Original Article

Safety and Immunogenicity of Hepatitis B Vaccine: a Study on Iranian Navy Personnel

Hanie Mahdiani[1], Delaram Doroud[1*], Mohammad Hosein Haghparvar[2*], Seyed Nezameddin Hoseini[1]

1 Department of Quality Assurance, Production and Research Complex, Pasteur Institute of Iran, Tehran, Iran.
2 Shahid Mahdavi Clinic, Navy Medical Corps Command of Islamic Republic of Iran, Tehran, Iran.

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ABSTRACT

Introduction: Preventing communicable diseases in the armed forces (AF) of a country is considered as a national interest and has a great importance. There are limited data on the efficacy of vaccination of the navy personnel. This study was designed to evaluate both the safety and the immunogenicity of a recombinant hepatitis B (HB) vaccine made by Pasteur Institute of Iran (IPI-rHB), in this target group. **Methods:** In this study, all members of two navy units were enrolled. After measuring their amount of primary antibody, the subjects were selected for vaccination. Finally, 108 male volunteers were surveyed who were all between 25 to 45 years of age. Three doses of IPI-rHB vaccine (1 ml containing 20µg of the recombinant antigen) were administered to the subjects who were serologically negative for HB markers. The vaccination was given intramuscularly via the deltoid muscle according to the 0, 1 and 6 months schedule. The subjects were carefully monitored to record any adverse reaction of the vaccine. Blood specimens were collected from each subject, 1 month after the second and third vaccinations, in order to determine the anti-HBs antibody response to the vaccine. The obtained serum was tested by ELISA to quantify anti-HBs antibodies raised in each subject. **Results:** The results showed that local pain, fever, erythema, induration, nausea, vomiting and headache were the significant side effects noted. Protective antibodies (anti-HBs) were detected with seroconversion and seroprotection rates of 52.9% and 47.1%, respectively after the second dose and seroconversion and seroprotection rates of 100% and 93.51% after the third dose of the vaccine. After completing the vaccination; in 101 (93.51%) of the participants, antibody level was greater than 10 mIU/ml and in 7 (6.48%) subjects, the antibody level was between 1 and 10 mIU/ml. After the vaccination, the geometric mean titer of anti-HB antibody was 132.85. The seroprotection rate was 93.51% among the vaccinated population and 6.48% of the participants showed seroconversion. In this study, 22 (20.37%) subjects had anti-HBs titer of 10-100 mIU/ml and 79% (73.15%) subjects had anti-HBs titer of greater than100 mIU/ml. Although immunity to HB was achieved to a protective level among all the participants, the assessment of the long term immunity in AF members after complete vaccination is recommended. **Conclusion:** the results emphasized the importance of HB vaccination in adults, especially the AF members. Moreover, it reinforced the fact that three doses of HB vaccine is necessary to increase the seropositivity rate of anti-HBsAg in this group.

KEYWORDS: Recombinant hepatitis B vaccine, immunogenicity, safety, seroprotection, armed forces

INTRODUCTION

Hepatitis B virus (HBV) infection is a global health problem which plays a significant role in endangering the public health [1, 2]. In Iran, hepatitis B (HB) infection has an intermediate endemicity [3] and the transmission pattern of the infection is mixed. The disease occurs at all ages [4] and 3-8% of the population are chronic carriers [5, 6].Chronic carriers are positive for HB antigens for years but they are negative for HB antibody. HB antibody will be raised in situations like previous infections, recovery from the infection and also when the immunity is acquired by HB vaccination. Although much progress has been made in medical sciences, there is still no effective treatment for HB infection. Therefore, immunization by means of recombinant vaccination is necessary to prevent the clinical disease, the progress of the carriers and the transmission of the HBV to high risk persons. To prevent HBV

***Corresponding Author:** Delaram Doroud, Ph.D; Quality Assurance Department, Production and research Complex, Pasteur Institute of Iran25th Km Tehran-Karaj Highway, 3159-91511.
Email: d_doroud@pasteur.ac.ir, d_doroud@yahoo.com
Tel/Fax: (+98) 2636100967
***Co-Corresponding Author:** Mohammad Hosein Haghparvar, Ph.D; Shahid Mahdavi Clinic, Navy Medical Corps Command of Islamic Republic of Iran.
Email: m.haghparvar@gmail.com
Tel/Fax: (+98) 21 77131204

infection, vaccination during the first year of life remains the most effective practice.

The high cost of available vaccines is a major limiting factor for a universal vaccination strategy, especially in the developing countries. Since IPI recombinant HB vaccine (IPI-rHB) is being manufactured according to Cuban Hebervac® technology transfer, the safety and immunogenicity of the vaccine has been previously established [7-9]. On the other hand, being used for many years in the national immunization program, the efficacy and safety of the vaccine is confirmed by Iranian national regulatory authorities.

Up to our knowledge, there are few reports about the immunity level and HBV immunity status in vaccinated Iranian military recruits [10, 11] and also other countries [12]. Hospitalizations and histories of blood transfusions were the main risk factors for HBV infection in this group [13]. Immunity to HBV and its proper immunization programs guarantees the health status of the military recruits in every country and maintains their ability to accomplish their missions. Therefore in this study, with regard to military health importance, our target group was selected from the navy personnel and we determined their serum HBs antibody levels and HBV immunity status, following HB vaccination. This is the first study on evaluation of safety and immunity level to HBV in a vaccinated army group in Iran. The results of this study may lead to better planning of future studies, investigating the efficacy (immunogenicity) and safety of this vaccine in other demographics of Iranian population.

MATERIALS and METHODS

This study was carried out in summer of 2013 at Shahid Mahdavi Medical Center (Navy Medical Corps Command). A total of 140 men from the navy personnel were evaluated, among them 108 healthy men, aged between 25 and 45 years, were admitted and enrolled in the study (Protocol No. 6345). All participants provided informed consent, allowing access to their HB antibody titer results. This study was approved by the appropriate ethics committees. All the subjects were asked to complete a self-administered, anonymous questionnaire containing: age, body mass index (BMI) and the time elapsed since their last vaccination. Heights and weights were measured to the nearest 0.1 cm and 0.1 kg, respectively, by the research team. BMI calculated as weight in kg divided by squared height in metric system (i.e. kgm-2) was determined for each subject. HBs-Ab titers were determined by enzyme linked immunosorbent assay (ELISA) using Bioelisa anti-HBs (Biokit, CE-0843, Spain). Based on their serum HBs-Ab levels, the subjects were classified as non-immune (less than 10 mIU/ml); moderately immune (between 10 and 100 mIU/ml) and fully immune (more than 100 mIU/ml) populations.

The participants who were HBsAg positive as well as subjects who did not receive HB vaccine or those who had incomplete vaccination against the HBV and subjects who had no tendency to enter this study were excluded. All of the collected data were entered in statistical software program SPSS (version 11.5) and were analyzed by descriptive statistics, chi-square and t-test. Other exclusion criteria applied to the subjects were history of prior exposure to HCV, history of blood transfusion and the presence of any systemic disease such as chronic renal failure, congestive heart failure, bleeding diathesis and malignancies.

All participants showed seronegativity for HBsAg and the screening tests were performed for markers of Anti-HBsAg by commercial diagnostic immunoassay kits. The primary amounts of anti-HBs antibodies were measured in order to exclude seroprotected subjects. The presence of Anti-HBs titers equal to 1 mIU/ml was considered as seroconversion and titers greater than 10 mIU/ml as seroprotection. Therefore, individuals with antibody titers greater than 10 mIU/ml. were excluded from the trial. Among the 108 cases, the blood sample of 86 subjects had no antibodies against HB and antibody levels in 22 patients between 1 and 10 mIU/ml suggested that they had higher levels than seroconversion level.

Vaccination protocol

The WHO classic HBV vaccination protocol was conducted on the 108 above-mentioned subjects which constituted of 3 doses of HB vaccine (14, 15). The first dose was administered for all individuals (time zero) and other two doses were administered at 1 and 6 months after the first dose. For all subjects, 1 ml of the vaccine containing 20 µg of IPI-rHB was administered intramuscularly in the deltoid muscle at each time. Subjects were repeatedly visited and tested for evidence of any side effect and asked to report any adverse events during the first week.

One month after the third dose, the serum anti-HBs titer was measured according to the kit' instruction using a 96-well microplate coated with highly purified HBsAg (ad and ay subtypes).

Test results after one month of vaccination were interpreted as follows:

- Seroconversion; presence of detectable anti HBs antibody greater than 1 mIU/ml
- Seroprotection; presence of detectable anti HBs antibody greater than 10 mIU/ml
    - Moderate responder; anti-HBs antibody titer of 10-100 mIU/ml
    - Good responder; presence of anti HBs antibody greater than 100 mIU/ml
- Unsatisfactory response; absence or presence of anti HBs antibody less than 1 mIU/ml
- hypo-response; presence of anti HBs antibody less than 10 mIU/ml

Vaccine safety estimated with the following concepts:

- Reactogenicity; nature and incidence of reaction or adverse events after each vaccination.
- Local and general symptoms including; fever, rash, redness, fatigue, headache, body ache, allergy, nausea and vomiting after each vaccination.

RESULTS

The mean age (±SD) of 108 male navy personnel enrolled in the study was 35±10 years. The subjects' characterizations are summarized in the Table 1. P value<0.05 is considered statistically significant using Pearson-chi square test.

According to serologic tests results, all participants showed seroconversion (geometric mean titers (GMT)=132.85). Among them, 101 subjects (93.51%) showed seroprotection (GMT=166.67) while 101 subjects, 79 (73.15%) were good responders and 22 (20.37%) were moderate responders. Mean age of the subjects in moderate responder group was 35±10 years and in the good responder group was 33±10 years (P>0.05). Furthermore, 83 subjects had normal weight

(18.5<BMI<24.9). Amongst them, 79 (95.18%) were seroprotected.

As shown in Fig. 1, the majority of the subjects were categorized as good responders. Therefore, the efficacy and immunogenicity of the vaccine and the vaccination protocol was well proven. There was no significant association between BMI and HBs-Ab level (Table.1.P>0.05).

Safety analysis was conducted on all 108 subjects. Overall, the vaccine was well tolerated. The main adverse effects that were followed for a short period of time included vomiting, diarrhea, fever, coughing, breathing problems, blocked nose, flu, stomach cramps, sneezing and irritability. Three vaccinated subjects showed a slight increase (mean 0.7ºC) in the body temperature on day 4. Because of the late onset of this slight fever, it was suggested by the clinical team that there was no relation between the vaccination and the fever. Erythema (average diameter of 3.6 cm) was not reported to appear around the injection site in any of the individuals. Headache was reported in 2 cases but the rate of headache was not significant (P>0.05). Other side effects like local pain, induration, nausea and vomiting were not observed in the subjects. All other post-vaccination reactions (if any) were mild or moderate and resolved without complications. No serious adverse effect was reported.

Table. 1. Characteristics of the subjects.

	Group individual	**unit**
Age (mean)	35±10	years
BMI (mean)	24±4	Kg/m^2
Gender	100% Male	Male/Female
Habitual History	-	Cigarette Alcohol Opium
Tattoo	-	
Diabetes	6	
HIV infection	-	

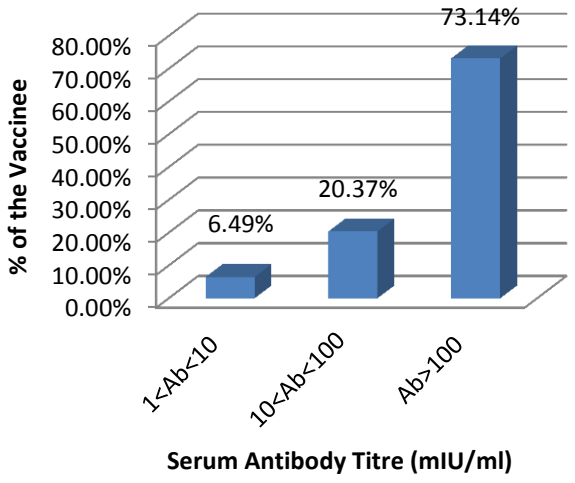

Fig.1. Antibody response [antibody titer (mIU/ml)] shown in percentages after administration of IPI-rHB vaccine to the subjects.

DISCUSSION

The Advisory Committee on Immunization Practices (ACIP) has issued recommendations for HB vaccination in adults who have known risks for acquisition of HB infection, populations where HB infection would have significant health impact, including persons with chronic liver disease or HIV infection. Also in health care evaluations that a high proportion of people have known risk factors for HB infection, the vaccination is recommended [14]. Iranian navy personnel need protection against the communicable infections that they may get exposed to, due to the nature of their activities during the trainings and overseas deployments. Today, HB immunization policy for the military personnel is considered important and HBV vaccination is practiced for enhancing their military readiness.

Post-vaccination HB antibody level testing and evaluations are not routinely performed for adults. In contrast, such testing should be considered for persons who have known ongoing risk for HB exposure, those with known diminished protective response to the vaccine and those whose clinical management depends on the knowledge of their HB immunity status.

The AF personnel are among the populations who are generally at risk for the infection. Therefore for these groups, post-vaccination testing is suggested be done 1-2 months after completion of the vaccination schedule where HB antibody levels of ≥10 mIU/mL is considered protective [14, 15].

This study demonstrated that IPI-rHB vaccine is safe and immunogenic for classic vaccination schedules. In addition, a decidedly immunogenic HB vaccine is desirable because it will reduce the number of non-responders which is a problem with most currently available HB vaccines. On the other hand, such a vaccine is prone to induce a heightened response with persistent anti-HBs titre of over 10 mIU/ml. Although HB vaccine boosting is not usually recommended for healthy adults who have been vaccinated, due to the potential risk of diminishing protective immunity over time and the chance of continued exposure to HB infection, the AF should consider annual testing of their personnel's HB antibody levels with administration of a booster dose of the vaccine when HB antibody levels fall below 10 mIU/mL.

In conclusion, IPI rHB vaccine manufactured in a governmental infrastructure can potentially be used as a low-cost HB vaccine. Therefore this affordable vaccine will help the government to protract HB immunization programs among the high risk populations.

Although the results of our study shows a good response to HBV vaccination in the navy personnel, as a high risk group, the vaccinated subjects are suggested to be monitored for HBs-antibody follow-ups. Furthermore, the continuation of anti-HBV vaccination and its practice in other branches of the Iranian AF is recommended.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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