

ORIGINAL ARTICLE

Hepatitis B seroepidemiology and booster vaccination in pre-clinical medical students in a Malaysian university

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Abstract

Introduction: Infant hepatitis B vaccination was introduced into the Expanded Programme on Immunisation (EPI) in Malaysia in 1989. This study aimed to investigate seroprevalence of hepatitis B among UKM pre-clinical medical students, born between 1991 and 1995, and had their infant vaccination more than 20 years ago. **Materials and Methods:** A prospective, cross-sectional study involving 352 students, comprising 109 (31.0%) males and 243 (69.0%) females. Blood specimens were tested for anti-HBs, where levels of ≥ 10 mIU/mL was considered reactive and protective. Students with non-reactive levels were given a 20 μ g HBV vaccine booster. Anti-HBs levels were tested six weeks after the first booster dose. Those with anti-HBs < 10 mIU/mL were then given another two booster doses, at least one month apart. Anti-HBs levels were tested six weeks after the third dose. **Results:** Ninety-seven students (27.6%) had anti-HBs ranging from 10 to > 1000 mIU/mL while 255 (72.4%) had anti-HBs < 10 mIU/mL. After one booster dose, 208 (59.1%) mounted anti-HBs ≥ 10 mIU/mL. Among the remaining 47 (13.3%), all except two students (0.6%) responded following completion of three vaccination doses. They were negative for HBsAg and anti-HBcore antibody, thus regarded as non-responders. **Conclusions:** Anti-HBs levels waned after 20 years post-vaccination, where more than 70% were within non-reactive levels. For healthcare workers, a booster dose followed by documenting anti-HBs levels of ≥ 10 mIU/mL may be recommended, to guide the management of post-exposure prophylaxis. Pre-booster anti-HBs testing may not be indicated. Serological surveillance is important in long-term assessment of HBV vaccination programs. No HBV carrier was detected.

Keywords: Hepatitis B vaccination, seroprevalence, serological surveillance, healthcare workers, medical students, anti-HBs

INTRODUCTION

Worldwide, an estimated 257 million people are infected with hepatitis B virus (HBV), which is defined as hepatitis B surface antigen positive. In 2015, hepatitis B resulted in 887,000 deaths, mostly from complications including cirrhosis and hepatocellular carcinoma.¹ The prevalence of HBV infection varies widely in different parts of the world. In high HBV endemic areas, the seropositivity of hepatitis B surface antigen (HBsAg) exceeds 8% of the total population. In regions of medium HBV endemicity, HBsAg seropositivity is between 2% and 7%, while regions with HBsAg seropositivity of less than 2% are considered as low HBV endemic regions.²

Vaccination against hepatitis B in early childhood is the most effective way of preventing infection. Safe and effective vaccines against hepatitis B have been available since 1982.³ Hepatitis B vaccines induce a protective antibody response to hepatitis B surface antigen (anti-HBs antibody) in about 95% of vaccines and confer long-lasting protection against HBV infection in immunocompetent individuals and prevents the development of chronic disease and liver cancer due to hepatitis B.^{4,5,6} In Malaysia, hepatitis B vaccine has been introduced in the Expanded Programme on Immunisation (EPI) under the Ministry of Health in 1989. All newborns are given three doses of HBV vaccine. The first dose is given within 24 hours of birth, and the two

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subsequent doses given at first and fifth month of age, respectively.

Hepatitis B is an important occupational hazard for health workers. The Centres for Disease Control (CDC) recommends that all healthcare personnel including students at risk for HBV infection be tested for anti-HBs and those found to be susceptible should receive the vaccine.^{7,8} A titer of anti-HBs of 10 mIU/mL was generally accepted to be the minimum level for protective immunity against HBV.^{8,9,10} Healthcare personnel who do not have protective concentration of anti-HBs of more than 10 mIU/mL after revaccination should be tested for HBsAg and anti-HBcore to determine their infection status.

This study aimed to determine the seroepidemiology of hepatitis B among our medical students who have been vaccinated more than 20 years ago, as part of serological surveillance for long-term assessment of HBV vaccination program. Response following a booster vaccination was also assessed and vaccine non-responders were assessed for possible chronic HBV infection status.

MATERIALS AND METHODS

This prospective, cross-sectional study was conducted from July 2013 until June 2015, involving first and second year (pre-clinical) medical students. Ethics approval was obtained from Universiti Kebangsaan Malaysia Medical Centre (UKMMC) Research and Ethics Committee prior to starting the study (UKM Fundamental Fund, project code FF-2013-418). The study objectives were explained to the students and consent was obtained. Those with contraindications to hepatitis B vaccines were excluded.

Two millilitres of blood were drawn from all participating students, collected into a plain tube and transported to virology serology laboratory, where samples were processed and tested for anti-HBs titre. The sera were analysed using fully-automated analyser, ROCHE Cobas® Elecsys e601, according to manufacturer protocol. Students with detected anti-HBs titre of ≥ 10 mIU/mL were considered immune to hepatitis B, thus excluded from booster vaccination and further testing.

Students with non-reactive or anti-HBs titre <10 mIU/mL were given one booster dose of HBV vaccine (20 μ g). Another 2 ml of blood were drawn from these students 4 to 6 weeks after the booster vaccination and tested for anti-HBs titre. For those with anti-HBs titre <10 mIU/mL, another 2 doses were given to complete a course of 3-dose booster vaccination. Retesting for anti-HBs level was done 4 to 6 weeks after completion of the third dose, to check for late anamnestic response. For non-reactive students after revaccination (completion of three doses of booster vaccination), HBsAg and anti-HBcore total antibody were tested to assess their status to determine whether they were chronic hepatitis B carriers or vaccine non-responders.

RESULTS

A total of 352 students consented to participate in this study, comprising 109 (31.0%) males and 243 (69.0%) females. The majority of the students were Malay (n = 258; 73.2%), followed by Chinese (n = 53, 15.0%), Indian (n = 38, 10.8%) and other ethnicities (n = 3, 0.9%). These students were born between year 1991 and 1995. The majority were born in the year 1993. Demographics of students in the study are shown in Table 1.

TABLE 1: Demographics of pre-clinical medical students in the study

		Number of students (n = 352)	Percentage %
Year of birth	1991	1	0.3
	1992	21	6.0
	1993	195	55.4
	1994	129	36.6
	1995	6	1.7
Gender	Female	243	69.0
	Male	109	31.0
Ethnicity	Chinese	53	15.0
	Indian	38	10.8
	Malay	258	73.3
	Other	3	0.9

TABLE 2: Anti-HBs antibody responses among the pre-clinical medical students

	Immune status	Anti-HBs level (mIU/mL)	Number of students	Percentage %
Screening (n = 352)	Non-immune (n = 255, 72.4%)	<2	226	64.2
		2-10	29	8.2
	Immune (n = 97, 27.6%)	>10-50	42	11.9
		51-100	11	3.1
		100-1000	22	6.3
		>1000	22	6.3
After one booster dose (n = 255)	Non-immune (n = 47, 13.3%)	<2	36	14.1
		2-10	11	4.3
	Immune (n = 208, 59.1%)	>10-50	36	14.1
		51-100	20	7.8
		100-1000	101	39.6
		>1000	51	20.0
Completed 3-dose booster vaccination (n = 47)	Non-responder (n = 2, 0.6%)	<2	2	4.3
		2-10	0	0.0
	Immune (n = 45)	>10-50	4	8.6
		51-100	2	4.3
		100-1000	13	27.7
		>1000	26	55.3

Anti-HBs antibody responses among the pre-clinical medical students are shown in Table 2. The summary of study flow and results is shown in Fig. 1. Pre-vaccination screening of anti-HBs revealed 97 out of the total 352 students (27.6%) were immune against hepatitis B. The remaining 255 students (72.4%) had antibody levels below 10 mIU/mL. These 255 non-immune students were given a booster of hepatitis B vaccine, after which 208 students (59.1%) had protective immunity with anti-HBs ≥ 10 mIU/mL while another 47 students (13.3%) still had anti-HBs level of less than 10 mIU/mL. All 29 students with measurable but low anti-HBs (2-10 mIU/mL) at the initial screening seroconverted after one booster HBV vaccination.

After one booster dose, among the 47 non-immune students, 76.6% (n = 36) had < 2.0 mIU/mL anti-HBs titre, and another 23.4% (n = 11) had anti-HBs titre between 2 and 10 mIU/mL. After completion of 3 doses of hepatitis B booster vaccination doses for these 47 students, 2 students remained as “non-responder”. Screening for HBsAg and anti-HBcore antibody were negative for both students.

Among the students with immunity who did not require booster vaccination (n = 97), 42 out of 97 (43.3%) claimed they have taken at least one dose of HBV booster vaccination before enrolling into medical school, but were unsure

or have not tested their anti-HBs. Half of them completed 3 booster doses while another half had one to two booster doses. Their anti-HBs levels ranged between 30.0 to >1000 mIU/mL (median 1000, mean 702.9 mIU/mL). While the remaining 55 out of 97 students with immunity (56.7%) either denied or cannot recall any HBV booster vaccination prior to enrolment into medical school. Anti-HBs levels among the latter group were lower, ranging between 10.1 to 591.3 mIU/mL (median 28.8, mean 64.5 mIU/mL).

DISCUSSION

Approximately 780,000 people die each year from HBV infection; 650,000 from cirrhosis and liver cancer due to chronic hepatitis B infection and another 130,000 from acute hepatitis B.¹¹ Hepatitis B vaccine is given to infants mainly to prevent infection and the development of chronic liver disease and liver cancer. By the end of 2016, infant vaccination had been introduced nationwide in 186 countries. In 2015, global coverage with 3 doses of hepatitis B vaccine reached 84% and was as high as 92% in the Western Pacific region. Furthermore, 101 countries introduced one dose of hepatitis B vaccine to newborns within the first 24 hours of life, and the global coverage with the birth dose of hepatitis B vaccine was 39%.¹²

In Malaysia in 2015, the incidence rate of

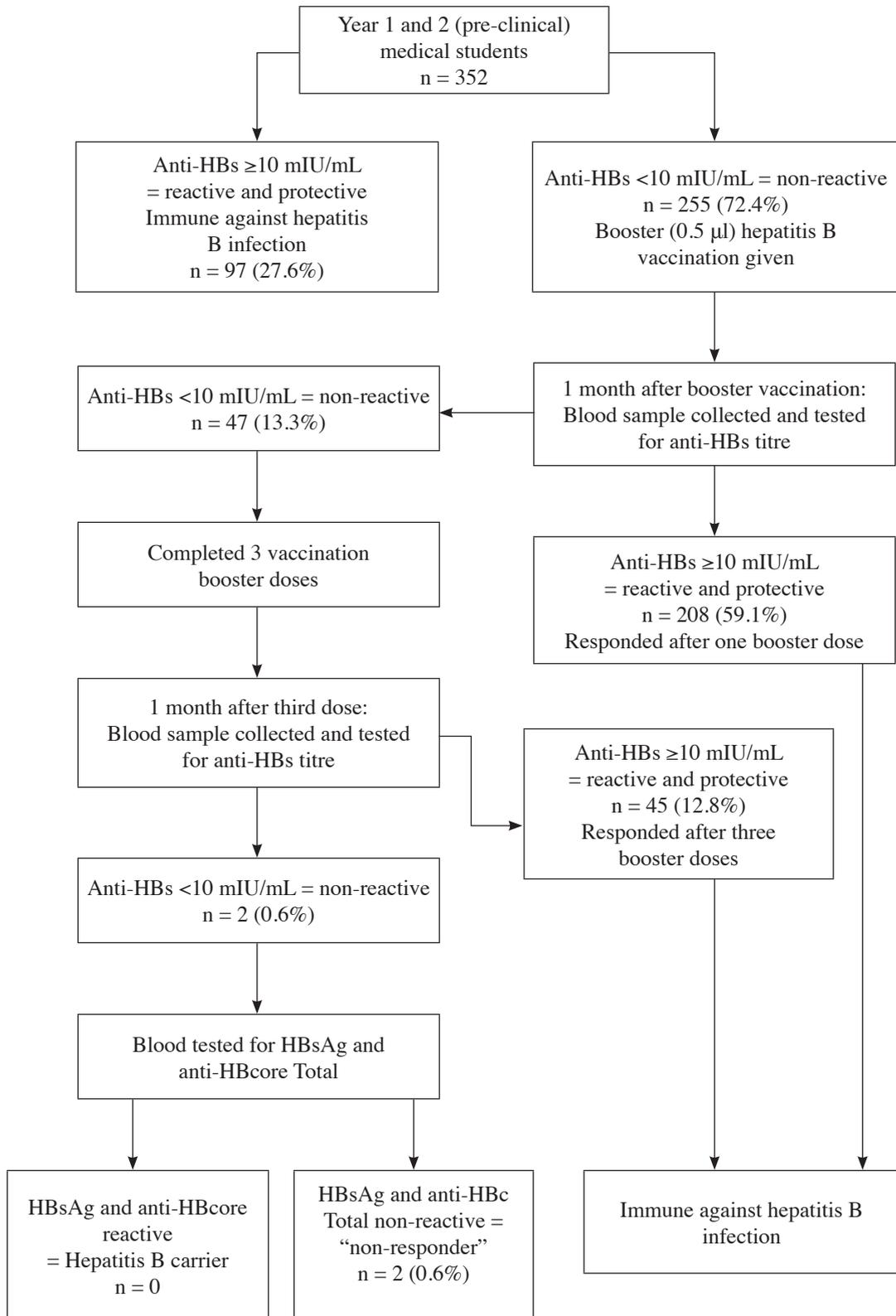


FIG. 1: Summary of study flow and results.

hepatitis B was 12.65 per 100,000 population, and mortality 0.13 per 100,000 population.¹³ Coverage for three doses of HBV vaccination in this country in the past ten years were between 96-99%, while coverage for birth dose of HBV vaccine ranged between 88-99%.¹⁴

The EPI was established in 1974 to ensure children worldwide would benefit from vaccines.¹⁵ Universal infant HBV vaccination was introduced in 1992,¹⁶ a strategy that is more feasible and cost-effective^{17,18} compared to targeting populations at high risk of HBV infection. Reduction in hepatitis B seroprevalence and hepatitis B-related deaths were observed in countries where universal infant vaccination against hepatitis B is in place, and the seroepidemiology of hepatitis B changes after EPI included HBV vaccination.^{2,6,19} Before nationwide vaccination initiation, Malaysia had an intermediate endemicity with HBsAg prevalence of 5-7%.²⁰ In the study by Yap SF *et al.* (1994), chronic HBV carrier rate varies in this country between < 1% to about 10% depending on the ethnic group studied.²¹ In another study among blood donors in Kelantan, Malaysia, there was a statistically significant difference in the prevalence of hepatitis B infection between regular (0.45%) and first time donors (1.83%).²²

The overall prevalence of HBsAg from 1997 to 2003 in Malaysian school children was reported at 0.6%, with a decrease from 1.6% in 1997 to 0.3% in 2003.²³ Among these school children aged 7 to 12 years, there was a dramatic reduction of HBsAg seroprevalence in those born after the implementation of universal infant HBV vaccination (0.4%) compared to 1.7% in those born before the implementation. This showed that the vaccination program has been effective in reducing vertical transmission of HBV. From the year of birth, all students enrolled in our study were born after the implementation of universal infant HBV vaccination which was initiated since year 1989 in this country. They received their childhood vaccination more than 20 years ago. No HBV carrier was detected, supporting recent reports of low endemicity in this country. However, this represents a very specific population among people who received their childhood vaccination.

Serological testing for documenting seroconversion in children is usually unnecessary. Studies have shown that the vaccine is more than 90% effective in preventing infection in immunocompetent individuals.^{4,5,6} Evidence indicates that successfully vaccinated individuals

whose antibodies decreased or disappeared over time usually show a rapid anamnestic response when boosted with an additional dose of vaccine given several years after the primary course of vaccination or when exposed to HBV. This means that the immunological memory for HBsAg lasts longer than the anti-HBs detection, which continues to provide long-term protection against acute disease and the development of an HBsAg carrier state.^{24,25}

The magnitude of the anti-HBs response is largely influenced by vaccine regimen, body mass, site and route of injection, immunosuppression, and age.²⁶ Anti-HBs concentration of 10 mIU/mL measured one month after completion of primary vaccination course is considered to provide long-term immunity and protection against infection.⁷⁻¹⁰ Poor or suboptimal responses to vaccination are well known among immunocompromised patients and certain groups such as end-stage renal disease on haemodialysis, chronic liver disease and human immunodeficiency virus (HIV) patients.²⁷ For immunocompromised patients, regular testing for anti-HBs, and a booster injection when the titre falls below 10 mIU/mL is advised.²⁷ Non-responders to a primary course should also be investigated.

In this study, 72.4% (255 out of 352) of those vaccinated at birth has non-reactive level of anti-HBs. We found that 59.1% of the population (208 out of 352) or 81.6% of those without protective antibody levels (208 out of 255) achieved protective anti-HBs levels after one HBV booster dose. All 29 students with measurable but low anti-HBs (2-10 mIU/mL) seroconverted after one booster HBV vaccination. A total of 12.8% of the population (45 out of 352) achieved protective anti-HBs levels after two additional doses. This adds up to a cumulative 71.9% who responded after one or three booster doses. In school children who belonged to the EPI group, 59.3% had anti-HBs detected when screened at 7 to 12 years after infant vaccination.²³ Another study by Ng KP *et al.* (2013) reported that among students in a Malaysian university who enrolled from 2005 to 2011, anti-HBs was not detected in 66.14% of those vaccinated at birth as compared to only 13.12% of those vaccinated voluntarily at an older age.²⁸

Zuckerman JN *et al.* (1998)²⁶ recommended testing for anti-HBs one month after the primary vaccination or booster to ensure protection against HBV infection and disease,

and emphasised the reliance on immunological memory rather than booster doses to protect against infection. However, Yoshida *et al.* (2000) suggested that regular boosters may be used to provide reassurance of protective immunity against breakthrough infection.²⁹ CDC recommended that revaccination is not needed for immunocompetent individuals with known response to a three-dose course of HBV vaccination, with anti-HBs of at least 10 mIU/mL, regardless of the decrease in anti-HBs later on. CDC also stated that pre-vaccination serologic testing is not indicated in persons who have been vaccinated except those at risk for HBV infection, such as healthcare workers and certain high-risk populations.^{7,8} Healthcare workers are required to have a documentation of a complete course of vaccination and anti-HBs testing with levels of ≥ 10 mIU/mL, after which no further periodic testing of anti-HBs or booster is required.^{8,9}

The European Consensus Group on Hepatitis B Immunity stated that the routine administration of booster doses of vaccine to immunocompetent children and adults who completed the full three-dose vaccination is not necessary for long-term protection. Boosters are therefore not required in immunocompetent individuals including healthcare workers and others at occupational risk of infection, in whom adequate immunological priming has been achieved, even if antibody titres to hepatitis B surface antigen (HBsAg) decline after vaccination.^{30,31} However, there may be policies of administering booster doses to certain risk groups. Medical students belong to the healthcare worker group, considered as a high-risk population. However, they lack records of anti-HBs screening after completion of the primary course of HBV vaccination. A booster dose followed by testing to record anti-HBs protective level achieved will guide the management of post-exposure prophylaxis. Pre-booster anti-HBs testing may not be indicated considering the majority will have non-reactive or low levels, but will mount anamnestic response after one booster dose.

Two students (0.6% of this population) with anti-HBs of <10 mIU/mL post-booster vaccination, had completed a full three-dose course of vaccination. However, their anti-HBs were still not detected. Tests for HBsAg and anti-HBcore antibody were negative, indicating no previous exposure to HBV. With the assumption that they had a complete course of infant vaccination, they can be regarded as

“non-responders”, that is defined as persons with anti-HBs <10 mIU/mL after ≥ 6 doses of HBV vaccine.⁸ They are considered as ‘susceptible’ and in the event of exposure to a HBsAg-positive patient, they should receive two doses of hepatitis B immunoglobulin (HBIG), a month apart as post-exposure prophylaxis.^{7,9}

Other methods for surveillance and monitoring of HBV vaccination program in the long term include data on the number of acute viral hepatitis cases and incidence rate by year, month, geographical area and age group. There should also be data on the proportion of all cases of chronic liver disease, cirrhosis and primary liver cancer that are HBsAg-positive or anti-HCV-positive. In Italy, HBV is intermediate endemicity and universal vaccination of infants and adolescents was initiated in 1991. From pre-vaccination to post-vaccination period, the incidence of acute hepatitis B decreased 24-fold and 50-fold in the 15–24-year and 0–14-year age groups respectively.³² In Taiwan, the incidence of hepatocellular carcinoma in children 6–9 years of age decreased 4-fold from $0.52/10^5$ in the cohort born before implementation of the universal vaccination program to $0.13/10^5$ in those born after the program.³³ Seroprevalence surveys are needed to measure the prevalence of viral hepatitis infection in the general population as part of long-term monitoring, which should continue to confirm the absence of clinically significant breakthrough episodes of hepatitis B and development of a carrier state.³⁴ This is particularly important in the special groups such as healthcare workers, blood donors, pregnant women, military recruits, patients with chronic liver disease and end-stage renal disease patients on dialysis.³⁴

In May 2016, the World Health Assembly adopted the “Global Health Sector Strategy on Viral Hepatitis, 2016-2021”. The strategy has a vision of eliminating viral hepatitis as a public health problem, with the global targets of reducing new viral hepatitis infections by 90% and reducing deaths due to viral hepatitis by 65% by 2030.³⁵

CONCLUSION

Anti-HBs waned after 20 years post-vaccination, where more than 70% were within the non-reactive level. Medical students belong to the healthcare worker group, who are considered within the high-risk group for HBV exposure in their clinical years and beyond. Even though completion of the primary course of vaccination

provides long-term immunity, a booster HBV vaccine dose followed by anti-HBs testing to document protective immunity against HBV is probably a good approach. This will guide the management of post-exposure prophylaxis in healthcare workers. Pre-booster anti-HBs testing may not be indicated considering the majority will have non-reactive or low levels, but will mount anamnestic response after a booster dose. Serological surveillance is an important component of the long-term assessment of HBV vaccination programs, which was initiated almost three decades ago in Malaysia. No HBV carrier was detected among this specific population group.

Conflict of interests: The authors declared no conflict of interest.

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