Evaluation of response to hepatitis B vaccine in Iranian 6–18-year-old students

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Background: Hepatitis B is a dangerous disease with high morbidity and mortality rates all around the world. Vaccination is the most important way to its prevention and control. This cross-sectional study was carried out to study the levels of immunogenicity to hepatitis B vaccine in students. **Materials and Methods:** Six hundred and forty-four students aged 6–18 years including 316 girls and 328 boys were selected from the Chaharmahal Va Bakhtiari province. Selected students had been received three doses of recombinant vaccine (0, 1, and 6 months). Blood samples were taken and the titers of hepatitis B surface antigen were studied. **Results:** From a total of 644 students, 396 (61.5%) had a titer lesser than 10 mIu/ml and 248 (38.5%) had a titer higher than 10 mIu/ml. Therefore, the level of respond to vaccine with 95% confidence was 38.5% (34.7%–42.4%). Levels of respond to vaccine were related to age, body mass index (BMI), and educational level and were not related to sex and habit of students. **Conclusion:** Reverse significant relation was seen between the respond to vaccine and age and BMI in a way which the titers of antibody were lower in students with higher age and BMI.

Key words: Hepatitis B, immunization, body mass index, age, hepatitis B surface antigen

INTRODUCTION

Despite universal vaccination against hepatitis B virus (HBV), an infection with this pathogen continues to be responsible for important morbidity and mortality all around the world.[1] It has been an international health problem estimated to lead to between 500,000 and 1.2 million deaths every year.[2] Incidence of infection with HBV estimates range between 0.1% and 0.7% in Northern, Western, and Central Europe, whereas those significantly higher in Southern and Eastern Europe.[3,4] In the Middle East and North Africa, the prevalence of HBV infection is different such as 7.4% in Iran, 9.8% in Egypt, 6.9% in Libya, 2.4% in Lebanon, 2.6% in Palestine, 8.6% in Israel, and 6.1% in Saudi Arabia.[5] In North America, 41% had anti-hepatitis B surface (HBs) levels of >10 mIU/ml 7–9 years after booster vaccination at birth,[6] even 51% had these protective levels 30 years after receiving the primary series without subsequent doses.[7]

Hepatitis B vaccine has been shown to be highly effective in preventing infection with HBV. The hepatitis B vaccine was introduced in the early 1980s. In 1991, the World Health Organization recommended that all countries introduce hepatitis B vaccination into their national immunization programs. In Iran, vaccination against HBV is recommended for all infants, previously unvaccinated children, and unvaccinated adults at high risk in an attempt to achieve lifelong protection against HBV infection.[8] Immunization with this vaccine starting at birth has dramatically reduced the subsequent development of chronic hepatitis B in young children from perinatal or early childhood exposure to HBV.[9-11]

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Various investigations have been reported that type of vaccine, the number of injections, site of injection, the use of adjuvant materials, and storage conditions of vaccines cause differences in the immunogenicity of the hepatitis B vaccine.[12] In keeping with this, the potential effects of the genetic, age, weight, and a history of alcohol, drugs, and narcotic materials consumption in the immunogenicity of hepatitis B vaccine should not be overlooked.[12] Approximately 4%–10% of healthy, immunocompetent individuals fail to mount protective levels of antibodies to recombinant HBs antigen (HBs Ag) after completing the standard HBV vaccination schedule.[13] However, the levels of immunity against hepatitis B vaccine in Iran and other sites of the world had a range of 70%–95%.[7,8] The previous investigation revealed that age of patients had a significant effect on the seroprevalence of hepatitis.[14]

There were no previously published data on the evaluation of the levels of immunity against hepatitis B vaccine in Iran. Therefore, the present investigation was carried out to evaluate the levels of response to the hepatitis B vaccine in Iranian 6–18-year-old students.

MATERIALS AND METHODS

Ethics

This study was approved by the Ethical Committee of Department of Pediatrics, College of Medicine, Shahrekord University of Medical Sciences, Shahrekord, Iran (Consent Ref Number: IR.SKUMS.REC. 1394.195). All samples were obtained after informed consent had been given; as patients were under age, approval was obtained from parents.

Samples

This cross-sectional study was conducted on 6–18-year-old students of different regions of the Chaharmahal Va Bakhtiari province, Iran on 2016 year. Samples were taken from students at different educational levels including primary (6–11-year-old including the students of first, second, third, fourth, fifth, and sixth educational levels), secondary (guidance) (11–13 years old including the students of seventh, eighth, and ninth educational levels), and tertiary students (14–18-years old including the students of first, second, third, and fourth educational levels).

The sample size was calculated based on assuming a 15% for nonrespond[6-8] to vaccine using a 95% confidence and assuming 20% relative error. The sample size obtained as 544 students. To increase the precision, 100 additional samples were considered, and finally 644 students were selected randomly and studied. All children received three doses of recombinant hepatitis B vaccine at the birth, after 1 month, and after 6 months. Three-stage random sampling (school selection, class selection, and student selection) was done. Five milliliters of venous blood was taken from each student by venipuncture into sterile tubes. The sera samples were separated using centrifugation (Hettich, Germany). Serum samples were then stored at −20°C until analyses were performed.

Evaluation of sera titers

All of the sera samples were analyzed for the titers of antibodies against HBV using HbsAb commercial kit (Diapro, Italy) according to the manufacture's instruction. Results were analyzed and those that had a titer lower than 10 mIu/ml were analyzed for the presence of HBV antigen using the HBsAg commercial kit (Diapro, Italy) according to the manufacture's instruction. The sera samples which were negative for the presence of HBsAg were analyzed for the M class antibodies against C antigen of the HBV using the HBcAb (IgM) commercial kit (Diapro, Italy) according to the manufacture's instruction.

Statistical analysis

Statistical analysis was done using the mean ± standard deviation for quantitative variables and the number and percentage for qualitative variables. Chi-square and independent *t*-test were used to study the relation between sex, location, age, and education level with respond to vaccination and spearman correlation coefficient was used to study the relation between titers of antibodies with age and body mass index (BMI) of students. The logistic regression was used to determine the relation between respond to vaccination and sex, BMI, and educational level of students. Statistical analysis was performed using SPSS/21.0 (SPSS Inc., Chicago,IL, USA) and significance was regarded at a $P < 0.05$.

RESULTS

Of 644 samples studied, 328 students (50.9%) were boy and 316 students (49.1%) were girl. Three-hundred and forty-nine students (54.2%) were from primary school, 148 students (23%) from secondary, and 147 students (22.8%) from tertiary schools. Samples were gathered from seven different regions of the Chaharmahal Va Bakhtiari province, Shahrekord county with 236 students (36.6%) had the most, and Kiar and Kohrang countries each one with 24 students (3.7%) had the least sample sizes. Totally, 248 students (38.5%) had a positive respond (responder) and 396 students (61.5%) had a negative respond (nonresponder) to the vaccination. Therefore, the level of respond to vaccine with 95% confidence was 38.5% (34.7%–42.4%).

Table 1 represents the levels of response to the hepatitis B vaccine in the students based on the sex and location. Totally, there were no statistically significant differences between the levels of response to hepatitis B vaccine and sex

and location of students. The highest percent of response to vaccine was found for girl students (39.6%) and also in the Kiar region (45.8%).

Table 2 represents the levels of response to the hepatitis B vaccine in the students based on the educational level, BMI category, and age. The highest levels of respond were found in secondary level (45.3%) and BMI less than 20 (41.1%) and the lowest levels of respond were found in the tertiary level (28.6%) and BMI higher or equal than 25 (24.5%). The mean age of the responder and nonresponder students was 11.4 ± 3.3 and 12 ± 3.4 years, respectively ($P = 0.016$). Spearman correlation coefficient showed the reverse significant relation between age of students and levels of antibody titer ($P = 0.03$ and $r = -0.086$).

Table 3 represents the levels of response to the hepatitis B vaccine based on the interaction of educational level and BMI. Spearman correlation coefficient showed the reverse significant relation between BMI and levels of antibody titer ($P = 0.011$ and $r = -0.101$). The highest levels of respond to vaccination (42.2%) was found in the secondary students with BMI ≤20 and also primary students with 20< BMI ≤25. The lowest levels of respond to vaccination (20.8%) were found in tertiary students with BMI ≥30.

Table 4 represents the result of the logistic regression model for respond to vaccination. The model estimation was done using backward: LR method. The variables of sex, BMI, education level, and the interaction of BMI and education level were entered into the model. At last step, the variables of BMI and education level remained in the model. These variables were significant on respond to vaccination. Responding to vaccination in secondary school was higher than tertiary school. Odds ratio for BMI was equal to 0.949. Therefore, with increasing 1 kg/m² in student's BMI, the ratio of chance for respond to nonrespond to vaccination decreases by 5%.

Results of the evaluation of the HBsAg test were negative in all of the sera samples with an antibody titers lower than 10 mIu/ml. Therefore, 84 samples were randomly selected from these sera samples and analyzed for the HBcAb. Results showed that all of the samples had a HBcAb (IgM) concentration lower than 0.9 and were recognized as negative. Only one serum sample had a concentration of 2.4 and was recognized as positive.

DISCUSSION

Hepatitis B was still a worldwide health problem. This disease as an acute and chronic communicable disease has been a worldwide health problem estimated to lead to between 500,000 and 1.2 million deaths every year through causing chronic hepatitis, cirrhosis, and hepatocellular carcinoma.[12] Vaccination is a good method to decrease

the risk of hepatitis B infection in human. However, many factors influencing response to hepatitis B vaccine.

The current study was focused on the analysis of the effects of age, sex, educational level, location, and BMI of

Table 1: Levels of response to the hepatitis B vaccine in the students of Chaharmahal Va Bakhtiari province based on the sex and location

Variations	Response to hepatitis B vaccine			P
	Yes (%)	No (%)	Total (%)	
Sex				
Boy	123 (37.5)	205 (62.5)	328 (100)	0.592
Girl	125 (39.6)	191 (60.4)	316 (100)	
Total	248 (38.5)	396 (61.5)	644 (100)	
Location				
Shahrekord	83 (35.2)	153 (64.8)	236 (100)	0.417
Boroujen	32 (37.6)	53 (62.4)	85 (100)	
Farsan	24 (32.9)	49 (67.1)	73 (100)	
Lordegan	71 (44.4)	89 (55.6)	160 (100)	
Ardal	19 (45.2)	23 (54.8)	42 (100)	
Kiar	11 (45.8)	13 (54.2)	24 (100)	
Koh Rang	8 (33.3)	16 (66.7)	24 (100)	
Total	248 (38.5)	396 (61.5)	644 (100)	

Table 2: Levels of response to the hepatitis B vaccine in the students of Chaharmahal Va Bakhtiari province based on the educational level, body mass index, and age

Variations	Response to hepatitis B vaccine			P
	Yes (%)	No (%)	Total (%)	
Educational level				
Primary	139 (39.8)	210 (60.2)	349 (100)	0.01
Secondary	67 (45.3)	81 (54.7)	148 (100)	
Tertiary	42 (28.6)	105 (71.4)	147 (100)	
Total	248 (38.5)	396 (61.5)	644 (100)	
BMI				
<20	172 (41.1)	247 (58.9)	419 (100)	0.062
20–25	64 (36.4)	112 (63.6)	176 (100)	
≥25	12 (24.5)	37 (75.7)	49 (100)	
Total	248 (38.5)	396 (61.5)	644 (100)	
Age				
6	13 (56.5)	10 (43.5)	23 (100)	0.016*
7	25 (45.5)	30 (54.5)	55 (100)	
8	23 (39)	36 (61)	59 (100)	
9	23 (39.7)	35 (60.3)	58 (100)	
10	23 (35.9)	41 (64.1)	64 (100)	
11	23 (37.1)	39 (62.9)	62 (100)	
12	21 (42.9)	28 (57.1)	49 (100)	
13	23 (44.2)	29 (55.8)	52 (100)	
14	25 (47.2)	28 (52.8)	53 (100)	
15	18 (35.3)	33 (64.7)	51 (100)	
16	14 (25.5)	41 (74.5)	55 (100)	
17	9 (21.4)	33 (78.6)	42 (100)	
18	8 (38.1)	13 (61.9)	21 (100)	
Total	248 (38.5)	396 (61.5)	644 (100)	

*Based on independent-samples *t*-test. BMI = Body mass index

Table 3: Levels of response to the hepatitis B vaccine in the students of Chaharmahal Va Bakhtiari province based on the interaction of educational level and body mass index

Educational levels × BMI*	Response to hepatitis B vaccine			P
	Yes (%)	No (%)	Total (%)	
Primary with BMI ≤20	110 (41)	158 (59)	268 (100)	0.05**
Guidance with BMI ≤20 or Primary with 20< BMI ≤25	68 (44.2)	86 (55.8)	154 (100)	
Secondary with BMI ≤20 or Primary with BMI ≥30	23 (29.5)	55 (70.5)	78 (100)	
Guidance with 20< BMI ≤25	21 (42)	29 (58)	50 (100)	
Guidance with BMI >25 or Secondary with 20< BMI ≤25	21 (30)	49 (70)	70 (100)	
Secondary with BMI ≥30	5 (20.8)	19 (79.2)	24 (100)	
Total	248 (38.5)	896 (61.5)	644 (100)	

**Based on Chi-square test *BMI = Body mass index

Table 4: Results of the logistic regression model for respond to vaccination in the students of Chaharmahal Va Bakhtiari province

Variable	Coefficient	SE	Significance	OR	95% CI for OR	
					Lower	Upper
Education level			0.025			
Education level (1)	0.309	0.231	0.182	1.362	0.865	2.144
Education level (2)	0.666	0.248	0.007	1.947	1.196	3.167
BMI	−0.052	0.024	0.031	0.949	0.905	0.995
Constant	0.192	0.543	0.724	1.211		

*Dependent variable is responding or not responding to vaccination. SE = Standard error; BMI = Body mass index; CI = Confidence interval; OR = Odds ratio

6–18-year-old students on the levels of response to hepatitis B vaccine. As far as we know, the present investigation is one of the most comprehensive studies on evaluation of the immunogenicity of hepatitis B vaccine in Iranian students. Findings obtained from this research revealed that only 31.5% of studied students had an antibody titer higher than 10 mIu/ml. To our best knowledge, the present research reported the lowest percent of respond to vaccination against hepatitis B vaccine in students all around the world.

We found that young children had a higher seroprotection rate to hepatitis B vaccine. The lower responsiveness to hepatitis B vaccine in older children might result from waning immunity with age. We also found that BMI had a significant effect on levels of response to hepatitis B vaccine. Low levels of response to hepatitis B vaccine in students with higher levels of BMI is maybe due to the distribution of vaccine in fat not in muscle which decrease its absorption. Significant effects of age and BMI on the levels of response to hepatitis B vaccine have been reported from Belgium,[15] China,[16] Turkey,[17] and Iran.[18]

Falah Khoshkholgh *et al.*[19] reported that 201 cases out of 219 students (91.8%) had positive anti-HBs antibody response and 18 participants (8.2%) were nonresponsive which was lower than our findings. They showed that the mean antibody titer in males and females was 395.72 ± 46.33

and 397.33 ± 27.85, respectively. Momeni *et al.*[20] reported that of 1538 vaccinated individuals, 55 (3.7%), 126 (8.4%), and 1309 (87.9%) had received one, two, and full three doses of vaccine, respectively. One hundred and seventy-six (11.5%) were nonimmune (anti-HBs <10 IU/mL) and 1362 (88.5%) were immune (anti-HBs >10 IU/mL). Statistically, the levels of anti-HBs were significantly associated with gender and age which was similar to our findings. A Recent study which was done by the Nashibi *et al.*[21] reported the relevant effect of age and irrelevant effect of BMI on the levels of response against hepatitis B vaccine. They showed that 14 cases (5.9%) were nonresponders, 37 (15.5%) were poor responders, and 188 (78.6%) were good responders. Nonresponders were older (>50 years) than the responders ($P = 0.0001$), whereas the BMI was not significantly different ($P = 0.37$) between them. Moradi *et al.*[22] studied the levels of response to the HBV vaccine in Iranian infants. They showed that 86% of children responded positively to the vaccination against hepatitis B, whereas thirty cases (14%) did not respond properly.

Reports of the Center for Diseases Control and Prevention[23] and American Academy of Pediatrics Infectious Diseases[24] showed that three times infusion of HBV vaccine caused the occurrence of protective antibody in 85%–100% of people which was higher than our report. The levels of immunity against vaccination with recombinant hepatitis B vaccine in a study which was conducted in Yemen[25] and those of Iran[26-28] had ranges of 50%–80% which was higher than our findings. Several studies have been reported that immune response to hepatitis B vaccine is depended on host factors such as sex, age, weight, genetics, and immunocompetent of individuals.[13,14] Previous research reported that concurrent application of tetanus-diphtheria and HBV vaccine could effectively enhance the protective level of HBsAb titer in low responder individuals.[2] Heravi *et al.*[27] indicated that there was not any significant relationship between the age and sex of student and levels of anti-hepatitis B antibodies. They mentioned that the time passed from the last vaccination had significant effects on the levels of anti-hepatitis B antibodies. Marinho *et al.*[28] showed that older and also male staffs showed a weak response to the

vaccination with hepatitis B vaccine. They revealed that the lower responsiveness to hepatitis B vaccines in older children might result from the fading immunity with age. Males had a higher contact with the polluted environment, and they always do harder works. Therefore, the risk of encounter with the HBV is higher in males than females. Higher levels of stress make the immune system of males weaker which decrease the levels of response to hepatitis B vaccine.

However, the main reason for the lower levels of response to the hepatitis B vaccine in our study is unknown, but it seems that difference in the fields of genetics, age, and BMI of students are probable reasons. Unfortunately, Iranian students always use from fast foods. This matter makes them obese, and the high BMI index is a known risk factor for lower levels of response to hepatitis B vaccine.[15-18,29,30] This explanation may be the probable reason for the lower levels of response to the hepatitis B vaccine in students of our study and those of other countries. Baba Mahmoodi[29] reported that smoking and obesity had negative effects on the production of antibodies after vaccination with hepatitis B. Young *et al*.[30] reported that obese individuals (BMI ≥ 30 kg/m²) were significantly more likely to be nonresponders following two recombinant hepatitis B vaccine doses (adjusted odds ratio of 8.75; $P = 0.043$) which was similar to our findings. They also showed that there was no observed association between vaccine responses and age (which was in contrast with our findings), method of contraception, or time from vaccination to antibody measurement. According to the high number of nonresponder students, it seems that obesity is not the only reason for the low levels of response to hepatitis B vaccine. Therefore, it seems that the students of our investigation had a lower capacity for antibody production due to the obesity and also other probable risk factors such as risky behavior such as smoking, presence of kidney disease such as dialysis and also metabolic diseases, and especially diabetes. Therefore, additional researches are needed to found the exact reasons for the low levels of response against hepatitis B vaccine in Iranian students.

CONCLUSIONS

Results of the present study showed that the prevalence of hepatitis B is dropped due to the vaccination programs in infants, children, and high-risk people. We found that response to the immunity against vaccination with hepatitis B vaccine is depending on the BMI, age, and educational level of students. In keeping with this, type of vaccine, site and numbers of injection, type of adjuvant in vaccine, and finally history of students in committing the high-risk behaviors can also reduce the levels of response to the hepatitis B vaccine. Injection of booster and weight loss can

increase the immunogenicity against hepatitis B vaccine in students.

Conflicts of interest

There are no conflicts of interest.

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