



## Hepatitis B immunity in adolescents and necessity for boost vaccination: 23 years after nationwide hepatitis B virus vaccination program in Taiwan

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### ABSTRACT

The first universal hepatitis B vaccination program for newborns in the world was launched in Taiwan in July 1984. Most studies on the effectiveness of hepatitis B vaccination focused on the seroprevalence of HBs Ag among children under 14 years old. Only few studies focused on the seropositivity of anti-HBs among adolescents aged 15–18 years old. The present study aimed to evaluate the impact of the nationwide hepatitis B vaccination program on the immunity to HBV infection and the necessity of boost among adolescents. In this study including eight annual seroprevalence surveys from 2000 to 2007, 2342 college entrants (1589 15-year-olds in group I and 753 18-year-olds in group II) and 1851 university freshmen (18-year-olds in group III) participated. Subjects identified anti-HBs, HBs Ag and anti-HBc negative were given boost three doses of HBV vaccine. The HBs Ag seroprevalence was 11.6%, 3.5% and 1.0% for participants who were born before 1984, 1984–1986 and after 1986. The anti-HBs-seropositive rates were significantly higher in group II (83.1%) than in group I (53.0%) and group III (53.5%). All 572 participants who were seronegative for anti-HBs, HBs Ag and anti-HBc became anti-HBs-seropositive after catch-up vaccination. It is concluded that the anti-HBs-seropositive rate decreased to 50% in 15 years after vaccination, and boost vaccination was 100% effective. The necessity and age for boost among anti-HBs negative adolescents and the timing of the first immunization should be further evaluated.

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### 1. Introduction

Hepatitis B is a worldwide disease and hepatitis B virus (HBV) infection is also an important cause of morbidity and mortality all over the world. It is estimated that presently two billion people have been infected with HBV at some time in their lives, and approximately 400 million are chronic HBs Ag carriers with risk for HBV-associated liver disease [1,2]. The HBV is transmitted through serum and even body fluids such as semen, saliva, sweat, tears, or breast milk. The majority acquire the infection perinatally. However, the currently acknowledged risk factors for infection by the HBV are sexual promiscuity, intravenous drug abuse, blood and derivatives transfusions, hemodialysis, and needle accidents

among health-care professionals. HBV infection can cause asymptomatic disease or acute or chronic hepatitis. The latter may evolve to cirrhosis, fulminant hepatitis with massive necrosis, or hepatocellular carcinoma [3]. Approximately a quarter of those who become chronic carriers go on to develop progressive liver disease leading to cirrhosis (some 60 million cases annually) and hepatocellular carcinoma (HCC) [4].

Before the nationwide hepatitis B vaccination program was launched in Taiwan, the overall incidence rate was 1.5–5% in Taiwan [5,6] and more than 90% of general population was infected by HBV, besides 15–20% of them became chronic carrier [5,7,8]. The prevalence of HBV (positive anti-HBc) found in Taiwan is much higher than in other parts of the world [9] and 67% of male HCC in Taiwan was related to HBV infection [10].

Because HBV-related cirrhosis and hepatocellular carcinoma (HCC) usually occur in adults who were infected with HBV as child, and carrier rate of HBs Ag was as high as 15–20% in Taiwan before nationwide hepatitis B vaccination [9]. Taiwan has been making inroads on the prevention of new infections by immunizing babies at birth since July, 1984. From July 1984 to June 1986, only newborns of HBs Ag positive mothers were vaccinated and

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after July 1986, all newborns were vaccinated at birth. Besides, the preschool children who did not receive vaccine at the neonatal stage were also vaccinated after July 1986. Before November 1, 1992, plasma-derived vaccine was used for this national program and after November 1, 1992, the vaccine was changed to a recombinant DNA vaccine [9]. The nationwide vaccination program in Taiwan was the first universal hepatitis B vaccination program for newborns in the world and the national vaccine coverage was over 90% [9].

Although nationwide hepatitis B vaccination has effectively prevented hepatocellular carcinoma (HCC) [11,12], reducing the prevalence of HBV infection and chronic carrier rates [13], most of the studies concerning the protective effectiveness of Hepatitis B vaccination in Taiwan were focused on the seroprevalence of HBs Ag among the vaccinees and the study subjects were focused on children under 14 years old [9,14]. There were very few studies focused on the seropositive rate of protective antibody, named anti-HBs, among adolescents especially aged 15–18. The goals of the present study were to investigate the currently HBV immunity status, the seropositive rate of anti-HBs antibodies, and seroprevalence of HBs Ag in adolescences who were immunized with hepatitis B vaccine at their birth in Taiwan. We also compared the seropositive rate of anti-HBs between students who were immunized at birth and at the preschool age to evaluate the timing of first vaccination. In addition, the necessity of additional doses of hepatitis B vaccine in their adolescent period was evaluated. To achieve the study purposes, we collected and analyzed the health examination data of adolescent students aged 15 and 18 years respectively for a successive period of 8 years. Health examination was performed upon enrollment in school: i.e., anti-HBs and HBs Ag in blood serum. Subjects identified to be anti-HBs, HBs Ag and anti-HBc negative were given boost doses of HBV vaccine and anti-HBs was tested 6 months after the last booster vaccination.

## 2. Materials and methods

### 2.1. Subjects and measurement

The study is a series of cross-sectional seroprevalence survey from 2000 to 2007, comprising a total of 4193 adolescents from the school enrollment health files of two schools. The study subjects of successive birth cohorts included all newly enrolled students admitted to a public college in central Taiwan from 2000 to 2007 and a private university in northern Taiwan in 2005. There were three groups of enrolled students in this study: freshmen of junior college (mean age =  $15.4 \pm 0.5$ ) and freshmen of college (mean age =  $18.5 \pm 0.5$ ) in the same public college, hereafter named group I (15-year-olds) and group II (18-year-olds) respectively. The numbers of subjects, all female, were 1589 aged 15 (group I) and 753 aged 18 (group II). In addition, 1851 university freshmen aged 18 (mean age =  $18.6 \pm 0.8$ ; male 65.5% and female 35.5%), named group III, were included. The public college in central Taiwan enrolled a wide variety of students, from the lowest to highest socioeconomic level, quite representative of adolescents in central Taiwan. The private university in northern Taiwan also enrolled a wide variety of student from all over Taiwan and could represent adolescents in Taiwan. The sample of the present study was therefore considered valid and unbiased. The age, gender, HBs Ag and anti-HBs of each individual were recorded, our database comprising the results of medical checkups performed by us.

The college students' data obtained in this study was from 2000 to 2007. The birth cohorts were 1985–1992 for junior college students (Table 1; group I), who attended junior college at age 15. The birth cohorts were 1982–1989 for college students (Table 1; group II), who attended college at age 18 after they finished nursing education from nursing occupational high school. For group III

(Table 1), university freshmen group, who entered university at the age of 18, only the birth cohorts of 1987 were included in the analysis.

### 2.2. Blood samples and immunoassays

Blood samples were collected from each freshman at their entrance into the college or university. Ten milliliters of blood were collected in non-anticoagulant sterile tubes, centrifuged within 90 min, and analyzed within 4 h. All blood samples were analyzed for HBs Ag and anti-HBs by enzyme-linked immunosorbent assay (ELISA) (model: Abbot I-2000, USA). The anti-HBs and HBs Ag samples were considered to be positive when they were higher than 10 mIU/ml and 1.1 S/CO respectively.

### 2.3. Booster immunization of recombinant HB vaccine

Five hundred and seventy-two seronegative of anti-HBs, HBs Ag and anti-HBc students, aged 15–18, were boosted with three doses of recombinant HB vaccine at beginning of immunization, 1 and 6 months later. Sera were collected 6 months after booster vaccination, and tested for antibody to hepatitis B antigen (anti-HBs) by ELISA described above.

### 2.4. Statistical analysis

Two sets of categorical variables between two different age groups were analyzed by  $\chi^2$ -test and confidence limit. Statistical analyses were performed using the Epi-Info program, version 6.0. Statcalc and EpiTables applications,  $p < 0.05$  was considered statistically significant.

## 3. Results

### 3.1. Seropositive rate of anti-HBs antibody in 15- and 18-year-old adolescents

The seropositive rates of anti-HBs from 2000 to 2007 were 72.4%, 72.4% (76.7% among students born before June 1986 and 45.2% among students born after July 1986), 52.3%, 43.1%, 48.3%, 45.1%, 45.3% and 44.7% respectively, with a total of 53.0% among 15-year-old junior college adolescents (group I) (Table 1). The seropositive rates of anti-HBs from 2000 to 2007 were 81.7%, 90.6%, 87.5%, 88.7%, 86.7%, 95.5%, 92.0% and 44.3% respectively, with a total of 83.1% among 18-year-old college adolescents (group II) (Table 1). For comparison, a group of university freshman, 18-year-old adolescents (group III), was included and the anti-HBs seropositive rate was 53.5% (Table 1). There was no different in anti-HBs seropositive rate between male (53.9%) and female (53.2%) among group III (data not shown).

### 3.2. Significant difference in anti-HBs seropositive rate found between group I and group II female adolescents but not found between tested adolescents with same birth cohort

As for Table 1, we further compared the difference of anti-HBs seropositive rates among junior college (group I), college (group II) and university (group III) entrants. There was significant difference in the seropositive rate of anti-HBs between 15-year-old (group I) and 18-year-old (group II) female adolescents (OR = 4.37, 95% CI: 3.51–5.45,  $p < 0.001$ ). There was no significant difference in the seropositive rate of anti-HBs between 15-year-old (group I) and 18-year-old university (group III) adolescents (OR = 1.02, 95% CI: 0.89–1.17). Since the seropositive rate of anti-HBs was different between 15-year old (group I) and 18-year old college adolescents

**Table 1**  
Seropositivity of anti-HBs in Taiwan adolescents.

Year of analysis	Junior college 15-year-olds (group I), n = 1589 [birth cohort]	College 18-year-olds (group II), n = 753 [birth cohort]	University 18-year-olds (group III), n = 1851 [birth cohort]
2007	44.7% (89/199) [1992]	44.3% (43/97) [1989]	ND <sup>a</sup>
2006	45.3% (92/203) [1991]	92.0% (80/87) [1988]	ND
2005	45.1% (88/195) [1990]	95.5% (85/89) [1987]	53.5% (990/1851) [1987]
2004	48.3% (97/201) [1989]	86.7% (85/98) [1986]	ND
2003	43.1% (85/197) [1988]	88.7% (86/97) [1985]	ND
2002	52.3% (102/195) [1987]	87.5% (84/96) [1984]	ND
2001 <sup>b</sup>	72.4% (147/203) [1986]	90.6% (87/96) [1983]	ND
2000	72.4% (142/196) [1985]	81.7% (76/93) [1982]	ND
Total	53.0% (842/1589)	83.1% (626/753)	53.5% (990/1851)

<sup>a</sup> ND, not detected.

<sup>b</sup> 48.4% (15/31) anti-HBs positive students born after July 1986, all newborns were vaccinated at birth and 76.7% (132/172) anti-HBs positive students born before June 1986, all were vaccinated at their preschool ages except those born by HBs Ag positive mother at their newborns.

(group II), we further evaluated the difference of anti-HBs positivity between 18-year college adolescents (group II) and university adolescents (group III). There was also significant difference in the seropositive rate of anti-HBs between 18-year-old college (group II) and 18-year-old university (group III) adolescents (OR = 4.29, 95% CI: 3.45–5.33,  $p < 0.001$ ). Such finding is interesting and we further compared the seropositive rate of anti-HBs between the same 1987 birth cohort (Table 1) in 15-year-old group (group I) and 18-year-old, the university group (group III), and found that there was no difference in anti-HBs seropositive rate between adolescents born of the same birth cohort in 1987 even they were detected at different year time (OR = 0.95, 95% CI: 0.70–1.30,  $p = 0.75$ ). This implied that the positive rate of anti-HBs did not decrease in 3 years between 15- and 18-year-old adolescents.

### 3.3. The seroprevalence rate of HBs Ag in Taiwan's adolescents

The mean of HBs Ag seropositive rates were 1.3% in 15-year-old junior college (group I), 5.2% in 18-year-old college (group II) and 1.3% in 18-year-old university freshmen (group III) respectively (Table 2). There was significant difference between 15-year-old junior college (group I) and 18-year-old college (group II) adolescents, and between 18-year-old college (group II) and 18-year-old university (group III) adolescents, but not between 15-year-old junior college (group I) and 18-year-old university (group III) adolescents in HBs Ag seropositive rate. After detail investigation, the higher HBs Ag seropositive rate found in group II was due to the higher positive rates found in 1982 and 1983 birth cohorts (14% and 9.4% respectively). When compared the 1987 birth cohort among group II and group III, both were 18-year-old at the tested time point, there were not obvious difference in HBs Ag seropositive rate. Since newborns before July 1984 were not vaccinated at their birth, from July 1984 to June 1986, only newborns of HBs Ag positive

mothers were vaccinated and after July 1986, all newborns were vaccinated at birth. We further analyzed the seroprevalence of the three birth cohorts, the HBs Ag seroprevalence was 11.6%, 3.5% and 1.0% for participants who were born before 1984, 1984–1986 and after 1986.

### 3.4. Significant difference in anti-HBs seropositive rate found between students born before and after July 1986 in group I adolescents

As we have described that all students, born before July 1986, were vaccinated at their preschool ages except those born by HBs Ag positive mother at their newborns and all students, born after July 1986, were vaccinated at birth. In order to evaluate the timing of the first immunization, we further analyzed the difference of anti-HBs seropositivity between these two subgroups of group I students. Data shown that marked difference appeared in seropositive rates of anti-HBs between students born after July 1986, who were all vaccinated at their birth, and those born before June 1986, who were vaccinated at their preschool age (Table 3; OR = 0.30, 95% CI: 0.23–0.39,  $p < 10^{-7}$ ). Although we did not have the data of anti-HBc seropositivity among subgroup 1B participants, the report of Su et al. in Taiwan have shown the HBV natural infection rates were 9.8% and 8.1% in 1984 and 1985 birth-year cohort respectively, with a mean of 8.7% (112/1290) [15]. The 1984 and 1985 birth-year cohorts were defined as HB vaccination of infants born to HBs Ag carrier mothers in the report of Su et al. In Table 3 we defined subgroup 1B as those participants born before June 1986 (in the Table 3 headline), actually those participants are mainly born in 1985 and partly born between January to June in 1986 in Table 1. The population of subgroup 1B was vaccinated as they entering primary school, except those born by HBs Ag carrier mothers. Thus, the population of subgroup 1B is similar to the population of 1984 and

**Table 2**  
Seropositivity of HBs Ag in Taiwan's adolescents.

Year of analysis	Junior college 15-year-olds (group I), n = 1589 [birth cohort]	College 18-year-olds (group II), n = 753 [birth cohort]	University 18-year-olds (group III), n = 1851 [birth cohort]
2007	2.0% (4/199) [1992]	3.1% (3/97) [1989]	ND <sup>a</sup>
2006	0.5% (1/203) [1991]	1.1% (1/87) [1988]	ND
2005	0.5% (1/195) [1990]	0% (0/89) [1987]	1.3% (24/1851) [1987]
2004	1.0% (2/201) [1989]	5.1% (5/98) [1986]	ND
2003	0.5% (1/197) [1988]	5.2% (5/97) [1985]	ND
2002	0.5% (1/195) [1987]	3.1% (3/96) [1984]	ND
2001	1.5% (3 <sup>b</sup> /203) [1986]	9.4% (9/96) [1983]	ND
2000	4.1% (8 <sup>b</sup> /196) [1985]	14% (13/93) [1982]	ND
Total	1.3% (21/1589)	5.2% (39/753)	1.3% (24/1851)

<sup>a</sup> ND, not detected.

<sup>b</sup> All were born before June 1986 and were vaccinated at their preschool ages.

**Table 3**

Comparison of anti-HBs among students born after July 1986 (subgroup IA and before June 1986 (subgroup IB) in junior college (group I) entrants.

Entrants (subgroup)	Anti-HBs (+)	Anti-HBs (–)	Total	OR <sup>a</sup> (95% CL)	p-Value
Subgroup IA (all vaccinated at birth)	568 (46.5%)	653	1221	0.30 (0.23–0.39)	<10 <sup>–7</sup>
Subgroup IB (all vaccinated at preschool age)	274 (74.5%)	94	368		

<sup>a</sup> Odds ratio with 95% confidence limits.

1984 birth year cohorts in Su et al.'s paper. In Table 3, the anti-HBs positivity was 74.5%, even if we ruled out the positivity of natural infection in this period in Taiwan (8.7%), the anti-HBs positivity was 65.8 (74.5 minus 8.7) in subgroup 1B. The OR is 0.34 (95% CL: 0.26–0.44) and is statistically significant.

### 3.5. Anti-HBs found in all of the anti-HBs negative adolescents receiving three doses of recombinant vaccine

Among the 722 junior college students (15-year-old group) who were not detected anti-HBs positive at their entrance, both of their HBs Ag as well as Anti-HBc were also negative. 678 (93.9%) of them were boosted by three doses of recombinant HB vaccine and seroconversion was demonstrated among this group, the seropositive rate of anti-HBs was 100% (Table 4). Since sera were collected 6 months after the third dose of booster vaccination as described in methods, there were 106 students who were vaccinated in 2007 but not tested anti-HBs present. There were only 572 students tested anti-HBs in this study.

## 4. Discussion

The seropositive rate of anti-HBs in our study was around 53% in the primary HB vaccination (group I and group III). This was similar to what was seen in a study of year 2004 where the seropositive rate of anti-HBs was 50.5% in those born after the vaccination program [16]. However, it is interesting to find that group II has a much higher anti-HBs positive rate of 83.1% (as shown in Table 1). Such finding was curious, as the junior college and college students (group I and group II respectively) were enrolled to the same school, and the ELISA test for anti-HBs was performed by the same public hospital laboratory. Why the seropositive rate of anti-HBs was higher in college students (18-year-old; group II) than in junior college students (15-year-old; group I) at 83.1% vs. 53.0% (Table 1) and with statistical significance. It was because that the college students attended to this school came from nursing occupational high schools at the ages of 15–18 during 2000–2006 and was given boosted HB vaccine once, if they were anti-HBs, HBs Ag and anti-HBc negative, before they practiced at hospital when they were in their nursing occupational high school. Therefore the seropositive rates were much higher in college students (18-year-old, group II) from 2000 to 2006 (Table 1). However, for the year of 2007 when the 18-year-old college students (group II) were from general high school where they did not get HB vaccine boosted, the seropositive

rate of anti-HBs was only 44.3% (Table 1). This was similar to what was seen in 15-year-old junior college students (group I) analyzed in 2007 (44.7%) (Table 1).

Since from July 1984 to June 1986, only newborns of HBs Ag positive mothers were vaccinated, most of the students were not vaccinated at their birth. They were only vaccinated at their preschool age after July 1986. All newborns were vaccinated at their birth after July 1986. The significant difference between students born after July 1986 and before June 1986 in junior college (group I) entrants (Table 3) implied that the production and persistence of anti-HBs may be dependent on the time of vaccination. Considering the carrier rates of HBs Ag and the persistence of anti-HBs, it should be re-evaluated that the optimal vaccination time of HB vaccine for children.

According to the result described above, comparing the year 1987 birth cohorts of junior college students (15-year-old; group I) and university students (18-year-old; group III), there was no significant difference between these two groups in anti-HBs seropositivity (52.3% vs. 53.5%;  $p = 0.75$ ), this is similar to what was seen in other reports in Taiwan where the prevalence of anti-HBs in birth-year 1987 students was 48.2% [17] and 50.5% detected in 2004 [16], indicated that the effectiveness of HBV vaccination was not different among adolescents all over Taiwan. It also implied that anti-HBs do not disappear from 15- to 18-year-old adolescents in Taiwan.

The vaccination coverage rate for hepatitis B for all targeted birth cohorts from 1984 to 2002 were more than 90% [9] and the overall protective effectiveness of the vaccines was 85% summarized from previous studies [9]. The effectiveness of universal hepatitis vaccination has been reported, including declined hepatitis carrier rates among children [18], decreasing pediatric fulminant hepatic failure [19], annual incidence of childhood hepatocellular carcinoma [20,21] and providing long-lasting protection against HBV carrier status [22]. In addition, the anti-HBs were detected in vaccinated children in their childhood [11]. However, our study revealed the disappearance of anti-HBs in their adolescent period and the boost of vaccine in 15-year-old adolescents could elevate the seropositivity of anti-HBs in this population. Furthermore, the present study shows that, after boosted recombinant HB vaccine for anti-HBs, HBs Ag and anti-HBc seronegative junior college students (group I), the anti-HBs were detected in almost 100% of the vaccinated student (Table 4). One report has revealed that vaccination at older age was associated with persistence of higher anti-HBs levels [23], which supported the effectiveness of booster in adolescents, rather than

**Table 4**

Seropositivity of anti-HBs after three doses of recombinant vaccine boost.

Year of analysis	Number of HBs Ag, anti-HBs and anti-HBc (–)	% of vaccination	% of anti-HBs seroconversion
2007	110	96.4% (106/110)	NDY <sup>a</sup>
2006	111	96.4% (107/111)	100% (107/107)
2005	107	95.3% (102/107)	100% (102/102)
2004	109	96.3% (105/109)	100% (105/105)
2003	103	98.1% (101/103)	100% (101/101)
2002	85	100% (85/85)	100% (85/85)
2001	53	98.1% (52/53)	100% (52/52)
2000	44	45.5% (20/44)	100% (20/20)
Total	722	93.9% (678/722)	100% (572/572)

<sup>a</sup> NDY, not detected yet.



younger age. Our result also indicated that the vaccine of HB is effective, and the boost of HBV vaccine is necessary for adolescents to increase the seropositive rate of anti-HBs in adolescents or adults.

In terms of protecting the susceptible population against HBV by vaccination, it has been shown that between 5% and 10% population fail to develop antibody to hepatitis B vaccine. This non-responsive state is largely genetically determined [24]. Non-responsiveness to hepatitis B virus (HBV) vaccine in adults is strongly associated with HLA-C4A\*Q0, DRB1\*0301, DQB1\*02 haplotype [25]. Other risk factors for HB vaccine non-responsiveness include obesity, smoking, gender (male) and older age and the presence of immunocompromising chronic disease [26,27]. Hepatitis B vaccine non-responsiveness represents a significant problem for individuals who, through their occupation, remain at considerable risk of infection with hepatitis B virus.

Seroprevalence of HBs Ag among children in Taiwan was 9.8% before universal infant hepatitis B vaccination and it decreased to 0.7% after universal infant vaccination 15 years later [21]. Recently, studies showed the HBs Ag seropositive rate was 4.5% among incomplete vaccinees and 1.3% among complete vaccinees [9]. Another report revealed the seropositive rate of HBs Ag was 1.2% in Taiwan [16]. In our study, seroprevalence of HBs Ag was 1.3% in 15-year-olds (group I) and 5.2% in 18-year-olds (group II) which were consistent with other studies [14,16] in Taiwan and 0.7% in Thailand [28]. However, the seroprevalence of HBs Ag is still higher in adolescents than in children [11] and also higher in group II (18-year-olds) than in group I (15-year-olds). This might imply that there were some persons whose anti-HBs decreased from their childhood to adolescent period and became susceptible to be infected by HBV.

Studies have shown that long-lasting protective efficiency of HBV vaccination till the age of 15 and 18 were demonstrated in Alaska, Taiwan and Saudi Arabia [23,29–31]. An adequate protection for HBV infection has also been reported up to 14 years of age in a long-term follow-up of HB vaccinated children and the annual decay rate of anti-HBs was 10.2% in children who did not receive a booster dose in Taiwan [32]. Results from multiple cohort studies revealed that the immunized persons are still protected against HBV infection even the level of anti-HBs declined to undetectable [33]. Other studies also indicated that giving a booster dose of hepatitis B vaccine to persons who have been vaccinated and the concentration of anti-HBs was below 10 mIU/ml, could develop a rapid rise in anti-HBs antibody [34]. Ninety-six percent of vaccinees had detectable anti-HBs in their serum after the third dose of vaccine [9]. In this paper we found that 100% of 15- to 18-year-old adolescents, after receiving booster doses of recombinant HBV vaccine, developed antibody. The study also indicated an immune memory responses occurred in those populations [35–38]. A report suggested that routine booster vaccination may not be required to provide protection against chronic HBV infection age 15 [29]. However, as shown in Hammit et al. [40] a booster dose of recombinant HB vaccine maybe unable to induce sufficient immunological response in adolescents who had undetectable residual anti-HBs titer [39]. In addition, half of the children who had received hepatitis B vaccine starting at birth did not have evidence of immune memory as measured by development of anamnestic responses to a booster dose vaccination 15 years after vaccination at birth [40], and three doses of revaccination to non-responders could develop a 100% seroprotective antibody response [41]. Besides, the higher the anti-HBs titers remained for individual subsequent to primary vaccination in their infancy, the greater the anamnestic response to a booster dose of HB vaccine observed [42]. Our study shows that completing three doses of booster with recombinant HB vaccine could induce anti-HBs in 100% of vaccinees. Therefore, children or those high risk groups for HBV infection fail to achieve producing the protective anti-HBs upon receiving normal courses of hepatitis B vaccine at newborn may be boosted at their adolescent.

There is limitation to the present paper. First, anti-HBc was not tested for all participants and the seropositivity of anti-HBs may not come from vaccination. It may partly be due to natural infection. However, we referred to Su et al. [15] in Taiwan, the HBV natural infection rate were 9.8% and 8.1% in 1984 and 1985 birth-year cohort respectively, with a mean of 8.7%. This is described in the result section. In addition, in this report we have checked the vaccination records at Taiwan's Center for Disease Control (CDC), and have found that the seropositivity of anti-HBs was 51.2% among our participants vaccinated at birth. This data was similar to our report in Table 1. Besides, the CDC records that we checked show no HBs Ag positive participant who has vaccinated at birth, which support the data in Table 2. Table 2 also shows that only 11 participants born in 1985 and 1986 (shown with \*) and vaccinated at preschool ages, were HBs positive. In other word, all participants vaccinated at birth were HBs Ag negative and the anti-HBs positivity may not come from natural infection. The result partially complements the deficiency of anti-HBc data of our participants.

In conclusion, while many studies consider the nationwide hepatitis B vaccination program in Taiwan is successful and effective, a recent report further suggested that universal booster is not indicated for the primary HBV vaccines before adulthood [16]. The booster of completing three doses of vaccine to anti-HBs, HBs Ag and anti-HBc negative adolescents may be essential for maintaining anti-HBs antibodies titer in their serum or getting good immunity to HBV and reducing the seropositive rate of HBs Ag. In addition, the significant difference between students born before and after July 1986 (Table 3) indicated that students vaccinated at their preschool age have higher anti-HBs positive rate than those vaccinated at their birth of newborns. Therefore, the optimal vaccination time of HB vaccine for children may be re-evaluated. However, the carrier rates of HBs Ag in newborns and the protective rate of anti-HBs in adolescents should also be considered. Besides, since all reports about seropositive rate of anti-HBs in adolescents over 15-year-old age in Taiwan so far were from vaccinees vaccinated by plasma-derived vaccine, it was worthy to continue monitoring the long-term seroprevalence of anti-HBs in vaccinees (born after November 1, 1992) after being immunized with recombinant DNA vaccine in Taiwan.

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