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Original Article

Evaluation of Immune Response towards Hepatitis B Virus Vaccination among Vaccinated Students of Ardebil University of Medical Sciences in Iran

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Background: Hepatitis B infection is a major public health problem worldwide. Given that immune response towards the vaccine is not perfect, we aimed to evaluate circumstances of immune response in vaccinated students.
Materials and Methods: In this study, 219 medical students of Ardabil University of Medical Sciences were recruited, who had been administered vaccine series for the first time, and booster doses after one and six months completely. The serum samples were extracted from whole blood of the participants. The concentration of Hepatitis B surface antigen (HBsAg) and anti-HBs antibody (HBsAb) was measured using a commercial ELISA kit.
Results: It was observed that 201 cases (91.8%) out of 219 cases had positive anti-HBs antibody response, and 18 subjects (8.2%) were nonresponsive cases. Level of HBsAb was significantly different between males and females as well as alcoholics and non-alcoholics. None of the cases was identified as positive for HBsAg.
Conclusion: Considering the results of the present and previous studies in other countries, it can be claimed that the mass vaccination has been effective, especially in medical students.

Keywords: Hepatitis B, Immunity, Anti-HBs antibody, HBsAg, Medical students

1. Background

HBV is a DNA virus and a member of *Hepadnaviridae* family. Humans are known as natural hosts for HBV which enters to the liver and crosses through the bloodstream, and it replicatses only in liver cells (1).The World Health Organization (WHO) has reported that approximately 2 billion people in the world have been contaminated with HBV, and about 350 million people suffer from HBV-induced chronic liver disease (2). More than 3% of the general population is affected in Iran, among them 200,000-300,000 cases have chronic hepatitis. However, the prevalence rate of Hepatitis B contamination in overall population of Iran has declined since 1977, but it has still been the most common cause of cirrhosis and hepatocellular carcinoma in Iran (3).

Hepatitis is a medical condition which is defined by the inflammation of the liver and characterized by the presence of inflammatory cells in the parenchyma of the organ. Hepatitis may occur with poor signs or asymptomatically but often leads to jaundice, anorexia, and malaise (4). Hepatitis is known as acute when its involvement lasts less than six months; however, its chronic form happens when the involvement persists longer than six months. Hepatitis viruses are the most common cause of this condition all over the world, but hepatitis can be caused by other elements typified by toxic substances (notably alcohol, certain medications, some industrial organic solvents, and plants) and autoimmune diseases. Excessive alcohol consumption is an important cause of hepatitis and liver damage (cirrhosis). Alcoholic hepatitis usually develops over long lasting exposure to alcohol. Alcohol intake more than 80 grams per day in men and 40 grams per day in women has been associated with the development of alcoholic hepatitis. The combination of HBV contamination and alcohol consumption accelerate the development of the cirrhosis (5).

In 1992, WHO made recommendation to consider Hepatitis B vaccine in the national immunization programs in all highly endemic countries by 1995 and all other countries by 1997 (1). The early and first licensed Hepatitis B vaccines were plasma-derived, which were built of purified HBsAg. In endemic countries, 80% of all the vaccines designed are almost plasma-derived (6). However, the commonly available Hepatitis B vaccines nowadays are produced by recombinant DNA technology (7). The currently used vaccine in Iran is a recombinant vaccine which is produced by cloning the S gene, encoding the HBsAg of virus. This vaccine is typically injected in 3 doses of 20 µg at 0, 1, and 6 months.

Without intervention, 15-40% of the chronic HBV contaminated individuals will develop cirrhosis, end-stage liver disease, hepatocellular carcinoma (HCC), or will require liver transplantation (8). Screening based upon Hepatitis B surface antigen (HBsAg), Hepatitis B core antibody (HBcAb), and Hepatitis B surface antibody (HBsAb) are recommended for surveying at risk individuals, and detection is suggested in order to ensure early diagnosis for treatment and monitoring (9). Because of reduction in anti-HBs level over the time, Infectious Disease Society of America (IDSA) recommends to measure HBs antibody

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levels in all high risk cases after administration of three doses of Hepatitis B vaccine (10).

2. Objectives

The aim of this study was to evaluate the post Hepatitis B vaccination immune response among medical students of Ardabil University of Medical Sciences. Moreover, the effects of behavior and lifestyle of participants were assessed on the quality of antibody response.

3. Materials and Methods

3.1. Study subjects

A total of 219 cases were recruited from medical students of Ardabil University of Medical Sciences, Ardabil, Iran over 2013 and 2015. Students were vaccinated in three doses at the times of zero, one and six months. At the time of sampling for the study, participants were experiencing less than 5 years since the first dose of their vaccination. But in 7 participants, it had taken more than 5 years since the first dose of their vaccination. Individuals had received no immunomodulatory therapy for at least 3 months before they were included in the study. The Human Research Ethics Committees from the Ardabil University of Medical Sciences approved this study. Written informed consent was taken from all the participants. About 10 mL blood of each subject was collected in EDTA-anticoagulated tubes using venipuncture.

3.2. Questionnaire form

A questionnaire was designed containing questions about susceptibility factors which possibly change the immunologic response such as alcohol consumption and the lasted time since last vaccination.

3.3. Preparation of samples

From 5 mLvenous blood of each volunteer, serums were collected through centrifugation at 3000 rpm. The serum samples were then sent to paraclinical laboratory of Imam Khomeini hospital in Ardabil city for further examinations, namely ELISA.

3.4. HBsAg and HBsAb titration measurement via ELISA

Samples were tested for anti-HBs antibody to gain titers of the antibody against HBsAg in order to detect immune response. ELISA test was applied to evaluate the levels of HBsAb and HBsAg in serum samples. Human Anti-Hepatitis B Surface Antigen IgG (anti-HBsAb-IgG) ELISA kit (Cat# 4200; Alpha Diagnostic Intl Inc., San Antonio, TX 78244, USA) for the measurement of anti-HBsAb after vaccination was applied according the manufacture's manuals. After determination of the antibody titer, these titers were categorized into four range groups according to the guidelines as follow: Group I: Non- responders (Titer<10 $mIU.mL^{-1}$); Group II: Low-responders (10≤ Titer <100 $mIU.mL^{-1}$); Group III: Appropriate-responders (Titer100≤Titer<1000 $mIU.mL^{-1}$); Group IV: Excellent-responders (Titer≥1000 $mIU.mL^{-1}$). Antibody titration levels ≥10 $mIU.mL^{-1}$ were considered as acceptable immune response and those levels of<10 $mIU.mL^{-1}$ were not acceptable. In cases in which the antibody responses were not acceptable, HBsAg titers were detected using Hepatitis B Surface Antigen (HBs-Ag, native or recombinant) ELISA Kit (Cat# 4110; Alpha Diagnostic Intl Inc., San Antonio, TX 78244, USA).

3.5. Statistical analysis

Data analysis was conducted using SPSS software version 21 (SPSS, Chicago, IL, USA). Scale variables were calculated for normality of distribution using the Kolmogorov–Smirnov test. By using the independent sample t-test, comparing the continuous variables of the groups were carried out. If the variables were not normally distributed, Mann-Whitney nonparametric test was conducted. To depict data by graphs, the GraphPad Prism version 5.00 for Windows (GraphPad Software, La Jolla California USA, www.graphpad.com) was used. All results were expressed as mean ± standard deviation (SD), and statistical significance was set at 5%.

4. Results

Of all the 219 participants, 71 cases (32.4%) were male and the remaining 148 volunteers (67.6%) were female. Mean age of male and female individuals were 22.2±2.7 and 21.3±3.4, respectively. Among subjects, 4 (1.8%) and 37 (16.9) cases were alcoholic and smoker, respectively. None of the subjects had a family history of immune based disease. It was observed that 201 cases (91.8%) out of 219 cases had positive anti-HBs antibody response, and 18 subjects (8.2%) were nonresponsive.

Among the subjects, 18 individuals (8.2%) were categorized into Group I (Non-responders), 36 individuals (16.4%) were categorized into Group II (Low- responders), 146individuals (66.7%) were categorized into Group III (Appropriate-responders), and finally 19 individuals (8.7%) were categorized into Group IV (Excellent-responders). The mean concentration of HBsAb for each group was 5.1±2.3, 54.66±4.3, 419.31±11.85, 1106.65±48.98, respectively. Moreover, none of the non-responder subjects were positive for HBsAg.

The mean antibody titer in males and females were 395.72±46.33 and 397.33±27.85, respectively. No significant difference was observed in antibody concentration between males and females (P=0.871; Table 1, Figure 1.A).No significant difference was observed in HBsAb titration level between smokers and non-smokers (P=0.151; Figure 1.C), while alcoholics (215.41±23.52) demonstrated decreased HBsAb concentration in comparison to non-alcoholic (577.59±41.33) individuals (P<0.001; Figure 1.B). Subjects with less than 5 years since the last vaccination (442.75 ± 21.7) developed significantly (P<0.001; Figure 1.D) more levels of HBsAb in comparison to those with more than 5 years since the last vaccination (350.25 ± 25.4).

Table 1. Comparison of anti-HBs antibody titration among various study groups.

Variable		Number	Mean anti-HBs antibody titer(mIU/mL)	*P*-value
Sex	Male	71 (32.4)	395.72 ± 46.33	0.871
	Female	148 (67.6)	397.33 ± 27.85	
Alcohol consumption	Yes	4 (1.8%)	215.41±23.52	<0.001
	No	215 (98.2%)	577.59±41.33	
Smoking	Yes	37 (16.9%)	384.55 ± 14.31	0.151
	No	182 (83.1%)	408.45 ± 34.11	
Time since last vaccination	>5 years ago	7 (3.2%)	350.25 ± 25.4	<0.001
	<5 years ago	212 (96.8%)	442.75 ± 21.7	

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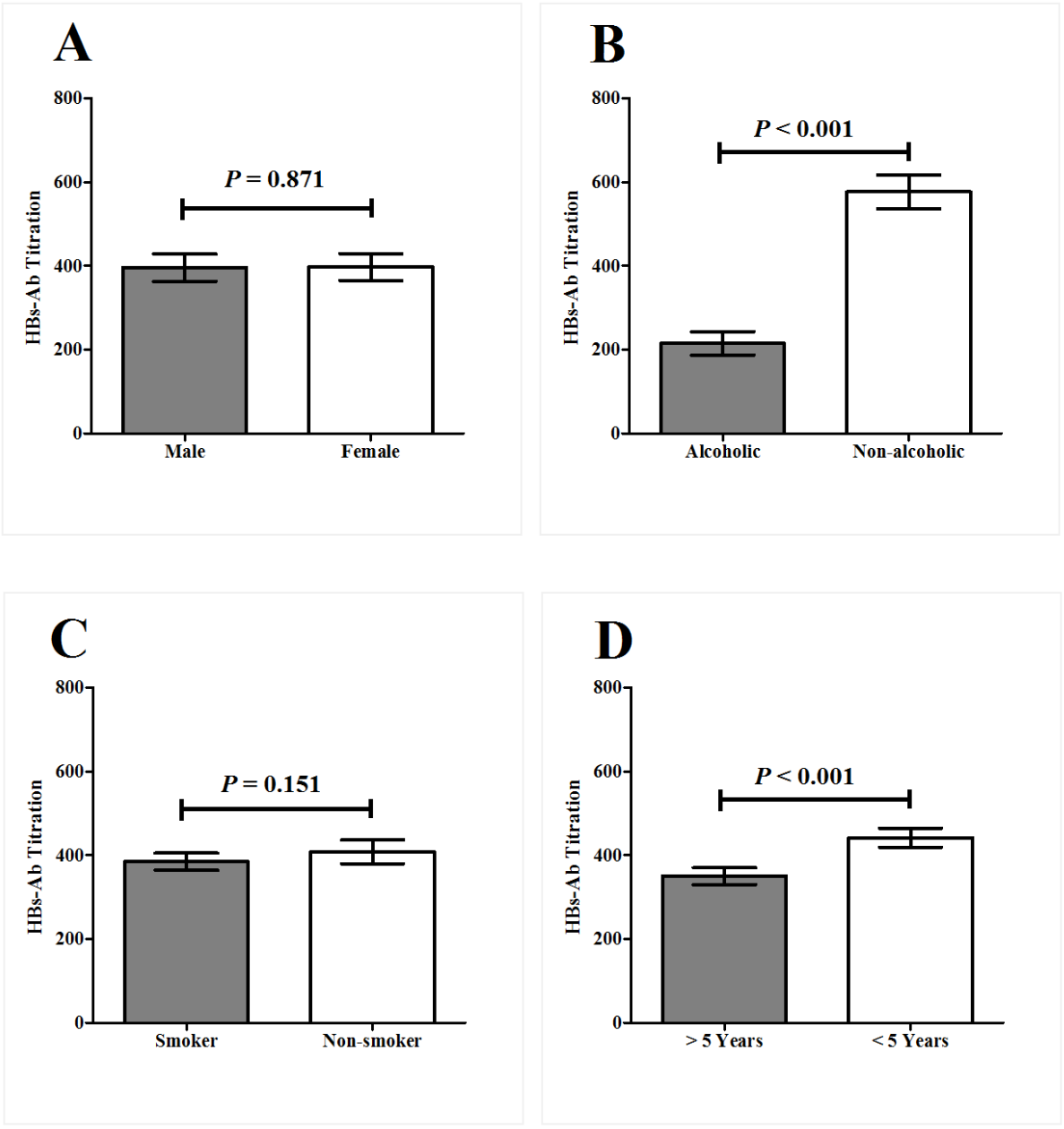

Figure 1. Comparison of antibody titration between various groups of (A) males and females, (B) alcoholics and non-alcoholics, (C) smokers and non-smokers, and (D) subjects with less and greater than 5 years since last vaccination.

5. Discussion

HBV infection is a universal challenging health issue, and HBV is one of the oncogenic viruses which are vaccine-preventable. HBV vaccine induces anti-HBs antibody response which can prevent HBV infection. Prevention of primary infection by vaccination is an important strategy to decrease the risk of chronic HBV infection and its subsequent complications. Studies have shown that childhood vaccination significantly could reduce the rate of chronic HBV infection (11). Medical students are high-risk group to be contaminated by HBV(12).

In our study, 91.8% of the subjects had protective level of anti-HBs antibody (Titer≥10 mIU.mL^{-1}). This result is almost similar to that of other studies performed globally as well as in Iranian adults (12, 13). The standard immune response rate towards HBV has been declared approximately 91.3% among general population (14). In a study on 89 medical students, 100% of the subjects had adequate antibody response after 6 months from the injection of the second dose of HBV vaccine (15).

In this study, it was found that 18 subjects (8.2%) did not develop protective antibody response to vaccination. The quality and quantity of immune response (Ir) to an antigen, which is recognized by T-lymphocytes, is regulated genetically, mainly through Major Histocompatibility Complex (MHC) associated Ir genes (16). Binding of peptides to MHC Class I and II molecules demonstrates a specific status ofparticularity. Some peptides are bound with higher affinity to the products of certain alleles, and this differential binding is important for the induction or suppression of an

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immune response to a specific antigen.. It has been shown that an individual's immune responsiveness to HBV is under genetic MHC control, and the course of the infection seems to be influenced by one's HLA phenotype (17, 18). On the other hand, human MHC-related Ir genes may be operative in regulating the T-dependent humoral antibody and T-cell mediated immune response to HBsAg (19). On the other hand, human MHC-related Ir genes may be active in regulating the T-dependent humoral antibody and T-cell mediated immune response to HBsAg (19). In a study, wide application of the HBV vaccination showed that a low percentage of the vaccinated people didn't raise protective antibodies (20).

In our study, no difference was observed in HBsAb titration between males and females. This observation was in accordance with the previous studies (21, 22) in which the influence of gender on vaccine response was not seen. Moreover, our study demonstrated similar results with Alavian *et al.* (23), as students with longer interval between their last vaccination and the first vaccination at the time of the study revealed lower level of antibody titration in comparison to those who their vaccination had been accomplished less than five years before the first vaccination at the time of the study. This observation is because of humoral immune system responses that had existed previously. Humoral arm of adaptive immune system is the specific defense against bacteria and viruses that cross through the body fluids. In this kind of immunity, the B cells (B lymphocytes) play the main role. When B cells respond to the antigen for the first time, they are activated, proliferated, and finally differentiated into plasma cells and memory B cells. Plasma cells produce and secret a protein combination that are called antibody. After the first antigen recognition, memory B cells also recognize the same antigen and mostly differentiate to plasma cells, and less to memory cells, resulting in further antibody production in larger amounts and more velocity (24).

The HBsAb titration was not significantly different between smoker and non-smoker subjects. However, nonalcoholics had developed powerful antibody response against vaccination. Cigarette smoking, heavy alcohol consumption, and HBsAg have been independently associated with increased risk of mortality from hepatocellular carcinoma (25). A number of studies has previously reported an association between Hepatitis B viral infection and alcohol consumption (26, 27). A randomized double-blind trial has evaluated the efficacy of a high-dose versus standard-dose Hepatitis B vaccine in alcoholic individuals. It has been reported that high- and accelerated-dose regimen of Hepatitis B vaccination leads to the improvements in the immune response in alcoholic individuals (28).

There are limitations in this study that need to be addressed; the effect of alcohol consumption on the quality and quantity of immune response against vaccine is dose-dependent. We did not have information about the dose of alcohol intake in the alcoholic cases of study subjects. Hence, we conclusively report that nonalcoholics had developed high-quantity response against vaccination compared with alcoholic subjects. Furthermore, considering the little number of alcoholic cases in our study subjects, we could not conclude strongly about the impression underlying alcohol intake on the development of antibody response.

6. Conclusion

The results of the present study show that immunogenicity to HBV is not perfect and decreases with the progression of time lasting from the third dose of vaccination. Alternately, alcohol intake significantly reduces the quantity of antibody response. According to the results, we suggest that the anti-HBs antibody evaluation to be conducted after the completion of vaccination program to ensure the attainment of adequate protective antibody levels. It would be useful for management of health policy and programs, although conducting other studies will be necessary to reach to the vigorous conclusions in Iranian population.

Conflict of Interests

There is no conflict of interest to declare.

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Authors' Contribution

Ebrahim Falah Khoshkholgh developed the protocol, performed the experiments, analyzed the data, and wrote the manuscript. Behrouz Mikailpour Ardabili interpreted the data and wrote the manuscript. Shahram Habibzadeh developed the original idea of the study and financially supported the investigation. Firouz Amani guided the data analysis. Effat Seyed Hashemi, Masoome Mamlooki, and Zahra Bakhshandeh participated in performing the experiments of the study.

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