Sero protection of Hepatitis B Vaccine in Dental Students Two Decades after Infant Immunization and the Possible Need for Revaccination

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Abstract

Objectives This study aimed to assess hepatitis B surface antibody (anti-HBs) persistence among dental students two decades after infant vaccination and immune response after revaccination or booster dose in nonimmune groups.

Materials and Methods A cross-sectional study was performed using laboratory reports for anti-HBs performed to dental students at the Umm al-Qura University from 2016 to 2020. Reports were classified according to baseline antibody titer into group I (titer < 10 mIU/mL), group II (titer 10 to < 100 mIU/mL), and group III (titer ≥100 mIU/mL). The basal antibody titer of each group was correlated to student’s gender and birth year and compared with postrevaccination or booster dose titer in groups I and II.

Statistical Analysis Data of baseline and postrevaccination anti-HBs antibody titers were analyzed using Statistical Package for Social Science (SPSS). One-way ANOVA was used to compare between different means for antibody titers of students as well as baseline and postrevaccination antibody levels for nonimmune students with nonprotective antibody titers. Pairwise multiple comparison of the difference between baseline and postrevaccination anti-HBs antibody titers were done by post hoc Tukey’s honestly significant difference (HSD) test. Chi-squared test was used for comparing between the frequencies. A p-Value of ≤0.05 was considered significant and p < 0.01 was considered highly significant.

Results A significant percentage (73.6%) of students had antibody titer < 10 mIU/mL, while only 4.8% had antibody titer ≥100 mIU/mL with nonsignificant difference between both genders (χ² = 3.784, p = 0.151). A statistically nonsignificant difference was also found between the three categories of anti-HBs antibody titers among students with different birth dates (χ² = 13.817, p = 0.182). After revaccination of nonimmune students, 100% of them showed strongly protective antibody titers with mean of 842.88 to 844.58 mIU/mL. A highly significant difference was observed...
between the mean baseline and postrevaccination antibody titers in both genders, with a higher mean of post revaccination ($p = 0.000$).

**Conclusion** Two decades after infant immunization, a significant percentage of dental students failed to maintain the anti-HBs seroprotective titer. So titer measurement should be made compulsory before they begin their clinical training and revaccination or booster dose should be given to nonimmune students to maintain a high protection level.

**Introduction**

Hepatitis B is a major public health problem all over the world. Health care professionals (HCP), whether they live in an endemic region or not, are considered high-risk groups to this disease. Consequently, dental professionals constitute one of the most vulnerable HCP to infection by such diseases due to their direct contact with blood and saliva as well as their high rates of contact with contaminated sharp equipment. In the 1970s, a vaccine against hepatitis B virus (HBV) was developed and it has been widely distributed since 1986. It is considered the main preventive method against hepatitis B and is reported to be highly reliable, accurate, and safe. Among dental practitioners, HBV vaccination is not only a self-care practice but also the most effective measure to protect patients’ health, reducing the incidence of the disease in their communities.

A hepatitis B vaccine program was started in Saudi Arabia in 1989. Since then, Saudi Arabia implemented a national strategy for the elimination of HBV infection by applying the universal administration of HBV vaccine to all infants. Furthermore, Saudi government conditioned the issuance of birth certificates upon completion of the first-year vaccination program to confirm the maximum coverage of vaccination against HBV. The vaccination regimen established include administration of the first dose of vaccine at birth, the second at 1 month of age, and the third at 6 months of age.

Implementation of infant vaccination program in Saudi Arabia resulted in significant improvements in the past 30 years in terms of decrease in HBV prevalence from 6.7% to be around 1.3%. Regarding different age groups, in spite of the low HBV infection rates in the younger Saudi populations, its prevalence in older people has not decreased, which emphasizes the effectiveness of infant vaccination programs.

Although the effectiveness of the hepatitis B vaccination is evident, the duration of protection after vaccination is not exactly known in addition to the fact that some people remain nonresponders to vaccine and do not develop adequate anti hepatitis B surface antigen titer (anti-HBs) essential for protection against HBV.

As determination of anti-HBs response after completion of infant vaccination is not in practice in Saudi Arabia, the aim of this study was to assess the seroprotection of hepatitis B vaccine in dental students at the Umm Al-Qura University two decades after infant immunization and to determine the immune response after adult revaccination among students with nonprotective antibody level.

**Materials and Methods**

A cross-sectional study was conducted on the laboratory reports of dental students’ anti-HBs antibody levels. Data were collected from the database of the Infection Control Unit, Faculty of Dentistry, Umm Al-Qura University, including student’s gender, birth year, and baseline anti-HBs antibody levels as well as postrevaccination levels for students who have a nonprotective baseline level. The reports included were for students of preclinical training who received a full course of infant vaccination for hepatitis B and did not receive a booster dose the following years. Laboratory reports of students whose infant immunization records were not available or those with immunocompromised disease were excluded from the study. Reports that were performed in laboratories other than the Umm Al-Qura university medical center or showing nonquantitative antibody levels were also excluded. Measurement of HB antibody levels at the university medical center was performed by the ELIZA technique using the VITROS Anti-HBs Quantitative Reagent Pack and the VITROS Anti-HBs Calibrators on VITROS ECi/ECiQ Immunodiagnostic Systems analyzer (Ortho-Clinical Diagnostics, Inc., Raritan, NJ, United States).

**Sampling Technique and Sample Size Calculation**

Sampling was done by the convenience sampling technique. The sample size was calculated using the online calculator at https://www.calculator.net/sample-size-calculator.html. The total population size was 35, using a 95% confidence interval, marginal error of 5%, and population proportion of 50%; the calculated sample size was 186.

**Data Collection and Management**

Data from laboratory reports available (from 2016 to 2020) were collected not showing any nominative information, identified by serial study code and initials. Two different persons performed data entry; data were transferred to statistical database directly after verification.

**Study Procedure**

Students’ reports were classified according to the baseline antibody level into three groups; group I: reports of students with nonreactive antibody titer (<10 mIU/mL); group II: reports of students with reactive low antibody titer (10 to
<100 mIU/mL) and relatively immune; and group III: reports of students with appropriate antibody titer (≥100 mIU/mL). The antibody level of each group was correlated to student’s gender and birth year. Then, antibody levels after receiving one booster dose for group II or postrevaccination for group I students were compared with their basal antibody titer (measured 6–8 weeks either after the booster dose or the 3rd dose).

Statistical Analysis Plan
Data of baseline and postrevaccination anti-HBs antibody titers were analyzed using Statistical Package for social Science (SPSS) software version 20. One-way analysis of variance (ANOVA) was used to compare between different means for antibody titers of students as well as baseline and postrevaccination antibody levels for nonimmune students with nonprotective antibody titers. Pairwise multiple comparison of the difference between the baseline and postrevaccination anti-HBs antibody titers by post hoc Tukey’s HSD test was. Chi-squared test was used for comparing between the frequencies. A p-value of ≤0.05 was considered significant and p < 0.01 was considered highly significant.

Ethical Part and Confidentiality
The study received ethical approval from the institutional review board of the Umm Al-Qura University (IRB approval no. HAPO-02-K-012–2021–04–648).

Results
Demographic Characteristics of Participants
A total of 292 laboratory reports fulfilling the selection criteria were included in the study. Female students were the majority (197; 67.5%). The least number/percentage of reports (24; 8.2%) included in the study were for students born in 1995 and the highest number/percentage (63; 21.6%) was for students born in 1999. With regard to gender, most of the male students (26) were born in 1999, and most females were born in 1998 (43). The number of reports for females were higher than those for males every year except the year 2000 (→ Fig. 1).

Categorization of Laboratory Reports according to Baseline Anti-HBs Antibody Titers in Relation to Gender
The available reports included data of 197 (67.5%) females and 95 (32.5%) males. The majority of students (73.6%) had baseline antibody titer less than 10 mIU/mL, while only 4.8% of them had antibody titer of 100 mIU/mL or more with a nonsignificant difference between both genders ($\chi^2 = 3.784$, $p = 0.151$; → Table 1).

Categorization of Laboratory Reports according to Baseline Anti-HBs Antibody Titers in Relation to Birth Year
The highest percentages of students with nonprotective anti-HBs antibody titers (<10 mIU/mL) were those born in 1998 and 2000 (75.5 and 75.6%, respectively). For those with protective antibody titers (≥100 mIU/mL), the highest percentage (11.1%) was among students born in 1996. A statistically nonsignificant difference was found between the three categories of anti-HBs antibody titers among students with different birth years ($\chi^2 = 13.817$, $p$-value = 0.182; → Fig. 2).

Anti-HBs Antibody Titers before and after Revaccination/Booster Dose of Nonimmune Students
The anti-HBs antibody titers mean was increased after revaccination in both male and female students where it increased in males from 10.08 to 844.58 mIU/mL and in females from 20.49 to 842.88 mIU/mL. Statistically, a highly significant difference was observed between mean baseline and postrevaccination antibody titers in both genders ($p = 0.000$; → Table 2).

| Gender | Baseline anti-HBs antibody titer (mIU/mL) | Total No. (%) |
|--------|------------------------------------------|---------------|
|        | >10 No. (%)                              | 10 to ≥100 No. (%) | ≥100 No. (%) | Total No. (%) |
| Males  | 76 (80%)                                 | 17 (17.9%)     | 2 (2.1%)     | 95 (32.5%)    |
| Females| 139 (70.6%)                              | 46 (23.4%)     | 12 (6.1%)    | 197 (67.5%)   |
| Total  | 215 (73.6%)                              | 63 (21.6%)     | 14 (4.8%)    | 292 (100%)    |

$\chi^2$ (p-value) = 3.784 (0.151)

Abbreviations: No., number of subjects.
Note: p-Value is significant at ≤0.05.
Multiple Comparison of the Difference between Baseline and Postrevaccination Anti-HBs Antibody Titers

A comparison of the differences between the mean values revealed that the mean baseline anti-HBs antibody titer was higher in female students than in male students, while the mean postrevaccination titer was higher in male students with a nonsignificant difference between both genders in either situation ($p = 0.977$ and $1.000$, respectively).

On the other hand, the mean postrevaccination anti-HBs antibody titers in both males and females was higher than the mean baseline titers with a highly significant difference between the means in both genders ($p = 0.000$; Table 3).

**Discussion**

HBV infection is still a significant public health burden in Saudi Arabia despite being the first country in the Middle East to adopt the HBV vaccine and achieving significant progress in prevention of the virus. Previous studies concluded that transmission of HBV in Saudi Arabia is mainly through blood and its derivatives, hemodialysis, or by intravenous or percutaneous means. Percutaneous injuries by needle sticks constitute a significant occupational hazard among dental professionals and students. Thus, their vaccination and maintenance of adequate antibody levels is of utmost importance. Anti-HBs antibody is the serological marker measured to assess immunity induced by the HBV vaccine. However, determination of the anti-HBs antibody titer is not in practice after completion of vaccination.

The aim of this study was to assess the persistence of anti-HBs antibody after two decades of infant vaccination among dental students and determine the immune response after revaccination of nonimmune participants.

Vaccination in Saudi Arabia was introduced in 1989 for all infants at birth, and in 1990 for all school children. So laboratory reports for anti-HBs antibodies investigated in the

**Table 2** Means and standard deviations of baseline and postrevaccination/booster dose anti-HBs antibody titers among nonimmune students

| Variable                                            | Gender          | Males (n=95) | Females (n=127) |           |           |           |
|------------------------------------------------------|-----------------|--------------|-----------------|-----------|-----------|-----------|
|                                                      | Males (n=95)    | Females (n=127) |                 |           |           |           |
|                                                      | Min | Max | SD | Mean | Min | Max | SD | Mean | Min | Max | SD |
| Baseline anti-HBs antibody titer                     |     |     |    |      |     |     |    |      |     |     |    |
| Postvaccination/booster dose anti-HBs antibody titer | 10.08 29.55 0.00 257.00 | 20.49 53.14 0.00 541.46 |
| $F$ ($p$-Value)                                      | 800.707 (0.000)* |     |     |    |      |     |     |    |      |     |     |    |

Abbreviations: Max, maximum; Min, minimum; no, subjects’ numbers; SD, standard deviation.

*p $< 0.01$ highly significant.

Note: $p$-Value is significant at $p < 0.05$.

**Table 3** Pairwise comparison of the difference between baseline and postrevaccination anti-HBs antibody titers by Tukey’s HSD test

| Comparison groups                                      | Mean difference | $p$-Value |
|--------------------------------------------------------|-----------------|-----------|
| Mean male baseline versus mean female baseline titers   | $-10.40850$     | 0.977     |
| Mean male baseline versus mean male postrevaccination titers | $-834.49640$ | 0.000*    |
| Mean female baseline versus mean female postrevaccination titers | $-822.38991$ | 0.000*    |
| Mean male postrevaccination versus mean female postrevaccination | $1.69799$       | 1.000     |

*p $< 0.01$ highly significant.

Note: $p$-Value is significant at $p < 0.05$. 

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**Fig. 2** Baseline anti-HBs antibody levels according to birth year.

**Table 2** Means and standard deviations of baseline and postrevaccination/booster dose anti-HBs antibody titers among nonimmune students

| Variable                                            | Gender          | Males (n=95) | Females (n=127) |           |           |           |
|------------------------------------------------------|-----------------|--------------|-----------------|-----------|-----------|-----------|
|                                                      | Males (n=95)    | Females (n=127) |                 |           |           |           |
|                                                      | Min | Max | SD | Mean | Min | Max | SD | Mean | Min | Max | SD |
| Baseline anti-HBs antibody titer                     |     |     |    |      |     |     |    |      |     |     |    |
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Seroprotection of Hepatitis B Vaccine Two Decades after Infant Immunization

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As there is no routine assessment of vaccine response by measuring the anti-HBs antibody titers shortly after infant vaccination, we do not know whether the students recruited with nonprotective antibody titer had previously developed antibody responses or whether they were nonresponders to the vaccine. A considerable number of these students had zero levels of anti-HBs antibodies.

In the present study, the total percentage of students showed protective antibody titers whether with low immunity titer of 10 to <100 mIU/mL or high immunity titer of ≥100 mIU/mL, support the previous assumption that the persistence of protective levels of anti-HBs antibody could happen due to adequate immune response to infant vaccination.19

The nonsignificant difference between the baseline anti-HBs antibody titers in students with different birth years observed in the present study could be due to closely similar number of students in different years. There were nonsignificant differences in the anti-HB antibody levels between both genders, supporting the previous reports that concluded that gender has no influence on seroconversion after HBV vaccination.24 However, higher percentages of males having nonprotective antibody titer observed could be explained by the previous finding that suggested the role of gender in the nonresponse to HBV vaccine among Asians. This was attributed to few immunological genes expressed on male Y chromosome in contrast to numerous genes on female X chromosomes that play a fundamental role in immune competence, accounting for vaccine response heterogeneity in both genders. But the authors recommend further studies to confirm this issue.26,27

The protocol established by the infection control unit at the Umm Al-Qura university dental teaching hospital obligates students with anti-HBs antibodies titers <10 mIU/mL to be revaccinated with three doses of the HBV vaccine in accordance with the guidelines of the Centers for Disease Control and Prevention (CDC).28 Furthermore, students with anti-HBs antibody titers of 10 to <100 mIU/mL, being a high-risk group of health care workers, are advised one booster dose of HBV vaccine according to the United Kingdom and Germany policy for health care workers.29 These policies consider an anti-HBs titer ≥100 mIU/mL a successful vaccination.

Highly significant differences were observed between the mean baseline and postrevaccination antibody titers in both genders in the present study with higher levels reported postrevaccination. The increased antibody titer after revaccination is immunologically explained by the previous existence of humoral immune responses induced by infant vaccination. This response is generated as part of adaptive immune defenses to foreign antigens. After initial exposure to the HB antigen, B lymphocytes are activated and differentiate into plasma cells and memory B cells. Plasma cells produce antibodies specific to the antigen for clearance, and memory B cells remain for faster antibody production with future exposures to the antigen (e.g., with revaccination).16

To accomplish long-term immunity after infant vaccination in dental students, being a high-risk group, the policy of
our dental college is to revaccine students with anti-HBs of <10 mIU/ml with three doses of vaccines and those with anti-HBs titer of 10 to <100 mIU/ml with one booster dose. By adhering to this policy, students in the present study reported 100% seroconversion of anti-HBs antibody with a highly significant difference between the mean values of pre- and postrevaccination antibody titer. This could be attributed to anamnestic response, which is defined as an increase in the anti-HBs concentration of four times or more after the booster vaccine.28

On the other hand, different policy of giving only one booster dose for those having anti-HBs of <10 mIU/ml, followed in other countries, revealed seroconversion in lower percentages of participants. Previous studies on health care workers in Brazil and Thailand reported seroconversion only in 74.1 and 85%, respectively, with considerable percentages of participants (14.7 and 15%, respectively) still having anti-HBs antibody level <10 mIU/ml after a booster dose.30,31

Wider scale studies on the general population in Italy and China support our policy, where 94.2 and 90% of participants, respectively, demonstrated increased anti-HBs post booster dose, while the titer remained <10 mIU/ml in 5.8 and 6.9%, respectively.12,33 These percentages with nonresponse to a booster dose are likely explained by the possible loss of immunologic memory 17 to 18 years after primary vaccination.34 Another important result that supports our policy is the percentage reduction of nonresponders (titer <10 mIU/ml after one booster dose) among Brazilian and Italian study participants with antibody levels decreased to 2.7 and 0%, respectively, after completing three vaccine doses.30,32

**Conclusion**

In conclusion, two decades after infant immunization, a significant percentage (73.6%) of dental students failed to maintain seroprotective antibody titer against HBV (<10 mIU/ml). But a small percentage of them still have a highly protective titer (≥100 mIU/ml). After revaccination of non-immune students, 100% of them showed a highly protective antibody titers.

Therefore, it is strongly recommended to measure anti-HBs antibody titer in all dental students to assess their level of protection before they start clinical training. Accordingly, either one booster dose or a full course of revaccination should be administered with reevaluation of the antibody level to maintain the desired long-term seroprotection in dental students. Moreover, the threshold for hepatitis B vaccine-induced immunity in dental students should be revisited to maximize the duration of protection from occupational exposure. In addition, a national strategy to study the threshold and elucidate the immune memory after infant immunization in Saudi Arabia is recommended to assist in reducing the number of administered booster doses of vaccine.

The strengths of the present study include verification of students’ HBV vaccination by vaccination records and post-vaccination follow-up with quantitative anti-HBs antibody titers. The main limitations of this study are small sample size of students in one region and nonselection by random sampling, due to which the results could not be generalized to other regions. However, the consistency of our results with those from previously published studies supports the validity of these results. The other limitation is that some factors affecting immune response to vaccine such as smoking and body mass index (BMI) were not addressed.

**Conflict of Interest**

None declared.

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