original article

Persistence of hepatitis B surface antibody and immune memory to hepatitis B vaccine among medical college students in Madinah

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BACKGROUND: Globally, about 300 million people are infected with hepatitis B virus (HBV). Among the effective approaches to fight HBV infection is immunization. In 1989, an obligatory hepatitis B vaccine program was launched in Saudi Arabia.

OBJECTIVE: Assess hepatitis B surface antibody (anti-HBs) levels among the medical students before and after receiving booster doses of HBV vaccine.

DESIGN: Cross-sectional.

SETTING: Taibah University.

SUBJECTS AND METHODS: Students born between 1993 and 1995 were recruited in this study from the Occupational Health Clinic. Students were screened for anti-HBs levels using chemiluminescent microparticle immunoassay (CMIA) before and after booster HBV vaccine doses.

MAIN OUTCOME MEASURES: Anti-HBs levels before and after booster doses.

SAMPLE SIZE: 335.

RESULTS: About half of participants (n=164, 49%) had protective anti-HBs levels (≥10 mIU/mL) to the original primary series of HBV vaccine and received no booster doses. In contrast, 171 (51%) participants were at risk of HBV infection since their anti-HBs levels were <10 mIU/mL, despite having received the original primary HBV vaccine series. The levels of anti-HBs were higher in female than in male students (P<.001). In addition, female students showed a stronger humoral immune response to the booster vaccine than male students (P<.001). When participants were given the three boosters, most participants (98.3%) showed anti-HBs levels of ≥10 mIU/mL. The results also showed a strong correlation between pre-booster and post-booster anti-HBs levels in the ≥10 mIU/mL group (r^2=0.52, P<.001) but not in <10 mIU/mL group (r^2= 0.003, P=.53).

CONCLUSION: A considerable portion of the participants (about 51%) were at risk of HBV infection since their anti-HBs levels were <10 mIU/mL. Booster doses significantly trigger memory immune response and this ensured their protection against the virus. Pre-booster anti-HBs level are a good predictive of post-booster anti-HBs levels in ≥10 mIU/mL group.

LIMITATIONS: The sample size was small. Shortage of collaborators.

CONFLICT OF INTEREST: None.

Hepatitis B virus (HBV) is a small DNA virus that belongs to the Hepadnaviridae family.[1] The replication cycle of HBV involves an RNA intermediate that can incorporate into the host genome and subsequently persist in infected cells.[2,3] Accurate diagnosis of HBV infection is based on a group of clinical, biochemical, histological, and serologic results.[4] Several viral antigens and their corresponding antibodies can be detected in blood after infection with HBV.[5] For example, a positive hepatitis B surface antigen means an active (acute or chronic) HBV infection.[6] The presence of hepatitis B e-antigen (HBeAg) indicates a high-level of HBV replication and infectivity.[7] These markers are also used to evaluate the responses of patients to HBV therapeutic agents.[8] Moreover, antibody to HBsAg (hepatitis B surface antibody, anti-HBs) is a marker of immunity.[6] The positivity of this marker indicates an immune response to HBV infection and immune response to vaccination. In the latter case, the titer can be measured to evaluate vaccine effectiveness.[9]

HBV is considered a global health problem that accounts for about one million deaths annually as it is linked to the development of liver cirrhosis and hepatocellular carcinoma.[10,11] A substantial decrease in the number of infections caused by HBV is attributed to HBV vaccination programs for infants, children, adolescents and at high-risk populations, and to the advances in the screening of blood products and safe injection techniques.[12,13]

Saudi Arabia has witnessed a significant decrease in the last two decades in the prevalence of HBV due to the implementation of the childhood vaccination program against the virus,[14] which was launched in 1989.[15] One year later, a catch-up vaccination program supplemented for children at school entry, healthcare workers, and other high-risk groups was implemented. Therefore, almost all Saudis aged 24 years or younger had been vaccinated either at birth, or at school entry.[16,17]

The effectiveness of vaccination programs in inducing anti-HBs has been evaluated in many countries.[18-22] In a significant portion of the populations, anti-HBs levels were not high enough to guarantee protection against HBV. Therefore, in the current study, anti-HBs levels in the plasma of medical college students in Almadinah city, Saudi Arabia were evaluated. In addition, levels of anti-HBs were measured after the students had received booster doses.

SUBJECTS AND METHODS

Subjects

Students from medical colleges at Taibah University were invited to participate in this study at the Occupational Health Clinic at Medical Center at the Taibah University in Almadinah city. All subjects were born between 1993 and 1995 and had completed the original primary series of HBV vaccine. Students were initially screened for plasma levels of anti-HBs as a requirement before starting the obligatory one-year internship. The students were then invited to receive the booster vaccine for HBV. Students who agreed to complete the study were asked to present certificates for having received booster dose/doses. The students who did not provide the certificate were excluded from the post-booster analysis. The students were given three boosters (0, 1 and 3 months, dose 10 µg recombinant vaccine) and plasma samples were obtained 4 weeks after each booster. Informed consent was obtained from all students after full explanation of the study objectives and procedures. In addition, the study was approved by the ethics committee of Taibah University. The study was conducted between 2015 and 2017.

Chemiluminescence assay

About 5 mL of venous blood was obtained from each participant in EDTA tubes. Samples were centrifuged at 3000 xg for plasma separation. Plasma samples were stored at –20° C until used. The ARCHITECT HBsAg assay was used in the current study for the quantitative determination of hepatitis anti-HBs in human plasma samples. The assay is based on chemiluminescent microparticle immunoassay technology. The assay kit was obtained from Abbott (Wiesbaden, Germany). The overall specificity of the assay was estimated to be 99.67%. The overall sensitivity was estimated to be 97.54%. The assay was performed according to the manufacturer manual using the ARCHITECT i1000SR System (Abbott Park, IL, USA). If the concentration of the specimen was greater than or equal to 10 mIU/mL, the specimen was considered reactive for anti-HBs; if it is less than 10 mIU/mL it was considered non-reactive. All tests were performed at Ministry of Health Regional Lab, King Fahad Hospital, Almadinah, Saudi Arabia.

Statistical analysis

All the calculations and statistical analysis were performed using GraphPad Prism statistical software (version 5, USA). Data were expressed as mean and standard deviation (SD). Two-group comparisons were performed using the Mann-Whitney test. Analysis that involved three groups or more was performed using ANOVA followed by the Tukey post hoc test.

The correlation between pre- and post-booster antibody levels was determined by the Spearman's rank correlation test. A $P<.05$ was considered significant for all tests.

RESULTS

Enrollment totaled 335 students (**Table 1**). The mean (SD) age in years was 22.8 (0.9). About 26% of the sample were males. In addition, all participants were Saudi, from the Almadinah area and received the primary HBV vaccine during infancy. Finally, the time since vaccination was 21.3 (0.97) years. The final number of students who completed the whole study was 295 (88% of the total). To verify the effectiveness of HBV vaccine to induce a humoral immune response, all students were tested for the detection of anti-HBs in plasma. About 51% of the sample had anti-HBs plasma levels of < 10 mIU/mL (**Table 2**). In addition, a significant difference in the distribution of participants according to anti-HBs levels (<10 mIU/mL versus ≥10 mIU/mL) was observed between male and female students (**Figure 1**, $P<.001$). When participants were given the three boosters, most participants (98.3%) showed anti-HBs levels of ≥10 mIU/mL. However, levels of anti-HBs were higher in female than male students ($P<.001$), indicating a stronger humoral immune response to the booster vaccine in females.

About 97.3% of the participants had anti-HBs levels of ≥10 mIU/mL after the first booster (**Table 3**), indicating that almost all of the participants acquired immunity against HBV directly after the first booster. Significant differences ($P<.01$) in the mean levels of anti-HBs suggested a stronger response after each booster (**Figure 2**). Finally, the correlation between pre-booster and post-booster anti-HBs levels in the <10 mIU/mL and ≥10 mIU/mL groups showed a strong correlation between pre-booster and post-booster anti-HBs levels in ≥10 mIU/mL group ($r^2= 0.52$, $P<.001$, **Figure 3B**) but not in <10 mIU/mL group ($r^2=0.003$, $P=.53$, **Figure 3A**). Thus, pre-booster anti-HBs seems to be -predictive of post-booster anti-HBs levels in the ≥10 mIU/mL group.

DISCUSSION

In the current study, anti-HBs levels among the medical

Table 1. Demographics of participants.

Parameter	
Age (mean and standard deviation), years	22.82 (0.85)
Gender	
Male	90 (26.1)
Female	245 (73.1)
Ethnicity (n, %)	
Saudi	335 (100)
Others	0 (0)
Residency (n, %)	
Al Madinah area	335 (100)
Others	0 (0)
Age at HBV vaccination	
In infancy (0-3 years)	335 (100)
Other ages	0 (0)
Time since vaccinations (mean and standard deviation), years	21.3 (0.97)

Table 2. Frequency of protective levels of anti-HBs and levels of anti-HBs before and after receiving the third booster.

	Pre-booster		Post-booster	
	<10 mIU/mL	≥10 mIU/mL	<10 mIU/mL>	≥10 mIU/mL
Anti-HBs status				
Total	171 (51.0)	164 (49.0)	5 (1.7)	290 (98.3)
Males	62 (68.9)[a]	28 (31.1)	2 (2.5)	79 (97.5)
Females	109 (44.5)	136 (55.5)	3 (1.4)	211 (98.6)
Anti-HBs concentration (mIU/mL)				
Total	6.39 (2.0)	236.4 (222)	4.2 (2.3)	427.4 (314)
Males	6.24 (1.9)	244.5 (208)	3.0 (1.4)	260.5 (243)[b]
Females	6.47 (2.1)	234.7 (225)	5.0 (1.5)	490.6 (315)

Values are n (%) or mean (SD) mIU/mL. [a]Significant difference from females, $P<.05$. [b]Significant difference from females, $P<.001$

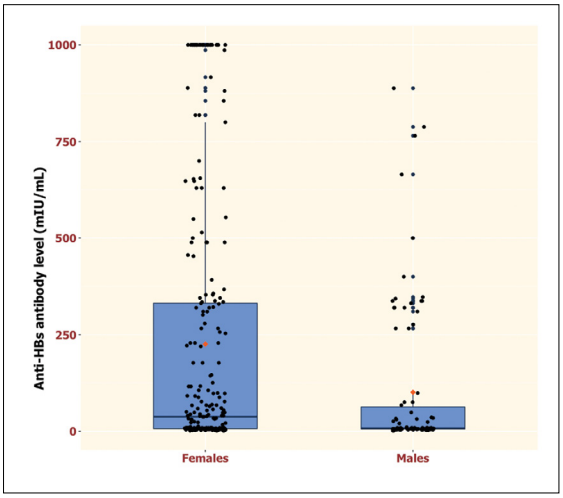

Figure 1. Anti-HBs concentrations by gender before the booster doses (n=245 females, n=90 males) (*P*<.001) (median, first and third quartiles, orange diamond: mean).

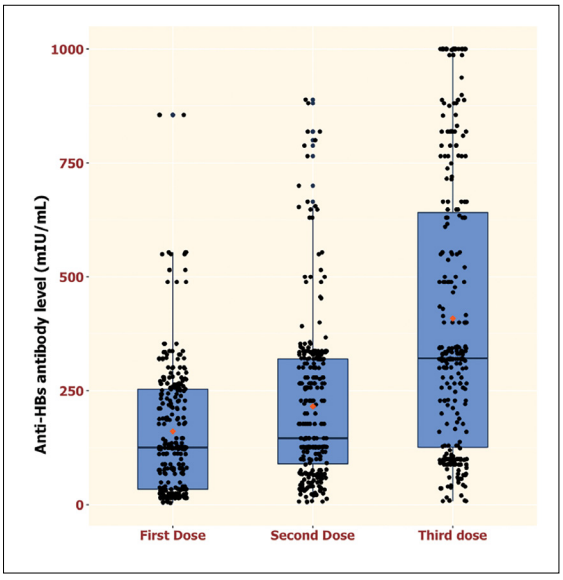

Figure 2. Dose-dependent immune responses of participants after additional doses of hepatitis B vaccine (*P*<.001, significantly higher after three doses) (median, first and third quartiles, orange diamond: mean).

students at Almadinah city, Saudi Arabia were examined. The results showed that about half of the students (51%) had a low protection level of anti-HBs (<10 mIU/mL), despite receiving the original primary HBV vaccine series. In addition, a spectrum of humoral immune response to HBV vaccine was also observed. A previous study from Egypt showed that about 40% of school children who received primary HBV vaccine series were not protected.[23] Similar percentages were reported in previous studies from countries such as Taiwan,[19,24] Europe,[25-27] and the United States.[18,28] However, 25% of medical students were not responsive in studies conducted in Italy.[20,21] The high percentage of students with anti-HBs <10 mIU/mL could be because they responded to the vaccine, but the levels of the anti-HBs declined with time.[29] This result is consistent with studies that showed decreased antibody level with increasing age.[23,29,30] In addition, our results are consistent with several studies that concluded that continuing protection against HBV infection depends on immunological memory, which seems to last 10-15 years in immunocompetent individuals who respond adequately to the primary vaccination.[31-33] Therefore, the scientific community and health authorities in Saudi Arabia should be urged to collaborate to do a larger long-term study similar to that by Bruce et al, 2016, which followed subjects for a period of thirty years.[34] This is important to assess the suitability of the current vaccine for the Saudi population.

About 98% of the students had an anamnestic response after they have received three boosters of the vaccine. Thus, 2% of the population seems to not respond to the current vaccine and may be at risk of developing HBV. Similar post-booster outcomes have been reported in other populations.[19,23,26] There was also a strong correlation between pre-booster anti-HBs level and post-booster anti-HBs status in the group with pre-booster anti-HBs levels of ≥10mIU/mL, but not in the non-responder group (<10mIU/mL). This indicates that pre-booster anti-HBs level can be used as a predictive factor for positive post-booster anti-HBs status.

Table 3. Frequency of protective levels of anti-HBs and levels of anti-HBs before and after the first, second and third booster

	Pre-booster (n=335)	First dose (n=295)	Second dose (n=295)	Third dose (n=295)
<10 mIU/mL	171 (51.0)	8 (2.7)	5 (1.7)	5 (1.7)
>10 mIU/mL	164 (49.0)	217 (97.3)[a]	290 (98.3)	290 (98.3)
Anti-HBs concentration (mIU/mL)	119 (193)[b]	138.9 (127.8)	289.4 (211.8)	516.2 (337.2)

Values are n (%) or mean (SD). [a]Indicates significant difference from prebooster group, *P*<.001; [b]Indicates significant difference between all groups, *P*<.01.

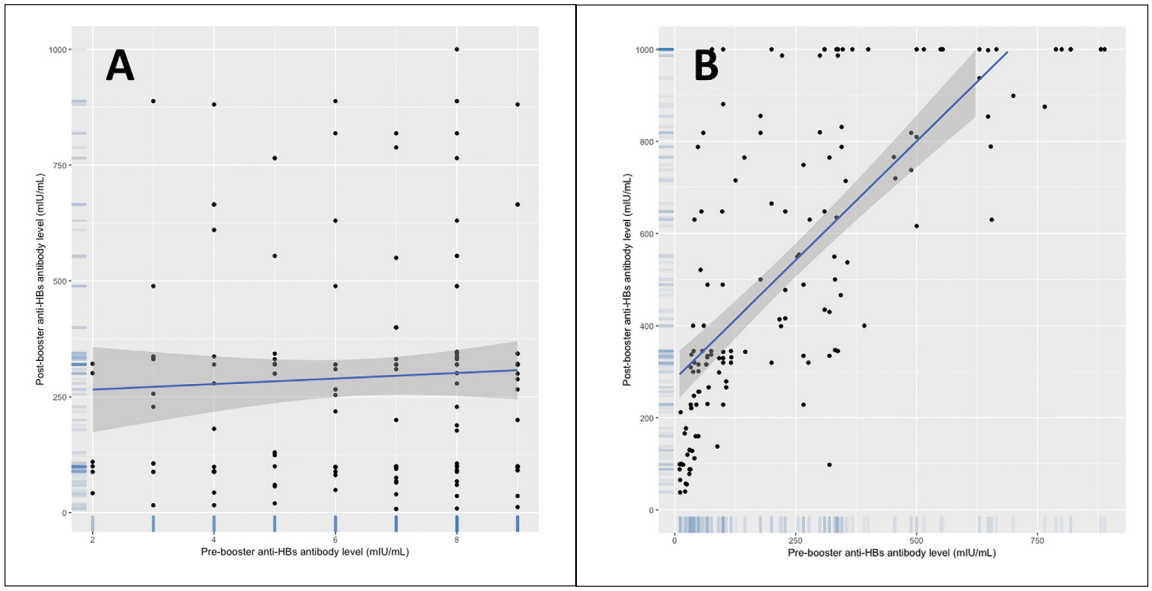

Figure 3. Correlation between pre- and post-booster anti-HBs levels for (A) < 10 mIU/mL group (r^2=.003, n=148, *P*=.53) and (B) ≥10 mIU/mL group (r^2 = 0.52, n=147, *P*<.001).

This finding is consistent with a studies conducted in Taiwan,[35] Egypt[23] and the United States.[28]

Females responded more strongly than male students, which is consistent with other studies that showed the same trend in terms of lower anti-HBs antibody titers in males compared with females.[36,37] Gender differences in the response to HBV vaccine were reported by some previous studies.[19,23] Similar findings were also reported in animal models.[38] Thus, in addition to pre-booster anti-HBs, female gender is another predictive factor for post-booster anti-HBs status. It has been postulated that gender differences might be due to the opposite effects of sex hormone androgen and estrogen.[36]

The inevitability of booster vaccination for HBV has been a subject of considerable argument as a decline in serum anti-HBs level essentially indicates a reduced protection and the need for a booster dose of the vaccine.[39,40] According to WHO recommendations, booster immunization for HBV is not recommended and the protection lasts at least 20 years, possibly lifelong. However, several studies, including the present one, highlight the importance of booster doses to trigger the memory immune system and maintain a higher protective level of anti-HBs.[41-43] Boosters will elicit an immune memory and offer reassurance of protective immunity against breakthrough infection. For immunocompromised patients, consistent testing for anti- HBs and a booster injection when the titer decreases below 10 mIU/mL are recommended.[44-48]

In conclusion, a considerable portion of medical students (about 51%) were at high risk of HBV infection as their anti-HBs levels were <10 mIU/mL. Booster doses significantly triggered memory immune response, which ensured protection against the virus. Finally, pre-booster anti-HBs seems to be predictive of post-booster anti-HBs levels in subjects with ≥10 mIU/mL anti-HBs.

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