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Original Article

Hepatitis B antibody titers in Indonesian adolescents who received the primary hepatitis B vaccine during infancy

Hartono Gunardi[1], Adra Firmansyah[2], Sri Rezeki S Harun[1], Sudigdo Sastroasmoro[1]

Abstract

Background Hepatitis B (HB) has been classified as moderate-to-highly endemic in Indonesia. HB vaccination, the most effective method to prevent HB viral transmission, induces protective antibodies against HB surface antigen (anti-HBs). However, these antibodies decline in titer over time. Studies on the duration of protection and the prevalence of non-responders in Indonesian adolescents have been limited.

Objectives To determine anti-HBs titers in 15-17-year old Indonesian adolescents given primary HB vaccine during infancy and the prevalence of non-responders after a HB vaccine booster dosage.

Methods This cross-sectional study was performed from February to September 2008 on adolescents aged 15-17 years in three senior high schools in Jakarta who received complete primary HB vaccines during infancy, based on parents' recall. Investigations included HB vaccination history, anthropometric measurements, and blood tests for anti-HBs before and 4-6 weeks after a booster dose of HB vaccine.

Results Of 94 subjects, 35 had protective anti-HBs and 59 had undetectable anti-HBs. A booster dose was administered to 58 of the non-protected subjects, of which 33 showed anamnestic responses. However, 25 subjects failed to generate protective anti-HBs. Taking into consideration the adolescents with protective anti-HBs before and after the booster dose, serologic protection was demonstrated in 73%. Non-responder prevalence was 27%. The high prevalence of non-responders may indicate bias of parents' recall.

Conclusion Protective anti-HBs is detected in less than half of Indonesian adolescents given primary HB vaccine during infancy. Following booster dosage, anamnestic responses are noted in one-third of subjects. The prevalence of non-responders is 27%, but confirmation with further study is needed. **[Paediatr Indones. 2013;53:160-6.]**

Keywords: *adolescent, anti-HBs, booster, hepatitis B vaccine, immunity*

Hepatitis B virus (HBV) infection persists as a worldwide public health problem.[1] Two billion people worldwide have serologic evidence of past or present HBV infection, and 360 million are chronically infected and at risk for HBV-related liver disease.[2-4] Indonesia has been classified as a moderate-highly hepatitis B (HB) endemic country, with a hepatitis B surface antigen (HBsAg) seroprevalence of 2.5-36.1% (mean 9.4%) and a carrier rate of 5-10% in the general population.[5,6]

Hepatitis B vaccination is the most effective method to prevent HBV transmission and its consequences.[7,8] In Indonesia, HB vaccination was introduced in 1987 and became national program in 1997.[9] Three doses of HB vaccine induces protective antibody to HB surface antigen (anti-HBs) in more than 95% of healthy infants, children and young adults.[4,10] However, anti-HBs decline over time and the vaccine-related protective duration is unknown. Studies conducted at 10 to 19 years after a completed primary HB vaccine series showed that 15 - 97% of subjects vaccinated as infants had low titers or undetectable anti-HBs.[11] In addition, a small

From the Department of Child Health,[1] University of Indonesia Medical School, Cipto Mangunkusumo Hospital, Jakarta, and, University of Indonesia Medical School,[2] Jakarta, Indonesia.

Reprint requests to: Hartono Gunardi, Department of Child Health, University of Indonesia Medical School, Jalan Salemba Raya No. 6, Jakarta, Indonesia. E-mail: h_gunardi@yahoo.com

proportion of healthy individuals who had received complete HB vaccinations did not achieve protective (≥10 mIU/mL) anti-HBs levels.[12]

Non-immune adolescents are potentially at risk of acquiring HBV infection due to horizontal transmission, in particular those who engage in risk-taking behaviors, such as sexual activity with multiple partners, tattooing, or injection-drug use.[13] Studies on immunity against HBV in adolescence have been reported from a few countries. However, information from Indonesia, a country with endemic hepatitis B, is limited. Furthermore, data on primary HB vaccinations have not been well documented, as it is often based on the parents' memory recall. We carried out a preliminary study aimed to determine the protection status against hepatitis B on 15 to 17-year-old adolescents from three high schools who had received the primary HB vaccine series, based on their vaccination history, and their response to a booster dose of HB vaccine given to individuals who had non-protective anti-HBs levels.

Methods

This cross-sectional study was done in Jakarta between February and September 2008, in adolescents from three public senior high schools (SMA 21, SMA 68, and SMA 77). Students were selected by consecutive sampling and given brief questionnaires regarding their history of HB vaccination, as well as presence of immunocompromised conditions (such as immunodeficiency diseases or taking immunosuppressive medicines), and chronic infections. Students who had received complete primary HB vaccine series during infancy based on their parents' recall through written documentation of the vaccination were included. Adolescents who had received a booster dose of HB vaccine or had an immunocompromised condition were excluded. Primary HB vaccine was defined as three or four doses of HB vaccine with appropriate dosage and intervals given according to the Indonesian Pediatric Society or Ministry of Health Immunization schedule. Subjects underwent anthropometric measurements and blood tests for anti-HBs, which was measured by a commercial microparticle enzyme immunoassay/ MEIA (AxSYM, Abbott Laboratories, Chicago, IL, USA) according to the manufacturer's instructions. A protective anti-HBs response was defined as an anti-HBs titer of more than or equal to 10 mIU/ mL.[3] A booster dose of 10 μg of a recombinant DNA HB vaccine (*HB Vax® II*; MSD, USA) was given by intramuscular injection in a deltoid muscle to subjects whose anti-HBs titers were less than 10 mIU/mL. Another blood specimen was taken 4-6 weeks after the booster dose of HB vaccine. An anamnestic response was defined as an increase in anti-HBs level to ≥10 mIU/mL after a HB vaccine booster dose.[11] A non-responder was defined as an individual who received the primary HB vaccine during infancy, but did not develop a protective anti-HBs titer after the booster dose.[14] The study protocol was approved by the Committee for Medical Research Ethics, University of Indonesia Medical School. Parental informed consent was obtained for all subjects.

Data analysis was performed using SPSS software version 12.0 (SPSS, Chicago, IL). Data was expressed as mean (SD) or median and range, depending on the normality of the test results. Paired t-test was used to analyze the difference of anti-HBs titers before and after a booster dose of HB vaccine for data showing normal distribution, otherwise the data was analyzed using Wilcoxon test. Independent T-test and ANOVA were used to analyze the differences between sex, age, and nutritional status groups showing normal distributions, otherwise the data was analyzed using Mann-Whitney and Kruskal-Wallis tests. Results were considered to be statistically significant for P values less than 0.05.

Results

During the study period, 94 adolescents who had received the complete primary HB vaccine series during infancy were included in our study. Subjects were students from three public senior high schools in Jakarta (15 subjects of SMA 21, 34 subjects of SMA 68, and 45 subjects of SMA 77). The majority of subjects were female (62%). The mean age at the time of study was 15.7 (SD 0.6) years. Mean body weight was 55.5 (SD 12.8) kg and mean height was 160.1 (SD 8.1) cm. Sixty-eight (72%) subjects had normal nutritional status, 6 (6%) subjects were below the 5th percentile of the BMI-for-age curve of

CDC-NCHS 2000 growth charts [15] (underweight), 12 (13%) subjects were between 85th - 94th percentile (overweight), and 8 (9%) subjects were equal or above the 95th percentile (obese). No subjects had received booster doses of HB vaccine. None had evidence of chronic infection, immunodeficiency disease, or were taking any immunosuppressive medicine at the time of study recruitment.

Subjects' serological status against HBV prior to the study was unknown. Based on blood test for pre-booster anti-HBs titers, subjects were classified into one of four categories: (1) titer < 10 mIU/mL, (2) titer between 10 and 99 mIU/mL, (3) titer between 100 and 999 mIU/mL, or (4) titer ≥ 1000 mIU/mL (**Table 1**). Thirty-seven percent of subjects showed protective anti-HBs with a median of 93.7 mIU/mL (range: 12.0->1000.0), while 63% showed undetectable anti-HBs.

Of the 59 subjects with non-protective anti-HBs titers, 58 subjects received booster doses of HB vaccine. One subject preferred to receive the HB vaccine from her own doctor and could not be analyzed further. No serious adverse events to the booster dose were reported. Four to six weeks after the booster, there was significant seroconversion among vaccinated subjects (P=0.000) (**Figure 1**). Thirty-three subjects (57%) achieved seroconversion to protective anti-HBs titers with a median of 188.9 mIU/mL (range: 12.8-1,001.0), while 25 subjects (43%) failed to generate protective anti-HBs titers, hence, they were categorized as non-responders. Subjects were classified into one of four categories of post-booster anti-HBs titers, as they were for pre-booster anti-HBs titers (**Table 1**).

Subjects were divided into groups according to age, gender, and nutritional status categories to determine anti-HBs responses after the HB vaccine booster doses among groups. There were no significant differences in post-booster anti-HBs titers among the different categories of age, gender, or nutritional status groups (P=0.551, P=0.279, and P=0.469, respectively). The results of post-booster anti-HBs titers for all subjects subdivided by age, gender, and nutritional status categories are shown in **Table 2**.

Prior to booster administration, 37% of subjects (35/94) had protective anti-HBs titers; and 4-6 weeks after booster administration, 57% (33/58) had protective anti-HBs titers. Therefore, a total of 73% of subjects (68/94) had protective anti-HBs, including both pre-booster and post-booster responders. However, after administration of the HB vaccine booster dose, 43% (25/58) of subjects failed to generate protective anti-HBs titers. Overall, the prevalence of primary non-responders was 27% (25/94).

Table 1. Pre- and post-booster anti-HBs titers in adolescents who received primary HB vaccines during infancy

Anti-HBs titers	Pre-booster n = 94 n (%)	Post-booster* n=58 n (%)
< 10 mIU/mL	59 (63)	25 (43)
10 – 99 mIU/mL	20 (21)	14 (24)
100 – 999 mIU/mL	12 (13)	14 (24)
≥ 1000 mIU/mL	3 (3)	5 (9)

*One subject withdrew from the study

Table 2. Post-booster anti-HBs titers according to age, gender, and nutritional status

Variables	n	Post-booster anti-HBs titer		P value
		Median, mIU/mL (range)	95% CI for median	
Age, years				
15	24	36.6 (0.0-1,001.0)	0.0 to 195.0	
16	30	15.0 (0.0-1,001.0)	0.0 to 79.3	0.551
17	4	320.6 (0.0-1,001.0)	0.0 to 1,001.0	
Gender				
Males	21	42.0 (0.0-846.2)	6.9 to 283.4	0.279
Females	37	12.8 (0.0-1,001.0)	0.0 to 60.4	
Nutritional status				
Underweight	5	280.0 (0.0-748.9)	0.0 to 748.9	
Normoweight	45	19.7 (0.0-1,001.0)	0.2 to 54.0	0.469
Overweight	5	188.9 (0.0-498.8)	0.0 to 498.8	
Obese	3	0.6 (0.0-180.3)	0.0 to 180.3	

Anti-HBs = antibody against hepatitis B surface antigen; CI = confidence interval

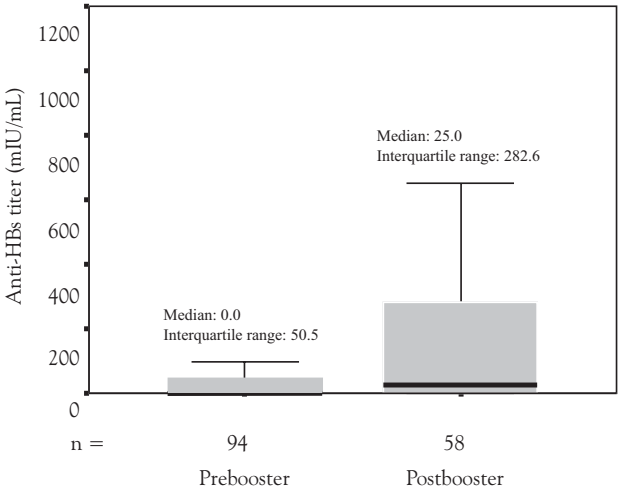

Figure 1. Anti-HBs titers in pre-booster and post-booster groups

Discussion

Hepatitis B vaccination programs are highly effective and have led to marked declines in chronic carrier rates and the incidence of hepatocellular carcinoma in moderate-to-highly HBV-endemic countries.[16] However, natural exposure to HBV continues in the general population. Without reliable long-term immunity, HBV infection may occur in adolescents at risk, such as by household contact with HBV carriers or due to risk-taking behaviors, especially tattoing or sexual relationships with multiple partners. The current priority is to ensure long-term protection of vaccinated adolescents who are at risk of HBV. To accomplish this, we assessed immunity to HBV in 15-17 year-old adolescents who had received complete primary HB vaccine series during infancy.

Protection against HBV infection was defined as the adequate presence of anti-HBs titer. This protection theoretically vanishes when anti-HBs concentrations fall below a value of 10 mIU/mL.[17] The duration of vaccine-induced protection in adolescents with complete primary HB vaccinations during infancy has an important implication on indications for booster vaccination. In a previous study of 10-12 year-old Indonesian children who had received complete primary HB vaccines in early infancy, protective anti-HBs titers were found in only 38% of the children.[18] Similarly, we found protective anti-HBs titers in 37% of our subjects. In addition, Lu *et al.* conducted a 15- to 18-year follow-up in Chinese vaccinated infants and found that 37% had protective anti-HBs titer in the long-term.[19] In a Thai study, Chinchai *et al.* reported a long-term follow-up in humoral immune parameters with 69.9% of subjects showing a protective anti-HBs titer after 18-20 years.[20] These various results indicate that policies for booster vaccinations should be based on epidemiological studies.

The possibility of waning immunity or eventual loss of the vaccine protectiveness should be investigated. If adequate protective anti-HBs titers are not detected a few years after the third dose of the vaccine, those with non-protective anti-HBs titers should be given a single HB vaccine booster dose, followed by anti-HBs titer testing 4 weeks later.[14] In our study, 59 subjects had undetectable anti-HBs. Of these, 58 subjects received booster doses of HB vaccine, of which 33 (57%) showed anamnestic responses at 4-6 weeks after administration of a booster doses. This result indicated that non-protective anti-HBs titers in most subjects were due to waning immunity.

A rapid increase in anti-HBs represented an anamnestic response and was considered to indicate the presence of HBsAg-specific immune memory.[11] Immune memory persists beyond the time at which anti-HBs titers may no longer be detectable, and protects against clinically apparent disease. In case of HBV exposure, the immune memory rapidly leads to a vigorous anamnestic response, which prevents acute infection, acute disease, prolonged viremia, and chronic infection.[21] The presence of HBsAg-specific memory after HB vaccination was suggested in a number of studies by epidemiological data showing the absence of disease in a vaccinated population and proven by demonstration of an anamnestic anti-HBs-response after revaccination.[21]

In our study, considering adolescents with protective anti-HBs titers and those who responded to the booster dose, protective anti-HBs responses were found in 73% of subjects. Samandari *et al.* reported that persistence of vaccine-induced immune memory among adolescents who had received primary HB vaccines 10-14 years earlier was demonstrated by an anamnestic increase in anti-HBs titers in 50-100% of these adolescents, 2-4 weeks after administration of HB vaccine booster doses.[11] The mechanism for

continued vaccine-induced protection is thought to be the preservation of immune memory through selective expansion and differentiation of clones of antigen-specific B and T lymphocytes. Bauer *et al*. suggested that individuals who had lost their protective anti-HBs still showed immunologic T cell memory and that these T cells were able to trigger anti-HBs production by B cells activated by revaccination. [21] This data indicated that a high proportion of vaccine recipients retained immune memory and would develop anti-HBs responses upon exposure to HBV.[8-]

In our study, we found unexpectedly high rates of undetectable anti-HBs in adolescents who had received 3 doses of HB vaccine as infants, 15 to 17 years prior. Forty-three percent of subjects with anti-HBs titers less than 10 mIU/mL did not respond to a booster dose of HB vaccine. These non-responders carry the greatest risk of getting HB infection. It was reported that about 2-15% of healthy vaccine recipients worldwide failed to produce protective anti-HBs titers after receiving a primary dose of HB vaccine.[22,23] The higher proportion of non-responders in this study may have been caused by uncertainty of the primary vaccine administration in the subjects, since no written documentation of vaccinations was obtained. Parents' memory of HB vaccinations might have been confused with other vaccination series. This recall bias may have led to overestimation of the prevalence of primary non-responders. The other cause that may play a role was HBV infection. HB surface antigen (HBsAg) also needed to be examined in non-responders to exclude definitely the possibility of HB virus infection. HB infection could not completely be rule out by history taking only.

Revaccination was recommended for those individuals whose post-booster HB vaccination anti-HBs titers were less than 10 mIU/ml. Those individuals should be given 2 further doses of HB vaccines at monthly intervals and should be re-tested for anti-HBs titers at least 4 weeks after each dose. Individuals who do not respond after two series (six doses) of HB vaccine should be regarded as true non-responders.[14] Non-responsiveness status is related to genetic factors, such as human leukocyte antigen (HLA).[24] HLA class II genotype, especially DRB1* alleles 3 and 7 is associated with suboptimal antibody response to full-dose HB vaccination.[24-26] This suboptimal antibody response was not caused by differences in peptide binding or by a shift in the Th1/Th2 profile but it is rather due to differences in the T cell recognition of peptide/MHC complexes as the critical event in T cell responsiveness to HBsAg.[27]

To our knowledge, this is the first study to explore long-term immunity of HB vaccinations in Indonesian adolescents. As with other health data, vaccination data was rarely kept by parents through the adolescent period, so parents' memory recall was used as a source of vaccination data. Analyses of vaccination coverage with recall and written vaccination data, have shown that recall may be used to estimate vaccination coverage in a population. Recall and written vaccination data were correlated (r= .71), but the magnitude of difference may have affected the identification of vaccination status of an individual child. Mothers tended to underestimate the number of doses actually received in older children.[28] In another study of 13-17 year-old adolescents, parent-reported vaccinations yielded 33% false positives for hepatitis B vaccinations in the recall only group.[29] The validity of a parent's recall depended upon the vaccine, and it decreased with increasing age of the child at vaccination and with an increasing number of vaccines that the parent had to remember.[30] Thus, to have more accurate results, written documentation of vaccination is needed to evaluate long-term HB vaccine-induced protective immunity.

In conclusion, we performed a preliminary study of HB immunity in adolescents who had received complete primary HB vaccines during infancy based on parents' recall. We detected protective anti-HBs titers in less than half of our subjects, as well as anamnestic response in one-third of subjects, and a 27% prevalence of non-responder adolescents after a booster dose of HB vaccine. Further study is needed to determine accurate HB vaccine-induced protection and non-responder prevalence with a larger sample size and written documentation of infant vaccinations.

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