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Effect of Tetanus-diphtheria Vaccine on Immune Response to Hepatitis B Vaccine in Low-responder Individuals

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ABSTRACT

Background: Conventional hepatitis B virus (HBV) vaccination fails to achieve efficient protection in about 5–10% of the world population. Hence, different strategies have been adopted to ameliorate HBV antibody titers. This study aimed to evaluate the concurrent application of tetanus-diphtheria (Td) and HBV vaccination on hepatitis B surface (HBs) antibody titer in low-responder healthy individuals.

Methods: This was a randomized clinical trial, which was implemented among 140 of medical staff working as health-care workers assumed as low-responders. The subjects were randomly allocated to either control or interventional groups. The control and interventional groups received HBV recombinant vaccine while the latter group was also vaccinated through Td. Enzyme-linked immunosorbent assay was applied to measure HBs antibody (HBsAb) titers just before and 6 months after the last vaccination. All data were entered into SPSS software. Independent *t*-test, paired *t*-test, and Chi-square or Fisher's exact test were applied for data comparison.

Results: Antibody titers of the subjects in the intervention and control groups soared from 49.08 ± 20.08 IU/L to 917.78 ± 204.80 IU/L and from 46.95 ± 18.55 to 586.81 ± 351.77 IU/L, respectively (both $P < 0.001$); nevertheless, by comparison with control group, variation of antibody titer in the interventional group was significantly higher ($P < 0.001$).

Conclusions: Concurrent application of Td and HBV vaccine could effectively enhance protective levels of HBsAb titers in low-responder individuals.

Keywords: Hepatitis B vaccine, low responders, tetanus-diphtheria vaccine

INTRODUCTION

Scientists following load reduction of hepatitis B virus (HBV) infection are still in a controversial dilemma, although many researchers have been implemented.[1] HBV infection commonly occurs in either subclinical or self-limited form; nevertheless, about 5% of the infections results in liver cirrhosis or hepatocellular carcinoma.[2] HBV has developed into chronic infection in about 240 million

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individuals worldwide, and about 1 million die annually due to the consequences of HBV infection.[3-5]

Vaccination has been successfully promoted in the majority of people; therefore, reduction of HBV load has been efficaciously established.[6,7] Nevertheless, vaccination failure in about 5–10% of the world population remains a controversial problem.[1,8,9] Various factors such as obesity, aging, male gender, immunodeficiency, renal failure, intragluteal vaccine administration, chronic diseases, as well as some human leukocyte antigen II (HLA II) molecules and single nucleotide polymorphism in chemokine receptor 5 (CXCR5) and chemokine ligand 13 (CXCL13) significantly contribute to this failure.[10,11] For example, HLA-DRB1*03, HLA-DRB1*07 and HLA-DQB1*02 loci are more likely to be assigned as low-responder for HBV vaccine.[1,12-14] Indeed, after conventional administration of recombinant HBV vaccines (after three injection), antibody response being above 100 IU/L is efficiently protective whereas antibody titers ranging from 10 to 100 IU/L is found in low-responders, who fail to protect themselves when exposure to high doses of HBV occurs.[1,15]

Different approaches have been adopted: Increase in antigen content, and PreS1/PreS2/S antigen application in either second or third generation vaccines; however, more clinical trials should be implemented.[1] Furthermore, other vaccines could efficiently induce protective immune response when they are co-administered with HBV vaccine. For example, Cardell *et al*. documented that combined HBV and hepatitis A virus vaccines resulted in efficient response rate (95–100%) in subjects, who were assigned to either nonresponders or naive individuals.[16] In a novel approach, humeral immunity was evaluated in low-responder dialysis patients when tetanus-diphtheria (Td) vaccine was simultaneously injected with recombinant HBV vaccine.[17] The study showed that Td vaccine could temporarily increase immune response, but hepatitis B surface (HBs) antibody titer did not remain for a long time. Nevertheless, the positive effects of Td vaccine on HBs antibody (HBsAb) titer in healthy individuals are not clear.

Therefore, we aimed to evaluate the concurrent application of Td and HBV vaccination on HBsAb titer in low-responder healthy individuals.

METHODS

Study design and subject

This study was a randomized controlled clinical trial, which was undertaken in Al Zahra Hospital of Isfahan University of Medical Sciences (Isfahan, Iran) in 2013. Iranian Registry of Clinical Trials (IRCT) number of this study was IRCT2014051015999N2 and it was approved by the Ethics Committee of the Isfahan University of Medical Sciences (reference number: 191049). Medical staff of hospital was assumed as our target population provided that their HBsAb titer ranges from 10 to 100 IU/L (low-responder). Importantly, all of these subjects previously received the conventional HBV vaccination schedule (at 0, 1, and 6 months).

Inclusion criteria were: (1) HBs antigen (HBsAg) negative, (2) low-responder individuals receiving three-dose series of recombinant HBV vaccine in a period of 6 months. Not met criteria comprised: (1) Different types of immunodeficiency, (2) corticosteroid therapy, (3) malignancies. One hundred and forty subjects (out of 2700 medical staff), whose medical staff were assessed through convenience sampling method, were selected. These subjects were randomly allocated either in control or interventional group. Each group comprised seventy individuals while they were matched in different variables encompassing age, sex, and vaccination records. All of the individuals were orally informed about the study, and informed consent was obtained for every subject.

Interventions and measurements

All of the subjects in both groups received HBV recombinant vaccine produced by Heber Company in Cuba. Each vial of the vaccine contained 40 μg of major HBsAg. HBV vaccine was administered through intramuscular rout into the deltoid muscle. Every subject, who was assigned in the interventional group, was also vaccinated by 0.5 ml of Td, which was produced in Pasteur Institute in Iran. Indeed, Td vaccination was implemented when the first dose of HBV vaccine was injected. We measured the primary titer of HBsAb just before the vaccination schedule and 6 months after the last vaccination. All of the antibody titers were determined through enzyme-linked immunosorbent assay method, which was implemented through a commercial kit (Medical Biological Service. Italy).

Statistical analysis

All data were entered into SPSS software (SPSS inc., version 18.0, Chicago, IL, USA). Continuous data were presented as mean ± standard deviation and median (range). Kolmogorov–Smirnov and p-p plot tests were performed to evaluate normality. Logarithmic transformation was done for skewed data. HBsAb titers were compared, within the groups, through paired *t*-test. Independent sample *t*-test was used to compare mean differences between groups. Response rate (level >100 IU/L for HBs-antibody titers) was compared through Chi-square or Fisher's exact test. The value of $P < 0.05$ was considered statistically significant.

RESULTS

Subject ages ranged from 22 to 60 years while 70% of them were females. The mean of the antibody titers in low responders assigned to interventional and control groups, just before and 6 months after the last vaccination, were determined. Table 1 shows antibody

Table 1: The levels of antibody hepatitis B surface titers in study groups

	Intervention group ($n=70$)		Control group ($n=70$)	
	Mean±SD (HBsAb [IU/L])	*P*	Mean±SD (HBsAb [IU/L])	*P*
Before vaccination	49.08±20.08 (43.5 [20-95])#	<0.001*	46.95±18.55 (43.5 [20-94])#	<0.001*
6 months after the last vaccination	917.78±204.80 (1000 [148-1000])#		586.81±351.77 (538 [67-1000])#	
Mean differences±SD	868.70±204.02		539.85±351.44	<0.001**

*Resulted from paired samples *t*-test for comparing before and 6 month after vaccination. **Resulted from independent samples *t*-test for comparing mean differences between two groups. #Values are median (range). HBsAb=Hepatitis B surface antibody, SD=Standard deviation

titers in the former and the latter groups. Before the vaccination program, antibody titers of the interventional and control group did not differ significantly ($P = 0.516$) whereas, 6 months after the last intervention, there was a significant difference between the antibody titers of the interventional and control groups ($P < 0.001$). Not only the antibody titers of the interventional group significantly were changed, but also significant variations of the antibody titers were found in control group ($P < 0.001$). Indeed, difference of the antibody titers in the interventional group was significantly higher ($P < 0.001$). Six months after the vaccination program, the antibody levels in all of the subjects allocated to the interventional group were more than 100 IU/L, whereas there was one individual, in the control group, whose antibody titer was <100 IU/L. However, the frequency of the subjects whose antibody titers were more than 100 IU/L did not significantly differ between the two groups.

DISCUSSION

This study revealed that the interventional group who was simultaneously vaccinated by HBV and Td vaccines showed more significant variations of the antibody titers.

Some healthy individuals as well as hemodialysis patients develop insufficient immune responses for HBsAb.[17,18] On the other hand, protective levels of this antibody in health-care workers, who are presumed as a potential high-risk group, are ultimately crucial. Many mechanisms seem to be involved in the insufficient immune responses. For instance, inappropriate antigen presentation, default of perfect repertoire in B and/or T cells, regulatory T cell dysfunction, lack of Th1 and/or Th2 cytokines, as well as B cell killing, which is undertaken by cytotoxic T cells, may be accountable for the insufficient HBsAb production.[19]

Therefore, different approaches aiming at promotion of HBV immune response, such as repeated doses of hepatitis B vaccines, have been under evaluation.[18] The current study was based on application of Td, as an adjuvant, for increase of HBV immune response in healthy individuals employed as health-care workers.

Results of this research showed that repeated vaccination program could efficiently ameliorate the levels of antibody titers. This finding was in accordance with some of the previous results documented revaccination strategy yielded amelioration of HBsAb in nonresponder healthy individuals, whose antibody titers were <10 IU/L.[20,21] Manuela Rosado *et al*. showed that HBV re-vaccination not only augmented memory B cells (from 11.3 in 10^6 cells to 28.2 in 10^6 cells), but also it induced high levels of HBsAb titers (from 2.9 to 284 mIU/ml). It seems that circulating memory B cells may be accountable for the immune response promotion.[22]

On the contrary, in another study, repeated vaccination failed to develop sufficient immune response in rare population of adult healthy individuals working as health care workers. Not only normal frequency of special memory B cells could be found in these subjects, but also *in vitro* stimulation of the corresponding B cells, which was performed through polyclonal antigens associated with special adjuvant (CpG), resulted in antibody production. Therefore, adequate titers of HBsAb production can be potentially achieved if appropriate adjuvant is applied.[10]

This study showed that 40 μg of HBV, which was assumed as a high antigen content, could enhance protective antibody titers in the healthy individuals. In a similar research, which was conducted in Chinese adults, booster vaccination in different antigen contents (10 μg, 30 μg, and 60 μg) was evaluated. The results of this study showed that the vaccine immunogenicity had a direct correlation with the vaccine antigen contents while the highest level of antigen was well tolerated.[21]

The results of this research showed that by comparison with the control group, subjects of the interventional group more frequently developed sufficient immune response although this difference was not significant. Similar results were found when Td vaccine was applied as an immunostimulator in nonresponder hemodialysis patients. To elaborate Shahidi *et al*. showed that Td vaccination led to higher frequency of the subjects efficiently responded to HBV vaccine; however, this higher response rate was not significant.[17]

Furthermore, positive effects of other adjuvants were demonstrated in different studies. Lin *et al*. documented that granulocyte-macrophage colony stimulating factor (GM-CSF) applied with HBV vaccines could

efficiently promote immune responses in the healthy individuals. In fact, 2 months after the third vaccination, significant seroconversion rate (64.7%) was found in subjects, who were treated with 150 μg GM-CSF before the HBV vaccination (20 μg). By comparison, control group, who only received the standard protocol of HBV vaccination (20 μg) had significantly lower seropositive rate (39.39%) ($P = 0.011$).[23]

In this study, comparison with the control group, not only the levels of HBV antibody titers in the subjects treated with Td significantly was higher, but also the interventional group developed much more increases in their antibody titers. The positive effects of Td in immune response promotion were previously documented. To elaborate, in another study, which was conducted by Shahidi *et al*., Td vaccine significantly boosted antibody production in hemodialysis patients.[17]

In addition, efficiency of tetanus toxoid (TT) adjuvant in immune response promotion was evaluated. For example, Sönmez *et al*. applied preS2 and S containing recombinant hepatitis with TT adjuvant in nonresponders for HBV vaccines. They divided their subjects into three groups including healthy individuals, pregnant women, and hemodialysis patients while everyone contained two subgroups, which they differed in TT receiving. This study documented that TT vaccination significantly enhanced seroconversion and antibody titer levels in healthy subjects allocated in each of the above-mentioned subgroups.[24]

CONCLUSIONS

In light of this study, the authors proposed that co-administration of Td and HBV vaccine can result in significant seroconversion and immune response being efficient for HBV protection.

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Conflicts of interest
There are no conflicts of interest

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