ORIGINAL ARTICLE

Seroprevalence of Hepatitis B Viral Markers Among Freshmen — 20 Years After Mass Hepatitis B Vaccination Program in Taiwan

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Background/Purpose: The nationwide hepatitis B vaccination program in Taiwan was well known for its efficacy in reducing the carrier rate of hepatitis B and the morbidity and mortality of hepatitis B-related diseases among children. The aim of this study was to investigate the seroprevalence of hepatitis B 20 years after this program was implemented.

Methods: A total of 7592 freshmen from one university in Northern Taiwan participated in this study during their school entry health exam in September 2003 and September 2004. Basic data including gender, birthday, family history and vaccination history of hepatitis B by self-reported questionnaire were collected. Hepatitis B serum markers, including hepatitis B surface antigen, antibody against hepatitis B surface antigen, and antibody against hepatitis B core antigen were all checked. The differences in the seroprevalence of hepatitis B between two groups of subjects born before July 1984 and after July 1984 were examined. Multiple logistic analyses were performed for identifying the odds ratio (OR) of family history and other variables for each hepatitis B serum marker.

Results: Subjects born after July 1984 were found to have a lower rate of hepatitis B surface antigen of 2.2% (95% confidence interval [CI], 1.8–2.6%) *vs.* 7.4% (95% CI, 5.9–8.9%), and core antibody against hepatitis B of 6.7% (95% CI, 6.0–7.3%) *vs.* 23.5% (95% CI, 21.1–25.9%), but a higher rate of surface antibody against hepatitis B of 74.3% (95% CI, 73.2–75.4%) *vs.* 69.1% (95% CI, 66.5–71.7%) compared with those born before July 1984 (all $p < 0.001$). Subjects with a family history of hepatitis B had higher risk of being infected by hepatitis B (OR, 4.07; 95% CI, 3.18–5.12) and becoming carriers (OR, 7.26; 95% CI, 5.05–10.44) after adjustment for sex, age, birth year, and self-reported hepatitis B vaccination history.

Conclusion: The seroprevalence of hepatitis B surface antigen continued to decline 20 years after neonatal hepatitis B vaccination program. It is strongly recommended that those who have a family history of hepatitis B should receive early check-up of hepatitis B status after complete vaccination or closely follow up their hepatitis B status after neonatal hepatitis B vaccination. [*J Formos Med Assoc* 2007;106(7):513–519]

Key Words: hepatitis B, seroprevalence, Taiwan, vaccination, youth

The carrier rate of hepatitis B surface antigen (HBsAg) in the general population of Taiwan was as high as 15–20% before the mass vaccination program was implemented.[1,2] Hepatitis B virus (HBV) infection was believed to cause 80% of adult-onset and nearly 100% of childhood cases of hepatocellular carcinoma (HCC) in Taiwan.[3–5] Therefore, the Taiwan government launched a

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nationwide hepatitis B vaccination program in July 1984. For the first 2 years, the program covered only neonates born to HBsAg-carrier mothers, but was later extended to cover all neonates by July 1986.[6,7] As a result, the carrier rate of HBsAg among children declined gradually as time went on.[8,9] The incidence of HCC in children aged 6–14 years and the corresponding rates of mortality from HCC also decreased.[10] Further, the mortality rate from fulminant hepatitis in children also decreased.[11,12] In a survey performed 15 years after nationwide vaccination had begun, more than 85% of all children had received at least three doses of the HBV vaccine. The decrease in HBV infection rate and HBsAg carrier rate was demonstrated 15 years after the launch of this nationwide vaccination program.[13] It has now been 20 years since the initiation of this program; thus, this study aimed to assess the seroprevalence of HBV serum markers 20 years after the implementation of the HBV vaccination program.

Subjects and Methods

Subjects

The studied university is a general university located in Taipei, Northern Taiwan. Freshmen were requested to receive routine school entry health examination at the beginning of the first semester, in September every year. For this study, those who entered the university in 2003 and 2004 were included since these students had been born just near the start of the mass HBV vaccination program in Taiwan. However, any students who were foreigners or who came from overseas were excluded from this study. All of the study subjects signed informed consent forms witnessed by the school health committee and the ClinicalTrials.gov identifier is NCT00173940.

Measures

Physical examinations, questionnaires, and blood tests were performed by medical staff from the university hospital and university health center. Basic data including gender, birthday, family history and vaccination history of hepatitis B by self-reported questionnaire were collected. HBV serum markers, including HBsAg, antibody against hepatitis B surface antigen (anti-HBs), and antibody against hepatitis B core antigen (anti-HBc) were all checked. The central laboratory of the university hospital changed some machines and kits in 2004. All serum samples in this study were checked for anti-HBc by microparticle enzyme immunoassay (AxSYM; Abbott, Germany). The other two serum markers, HBsAg and anti-HBs, were checked by enzyme immunoassay (BepIII; DADE Behring, Germany) for freshmen in 2003, and by microparticle enzyme immunoassay (AxSYM; Abbott) for freshmen in 2004. Samples with anti-HBs < 10 mIU/mL were interpreted as non-reactive. Throughout the study, we took HBsAg to be the marker of the chronic carrier stage, anti-HBs to be the marker of immunity, either from vaccination or infection, and anti-HBc to be the marker of past infection. Seronegativity was defined as being negative for all three hepatitis B markers HBsAg, anti-HBs and anti-HBc. With regards to anti-HBs(+), we took anti-HBs(+) and anti-HBc(–) to be markers of vaccination, whereas anti-HBs(+) and anti-HBc(+) were markers of past infection. We defined family history of hepatitis B as having any family members who have HBsAg positivity.

Statistical analysis

SAS version 9.1 (SAS Institute Inc., Cary, NC, USA) was used for statistical analysis. The differences in the seroprevalence of hepatitis B between the two birth groups were examined by χ^2 test. Multiple logistic analyses were performed for identifying the odds ratio (OR) of family history and other variables for hepatitis B serum markers. A p value less than 0.05 was considered statistically significant.

Results

A total of 8473 freshmen entered the university in 2003 or 2004 and received the entrance health examination; 881 foreigners, overseas students and

subjects with incomplete data were excluded. Therefore, 7592 freshmen were included in the analysis; 6388 (84.1%) subjects were born after July 1, 1984. Most of them (5805, 90.9% of subjects born after July 1, 1984) were born during the first 2 years of the mass vaccination program. The male to female sex ratio was 1.15, and mean age was 19.8 ± 2.7 years (range, 16.1–54.2 years). The majority (5135, 67.6%) of subjects reported previous hepatitis B vaccination history, and 661 (8.7%) subjects reported family history of hepatitis B (Table 1).

The prevalence of positive HBsAg, positive anti-HBs and positive anti-HBc among freshmen were 3.0% (95% confidence interval [CI], 2.6–3.4%), 73.5% (95% CI, 72.5–74.5%) and 9.3% (95% CI, 8.7–10.0%), respectively. The frequency distribution and 95% CIs of the HBV markers for freshmen born before July 1984 and after July 1984 are shown in Table 2. Compared with the subjects who were born before July 1984, the freshmen born after July 1984 had a lower rate of HBsAg seropositivity of 2.2% (95% CI, 1.8–2.6%) *vs.* 7.4% (95% CI, 5.9–8.9%) and anti-HBc seropositivity of 6.7% (95% CI, 6.0–7.3%) *vs.* 23.5% (95% CI, 21.1–25.9%), but a higher rate of anti-HBs seropositivity of 74.3% (95% CI, 73.2–75.4%) *vs.* 69.1% (95% CI, 66.5–71.7%) (all $p < 0.001$). Among freshmen born before 1984 with anti-HBs antibody, 77.5% (95% CI, 74.7–80.4%) were found to be anti-HBc negative. The prevalence of seronegativity for all three HBV serum markers was similar in the two groups: 23.2% (95% CI, 22.2–24.2%) *vs.* 22.8% (95% CI, 20.5–25.2%).

All seronegative subjects were classified into subgroups according to birth year and self-reported vaccination history. The seronegative rate was 21.5% in subjects with self-reported hepatitis B vaccination history, and 38.9% in those without self-reported hepatitis B vaccination history. In addition, the seronegative rate of subjects born before July 1984 and after July 1984 was 19.2% and 21.8% in subjects with self-reported hepatitis B vaccination history, and 33.5% and 41.5% in those without self-reported hepatitis B vaccination history, respectively ($p < 0.001$).

Multiple logistic regression analyses were performed to determine the association of hepatitis B family history and HBsAg seropositivity among freshmen (Table 3). After adjusting for gender, age,

Table 1. Characteristics of the study population

Characteristics	Total *n* (%)	Before July 1984 *n* (%)	After July 1984 *n* (%)	*p*
Year of school entry				< 0.0001
2003	3813 (50.2)	820 (68.1)	2993 (46.9)	
2004	3779 (49.8)	384 (31.9)	3395 (53.1)	
Sex				0.02
Female	3539 (46.6)	525 (43.6)	3014 (47.2)	
Male	4053 (53.4)	679 (56.4)	3374 (52.8)	
Family history of hepatitis B*				0.9
Yes	661 (8.7)	106 (8.8)	555 (8.7)	
No	6926 (91.2)	1098 (91.2)	5828 (91.2)	
Unknown or not available	5 (0.1)	0 (0.0)	5 (0.1)	
Self-reported vaccination history				< 0.0001
Yes	5135 (67.6)	657 (54.6)	4478 (70.2)	
No	501 (6.6)	161 (13.4)	340 (5.3)	
Unknown or not available	1956 (25.8)	386 (32.1)	1565 (24.5)	
Total	7592 (100.0)	1204 (15.9)	6388 (84.1)	

**Family history of hepatitis B refers to any family members who have HBsAg positivity.*

Table 2. Seroprevalence of hepatitis B viral markers for freshmen

	Total ($N=7592$)		Before July 1984 ($N=1204$)		After July 1984 ($N=6388$)		p^*
	n (%)	95% CI	*n* (%)	95% CI	*n* (%)	95% CI	
HBsAg							<0.001
Positive	229 (3.0)	2.6–3.4	89 (7.4)	5.9–8.9	140 (2.2)	1.8–2.6	
Negative	7363 (97.0)	96.6–97.4	1115 (92.6)	91.3–94.1	6248 (97.8)	97.5–98.2	
Anti-HBs							
Positive	5577 (73.5)	72.5–74.5	832 (69.1)	66.5–71.7	4745 (74.3)	73.2–75.4	<0.001
Anti-HBc(+)	457 (8.2)	7.5–8.9	187 (22.5)	19.6–25.3	270 (5.7)	5.0–6.4	<0.001
Anti-HBc(–)	5120 (91.8)	91.1–92.5	645 (77.5)	74.7–80.4	4475 (94.3)	93.7–95.0	
Negative	2015 (26.5)	25.6–27.5	372 (30.9)	28.3–33.5	1643 (25.7)	24.7–26.8	
Anti-HBc							<0.001
Positive	708 (9.3)	8.7–10.0	283 (23.5)	21.1–25.9	425 (6.7)	6.0–7.3	
Negative	6884 (90.7)	90.0–91.3	921 (76.5)	74.1–78.9	5963 (93.4)	92.7–94.0	
Seronegative†							0.786
Yes	1757 (23.1)	22.2–24.1	275 (22.8)	20.5–25.2	1482 (23.2)	22.2–24.2	
No	5835 (76.9)	75.9–77.8	929 (77.2)	74.8–75.9	4906 (76.8)	75.8–77.8	

*χ^2 test; †seronegativity was defined as being negative for all three hepatitis B markers HBsAg, anti-HBs and anti-HBc.

birth year, and self-reported hepatitis B vaccination history, subjects with family history of hepatitis B were found to have 7.26 times (95% CI, 5.05–10.44) higher risk of HBsAg positivity, 4.07 times (95% CI, 3.18–5.21) higher risk of anti-HBc positivity, and 0.63 times (95% CI, 0.52–0.77) risk of anti-HBs positivity compared with those without family history of hepatitis B ($p<0.001$).

Discussion

Previous studies have disclosed the significant decrease in HBsAg carriers and anti-HBc seropositivity among children and adolescents after the mass hepatitis B vaccination program.[6,9,14–16] This study, an epidemiologic survey extending over 20 years after the introduction of hepatitis B vaccination, adds additional information regarding the changing pattern of hepatitis B following vaccination in a previously hyperendemic country. The participants in the present study included freshmen who lived across Taiwan, thus they could be considered to be a distributed sample of the general population at about 20 years old. The main results delineated a significant decrease of both HBsAg and anti-HBc antibody as markers of HBV infection in students born after the implementation of the vaccination program compared to those born before the introduction of vaccination. In addition, the risk of having HBV markers of infection was 7.26 times (95% CI, 5.05–10.44) higher in subjects with a family history of hepatitis B.

Our study showed that the HBV carrier rate dropped to 2.2% after the initiation of hepatitis B immunization. Comparing the results between freshmen born before and those after July 1984, the HBsAg and anti-HBc positive rates decreased by 70.3% and 71.5%, respectively. The HBsAg positive rate (7.4%) in this cohort was lower than that of the general population (15–20%) and the university study done by Liu et al (14.4%),[17] revealing the effectiveness (84.7%) of this program. Despite the effectiveness of hepatitis B immunization, natural infection of hepatitis B was still detected (5.7%). The reasons may be incomplete vaccination coverage, incomplete hepatitis B immunization doses, or poor response to hepatitis B immunization.

The HBsAg positivity in this study (2.2%) was lower than that of a 15-year-old cohort from a rural township (11.4%)[15] and a small city (4.1%),[14]

Table 3. Multiple logistic regression analyses for the seropositivity of hepatitis B

		OR	95% CI	*p*
HBsAg(+)/HBsAg(–)				
Sex	Male/Female	1.08	0.76–1.52	0.68
Age	Continuous variable	1.04	0.99–1.09	0.13
Birth year	Before/After July 1984	2.06	1.30–3.27	0.002
Family history of hepatitis B*	Yes/No	7.26	5.05–10.44	<0.0001
Hepatitis B vaccination history	Yes/No	0.24	0.16–0.37	<0.0001
Anti-HBs(+)/Anti-HBs(–)				
Sex	Male/Female	0.70	0.62–0.79	<0.0001
Age	Continuous variable	1.00	0.98–1.04	0.77
Birth year	Before/After July 1984	0.98	0.79–1.21	0.84
Family history of hepatitis B*	Yes/No	0.63	0.52–0.77	<0.0001
Hepatitis B vaccination history	Yes/No	3.12	2.57–3.79	<0.0001
Anti-HBc(+)/Anti-HBc(–)				
Sex	Male/Female	1.02	0.83–1.24	0.87
Age	Continuous variable	1.13	1.09–1.18	<0.0001
Birth year	Before/After July 1984	1.96	1.48–2.62	<0.0001
Family history of hepatitis B*	Yes/No	4.07	3.18–5.21	<0.0001
Hepatitis B vaccination history	Yes/No	0.43	0.33–0.57	<0.0001

**Family history of hepatitis B refers to any family members who have HBsAg positivity. OR = odds ratio; CI = confidence interval.*

but higher than that from an urban area (0.5%).[13] The same trend in different areas could be seen in anti-HBc seroprevalence. The difference in the HBsAg positive rate may have several possible explanations. First, the hepatitis B seroconversion took place during these years, but this is unlikely because immune clearance rate before 20 years old was reported to be low.[18] Second, the baseline HBsAg positivity might be different among these studies, but past study has shown a similar HBsAg positivity and highly infectious rate of pregnant mothers from different counties between 1984 and 1985.[6] Third, different vaccine completion rates, defined as receiving at least three doses of hepatitis B vaccine, may contribute to these differences. For this birth cohort, only those who were born to HBsAg carrier mothers with positive HBeAg or serum HBsAg titers ≥2560 received vaccination at birth. Most of them received vaccination in the preschool or school-age period as the program extended. Since 67.4% of subjects of this birth cohort in our study were positive for anti-HBs and negative for anti-HBc, it might be inferred that these subjects have previously received hepatitis B immunization. Reviewing past studies, the vaccine completion rate was 11.8% at 15 months old for all, 60.7% at 6 years old for nationwide sampling, <72% at 15 years old in the rural area, and 80% at 15 years old in the urban area in Taiwan. Therefore, the vaccine completion rate in different areas was the most likely contributing factor to these differences.[6,9,13,15]

Subjects who are seronegative for all three HBV serum markers are more susceptible to hepatitis B infection. In our study, 21.8% of subjects who were born after the vaccination program and had self-reported hepatitis B vaccination history were still seronegative. The possible explanations included initial vaccination failure, decline in the efficacy of vaccination with time, or recall bias. It brings to attention the need for further booster shots and the confirmation of hepatitis B viral markers. At present, booster shots for waning immunity in fully immunized individuals younger than 15 years of age are not necessary except in health care workers or immunocompromised individuals.[19,20]

However, whether booster shots are needed for subjects older than 15 years is still under debate.[21]

After controlling the variables including gender, age, birth year, and hepatitis B vaccination history, subjects with family history of hepatitis B still had 7.26 (95% CI, 5.05–10.44) times higher risk of being infected by hepatitis B and becoming a carrier. Several studies suggested HBIG administration within 24 hours after birth for those born to high-risk mothers to reduce this risk.[22–24] Our results reinforced the importance of family history of hepatitis B as an independent variable. Further measures may be needed for those who have family history of hepatitis B, such as early check-up of hepatitis B status after complete vaccination or close follow up of their hepatitis B status. HBV eradication or treatment to lower HBV activity among pregnant women should be considered, taking cost-effectiveness and safety into account.

Some limitations of this study should be addressed. First, regarding the validity of vaccination history or family history of hepatitis B, some recall bias may have occurred since they were self-reported data. However, since the questionnaires were completed before the blood examinations, the bias would have been non-differential. To diminish the potential recall bias of the vaccination and family history of hepatitis B, we reminded subjects to ask their parents for confirmation of vaccination history and family history of hepatitis B. The status of family history of hepatitis B was assumed to be stationary among the study subjects due to limited age differences (mean age, 19.8 ± 2.7 years). We compared the data of family history of hepatitis B between birth year before July 1984 and after (Table 1). The results showed no difference (8.8% *vs.* 8.7%), so the validity of self-reported vaccination history can be assumed to be good. Second, with regards to the relationship between the concentration of anti-HBs titer and HBV vaccination, there is limited quantitative data on the anti-HBs titer in our study. Further study regarding this part is needed.

In conclusion, this study provides the seroprevalence data of hepatitis B status 20 years after the introduction of a mass vaccination program. Whether seronegative cases need boosters deserves further clinical trials to confirm. Those who have a family history of hepatitis B may require closer follow up of their hepatitis B status after hepatitis B vaccination.

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