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ORIGINAL ARTICLE

Investigation the longevity of hepatitis B surface antibody in vaccinated students of Hormozgan university of medical sciences

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ABSTRACT

Background and Objectives: Hepatitis B is a common chronic viral infection in humans. Universal use of hepatitis B vaccine is crucial for controlling the infection, but the duration of vaccine-induced immunity remains uncertain. This study aimed to assess hepatitis B antibody levels (anti-HBs) after vaccination in infancy and adolescence, and explore the relationship between immunity levels and variables such as age, sex, BMI, place of birth, and duration since last vaccination among students at Hormozgan University of Medical Sciences from 2019 to 2021.

Materials and Methods: The study included 1134 students who completed a questionnaire and provided blood samples for ELISA-based measurement of antibody titers.

Results: The findings revealed that 727 students (64.1%) had no protective antibody level (anti-HBs <10 mIU/ml), 299 (26.4%) had partial immunity (anti-HBs 10-100 mIU/ml), and 108 (9.5%) had complete immunity (anti-HBs >100 mIU/ml). No statistically significant relationships were observed between anti-HBs titer and age, sex, or BMI. However, antibody titer decreased with increasing time since last vaccination (P<0.001).

Conclusion: This study highlights the decline in antibody titer over time following primary vaccination. Sustained immunity against hepatitis B virus relies on antibody durability or robust immunological memory, suggesting the importance of timing booster vaccinations.

Keywords: Vaccines; Hepatitis B; Anti-hepatitis B antigens; Medical students

INTRODUCTION

Hepatitis B infection is a major public health problem worldwide. The course of the disease varies from an acute infection to a chronic disease (1). Acute and chronic hepatitis B infections cause between 500,000 and 1,200,000 deaths each year (2). Certain groups are at higher risk of hepatitis B infection, including infants born to mothers with chronic hepatitis B infection, people who regularly receive blood prod-

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ucts, hemodialysis patients, injecting drug users, and healthcare who handle blood products contaminated with hepatitis B virus (3). It appears that the prevalence of hepatitis B in the society can be reduced to a large extent through planned and targeted vaccination. In general, 85 to 90% of deaths caused by HBV can be prevented by vaccination (4).

Hepatitis B vaccine has been officially included in the childhood immunization program in Iran since 1993, with infants receiving the vaccine at birth and at 2, 4 and 6 months of age. In addition to infants, high-risk groups, particularly health care workers, have received the hepatitis B vaccine since 1993. In addition, vaccination of adolescents under 18 years of age has been included in the program since 2006 (5). Before universal vaccination of Iranian infants against this virus in 1993, the rate of infection was close to 30% and about 3% of the population were carriers of the virus. The vaccination program reduced the infection rate to less than 20% (5). A more recent retrospective cohort study by Moghadami et al. reported that the prevalence of hepatitis B carriers in Iranian population decreased to 0.9% during 1993-2018 which is a great achievement (6). After hepatitis B vaccination, anti-HBs antibodies appear in serum which is the serological marker associated with vaccine-induced protection. The anti-HBs titer required to achieve vaccine-induced protection is greater than 10 IU/ml (7).

Due to the decrease in the anti HBs levels over time and the high prevalence of needlestick injuries among medical students and hospital staff resulting in unnecessary vaccination and immunoglobulin (8). Determination of anti-HBs after vaccination should be routinely performed in all at-risk individuals. Therefore, this study aimed to determine the effect of complete hepatitis B vaccination on serum levels of anti-HBs among students of Hormozgan University of Medical Sciences in 2019, 2020 and 2021 and to evaluate the relationship between the level of immunity and various variables such as age, sex, body mass index, duration after vaccination and birthplace.

MATERIALS AND METHODS

This cross-sectional study was conducted on 1134 students of Hormozgan University of Medical Sciences during the period 2019-2021. Firstly, informed consent was obtained from the students and a questionnaire was prepared which included information about age, sex, BMI, birthplace, time elapsed since the last vaccine dose and history of contact with HBs Ag positive person. Blood samples were then taken and an ELISA test (Pishtaz Teb, Anti-HBs ELISA Kit, Iran) was performed to measure the antibody titer against HBs Ag. Subjects with the level of anti-HBs antibody titer less than 10 mIU/ml after two complete series of the hepatitis B vaccine were considered as non-responders. According to the questionnaire, students who received the vaccine and whose last vaccination was 2 months ago were included in the study, and those with a family history of HBV and immunodeficiency disease were excluded.

This study was approved by ethics committee of Hormozgan University of Medical Sciences (IR.HUMS.REC.1400.412).

Statistical analysis. All participants were categorized by sex, BMI, birthplace, last vaccination. Data were presented as mean ± standard deviation (SD) and the frequency (percentage) for continuous and categorical variables, respectively. For quantitative variables, the Kolmogorov–Smirnov test was performed with a P-value of less than 0.05 for all variables and showed a non-parametric frequency distribution. Therefore, comparisons of the quanti-ta¬tive data between two and more than two groups were made using Mann-Whitney's and Kruskal-Wallis tests, respectively. For qualitative data, the Chi-squared test was used for the comparison. The association between anti-HBs titers and age, BMI and time after vaccination was analyzed using Spearman's test. Multiple Cox regression model was used to investigate variables effecting antibody levels. P-values less than 0.05 were considered statistically significant.

RESULTS

Of the1134 students 636 (56.1%) were female and 498 (43.9%) were male. Their age ranged from 17 to 39 years with a mean age of 20.7 ± 2.2 years. Of the total participants, 1053 (92.9%) were vaccinated in infancy, 13 (1.1%) were vaccinated at age of 18 years or older and 68 students (6%) had also received a booster dose. Students who received a booster dose were vaccinated in infancy and/or at over 18 years of age. Regarding vaccine dose, 1093 (96.2%) had received 3 doses of recombinant vaccine, 31 (2.7%) had received

2 doses of vaccine and 10 (0.9%) had received only 1 dose of vaccine. The mean level of antibody titer against hepatitis B was 43.7 ± 136.9 mIU /ml and the mean duration after vaccination was 19.3 ± 4.8 years. The descriptive characteristics of the study are summarized in Table 1.

Overall, 64.1% of the vaccinated students had no protective response to the HBs antigen in the vaccine (anti-HBs <10 mIU/ml), 26.4% had an antibody titer between 10-100 mIU/ml and the others (9.5%) had antibody levels greater than 100 mIU/ml (Table 1). Among those vaccinated in infancy, 724 (68.8%) had anti-HBs titers <10mIU/ml. Among those vaccinated in adolescence 1 (7.7%) had antibody titers <10mIU/ml. Of the 68 booster vaccinees, 3 (4%) had antibody titers below 10 mIU / ml and were identified as non-responders (Table 2).

Table 1. The descriptive characteristics in study students.

Characteristics	All subjects (n=1134)
Age	20.7 ± 2.2 (17-39)
Sex	
Male	498 (43.9%)
Female	636 (56.1%)
Birthplace	
Hormozgan	443 (39.1%)
Kerman	86 (7.6%)
Fars	320 (28.2%)
Others	285 (25.1%)
BMI index (kg.m2)	22.1 ± 3.7 (2.3-35.3)
Normal (18.5-24.9)	652 (61.5%)
Skinny <18.5	187 (17.6%)
Fat ≥25	221 (20.8%)
Vaccinated status	
In infancy	1053 (92.9%)
In adolescence	13 (1.1%)
Booster	68 (6%)
Last dose of vaccine	19.3 ± 4.8 (1-28)
1-10 years	77 (6.8%)
11-20 years	603 (53.2%)
>20 years Dose of vaccine One dose	454 (40%) 10 (0.9%)
Two doses	31 (2.7%)
Three doses	1093 (96.2%)
Anti-HBs titer (mIU/ml)	43.7 ± 136.9
<10 mIU/ml	727 (64.1%)
10-100 mIU/ml	299 (26.4%)
>100 mIU/ml	108 (9.5%)
Last vaccination (year)	19.3 ± 4.9

Data are mean ± SD (standard deviation), n (%), or n/N (%), where N is the total number of patients with available data. BMI: body mass index.

Among those with an antibody titer <10, 68.8% were those who had been vaccinated at birth and had received three doses of the vaccine. Of the total cases with anti-HBs greater than10 mIU/ml, 33.5% had received three doses of the vaccine, and in 97.4% of them less than 10 years had elapsed since the last vaccination. Among those who had received the booster vaccine, the mean level of antibody titer was 357.6 ± 375.4 mIU /ml and 47 (62.7%) had an anti-HBs >100 mIU/ml. It was also shown that administration of the first dose resulted in a protective antibody response with a mean of 85.6 ± 27.4 mIU/ml. However, the level of anti-HBs antibodies was significantly lower in subjects vaccinated more than 5 years after the last booster dose compared to those vaccinated less than 5 years ago (P=0.01).

Table 2 shows the frequency distribution of the protective level of anti-HBs in the different study groups. There were no significant differences in the serum anti-HBs levels between age, sex and BMI groups (P=0.105, P=0.526, P=0.587). Comparison of the serum antibody levels between residents of different provinces showed no significant differences (P=0.345). However, the anti-HBs antibody titer showed a significant decrease in students vaccinated in more than 10 years ago compared to those vaccinated in less than 10 years ago (P<0.001). The percentage of protective antibody responses was similar between males (46%) and females (48.3%). No significant association was found between age, BMI, birthplace group and protective levels of anti-HBs response (P=0.161).

On the other hand, there was an inverse correlation between the level of anti-HBs and the time since vaccination, such that the antibody titer decreased with increasing time since vaccination (r = -0.27, P <0.001, respectively) (Fig. 1). In the multiple Cox regression model, only time since vaccination was significantly associated with anti-HBs antibody levels. However, age and BMI were not associated with antibody titers.

DISCUSSION

The aim of this study was to determine the levels of anti-HBs antibodies in medical sciences students and

Table 2. Frequency distribution of protective level of anti-HBs in different groups of study variables.

Variables	Anti-HBs <10 mIU/ml	Anti-HBs 10-100 mIU/ml	Anti-HBs >100 mIU/ml	P value
All students	727 (64.1%)	299 (26.4%)	108 (9.5%)	
Age				0.155
<20	201 (65.3%)	86 (27.9%)	21 (6.8%)	
≥20	526 (63.7%)	213 (25.8%)	87 (10.5%)	
Sex				0.381
Male	318 (63.9%)	138 (27.7%)	42 (8.4%)	
Female	409 (64.3%)	161 (25.3%)	66 (10.4%)	
Birthplace				0.124
Hormozgan	293 (66.1%)	112 (25.3%)	38 (8.6%)	
Kerman	52 (60.5%)	28 (32.6%)	6 (7%)	
Fars	201 (62.8%)	93 (29.1%)	26 (8.1%)	
Others	181 (63.5%)	66 (23.2%)	38 (13.3%)	
BMI index (kg.m2)				0.161
Normal (18.5-24.9)	441 (63.3%)	187 (26.8%)	69 (9.9%)	
Skinny <18.5	133 (66.2%)	48 (23.9%)	20 (10%)	
Fat ≥25	153 (64.8%)	64 (27.1%)	19 (8.1%)	
Vaccinated status				<0.001
In infancy	724 (68.8%)	270 (25.6%)	59 (5.6%)	
In adolescence	1 (7.7%)	8 (61.5%)	4 (30.8%)	
Booster	2 (2.9%)	21 (30.9%)	45 (66.2%)	
Last dose of vaccine				<0.001
1-10 years	2 (2.6%)	27 (35.1%)	48 (62.3%)	
11-20 years	399 (66.2)	170 (28.2%)	34 (5.6%)	
>20 years	326 (71.8%)	102 (22.5%)	26 (5.7%)	
Dose of vaccine				<0.001
One dose	1 (10%)	7 (70%)	2 (20%)	
Two doses	0 (0%)	16 (51.6%)	15 (48.4%)	
Three doses	726 (66.5%)	276 (25.2%)	91 (8.3%)	
Booster dose				<0.001
Yes	2 (2.9%)	21 (30.9%)	45 (66.2%)	
No	725 (68%)	278 (26.1%)	63 (5.9%)	

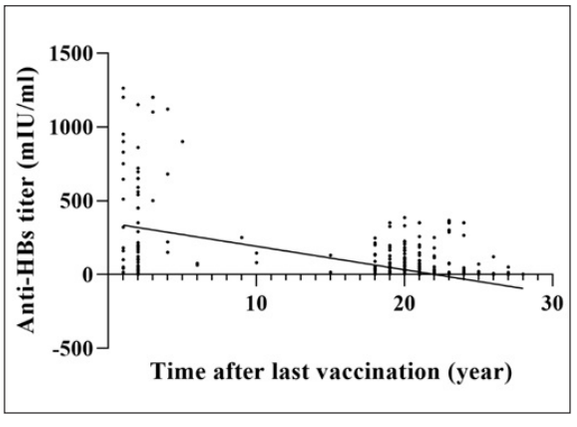

Fig. 1. Spearman correlations between Anti-HBs antibody response and postvaccination time.

the results showed that 31.2% of those vaccinated in infancy had a protective antibody response after two decades after vaccination, whereas, the prevalence of this response increased to 92.3% in those vaccinated in adolescence 10 years after vaccination. There was also a direct correlation between the protective antibody response and the time since vaccination.

Several studies have measured the level of antibodies to hepatitis B virus surface antigen in vaccinated individuals to assess the safety status and efficacy of the vaccine in different countries. Similar observations to our study have been reported in previous studies from American Samoa, China, Taiwan and Saudi Arabia (9-12). Some studies on the serum

prevalence of anti-HBs after infant vaccination have been performed in Iran. Aghakhani et al. reported a decline in protective antibody response from 65% in children one year after vaccination to 24% in 15 years after vaccination has been reported (13). A similar observation was reported by Norouzirad et al. The protective level of anti-HBS had a significant decreasing trend from 90% to 48.9% in 1 and 18-year old vaccinated children (14). Another study showed that 47.9% of 10- year-old children did not develop an adequate antibody titer after primary vaccination (15). A study from Taiwan showed that the percentage of protective responses was significantly lower in children aged 12 years (37.4%) compared with those aged 7 years (71.1%) (9). In addition, in a more recent study of 825 medical students reported that approximately more than half of the students vaccinated in childhood had anti-HBs titers <10 mIU/ml 18 years after vaccination, which is comparable to our study (16).

As reported in a number of previous studies and observed in our study in a large sample, immunity against HBV seems to decline over 10 years after vaccination. Therefore, it seems that it will be necessary for vaccinated people to assess their antibody titer 10 years after their primary vaccination. However, some other studies have shown conflicting results. Norrozi et al. and Rezaei et al. found that in 79.2% and 74%, in respectively maintained protective antibody levels for at least 15-18 years after childhood vaccination (17, 18). This discrepancy could be explained by issues such as the type of kit used to measure HBs antibody, genetic differences between subjects, geographical and regional differences, type or method of vaccine injection.

The present study also showed that for booster vaccination, even the first dose injection was sufficient to induce a protective antibody response in participants with anti-HBs <10 mIU/ml. Since protection against hepatitis B virus is induced by antigenic stimulation of memory cells and the production of specific antibodies after the initial vaccination (19). The emergence of an enhanced response to the booster dose indicates a memory response and possibly protection against hepatitis B infection. Therefore, it can be hypothesized that the substantial antibody response to the booster dose in individuals without protective antibody levels indicative of long-term immune memory, even after more than 18 years of infant vaccination. However, to support this hypothesis, we needed to test infection markers such as hepatitis B surface antigen (HBs-Ag) and hepatitis B core antigen antibody (HBc-Ab) as well as memory T cells in all participants, especially in those who did not have anti-HBs titers >10mIU/ml, which is the limitation of our study.

The results of the previous studies on the effect of demographic characteristics on anti-HBs antibody titers are controversial. Hashemi et al. reported a negative relation between age and antibody response to HBV vaccination, with a significant decrease in antibody titer with increasing age (16). In the study by Mahallawi et al. age and sex were correlated with anti-HBs antibody titer. Higher antibody levels were observed in female students compared to male students and decreased antibody titer was associated with increasing age (11). While some other reports on medical students indicated that there was no significant difference in the antibody response rate to hepatitis B vaccine based on age and sex (20, 21).

In the present study, sex, age and BMI were not significantly associated with anti-HBs titer after vaccination. In addition, the serum anti-HBs antibody levels did not show significant changes in students who were born in the different cities with different prevalence of the disease. Based on our results, a longer period of time after vaccination was the only factor influencing the lower antibody titer. In agreement with our results, a recent systematic and meta-analysis showed that among demographic and clinical variables, post-vaccination time had a significant inverse correlation with the protective levels of anti-HBs (22).

CONCLUSION

It is not known to what extent hepatitis B vaccine confers immunity. However, according to the literature and data from this study, approximately 90% of vaccinated individuals with a competent immune system have anti-HBs antibodies (>10 mIU/ml) for at least 5 years, after which even if no antibodies can be detected, memory cells in cellular immunity could provide immunity against this virus. Therefore, it is suggested that future studies be conducted in older age groups, with a large sample size in different regions of the country and evaluate the humoral and cellular immune responses in vaccinated individuals.

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