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Anti-HBs Antibody Levels and Anti-HBc Detection Among HBV-Vaccinated Freshmen Enrolled in the Department of Laboratory Sciences, Shiraz University of Medical Sciences, Iran

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Abstract

Background: There are few studies indicating the post-neonatal HBV vaccination level of anti-HBs antibody in first-year enrolled university students in Iran. In addition, anti- HBc antibody detection, which is a good indicator of virus exposure, has not been reported in vaccines. Hence, this study was conducted to determine the level of anti-HBs and anti-HBc antibodies in the serum sample of medical laboratory students who had received primary infantile HBV vaccination.

Methods: This study was conducted on first-year students enrolled in the department of laboratory sciences at Shiraz University of Medical Sciences, Iran. For determining anti-HBs and anti-HBc titers, 5 mL of venous blood was aseptically collected. Anti-HBs and anti-HBc antibody levels were determined by enzyme-linked immunosorbent assay. HBV DNA was also performed on DNA extracted from individuals positive for an anti-HBc antibody test.

Results: Of the 257 vaccinated individuals (188 females and 69 males) who participated in this study, 36.2% showed a non-protective anti-HBs response (anti-HBs < 10 mIU/mL) and 164/257 individuals (63.8%) showed a protective anti-HBs response (anti-HBs ≥ 10 mIU/mL). Significant numbers of females had protective levels of anti-HBS antibody in serum samples in comparison with the males ($P < 0.001$). Anti-HBc antibody was detected in 3 participants; however, HBsAg was not detected in any of the cases. HBV DNA was found in two.

Conclusions: Our results indicate that a substantial number of our study population vaccinated against HBV during childhood showed non-protective anti-HBs antibody level. Therefore, a booster dose of vaccine needs to be scheduled for students with anti-HBs level < 10 mIU/mL prior to the start laboratory internship.

Keywords: Vaccination, Students, Iran

1. Background

Vaccination against the hepatitis B virus (HBV) as a prophylaxis in prevention of infection is essential for the medical and paramedical students, who are at risk of exposure to the virus infection, especially during their practical training. According to the ministry of health and medical education, vaccination for all newborns in the first year of life is mandatory in Iran. However, people at risk of HBV infection, including healthcare workers (HCWs), are also subjected to vaccination if they tested negative for protective anti-HBs antibody titer (1).

Based on a recommendation by the world health organization (WHO) and the national project for implementing the pilot study of hepatitis B vaccination in Zanjan and Semnan in the year 1989, hepatitis B vaccination was integrated into the neonatal national expanded program of immunization (EPI) since 1993. However, post-vaccination titer testing (anti-HBs), 4 - 8 weeks after the third dose of the Hepatitis B vaccine, is recommended. Usually, individuals with anti-HBs ≥ 10 mIU/mL do not require further testing (2). Furthermore, different studies have indicated that immunologic memory remains intact for more than 25 years and confers protection against HBV infection, even if anti-HBs levels are reduced or declined below detectable levels.

However, if the post-vaccination titer is less than 10 mIU/mL it is recommended to repeat the vaccination series and recheck the anti-HBs titer 4 - 8 weeks following the completion of the second series. In the event that the titer is still negative after the second vaccine series, the individual should be considered as a non-responder to the hepatitis B vaccination and he will be considered to be susceptible to the HBV infection, if exposed (3).

HBV vaccine consists of the highly immunogenic surface antigen (HBsAg) protein. Studies have shown that immunogenicity of the vaccine is approximately 10 - 31 years after the primary vaccination (3, 4). However, the reason for why immunity persists, as defined by titers of HBsAb greater than 10 mIU/mL that may last for several decades after a single round of vaccination, is still not entirely clear (5).

Despite the high efficacy of the HBV vaccine, nearly 5% of immunocompetent individuals fail to respond to the primary HBV series. The reason for this is not clear, however, genetic predisposition may play an important role in non-responder individuals (6, 7).

Until now, the duration of protection after the hepatitis B vaccination of infants is unknown. After the primary vaccination, the level of anti-HBs titers declines over time, and regular mean titers of serum anti-HBs antigen rapidly decrease during the first few years (8). However, studies have shown that the anti-HBs titers remains protective for 2 - 4 years (9), or even more (10), after the primary vaccination in virtually all infants had responded to the primary course of vaccination.

In the present study, we investigated the non-protective rate of anti-HBs among HBV-vaccinated students enrolled in the department of laboratory medical sciences who were not tested after neonatal or even the second immunization with hepatitis B vaccine. Serum anti-HBc antibody titers were also determined.

2. Methods

2.1. Participants

This study was conducted at the Shiraz University of Medical Sciences, Shiraz Iran, during September to October in 2013 to 2016. Overall, 257 first-year medical laboratory sciences students who had received at least one complete vaccination series consists of three doses of vaccine against HBV during their infancy were included in this study. Their ages ranged between 18 - 25 years (mean, 21.1 ± 3.1); 69 were male, and 188 were female. Among them, 71 subjects had received at least one more single booster dose of vaccine during their adulthood.

All the students were enrolled in the study as volunteers. The exclusion criteria included underlying disease, immunosuppressive drug use, immune deficiency status, blood as well as blood products transfusion, and immunoglobulin received before vaccinations.

2.2. Specimen Collection and Serologic Testing

Demographic information from all individuals was collected at the time of specimen collection.

Five millilitres of peripheral blood samples from each individual was collected in tubes without anticoagulant and was allowed to clot at room temperature. Serum was removed by centrifugation and aliquots into 1.5 mL Eppendorf tubes and stored at -20°C until the serologic tests were undertaken.

Anti-HBs were determined quantitatively with an enzyme immunoassay (EIA) kit from Dia.Pro Diagnostic Bioprobes (Milan, Italy), according to the manufacturer's instructions. Anti-HBs levels were considered positive if > 1.0 mIU/mL and protective when > 10 mIU/mL.

All the sera were tested for antibody against hepatitis B core antigen (anti-HBc Ab) using ELISA kit (Dia.pro). If the results were positive, we would have tested the sera for hepatitis B surface antigen (HBs Ag) (Dia.pro) as well. All the procedures were performed according to the kits' manual. All the tests were performed in duplicates.

2.3. DNA Extraction and PCR Amplification Assay

Positive samples for isolated anti-HBc antibody were also tested for the presence of HBV-DNA by polymerase chain reaction assay according to the method described previously (11).

Ethical approval for the study was obtained from the local committee.

2.4. Statistical Analysis

Since the antibody titer was not normally distributed, differences by level of antibody titer were analyzed by the chi square test. Level of significance was set at $P < 0.05$. Statistical analysis was carried out through the package/program SPSS for Windows.

3. Results

3.1. Participants and Anti-HBs Levels

The study included 257 HBV vaccinated medical laboratory students; 188 (73.1%) females and 69 (26.9%) males. The study participants were divided into two separate groups based on the number of time receiving HBV vaccination. Group one consists of 186 students (136 females and 50

males) who only received a complete single dose of neonatal HBV vaccine (Table 1). Among them, 86/186 (46.2%) had anti-HBs antibody < 10 mIU/mL and the remaining 100/186 (53.8%) had protective anti-HBV antibody level > 10 mIU/mL. Results indicated that a significant numbers of females had protective levels of anti-HBS antibody in serum samples in comparison with the males (P < 0.001) (Table 1).

Table 1. Anti-HBs Antibody Levels in Serum Sample of Students Without Receiving Vaccine Booster Dose After Primary Childhood Vaccination[a]

Group	Anti-HBs, mIU/mL			P Value
	I	II	III	
Anti-HBs level	0.00 - 0.99	1.00 - 9.99	> 10	< 0.001
Female (n = 136)	9 (6.6)	44 (32.4)	83 (61)	
Male (n = 50)	12 (24)	21 (42)	17 (34)	
Total	21/186 (11.3)	65/186 (35)	100/186 (53.8)	
Overall anti-HBs level	< 10: 86/186 (46.2)	> 10: 100/181 (53.8)		

[a]Values are expressed as No. (%).

On the other hand, in group two, which consisted of 71 students (52 females and 19 males) who received a booster dose during adulthood and post neonatal vaccination, no significant differences was found between the level of protective antibody in both male and females (P = 0.1) (Table 2). Results indicated that 64/71 (90.1%) of participants in this group had anti-HBs antibody level > 10 mIU/mL.

Table 2. Anti-HBs Antibody Levels in Serum Sample of 71 Students Who Received Vaccine Booster Dose After Primary Childhood Vaccination[a]

Group	Anti-HBs, mIU/mL			P Value
	I	II	III	
Anti-HBs level	0.00 - 0.99	1.00 - 9.99	> 10	0.1
Female (n = 52)	1 (1.9)	2 (3.8)	49 (94.3)	
Male (n = 19)	2 (10.5)	2 (10.5)	15 (79)	
Total	3 /71 (4.3)	4/71 (5.6)	64/71 (90.1)	
Overall anti-HBs level	< 10: 7/71 (9.9)	> 10: 64/71 (90.1)		

[a]Values are expressed as No. (%).

Overall, 93/257 subjects (36.2%) showed a non-protective anti-HBs response (anti-HBs < 10 mIU/mL) and 164/257 individuals (63.8%) showed a protective anti-HBs response (anti-HBs ≥ 10 mIU/mL) (Table 3). Although 241 (93.7%) out of 257 participants had detectable anti-HBs (minimum, 0.1 mIU/mL; maximum, 250 mIU/mL), only 164 (63.8%) had protective levels of ≥ 10 mIU/mL (Table 3).

Table 3. Overall Results of Laboratory Characteristics of the Vaccinated Student[a]

Subjects (n = 257)	Anti-HBs > 10 mIU/mL	Anti-HBs < 10 mIU/mL	P Value
Females (n = 188)	132 (70.2)	56 (29.8)	< 0.001
Males (n = 69)	32 (46.4)	37 (53.6)	
Total	164/257 (63.8)	93/257 (36.2)	

[a]Values are expressed as No. (%).

3.2. Isolated Anti- HBc Antibody and DNA Detection

Among those with the seroprotective level of anti-HBs, 60/164 (36.6%) had received a booster injection of vaccine after their infancy. Anti-HBc antibody was detected in 3 subjects (one male and two females) who also had anti-HBs > 10 mIU/mL, however, HBsAg was not detected in any cases in Table 2. One of the 3 individuals with anti-HBc positive was also positive for the rheumatoid arthritis test as well. Additionally, serum samples of the three students remained positive for anti-HBc antibody three months after the first test. HBV DNA was detected in 2 serum samples of individuals with isolated anti-HBc antibody (Table 4).

4. Discussion

Neonatal hepatitis B vaccination has been implemented in Iran since 1993. However, the duration of protection against this infection remains uncertain. Although the post-vaccination level of anti-HBs antibody in vaccinated neonates have been reported from some ethnic and geographical regions of Iran (12), few studies have been conducted to investigate the persistence of anti-HBs seroprotection after vaccination during infancy (13-15).

The present study aimed to assess anti-HBs level in serum samples of medical laboratory students who were enrolled in the department of laboratory sciences. All students had HBV immunization in their infant's medical records. Of the 257 vaccinated individuals, 71 (25.8%) had received a booster dose after the primary immunization and 11 of them (15.5%) had anti-HBs antibody level < 10 mIU/mL. Anti-HBs antibody was not detected in 6.3% of the vaccine. Moreover, non-protective anti-HBs antibody titer < 10 mIU/mL was detected in 29.9% of the students, indicating that overall, 36.2 % of the freshman who had enrolled in the department of Laboratory Sciences should receive a booster dose before their hospital internship program. Individuals found to have anti-HBs levels of > 10 mIU/mL

Table 4. Anti HBc Antibody and HBV DNA Detection in 257 Vaccinated Students[a]

Subjects (n = 257)	Anti-HBc Positive	Anti-HBc Negative	P Value	HBV DNA Positive	HBV DNA Negative	P Value
Females (n = 188)	2 (1)	186 (99)	1	1 (0.5)	187 (99.5)	0.46
Males (n = 69)	1 (1.4)	68 (98.6)		1 (1.4)	68 (98.6)	
Total	3/257 (1.1)	254/257 (98.8)		2/257 (0.8)	255/257 (99.2)	

[a]Values are expressed as No. (%).

after the primary vaccine series are considered to be immune.

Since routine postchildhood vaccination testing is used to determine the seroprotective level of anti-HBs, the antibody has not been recommended simply since it is expensive. It is unclear whether the undetectable or low level of anti- HBs antibody (< 10 mIU/mL) is due to a decline in antibody titer after a while or individuals are actually nonresponders to the recombinant antigen. In addition to the ability of each individual immune response to the antigen, the variation of vaccine brand and manufacturing company could be the other cause of differences (16).

The percentage of seroprotective individuals among female participants was higher than the males (70.2% vs. 46.4%) (P < 0.001). Overall, immune response to HBV vaccine among females is significantly higher than males (15, 17).

One explanation is the higher number of female participants in comparison to the males (188 vs. 69). On the other hand, females' exhibit elevated hormonal and cell-mediated immune responses to vaccination compared to males. Besides, immunological, hormonal, genetic, and microbiota differences between males and females may also affect the outcome of vaccination (16).

Although neonatal HB vaccination has had a significant improvement in protecting against HBV infection in childhood, some vaccines were found to be HBsAg negative, but anti-HBs and anti-HBc positive as well (18). HBV DNA was also detected in a number of sample sera by using PCR assay (19, 20). In our study, three students with a protective level of anti-HBs antibody (> 10 mIU/mL) were found positive for anti-HBc antibody as well. However, the assay was repeated 3 months later and still remained positive for the anti-HBc antibody.

For the evaluation of specificity, the specimens were tested for the rheumatoid factor as well. One out of three samples was positive for rheumatoid factor; the others were negative. HBV DNA was found in both samples to be positive for anti-HBc antibody, indicating occult hepatitis in vaccinated individuals. Based on the family history of the HBV infection in one of the students who was positive for HBV-DNA, the student was most likely exposed to HBV prior to vaccination. However, the other student had no risk factor such as family history of the HBV infection. Overall, the prevalence of isolated anti-HBc among the vaccinated students was found to be about 1.1%.

On the contrary, in another study, the prevalence was reported to be 5% among vaccine with occult hepatitis (21).

In conclusion, our results indicate that seroprotective level of anti-HBs antibody in students who received 3 doses of HBV during their childhood vaccination was 63.8%; however, if we exclude those who received a booster in-between or before enrollment, the percentage will decrease to 37.8%. The prevalence of isolated anti-HBc was also found to be 1.1%. However, the persistent of seroprotective level of anti-HBs antibody is different in each individual, even with similar vaccination schemes. By considering the fact that medical laboratory students are an at risk population, it is necessary to schedule the determination of serum anti-HBs antibody titer to improve the immunization programs and to decrease the risk of infection before internship program.

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Footnote

Conflict of Interests: The authors declare no conflict of interest.

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