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Immunization Status Against Hepatitis B Among Iranian Junior Medical, Nursing, and Obstetrics Students With Different Vaccination Patterns

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Background: Since the protection time by hepatitis B (HB) vaccination is unclear, the strategy of immunization of junior students who previously received hepatitis vaccine is controversial.
Objectives: This study aimed to determine the status of immunity to hepatitis B in junior medical, nursing and obstetrics students with different hepatitis B virus (HBV) vaccination patterns.
Patients and Methods: In an analytical cross-sectional study, 255 junior medical sciences students were tested for quantitative antibodies to hepatitis B surface antigen (anti-HBs). The proportion of protective immunity was compared in different vaccination patterns.
Results: Vaccination coverage rates were 74.1%. About half the participants didn't show serological evidence of protective immunity; 68.9% had their last shot more than 10 years ago and 30.4% had a vaccination history of five years or less ($P < 0.001$). Geometric mean level of anti-HBs titer among students, who had received a primary series vaccine at birth, was significantly lower than students who had started vaccination at an older age ($P < 0.001$). Also, analysis of variance for geometric mean of anti-HBs titer showed significant differences between groups based on injection time from the last shot ($P < 0.001$) (post hoc comparisons resulted in a P value of < 0.001 for birth versus < 5 year group, and $P < 0.001$ for the 5 to 10 year group). The lowest rate of non-protective level belonged to participants with complete three doses and a booster additional shot (27.1%). The final model for independent predictors of anti-HBs positive status was made by a binary logistic regression analysis. The model included presence of a booster dose, injection time from last shot, and discipline of study.
Conclusions: This study shows lower anti-HBs among students who were vaccinated at infancy compared to those vaccinated at older childhood or adolescence. Also, subsequent measurement of anti-HBs level at the time of entrance to university is recommended for all previously immunized students.

Keywords: Hepatitis B; Vaccination; Medical Student; Antibody; Protective Immunity

1. Background

Hepatitis B virus (HBV) infection can be effectively prevented by hepatitis B (HB) vaccination, therefore, the world health organization (WHO) has recommended that all infants should receive hepatitis B vaccine (1). Routine HB vaccination has been provided to all Iranian newborns since 1993, although it had already started in two provinces as a pilot since 1989 (2). Before establishment of routine hepatitis B vaccination programs, HBV infection was common among the Iranian population with a prevalence of 3% for HBV chronic carriers (3, 4). Hepatitis B vaccination programs have resulted in a significant decrease in acute and chronic HBV infections among children and adolescents in Iran (5). In 2007, catch-up vaccination of all 17-year-old Iranian adolescents, who visited public healthcare providers was implemented (6). At that time, a three-injection schedule was applied for vaccination (6, 7). Although vaccination against HBV has been routinely performed for high-risk groups (such as healthcare workers) since 1992, yet nationwide population-based studies on HBV immune status of healthcare workers (HCWs) and its related factors are sparse in Iran (8). Studies from other counties have shown substantial reductions in the prevalence of chronic infection and the incidence of acute hepatitis B among HCWs in populations where routine infant vaccination has been implemented (9). Protection time after infancy is unknown (10, 11). Studies have shown low or undetectable levels of antibodies to hepatitis B surface antigen (anti-HBs) in a range of 15 to 97% of infants between 10 to 19 years after primary vaccination (12-16). Undetectable anti-HBs (serological marker for protection after primary vaccination) does not necessarily show lack of immunity (17, 18). In low risk populations, proving long-term protection of vaccine is difficult and not recommended (19). However, in high-risk groups (i.e. HCWs), HBV immune memory must be proved by measuring anti-HBs concentration (20). Immunity status of junior students who were vaccinated during infancy or

adolescence is important for avoiding occupational HBV transmission. Despite education and interventions, a recent study reported a rate of 53% for needle stick injury in nurses, at least once in the past year (21). Although occupational hepatitis seroconversion is relatively rare, risks and associated costs of a blood exposure are serious and real. These costs include initial and follow-up treatment of the exposed person (22, 23), fear and anxiety about possible consequences of an exposure and absence from work (24).

Romano et al. suggested that booster vaccination may be needed for situations where risks of contracting Hepatitis B virus (HBV) infection and becoming chronic are high (25). Also, failure to develop post-booster anamnestic response has been reported (as immune memory may wane during the second decade post-vaccination) (26). In addition, one sixth of vaccines were unable to respond to booster vaccination, having lost immunological memory (27). Individuals who lost immunological memory may become vulnerable to HBV infection, especially in highly endemic regions where HBsAg carriers are often positive for HBeAg, thus, highly infectious. Therefore, the need for a booster in this setting, where risk of acquiring infection and becoming chronic is high, should be considered (25). If this was the policy, booster should be given before immunological memory loss occurs. Due to a higher risk of transmission during the clinical education period, HBV immune status should be evaluated before any contact with blood and could be offered when students start their education. At the same time, junior students have several different patterns of hepatitis immunization.

2. Objectives

This study aimed to determine the status of immunity to hepatitis B in junior medical, nursing and obstetrics students with different prior HBV vaccination patterns.

3. Patients and Methods

In an analytical cross-sectional study in 2012, we checked anti-HBs status in junior medical, nursing, and obstetrics students, who had not yet started their in-hospital clinical education. All students were asked about receiving blood products, living with chronic HBV infected parents or siblings, immunosuppression or taking immunosuppressive medications, as well as their HBV vaccination status (time of receiving the initial dose, time of the last dose and more shots after primary vaccination), weight, height, and smoking status. We categorized the participants to six groups based on injection time from last shot (not-vaccinated (none), vaccinated at birth only (at birth), more than 10 years (> 10), five to 10 years (5 to 10), less than five years (< 5), and a group who couldn't recall their history of vaccination (can't recall)). The study received approval from the Qazvin University of Medical Science ethical board. The participants were enrolled after obtaining a written consent, and additional doses were offered to all participants who didn't have anti-HBs of > 10 mIU/mL. Two hundred and seventy students were asked to complete the questionnaire and return it within four days. With a response rate of 83%, 255 out of 270 students returned the questionnaires. Blood samples were collected from all participants. Serum was separated and stored at -20°C. Next, anti-HBs was measured by anti-HBs enzyme-linked immunosorbent assay (ELISA) kit (Pishtaz Teb-HBs-Ab-96). Serum samples with different concentrations of anti-HBs antibody were repeatedly tested both for intra-assay (within one kit) and inter-assay (between kits). Intra-assay variability (coefficient of variation presented by percentages) was 3.9 to 10% and inter-assay variability was 3.7 to 9.1% (28). Participants were considered immune if they had an anti-HBs of > 10 mIU/mL. Descriptive and analytical tests were performed using the SPSS® software version 19 (SPSS Inc., Chicago, Ill, USA). Continuous variables were summarized as means (or geometric mean) ± standard deviation (SD) and categorical variables as frequencies and percentages, unless otherwise stated. The chi square or Fisher's exact tests were used for nominal categorical variables. Analysis of variance (standard or nonparametric, as required) was used to test for the association between continuous outcomes and nominal categorical variables. Post hoc analysis (Bonferroni test) was used to determine the specific nature of significant analysis of variance (ANOVA) results. A binary logistic regression analysis was used to identify independent predictors of anti-HBs positive status (method = enter) among the students. A variable was entered into the model if the significance level of its F value was less than 0.05 and was removed if the significance level was greater than 0.1. A P value of < 0.05 was considered statistically significant.

4. Results

A total of 255 participants including 96 medical, 82 nursing and 77 obstetrics students were enrolled. Only 6.3% of the participants had previous contact with a chronic HBV infected person. None had ever received blood products and had evidence of immunosuppression and no one was taking immunosuppressive medications at the time of study (Table 1). Vaccination coverage rate (defined as the percentage of students who had received three or more doses of HB vaccine) was 74.1%, while 94.5% had received at least one shot.

About 41% of students were immunized at birth and 34.1% (87) received primary series vaccination during the recent five years (Table 1). Almost all (except one) students who were immunized at birth, completed three doses, among them 3% received a booster dose after the primary series. In the other participants, 18 out of 151 (12.0%) received only one shot and 22 (14.6%) received two doses (26.5% were determined as having received incomplete vaccination) (Table 2).

Table 1. Distribution of Protective Immunity Status Among the Two Hundred and Fifty-Five Iranian Students

Characteristics	Immune Status (Anti-HBs > 10) Count, %			P Value (Chi-Square Test)
	Positive	Negative	Total	
Age group, y				0.004 [a]
18 - 20	69	91	160	
≥ 21	58	36	94	
Sex				0.65
Male	31 (25.0)	36 (27.5)	67 (26.3)	
Female	93 (75.0)	95 (72.5)	188 (73.7)	
Overweight (based on BMI)				0.44
Yes	24	29	53	
No	99	92	191	
Marital status				0.46
Single	111 (89.5)	114 (87.0)	225 (88.2)	
Married	13 (10.5)	17 (13.0)	30 (11.8)	
Number of individuals in the family				0.20 [b]
3 - 4	38 (34.5)	47 (39.8)	85 (37.3)	
5	28 (25.5)	39 (33.1)	67 (29.4)	
6	21 (19.1)	11 (9.3)	32 (14.0)	
7	12 (10.9)	10 (8.5)	22 (9.6)	
≥ 8	11 (10.0)	11 (9.3)	22 (9.6)	
Discipline of study				0.02 [a]
Medicine	57 (46.0)	39 (29.8)	96 (37.6)	
Nursing	32 (25.8)	50 (38.2)	82 (32.2)	
Obstetric	35 (28.2)	42 (32.1)	77 (30.2)	
History of contact				0.40
Yes	10 (8.1)	6 (4.6)	16 (6.3)	
No	102 (82.3)	107 (82.3)	209 (82.3)	
Can't recall	12 (9.7)	17 (13.1)	29 (11.4)	
Injection time from last shot				0.00 [a]
None	1 (0.8)	4 (3.1)	5 (2.0)	
At birth	33 (26.6)	71 (54.2)	104 (40.8)	
> 10	4 (3.2)	11 (8.4)	15 (5.9)	
5 to 10 years	23 (18.5)	5 (3.8)	28 (11.5)	
≤ 5 years	57 (46.0)	30 (22.9)	87 (34.1)	
Can't recall	6 (4.8)	10 (7.6)	16 (6.3)	
Booster dose				0.00 [a]
Yes	81 (63.3)	42 (33.1)	123 (48.2)	
No	42 (32.8)	76 (59.8)	118 (46.3)	
Can't recall	5 (3.9)	9 (7.1)	14 (5.5)	

[a] The chi-square statistic is significant at the 0.05 level.
[b] Chi-square for trend.

Table 2. Geometric Mean Level of Anti-HBs Titer of Students Based on Injection Time From Last Shot and Booster Dose (Forgetful Students Were Excluded)

Time From Last Shot	Booster Dose						P Value
	Yes		No		Total		
	Count, %	Mean (SE) [a]	Count, %	Mean (SE)	Count, %	Mean (SE)	
None	0 (0.0)	-	5 (4.3)	0.401 (0.40)	5 (2.1)	0.401 (0.40)	-
At birth	3 (2.5)	0.672 (0.58)	101 (86.3)	0.836 (0.10)	104 (43.5)	0.831 (0.10)	0.781
> 10 year ago	13 (10.7)	0.854 (0.31)	2 (1.7)	0.887 (0.07)	15 (6.3)	0.858 (0.27)	0.968
5 to 10 years	24 (19.7)	1.889 (0.18)	4 (3.4)	2.396 (0.21)	28 (11.7)	1.962 (0.16)	0.274
≤ 5 year	82 (67.2)	1.727 (0.12)	5 (4.3)	0.627 (0.55)	87 (36.4)	1.664 (0.12)	0.031 [b]

[a] Mean: geometric mean level of anti-HBs titer, SE: standard error.
[b] The t test statistic was significant at the 0.05 level.

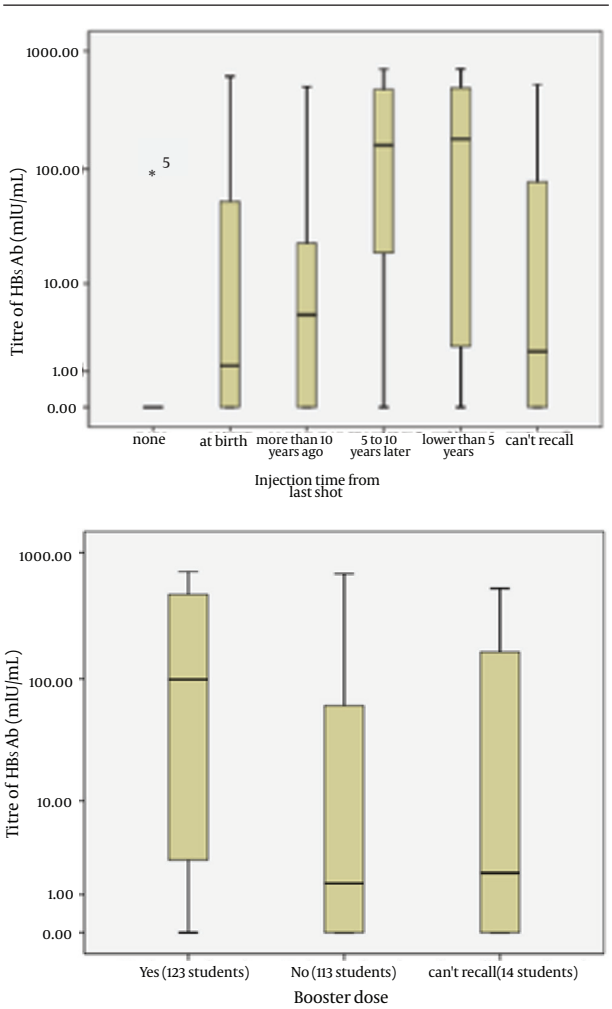

Figure 1. Comparison of Geometric Mean of Anti-HBs Titer According to Two Important Dependent Variables

Younger students (18 - 20 years) had significantly lower anti-HBs positivity rate than the older healthy adult group (≥ 21 year); 69/160 (43.1%) versus 58/94 (61.7%), P = 0.004 (Table 1). About half of the participants didn't show serological evidence of protective immunity. Low or undetectable anti-HBs level were found in 82/119 (68.9%) of students who had their last shot more than 10 years ago (including at birth) and 35/115 (30.4%) with a vaccination history of five years or less (P value < 0.001) (Figure 1).

Anti-HBs of lower than 10 mIU/mL was found in 67/104 (64.4%) of students who were immunized at birth. Also, geometric mean level of anti-HBs titer among students who received a primary series of vaccine at birth (mean = 0.83, SD = 1.01) was significantly lower than students who had started vaccination at an older age (1.52 ± 1.14) (mean difference = -0.69, P < 0.001). Also, ANOVA for geometric mean of anti-HBs titer showed significant differences between the six groups based on injection time from last shot (P < 0.001) (post-hoc pairwise comparisons showed that the "geometric mean of anti-HBs titer" had increased at < 5 year group (P < 0.001) and the 5 to 10 year group (P < 0.001) compared to at birth, also it was different at < 5 year group compared with the 5 to 10 year group (P < 0.001). It shows that the "geometric mean of anti-HBs titer" persistently decreased after birth (Table 3).

The lowest rate of non-protective level belonged to participants with complete three doses and a booster additional shot (27.1%). The ANOVA for geometric mean of anti-HBs titer showed significant differences among the three college students (P < 0.001); post hoc comparisons indicated a P value of < 0.001 for medicine versus nursing students, and 0.02 for medicine versus obstetric students (Table 3). Also, protective immunity proportion of nursing and obstetrics students was significantly lower than medical students (Table 1). The final model for independent predictors of anti-HBs positive status was made by a binary logistic regression analysis. The model included presence of a booster dose, injection time from last shot and discipline of study (Table 4).

Table 3. Multiple Comparisons of Geometric Mean of Anti-HBs Titer (mIU/mL) With Bonferroni Test

Variables	Mean Difference	Std. Error	P Value	95% Confidence Interval	
				Lower Bound	Upper Bound
Discipline of Study					
Medicine					
Nursing	0.639 [a]	0.166	0.000	0.24	1.04
Obstetric	0.462 [a]	0.169	0.020	0.05	0.87
Nursing					
Medicine	-0.639 [a]	0.166	0.000	-1.04	-0.24
Obstetric					
Nursing	0.176	0.175	0.941	-0.24	0.60
Time From Last Shot					
At birth					
≤ 5 years	-0.833 [a]	0.151	0.000	-1.28	-0.38
5 to 10 years	-1.130 [a]	0.222	0.000	-1.78	-0.47
> 10	-0.027	0.288	1.000	-0.88	0.82
None	0.430	0.478	1.000	-0.98	1.84
Can't recall	-0.071	0.280	1.000	-0.90	0.75

[a] The mean difference was significant at the 0.05 level.

Table 4. Independent Variables Associated With Anti-HBs Positive Status (Method = Enter)

Model	95% Confidence Interval for EXP (B)		P	Exp (B)
	Lower	Upper		
Step 1 [a]				
Discipline of study			0.022 [b]	
Discipline of study (1)	0.241	0.973	0.042 [b]	0.484
Discipline of study (2)	0.611	2.571	0.537	1.254
Booster dose (1)	0.344	2.138	0.742	0.858
Injection time from last shot			0.028 [b]	
Injection time from last shot (1)	0.157	41.599	0.510	2.557
Injection time from last shot (2)	1.399	8.841	0.008 [b]	3.517
Number of doses of vaccines	0.353	0.938	0.027 [b]	0.576
Age (1)	0.891	3.411	0.105	1.743
Constant			0.391	2.123

[a] Variables entered in step 1: age, booster dose, injection time from last shot, discipline of study and number of doses of vaccines.
[b] $P < 0.05$.

5. Discussion

In our study, only half the participants showed serological evidence of protective immunity. Also, booster dose, injection time from last shot, and discipline of the study have been identified as the most important independent predictors of anti-HBs positive status.

Numerous studies on various Iranian populations have been conducted over the past few years to investigate the protection provided by HB vaccine, yet the participants were not students of medical sciences (29-32). The results of this study are important in determining the ongoing and long-term protection provided by HB vaccine and selecting the best strategy for medical students' vaccination. The coverage rate was 74.1% in our study. This rate was based on the number of students who received vaccine at birth time, throughout a campaign or from other sources such as private centers. After 20 years of imple-

mentation, coverage rate for the cohort of adolescents who were born from 1989 to 1992 has reached 62% in 1993, and 74% at present (32, 33). Similarly, Alavian et al. in 2009, using administrative data estimated the vaccination coverage rate as 70%. They also indicated that 74.5% and 78.3% received at least two doses and one dose of the vaccine, respectively (6). The current study showed considerable rates of one or two doses of vaccine (94.5% and 82.7% of students, respectively) that were higher than the mentioned study.

Although many studies have shown a reverse association between obesity and immune response to hepatitis B vaccination (34, 35), our study did not reveal such finding. A possible explanation may be high body mass index (BMI) in some students at the time of study while they had normal BMI at the time of vaccination.

Our study found low or undetectable anti-HBs in 64.4% of junior students who were immunized at birth. Also, anti-HBs levels among students who received a primary series of vaccine at birth were significantly lower than students who had started vaccination at an older age; this may be due to a longer time between seroconversion and measurement in the first group. Results of some other studies were similar (20). It is also important to consider that the function of the immune system in infants is less developed than in older children and adults (19). Another explanation for this difference is "vaccination boosting" (36). In our study, the highest protective levels belonged to students who received the complete three doses and an additional shot of vaccine. This finding enforces the validity of late suggestion. Evidence of natural boosting has been demonstrated in HBV endemic areas (37). In this study, pre-entrance exposure to HBV-infected people (in the household and community) was low. Thus, opportunity for stimulation of immune memory by "natural boosting" were likely uncommon. In this study, vaccinated students who received the three-dose series and an additional shot had the highest anti-HBs levels. This finding is in agreement with other reports (34, 38). As our study indicated, medical students had a better HB immune status than other students. Enrolling students to medical faculties in Iran is based on passing a competitive nationwide entrance exam for high school graduates (39). The difference between medical students and other disciplines might be explained by differences in their socio-economic situations including their place of birth and education that had a systematic difference (most of the medical students were based in Tehran while others were mostly from Qazvin and other provinces). More studies are needed to determine other affecting factors.

This study had some limitations. The exact dosage, brand, and type of primary series vaccines of our participants were unknown, and it has been widely accepted that anti-HBs levels could be affected by these factors. Also, we collected BMI data to find the probable association between obesity and poor HB vaccine response, yet in the end this was not possible because vaccination was done in the past, for which we didn't have the BMI data.

This study showed a lower anti-HBs level among students who were vaccinated during infancy compared to those vaccinated as older children or adolescents. Although, serological testing should be performed for all trainees who are at risk of occupational exposure (to guide post-exposure prophylaxis) yet anti-HBs testing after administration of an additional dose of vaccine may be better and more cost-effective than a preliminary serological testing. This is more important for students with a history of vaccination for more than ten years, especially those immunized at birth (due to lack of serological evidence of protective immunity). Therefore, we offer an early administration of vaccine at the time of entrance to university and subsequent measurement of anti-HBs levels one to two months later. Also, trainees with an incomplete vaccination and high risk activities for exposure to blood or other infectious body fluids may not be protected and should complete the vaccination series.

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Authors' Contributions

Abbas Allami developed the original idea and the protocol, abstracted and analyzed the data, wrote the manuscript and was the guarantor. Navid Mohammadi and Azadeh Najar contributed to the development of the protocol, abstracted the data and prepared the manuscript.

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