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Original

Results of Hepatitis B Vaccination 3 Years After a Primary Vaccine Series in Medical Students

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SUMMARY

Objective : To investigate the significance of additional hepatitis B (HB) vaccination in medical students who were unresponsive to a primary vaccine series or those who had lost antibody to hepatitis B surface antigen (anti-HBs).

Methods : Subjects were followed up for 3 years after completion of a primary HB vaccine series. One additional dose was given to those who lost the anti-HBs within 3 years after the initial series, while 3 doses were given to those who had not responded to the initial vaccination. Subjects : 100 medical students (59 men and 41 women ; mean age on admission to university, 19.4 ± 1.6 years) enrolled at the School of Medicine, Dokkyo Medical University in April 2012.

Results : The rate of positivity for anti-HBs was 98% soon after completion of the primary HB vaccine series and decreased without the need for additional vaccination to 79% , 61% , and 55% at 1, 2, and 3 years after the primary series, respectively. Eighteen vaccinated subjects (18%) lost the anti-HBs 2 years after the primary series, and all of them responded to 1 additional dose. Another 18 successfully vaccinated subjects (18%) were anti-HBs negative both 1 and 2 years after the primary series ; 17 of them responded to 1 additional dose. As for 2 subjects (2%) who were unresponsive to the primary series, 1 became anti-HBs-positive for the first time after 3 additional doses given 2 years after the primary series.

Conclusion : A number of students became or remained anti-HBs negative after the primary HB vaccination, indicating that its timing and dose of additional vaccination need to be studied further to evaluate its utility.

Key Words : hepatitis B virus (HBV), hepatitis B (HB) vaccine, anti-HBs, additional vaccination, medical students

INTRODUCTION

One of the primary purposes of vaccination against hepatitis B virus (HBV) in adults is prevention of fulminant hepatitis as part of preventive measures against for those who are associated to healthcare and with occupational hazards[1~3]. Given the increasing incidence of horizontally transmitted acute hepatitis,

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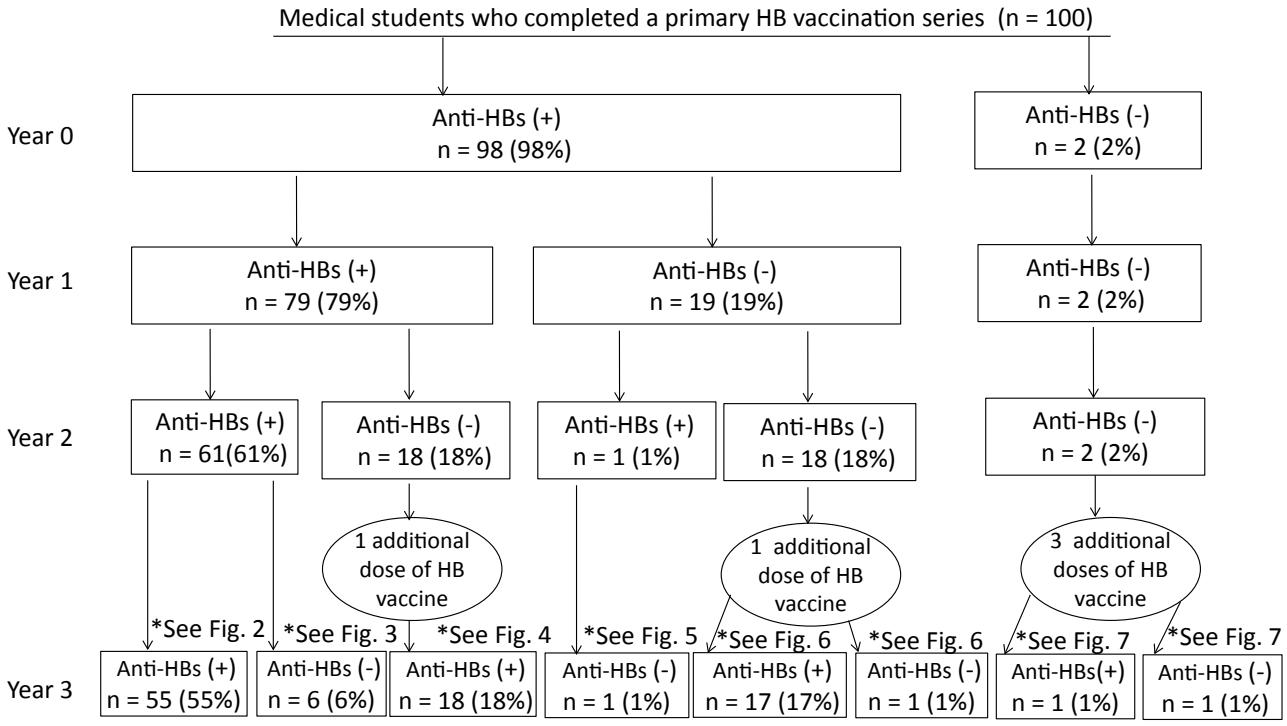

Figure 1

Grouping of subjects by anti-HBs status (n=100). Anti-HBs status in 100 medical students was monitored for 3 years after completion of the primary vaccine series. This flowchart depicts the grouping. hepatitis B : HB ; antibody to hepatitis B surface antigen : anti-HBs.

prevention of acute hepatitis caused by genotype A, which has a tendency for chronicity, is of interest[4,5]. It is particularly necessary to prevent the onset of *de novo hepatitis B*, which carries a poor prognosis and often occurs after administration of immunosuppressive therapy or chemotherapy in patients with a previous history of HBV infection[6,7]. HB vaccination is also important in the prevention of post-transfusion hepatitis.

In this study, we examined the levels of anti-HBs during a 3-year period after completion of a primary HB vaccine series in medical students enrolled at the School of Medicine, Dokkyo Medical University. Additional HB vaccination was given to those who appeared to require revaccination before starting the clinical training in their fifth year at our medical university and the benefits of additional HB vaccination was assessed.

MATERIALS AND METHODS

Subjects were 100 medical students (59 men and 41 women ; mean age on admission to university, 19.4 ±1.6 years) who were negative for both hepatitis B surface antigen (HBsAg) and anti-HBs at the time of enrollment at the School of Medicine, Dokkyo Medical University in April, 2012. They underwent a primary HB vaccine series (3 shots : 0 months, 1 month, and then 5 months) and were followed for a 3-year period after the primary vaccine series.

Additional doses were given to fourth-year students who required additional vaccination before starting clinical training in their fifth year. Furthermore, 1 additional dose was given to those who had tested positive for anti-HBs after the primary vaccine series but who then became negative year later and remained negative 2 years after the primary series, or to those who lost anti-HBs 2 years after the primary series. Also three additional doses were given to those who had not responded to the initial vaccination, and had remained unresponsive for 2 years.

All subjects received Bimmugen® (The Chemo-Sero-Therapeutic Research Institute, Kumamoto, Japan), a yeast-derived recombinant, adsorbed HB vaccine. A series of 3 doses (10 μg in 0.5 mL/dose) was administered subcutaneously, and the rates of anti-HBs positivity were examined. Additional vacci-

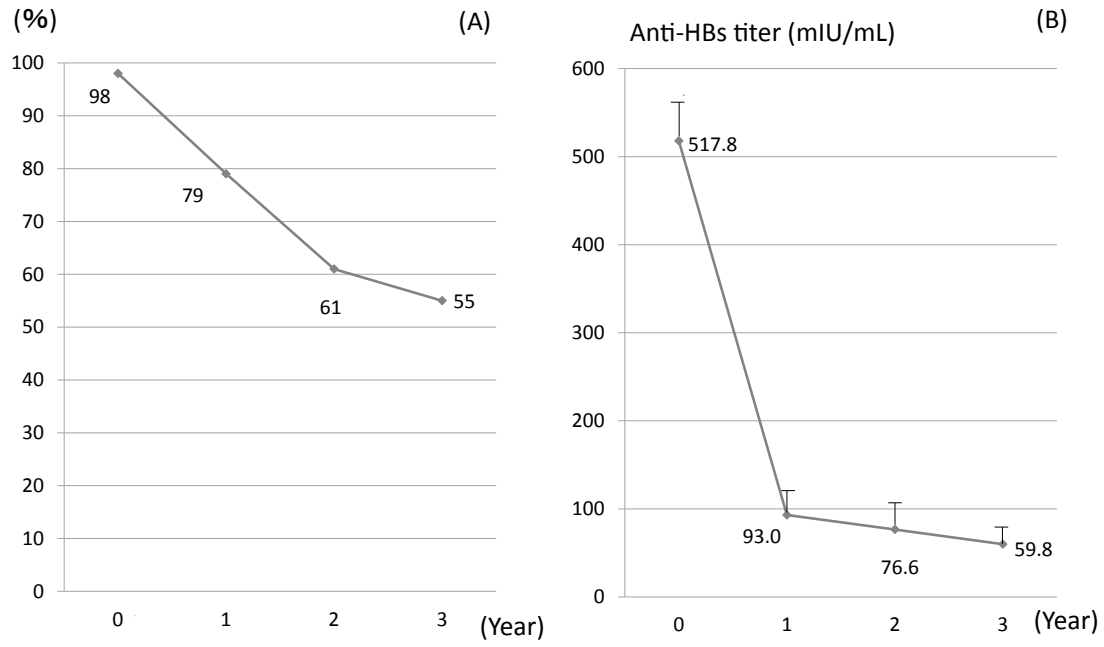

Figure 2

Changes in the HBs-positive rate (**A**) and HBs titer (**B**) in responders to the primary vaccine series who remained anti-HBs positive for 3 years after completion of the primary vaccine series.
Cut-off value of anti-HBs titer : 10 mIU/mL. Anti-HBs titers expressed as median ± standard error of the mean (SEM).

nation were administered in a similar manner if necessary when the students were in their fourth year. Chemiluminescent immunoassay (CLIA) was performed to determine the levels of anti-HBs at 4 months, 1 year, 2 years, and 3 years after the primary vaccine series. Levels of anti-HBs ≥ 10 mIU/mL were considered positive. Results were expressed as mean ± standard deviation (SD) or median ± standard error of the mean (SEM).

This study was approved by the institutional review board of Dokkyo Medical University, and all study participants had provided their informed consent. All procedures were in accordance with the Declaration of Helsinki.

RESULTS

1. Grouping of subjects by anti-HBs status and additional HB vaccine dose are shown in Figure 1.

Anti-HBs status in 100 medical students was monitored for 3 years after completion of the primary vaccine series. This flowchart depicts the grouping process.

2. Figure 2 shows the changes in HBs-positive rate (Figure 2A) and HBs titer (Figure 2B) in primary vaccine series responders who remained anti-HBs positive for 3 years after completion of the primary vaccine series are shown here.

The rate of positivity for anti-HBs was 98% in the same year of the primary HB vaccine series and then decreased without the need of additional vaccination to 79% , 61% , and 55% at 1, 2, and 3 years after the primary series, respectively. Thus, 55% of subjects retained their seropositive status after the primary series and retained their anti-HBs-positive status throughout a 3-year follow-up period (Figure 2A). Changes in anti-HBs titer were shown in Figure 2B.

3. Figure 3 shows the changes in HBs titer in 6 responders to the primary vaccine series who remained anti-HBs positive for 2 years but became anti-HBs negative on 3rd year after completion of the primary vaccine series.

4. Figure 4 shows the changes in HBs titer in responders to the primary vaccine series who became anti-HBs negative and had received one additional dose 2 years after completion of the primary vaccine series. All 18 subjects responded to the additional

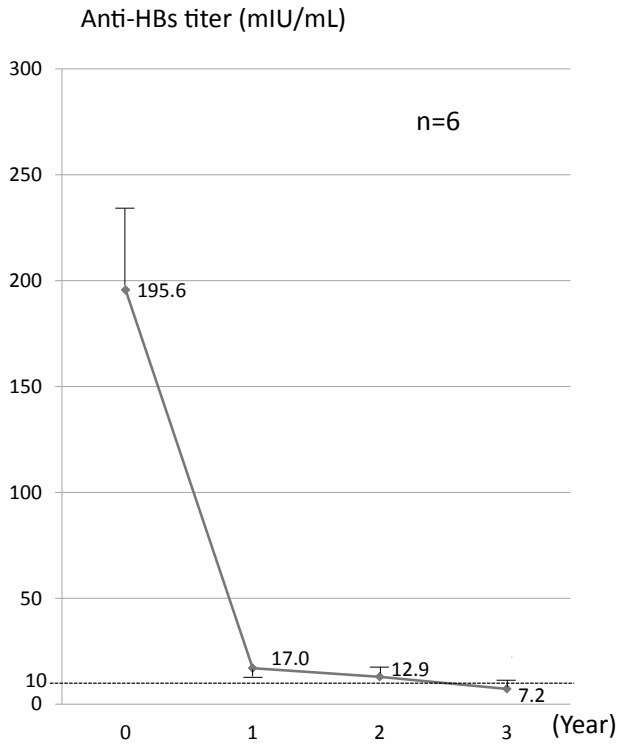

Figure 3

Changes in HBs titer in 6 responders to the primary vaccine series who remained anti-HBs positive for 2 years but became negative on 3rd year after completion of the primary vaccine series. Cut-off value of anti-HBs titer : 10 mIU/mL. Data expressed as median ± SEM.

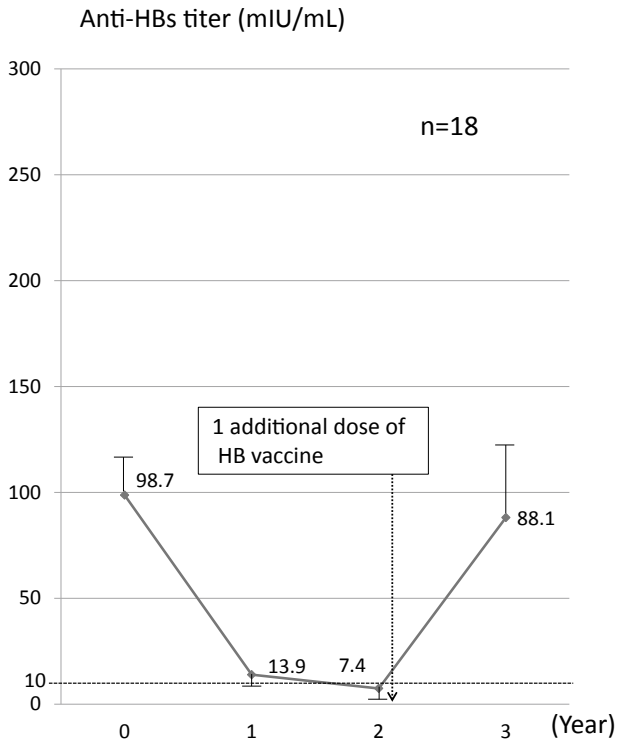

Figure 4

Changes in HBs titer in 18 responders to the primary vaccine series who became anti-HBs negative and had received one additional dose 2 years after the completion of the primary vaccine series. All 18 subjects responded to the additional dose and seroconverted to become anti-HBs positive again. Cut-off value of anti-HBs titer : 10 mIU/mL. Data expressed as median ± SEM.

dose and seroconverted to anti-HBs positive again.

5. One male subject became anti-HBs-positive (159.4 mIU/mL) after the primary vaccine series, but switched between positive and negative at each test time point : negative (8.1 mIU/mL), positive without an additional dose (21.3 mIU/mL), and then negative (3.9 mIU/mL) at 1, 2, and 3 years after the primary series, respectively as shown in Figure 5.

6. Figure 6 shows the changes in HBs titer in responders to the primary vaccine series who became anti-HBs negative in the following year, remained negative, and received an additional dose 2 years after completion of the primary vaccine series. Seventeen of 18 subjects responded to the additional dose and seroconverted to anti-HBs positive again (Figure 6A). Conversely, one male subject became anti-HBs-positive (25.7 mIU/mL) after the primary series, reverted to negative (4.7 mIU/mL) 1 year after the primary vaccine series, and then remained negative (1.4 mIU/mL) the following year. The subject did not respond to 1 additional dose, and remained anti-HBs-negative (8.4 mIU/mL) 3 years after the primary series (Figure 6 B).

7. Figure 7 shows the changes in HBs titer in non-responders to the primary vaccine series who remained anti-HBs negative for 2 years and then received 3 additional doses.

One male subject remained anti-HBs-negative in the same year of the primary vaccine series (5.9 mIU/mL), and at 1 and 2 years after the primary series (1.4 mIU/mL and 1.0 mIU/mL, respectively). He became seropositive for the first time at 3 years after 3 additional doses (45.2 mIU/mL) (Figure 7, case 1). However, HBs titer remained negative even after 3 additional doses were administered in the other subject (Figure 7, case 2).

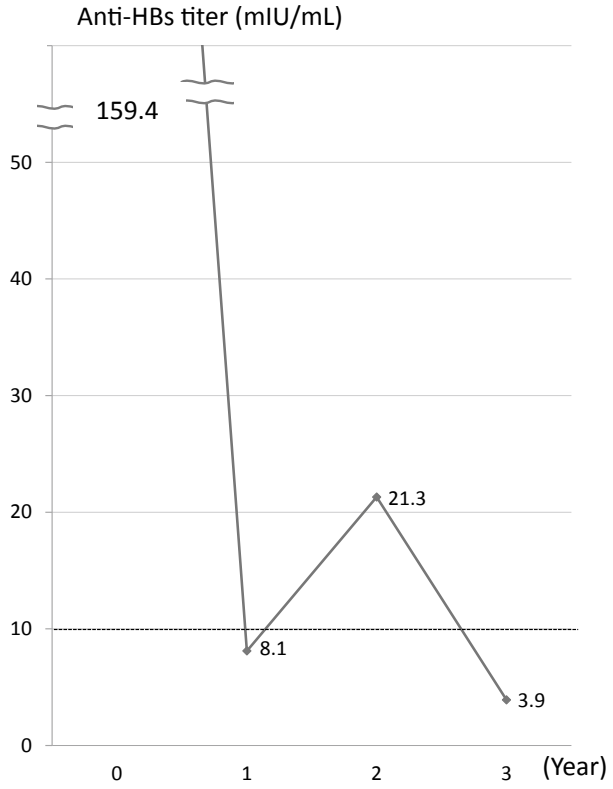

Figure 5

One male subject who lost anti-HBs 1 year after primary vaccine series, regained antibodies without an additional dose at 2 years, and then lost his positive status again at 3 years. Cut-off value of anti-HBs titer : 10 mIU/mL.

DISCUSSION

HB vaccination of infants born to mothers who are HBV carriers is crucial in preventing vertical transmission[8,9]. Also, given the risks of horizontal transmission present in daily life settings, such as intrafamilial transmission (including father-to-child transmission) and mass infection in nurseries during infancy[10]. It is therefore, the need of HB vaccination is an essential preventive measure. Risk of transmission of HBV, hepatitis C virus (HCV), and human immunodeficiency virus (HIV) was 30% , 1.8% , and 0.3% , respectively, clearly showing that the risk of HBV transmission is remarkably high[1,2].

Universal vaccination of infants <1 year old against HBV was finally introduced in Japan in 2016. However, voluntary HB vaccination programs for adults in high-risk groups are necessary until all citizens have benefited from this newly introduced vaccination program.

HB vaccination in adults is an essential preventive measure against healthcare-associated HBV infections in people, who are directly involved in healthcare (such as doctors and nurses), and against occupational infections in those at risk of exposure to infected blood and/or other body fluids (including hospital housekeeping, room cleaning, and laundry staff)[10]. It is also important in preventing post-transfusion hepatitis B. With the trend of increasing cases of horizontally transmitted acute hepatitis B, prevention of acute hepatitis caused by genotype A with its tendency for chronicity is particularly necessary[11~15]. Also, it is important to prevent the onset of *de novo hepatitis B*, which carries a poor prognosis and usually occurs after immunosuppressive therapy or chemotherapy in patients with a previous history of HBV infection[16~18].

Changes in anti-HBs levels after HB vaccination were previously examined[19~21]. The minimum protective anti-HBs level is 10 mIU/mL as determined by the World Health Organization (WHO) reference preparations, and this was the threshold value used in this study.

Our study showed that the primary vaccine series achieved an anti-HBs positivity rate of 98% ; the rates of positivity after year 1, 2 and 3 were 79% , 61 % , and 55% , respectively. Thus, 55% of the subjects remained anti-HBs positive throughout the 3-year follow-up period (Figure 2A). Variation in immune response is thought to have been a major contributing factor to the difference between those who retained anti-HBs positivity and those who lost this status[22]. There are several possible types of changes in anti-HBs levels including the persistent high level, the high-to-low level, the persistent low level, and the low-to-negative level types. We will continue to monitor anti-HBs levels at routine health checks to observe the changes in vaccinated students.

In this study, 1 male student lost, regained, and then lost anti-HBs -positive status 1 year, 2 years, and 3 years after the primary vaccine series, respectively (Figure 5). Although the possibility of a new infection cannot be eliminated, fluctuation of anti-HBs measurements may not be negligible around the positive/negative threshold.

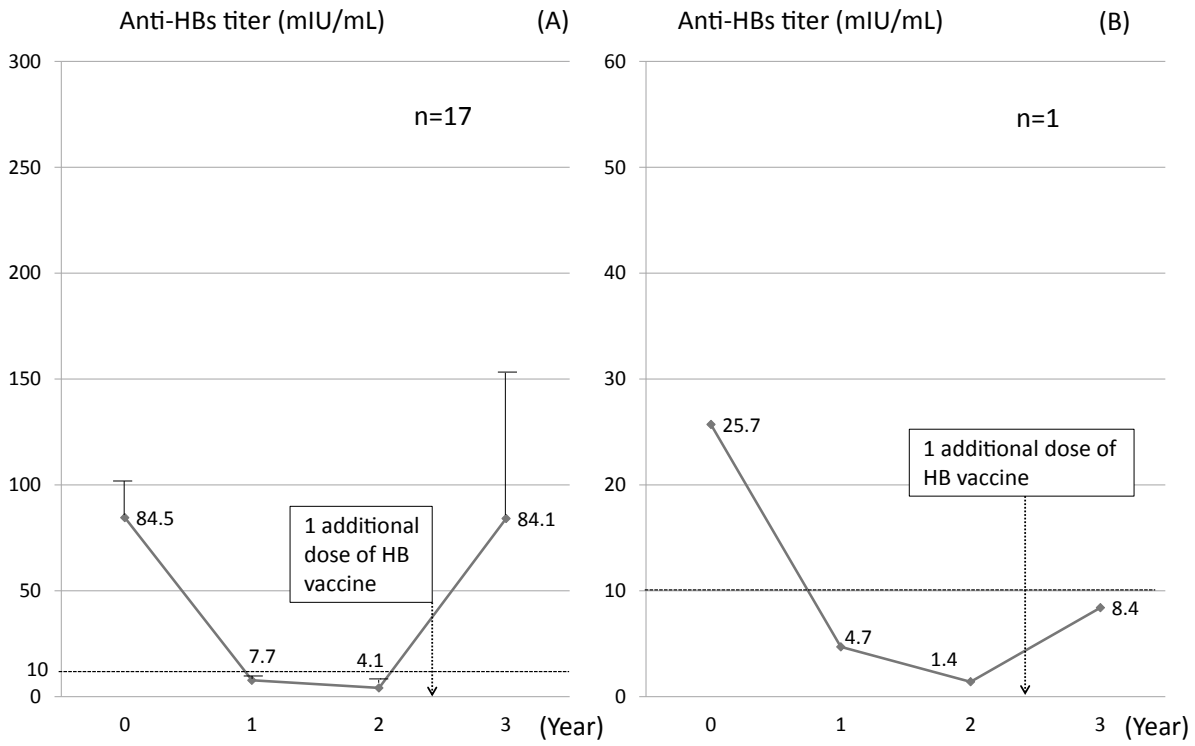

Figure 6

Changes in HBs titer in responders to the primary vaccine series who became anti-HBs negative the following year and remained negative and received an additional dose 2 years after completion of the primary vaccine series.

A : Changes in HBs titer in those who became anti-HBs positive after the additional dose (17 cases).

B : Changes in HBs titer in those who remained anti-HBs negative after the additional dose (1 case).

Cut-off value of anti-HBs titer : 10 mIU/mL. Data expressed as median ± SEM.

We found 3 additional dose patterns. The first pattern involved a single additional dose given to 18 subjects (18%) who retained vaccine-induced anti-HBs positivity 1 year after, but lost it 2 years after the primary vaccine series. All 18 subjects regained the anti-HBs positive status after receiving one additional dose (Figure 4). The second pattern was that of a single additional dose given to 18 subjects (18%) who lost their anti-HBs positive status 1 year after, and still remained negative 2 years after the primary series. Seventeen (17%) of these subjects regained the anti-HBs positive status after a single additional dose (Figure 6A), but 1 (1%) did not respond (Figure 6B). A single additional dose may not be sufficient to regain immunity against HBV in those with anti-HBs levels around the lower range of positivity after the primary series. The third pattern was of 3 additional doses given to 2 subjects (2%) who did not test positive for anti-HBs during the 2-year period after the primary series. One of these subjects became anti-HBs-positive for the first time after receiving 3 additional doses (Figure 7, case 1), suggesting the benefit of additional doses to those who were previously unresponsive. However, another subject still remained anti-HBs negative (Figure 7, case 2). Further study is warranted to determine the difference between case 1 and case 2.

In non-responsive vaccinated students who did not become seropositive, general countermeasures applicable to those unresponsive to HB vaccination consist of giving a normal 3-shot HB vaccine series a year later, changing the route of administration from subcutaneous to intramuscular, or changing the type of vaccine[23~25]. However, the most effective method has not yet been identified, and this needs to be examined in future.

55% of students who acquired anti-HBs-positive after the primary vaccine series retained antibodies

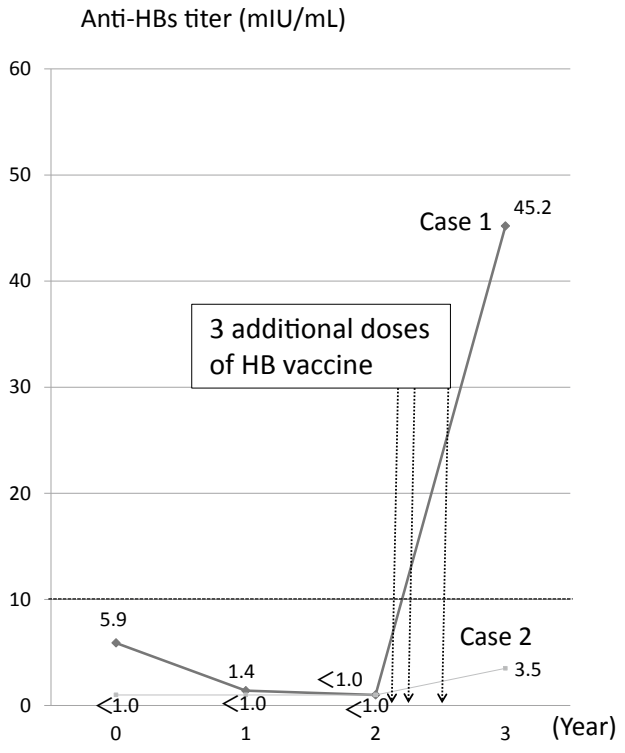

Figure 7

Changes in HBs titer in non-responders to the primary vaccine series who remained anti-HBs negative for two years and then received three additional doses.
Case 1 : One male subject who remained anti-HBs-negative throughout the 2-year post-primary series, but became positive for the first time after 3 additional doses given 2 years after the primary series.
Case 2 : One subject who remained anti-HBs-negative even after 3 additional doses.
Cut-off value of anti-HBs titer : 10 mIU/mL.

throughout a 3-year observation period. The United States Centers for Disease Control and Prevention (CDC) does not include individuals who lost acquired antibodies in the group requiring booster doses[26]. However, in 2011, Stramer et al.[27] reported that patients who had received HB vaccination tested negative for HBsAg and showed no increase in alanine aminotransferase (ALT) level ; however, they consistently tested positive for HBV DNA and immunoglobulin M antibody to hepatitis B core antigen (IgM anti-HBc) after secondary exposure to HBV, suggesting a new infection in individuals with low levels of anti-HBs.

In conclusion, as medical students are at high risk of HBV infection, especially during clinical training, seropositive status via HB vaccination is needed. Either three additional doses or one additional dose were given to fourth-year medical students who did not respond to the primary vaccine series or who responded but became anti-HBs negative before starting their fifth-year clinical training, respectively. After the additional doses, most of these students became anti-HBs positive. These findings suggest that additional doses of HB vaccine are necessary. However, 9 fifth-year students were anti-HBs negative (Figure 3 : 6 cases ; Figure 5 : 1case ; Figure 6B : 1 case ; Figure 7 : 1 case), indicating that the timing and dose of additional HB vaccination needs to be studied further to evaluate its efficacy.

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Conflicts of interest

The authors state that they have no conflicts of interest.

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