recommended to take a 3-dose hepatitis B vaccination to protect them from HBV infection. However, additional studies are needed to confirm the conclusions, which may aid in establishing more efficient immune booster strategies.
Materials and methods

Study population

The present study conducted at 6 centers in the Zhejiang Province in China, aimed at evaluating the long-term efficacy of hepatitis B vaccine booster among children. The samples of subject were collected on school enrollment.

Children born between 1993 and 2004 and those that received a course of 3-dose monovalent recombinant hepatitis B vaccine in the first years of life were selected. The age of the subject was calculated by the survey date and then rounded to the nearest whole number in years. We randomly sampled 1022 subjects aged 5–15 y who qualified for this study, including 841 children aged 11–15 y old in the earlier study. These 1022 subjects who had previously received booster vaccinations, as well as those who tested positive for HBsAg, anti-HBs, or anti-HBc antibodies, were excluded from the study.

We get the immunization information of all the children by the child’s immunization certificate kept by the parents or by review of the child’s immunization card kept at the township hospital. All the participants’ parents completed a questionnaire that included information about the child on gender, date of birth, HBV vaccination history, telephone number, and home address. The study was approved by the Institutional Review Board of Zhejiang Center for Disease Control and Prevention, and written informed consent was obtained from each parent before conducting any study procedures.

Specific inclusion criteria were as follows:
1. Born between May 1, 1993 and September 30, 2003, and vaccinated against HBV at 0, 1, and 6 months;
2. Never received a hepatitis B vaccine booster after primary vaccination;
3. Parental willingness to participate in the follow-up study and to allow the withdrawal of blood sample from the children after vaccination;
4. No acute illness within the previous 7 d, no fever within the past 3d (axillary temperature ≥38 °C), and no allergies or severe reaction to vaccination;
5. All the information regarding the study was provided by the subjects’ parents and the consent form signed by them.

Blood sample collection

We collected 3 mL blood sample from each participant. Booster vaccinations of recombinant hepatitis B vaccine, (CHO lot number: 200802A21 (01-05), dosage: 20 μg; NCPC GeneTech Biotechnology Pharmaceutical Co., Ltd.) were administered by intramuscular injection in the upper arm deltoid at months 0, 1, and 6, according to the immunization procedures. One month after the first and the third dose of booster vaccine injections, 3 mL blood samples were collected from each subject and preserved for testing. One year and 5 years after the third dose, 2 mL blood samples were collected from the follow-up participants and tested.

Lab testing and sample processing

A chemiluminescence immunoassay (CLIA) is used for the quantitative detection of HBsAg, anti-HBs, and anti-HBc in the frozen serum samples (from blood).

Apparatus and reagents

An Architect-i2000 (Abbott, USA) was used for CLIA. The commercial reagent (70318HN00) was used for the HBsAg tests, and a signal to noise (S/N) ratio ≥0.05 was considered to be positive. The anti-HBc test (reagent, 72448M100) at ≥1 mIU/mL was considered to be positive. The reagents, 75684M100, for the anti-HBs tests (one month after the first and third dose) and 10259F00 (one year and 5 years after the third dose) were used, with the criterion that an anti-HBs titer ≥10 mIU/mL was considered as positive and defined as exerting protective effects against the HBV infection. The samples with anti-HBs ≥1000 mIU/mL were diluted for further testing, while samples with antibody titer >15000 mIU/mL were excluded from further testing and recorded as >15000 mIU/mL.

Data analysis

The participants were divided into 3 groups according to their anti-HBs levels prior to administration of the boosters: Group I, <0.1 mIU/mL, Group II, 0.1 to <1.0 mIU/mL, and Group III, 1.0 to <10.0 mIU/mL. We established a database for these groups using EpiData3.2 (EpiData; Norway and Denmark), and statistical analysis was performed using SPSS 18.0 and Excel 2003. One-way ANOVA test, Chi-squared test, and Fisher’s exact test were used for comparisons among the study groups. The relationship between the anti-HBs level at different time points was compared using a bivariate correlation test. A p < 0.05 was considered statistically significant. We also calculated the geometric mean titer (GMT) and 95% confidence intervals to evaluate trends in the levels of anti-HBs due to the skewed distribution of anti-HBs levels. The participants with undetectable anti-HBs were assigned a nominal value of 0.01 mIU/mL.

Disclosure of potential conflicts of interest

The authors declare that they had no conflicts of interest.

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