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Study on Anti-Hepatitis B Surface Antibody Titer and Specific Interferon Gamma Response Among Dentists

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Abstract

Background: Hepatitis B virus (HBV) is a major problem for healthcare workers worldwide, and among them, dentists are at risk of acquiring HBV infection. The prevalence of HBV infection has been reported among the dentists in different regions of the world. Since none of the available drugs can clear HBV infection, the presence of effective immunity against HBV infection is important to prevent HBV infection.

Objectives: This study aimed at determining HBs antibody and specific HBV gamma interferon among the dentists, who received hepatitis B vaccine.

Methods: The blood samples were collected from 40 dentists, including 7 endodontics, 2 oral and maxillofacial radiologist, 4 periodontics, 11 oral and maxillofacial surgeons, 6 implantologists, 3 orthodontics, 1 oral and maxillofacial pathologist, 2 esthetic and restorative dentists, and 4 doctors of dental surgery (DDS) at from dental college of Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran during December, 2013. Overall, 31 (77.5%) dentists had already received 3 doses of recombinant hepatitis B vaccine, and 9 (22.5%) had received only two doses of the vaccine. Their sera were tested for HBsAb and anti-HBc-IgG by the Enzyme Linked Immunosorbent Assay (ELISA) test. The lymphocyte of individuals was separated from their blood sample by Ficoll-Hypaque, cells were washed with phosphate buffered saline (PBS) by centrifugation, and finally the pellet cells was resuspended in RPMI-1640 media. Separated cells were exposed to 2.5 μg of purified recombinant HBs antigen, and supernatants were collected after 72 hours and tested for detection of specific interferon γ level by ELISA test.

Results: Overall, 97.5% of dentists showed positive HBs antibody test results while 36 showed (90%) positive test results for specific interferon γ against hepatitis B virus infection.

Conclusions: High coverage of 97.5% immune response against hepatitis B infection was found, indicating high efficacy of recombinant HBV vaccine among the dentists.

Keywords: Hepatitis B Virus, Hbsab, Interferon Gamma, Health Care Workers

1. Background

Hepatitis B virus (HBV) is a major public health problem worldwide. It is a DNA virus, from the family Hepadnavirus. Estimates indicate that more than two billion of the world population have been infected with HBV, and more than 350 million have chronic hepatitis B virus (HBV) infection (1). Hepatitis B virus accounts for acute and chronic hepatitis, the persistence of HBV may result in cirrhosis and Hepatocellular Carcinoma (CHC) (2). The prevalence of HBV infection varies from 0.1% to 15.0% in different parts of the world (3). The prevalence of HBV in Iran is varies from 1.6% to 5%, and Iran has been classified as an intermediate endemic area (4). Dentists can occupationally become infected with HBV through needle sticks or percutaneous and mucosal exposure to blood and other body fluids (5). Hepatitis B virus remains a prominent agent of morbidity and mortality among the health care workers worldwide (6). The most effective way to prevent HBV in-

fection is vaccination in order to stimulate the production of an-tibodies of anti-HBs. Antibody protection for the general population is recognized with a titer of > 10 mIU/mL (7). Individuals with an anti-HBs titer < 10 mIU/mL are defined as non-response, and those with anti-HBs titer > 10 and < 99 mIU/mL are defined as hypo responders, they usually show shorter period of detectable antibody, called "Waning Antibody" or "Waning Immunity" (8).

Another effective form of immunity against hepatitis B virus infection is the specific Interferon gamma (IFN-γ) (9). The humoral HBsAb response contributes to the clearance of circulating virus particles and the prevention of viral spread within the host, whereas the presence of cellular immune response eliminates infected cells (10). The expression of antiviral, Th1 cytokines, such as Interferon gamma (IFN-γ), and Tumor Necrosis Factor Alpha (TNF-α) can control Hepatitis B infection (11). Hepatitis B vaccine can trigger the immune system to produce both HBsAb and specific HBs gamma interferon (12). Thus, the present study was conducted to determine anti-HBs antibody and specific interferon γ response in dentists, who received HBV vaccine.

2. Methods

The blood samples were collected from 40 dentists, including 7 endodontics, 2 oral and maxillofacial radiologist, 4 periodontics, 11 oral and maxillofacial surgeons, 6 implantologists, 3 orthodontics, 1 oral and maxillofacial pathologist, 2 esthetic and restorative dentists, and 4 doctors of dental surgery (DDS) at dental college of Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran, during August to December, 2013. The serum of each individual was tested for anti-HBs antibody titration and anti-HBc antibodies using the enzyme linked immunosorbent assay (ELISA) kit (Diapro, Italy).

2.1. Separation of Peripheral Blood Mononuclear Cells From Blood

An amount of 5 mL of fresh blood sample from each participant was collected in a tube containing EDTA. Next, 3 mL of Ficoll-Hypaque (Baharafshan, Iran) was added slowly over the blood sample, followed by centrifugation at 2500 RPM for 20 minutes. The mononuclear cells were collected and washed with PBS buffer to remove any Ficoll residue. The cells were resuspended with RPMI 1640 medium, 200 μL of each sample containing 10^7 cells, 2 mmol/L 1-glutamin, 1 mmol/L sodium pyruvate, 100U/mL penicillin, 100 U/mL streptomycin, and amphotericin B 2.5 μg, and then 2.5 μg of purified recombinant HBs antigen was added to each well of the 24-well plate (Nunc, Denmark). The plate was incubated with 5% CO_2 at 37°C for 72 hours. The supernatant was then collected from each well and tested for detection of interferon γ by ELISA (eBioscience, Vienna, Austria), according to the manufacturer instructions.

The proposal of this study was approved by the ethic committee of the Ahvaz Jundishapur University of Medical Sciences. Consent was obtained from each participant registered in this study.

2.2. Statistical Analysis

Data are presented as means, standard deviations (SD), and percentages. The data were analyzed by SPSS, version 15. The t test was performed to compare age contraction between the two genders. Chi-quare test was used to determine the homogeneity proportion among different age groups.

3. Results

Overall, 13 out of 40 (32.5%) participants were female and 27 (67.5%) were male, and mean age of participants was 37.74 $\pm$ 6.6 years. The youngest was 25 and the oldest 50 years. The elapse time of vaccination varied from 1 to 20 years, with mean of 8.22 $\pm$ 3.64 years. The sera of 39 (97.5%) participants showed negative HBc IgG test results; only 1 (2.5 %%) was positive for anti-HBc antibodies with low anti-HBs titer < 10 mIU/mL. In total, 32 (80%) of the subjects had received 3 doses, 6 (15%) had received 2 doses, and 2 (5%) had received 1 dose of the vaccine. The rate of humoral antibody response against HBV vaccine is presented in (Table 2).

Table 2 reveals that the distribution of HBsAb titer below 10 mIU/mL, 10-100mIU/mL and above 100mlU/mL among males and females was not significant (P = 0.42).

Table 3 shows the high rate of positive and negative IFN/gamma among different age groups; the table shows that positive cases were found within two age groups of 20 to 29 year-olds and 30 to 39 year-olds, and negative cases were found in 40 to 49, and > 50 year-old group (P = 0.000).

Table 4 shows that the number positive and negative IFN- γ among males and females was not significant (P = 0.702).

4. Discussion

Dentists are a high risk group exposed to HBV infection because of their routine work with sharp instruments in exposure-prone procedures (6). Thus, periodic examination of the level of immunity against HBV infection in dentists has been recommended (7-9, 13). The level of anti-HBs titer among dentists has been reported in Iran. In a study

Table 1. Number of Doses of Vaccine According to Age and Gender[a]

Age Group	Male	Female	Frequency	Receiving 3 Doses Vaccine	Receiving 2 Doses Vaccine	Receiving 1 Doses Vaccine
20 - 29	3 (7.5)	7 (17.5)	10 (25)	10 (25)	-	-
30 - 39	17 (42.5)	5 (12.5)	22 (55)	22 (55)	-	-
40 - 49	5 (12.5)	1 (2.5)	6 (15)	-	6 (15)	-
> 50	2 (5)	-	2 (5)	-	-	2 (5)
Total	27 (67.5)	13 (32.5)	40 (100)	22 (80)	6 (15)	2 (5)

[a]Value are expressed as N. (%).

Table 2. Titration of HbsAb Among the Male and Female Dentists[a]

Variable	HBs Ab Titer mIU/mL			Odd's Ratio (CI 95%)	P Value
	< 10	**10 - 100**	**> 100**		
Gender					
Male	1	5	21	0.536, 0.116 - 2.47	0.42
Female	-	4	9		
Total	1 (2.5)	9 (22.5)	30 (75)		
Age group					
20 - 29	-	0	10 (25)	$X^2 = 27.061$, df = 2	0.000 a[b]
30 - 39	-	2 (5)	20 (50)		
40 - 49	-	6 (15)	0		
> 50	2 (10)	-	-		

[a]Value are expressed as N. (%).
[b]The P-value related to rate of anti HBs 10 to 100 and 100 to 1000 IU/mL, among the age group was significant; this indicates high protection among the age group of 20 to 29 years while lower protection was found in the age group of 40 to 49(P = 0.000).

Table 3. Distribution of Interferon Gamma Among Different Age Groups[a]

Age group	IFN-γ		
	Positive	**Negative.**	**P Value**
20 - 29	10 (25)	-	0.000
30 - 39	22 (55)	-	
40 - 49	3 (7.5)	3 (7.5)	
> 50	-	2 (5)	

[a]Value are expressed as N. (%).

conducted by Joukar et al. (2016), on 1010 HCWs, who had received the hepatitis B vac-cine, 91 (9%) subjects showed non-protective anti-HB levels (9% of all HCWs) (14). In our study, the sera of 39 out of 40 (97.5%) subjects showed positive anti-HBs antibody, indicating the high efficacy of HBV vaccine against HBV infection. Only 1 (2.5%) dentist showed a low anti-HBs antibody titer of < 10 IU/mL; positive test results for anti-HBc antibodies revealed previous contact with HBV infection, however, further investigation for detection of HBVDNA by real time Polymerase Chain Reaction (PCR) or nested PCR is required. Sarmast et al. reported (2015) on 22 (56.4%) health care workers, who had received their last dose of vaccine 6.6 $\pm$ 4.3 years ago with a titer of HBsAb above 100IU/mL. Seventeen (43.6%) subjects, who had received their last dose of vaccine 10 $\pm$ 4.06 years ago, exhibited HBs titers lower than 100 IU, and 3 (7.7%) health care workers were positive for HBc-IgG and HBsAb, yet, negative for interferon γ (15). in the present study, the elapse time of vaccination varied from 1 to 20 years with mean of 8.22 $\pm$3.64 years, which are in accordance with findings reported by Sarmast et al. (15).

In our study, 20 to 39 year-olds showed 75% anti-HBs titer > 100 IU/mL, while the age group of > 40 years exhibited 25% anti-HBs titer < 100 IU/mL. In our previous study, the age group of 30 to 39 year-olds also showed 63.6% anti-HBs titer while the group of 20 to 29 year-olds displayed 37.5% anti-HBs titer (15). There was no significant difference in the rate of high anti-HBs titer (> 100 IU/mL) between fe-

Table 4. The Rate of Interferon Gamma Among Males and Females[a]

Gender	Positive IFNγ Pgr/μL	Negative IFN Pgr/μL	Odd's Ratio (CI 95%)	P Value
Male	24 (60)	3 (7.5)	1.445, 0.212 - 9.984	0.702
Female	11 (30)	2 (5)		
Total	35 (87.5)	5 (12.5)		

[a]Value are expressed as N. (%).

males and males (P > 0.05).

In-terferon γ was found to play an important role in the prevention of HBV infection in the presence of low titer of HBsAb (16). Bertoletti et al. (2009) described an increase in interferon γ expression in accordance with CD8 and CD4 T cell level and complete virus clearance (17). Dimitropoulou et al. (2013) indicated that the increase in interferon γ concentration leads to a decrease in serum hepatitis B viral load. This means that hepatitis B viral load and interferon γ level have a negative correlation (18).

In our study, 87.5% of dentists showed positive specific IFN-γ while 12.5% of the dentist had a negative IFN-γ response. In the present study, 5 (12.5%) dentists, who received 2 doses of HBV vaccine, showed negative interferon γ response. It is estimated that about 5% to 7% of the population are non-responsive to HBV vaccine. It has been found that HLA antigens, such as A1, B15 A2, B8, and B54 show negative effects on vaccination outcome, especially on Interferon γ (19). In our study, HLA antigens had not been investigated among dentists, and further investigations in this regard is required.

In conclusion, high coverage of 97.5% anti-HBs antibody and 87.5% specific INF- γ response have been found among the dentists, who received three doses of HBV vaccine, although, a booster dose of HBV vaccine requires individuals to have an anti-HBs antibody titer of < 100 IU/mL. Finally, the recombinant HBV vaccine was found to have good humoral as well as cell-mediated immunity against hepatitis B infection.

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Footnotes

Authors' Contribution: Manoochehr Makvandi obtained funding for the study. Dawood Khalafkhani and Rahil Nahid Samiei wrote the manuscript. Mojtaba Rasti and Shahram Jalilian performed the experiments. Nasrin Rastegarvand, Toran Shahani and Abdulnabi Shabani collected the samples. Mehrdad Sadeghi Haj and Mohammad Karimi Babaahmadi performed the statistical analysis. Mehran Varnasari prepared the manuscript. Mohammad Hosein Sarmast and Ahmadi revised and edited the manuscript.

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