ORIGINAL ARTICLE

Effect of tetanus-diphtheria (Td) vaccine on immune response to hepatitis B vaccine in healthy individuals with insufficient immune response

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Background: Hepatitis B virus (HBV) fails to produce appropriate immune responses in some healthy individuals; thus, different strategies have been adopted to promote immune responses. The current study aimed at evaluating the efficacy of HBV vaccine coadministered with tetanus-diphtheria (Td) vaccine compared with HBV vaccine in healthy individuals through measuring hepatitis B surface antibody (HBsAb) levels. **Materials and Methods:** This was a randomized controlled clinical trial, which was implemented in Isfahan, Isfahan Province (Iran) in 2013. One hundred and forty healthy individuals, whose HBsAb titers were less than 10 IU/L were recruited. The subjects were randomly assigned to either in intervention or control trials. The control group received 40 μg of recombinant HBV vaccines intramuscularly injected at 0, 1, and 6 months; however, the intervention group was simultaneously vaccinated by Td with the first dose of HBV vaccine. HBV antibody levels (titer) were measured before the vaccination and 6 months after the last vaccination. **Results:** Antibody titers of the subjects in the intervention and control groups increased from 5.07 ± 2.9 IU/L to 744.45 ± 353.07 IU/L and from 4.45 ± 3.4 IU/L to 589.94 ± 353 IU/L, respectively (both $P < 0.001$). Also, the mean difference of antibody titer was significantly different between the two groups ($P = 0.011$). **Conclusion:** Td vaccination can be applied as a feasible approach to promote efficient and persistent immunity in healthy individuals with insufficient HBsAb titers.

Key words: Hepatitis B surface antibody (HBsAb) titer, hepatitis B vaccine, tetanus-diphtheria (Td) vaccine

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INTRODUCTION

Hepatitis B virus (HBV) is recognized as a causative factor of chronic hepatitis B, which potentially results in cirrhosis, hepatic failure, and hepatocellular carcinoma.[1,2] Epidemiological studies showed that approximately 350 million individuals are diagnosed with HBV, whereas 20% of them who are affected by chronic hepatitis, die from liver-related diseases.[3]

In the USA, HBV prevalence, which is determined through hepatitis B surface antigen (HBsAg), ranges from 0.3% to 0.5%.[3] This low prevalence, which has been endorsed by the World Health Organization (WHO) since 1992, has been ascribed to HBV vaccination. This preventive strategy includes infant vaccination at birth, which is sought by a two-dose vaccine series by 6 months of age.[4-6]

Every vial of vaccine commonly contains 10 μg of antigen; however, this vaccination strategy fails to protect adequately in 5-10% of individuals.[7,8] These percentages vary in accordance with the types of vaccine administrations as well as the patient's characteristics.[8]

Different strategies have been evaluated to promote immune response to HBV vaccine. For example, third

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generation vaccines, which contain Pres1/Pres2/S antigens derived from mammalian cells, could partially enhance immune response.[9] Furthermore, immune response enhanced when Chinese adults were immunized with more antigen contents.[8] In another study, the potency of HBV DNA vaccines delivered with mesoporous silica as core and layered double hydroxide (LDH) as shell was endorsed.[10] Not only some nanoparticles such as glycol chitosan (GC), which were administered by intranasal rout enhanced mucosal immune response but also immunomodulator drugs such as levamisoles induced further seroprotective levels of HBV antibody.[11] Nevertheless, these strategies are complicated and have not successfully passed all of the stages of randomized clinical trials (RCTs).[8-10] Recently, the concurrent application of tetanus-diphtheria (Td) and HBV vaccine have been assessed in nonresponder dialysis patients. This clinical trial showed significantly increased responses in nonresponder individuals.[12] However, the effects of Td and HBV vaccination on the persistent production of hepatitis B surface antibody (HBsAb) are ambiguous. Thus, we aimed to evaluate the positive influence of Td, as an immune stimulator for HBV vaccine, on HBsAb levels in healthy and HBV-vaccinated individuals whose HBsAb titers were under 10 IU/L (individuals with insufficient immune response).

MATERIALS AND METHODS

Study design and participants

This was a randomized controlled clinical trial [Iranian Registry of Clinical Trials (IRCT) number: IRCT2014051015999N2), which was implemented at Alzahra Hospital (Isfahan University of Medical Sciences (IUMS), Isfahan, Isfahan Province, Iran) in 2013. Ethical approval for the study was granted by IUMS (reference number: 191049).

The study population contained the medical staff of Alzahra Hospital assigned as healthy individuals showing insufficient immune responses for HBV vaccines. The age range of the participants was between 22 years and 60 years. The female-to-male ratio was 70:30.

Inclusion criteria were those with negative HbsAg as well as those subjects who had insufficient immune response when their HBV antibody titers were lower than 10 IU/L. Exclusion criteria included malignancies, different types of immunodeficiency, and corticosteroid therapy. One hundred and forty subjects were selected according to inclusion and exclusion criteria through convenience sampling methods and were randomly assigned to the control group and intervention group. Participants in both the groups were matched based on age, sex, and history of HBV vaccination. Informed consent was obtained from each participant.

Interventions and variables assessment

Participants in the control group were vaccinated only against HBV. The vaccination schedule was implemented through Cuban HBV vaccines, which were produced by Heber Company in Cuba. All of these subjects who had insufficient immune responses for HBV vaccines intramuscularly (IM) received 40 μg of the abovementioned recombinant vaccine at 0, 1, and 6 months.

Participants in the intervention group were vaccinated using a combination of HBV vaccine and Td in which the participants in the intervention group were administered with 40 IU of Td with the first dose of HBV vaccine; Td and HBV vaccination were conducted at different sites. The same HBV vaccination protocol was conducted in both the intervention and control groups. HBsAb levels were measured before the vaccination schedules and 6 months after the last vaccination; HBsAb titer was evaluated by enzyme-linked immunosorbent assay (ELISA) using a commercial kit (Diagnostic Bio probes s.r.j, Milan, Italy).

Statistical analysis

Statistical Package for the Social Sciences (SPSS) software (version 18.0, Chicago, Il, USA) was used for the statistical analysis. Quantitative variables were expressed as mean ± standard deviation (SD) and median (range) and normality of variables were assessed using Kolmogrove-Smirnov and P-P plot approaches. For nonnormal (positive skewed), logarithmic transformation was conducted. Antibody titer levels were compared from the baseline assessment to 6 months after intervention using paired samples *t*-test while differences between the groups based on mean differences were evaluated using independent samples *t*-test. *P* values being under 0.05 were considered to be statistically significant.

RESULTS

There was no significant difference in antibody titers of both the groups before the intervention ($P = 0.26$); however, after 6 months, significant increases were observed in both the groups ($P < 0.001$) (see results of within-groups analysis). The observed increments were significantly higher in the intervention group in which the mean differences (changes from baseline values) were notably higher in Td combination with the HBV vaccination group ($P = 0.011$) [Table 1].

DISCUSSION

This study showed that Td vaccination can be applied as a feasible approach to promote efficient and persistent immunity in healthy individuals with insufficient HBsAb titers.

Immune response to HBV personally varies due to complex biological structures being accountable for the

Table 1: The levels of HBsAb titers in study groups

	Intervention group (n = 70)		Control group (n = 70)	
	Mean ± SD† HBsAb‡ IU/L	*P* value	Mean ± SD HBsAb IU/L	*P* value
Before the vaccination	5.07±2.9 4(1-11)#	*P*<0.001*	4.45±3.4 3(1-10)#	*P*<0.001*
6 months after the last vaccination	744.45±353.07 990(11-1000)#		589.94±353 500(67-1000)#	
Mean differences ±SD	739.38±352.37		585.48±352.98	*P*=0.011**

*Resulted from paired samples *t*-test for comparing before and 6 months after vaccination; **Resulted from independent samples *t*-test for comparing mean differences between the two groups; #Values are median (range); †SD = Standard deviation; ‡HBsAB = Hepatitis B surface antibody

level of immunogenicity.[13,14] For example, different studies demonstrated that variation occurred in class II human leukocyte antigen (HLA II), and interleukin 4 (IL-4) genes strongly correlated with HBV antibody levels.[13,15,16] Furthermore, other genes such as interferon gamma (IFNG), mitogen-activated protein kinase 8 (MAPK8), interleukin-10 receptor subunit alpha (IL10RA), integrin alpha L(ITGAL), interleukin 4 receptor (IL-4R), interleukin 10 (IL-10), tumor necrosis factor (TNF), interleukin-12 beta chain (IL12B), and FOXP1 as well as epigenomic modifications play some crucial roles in protective immunity.[13,15-20] Thus, it seems plausible that single-nucleotide polymorphisms (SNPs) befell some subjects and probably contributed to part of the immune failure.[13,15,19]

Different studies aiming to promote immune responses in immunocompromised subjects have been implemented. To elaborate, greater dose (40 μg) and more repeated vaccination series have been undertaken; nevertheless, the vaccination success rate did not get completely ameliorated.[21] On the other hand, the persistence of antibody titers in subjects vaccinated in adulthood is somewhat ambiguous.[22,23] The present study evaluated impact of Td as an adjuvant when simultaneously applied with HBV vaccine.

This study demonstrated that antibody titers of both the groups were significantly augmented when high and frequent booster doses of HBV vaccine were applied. This finding was in compliance with previous studies, which documented appropriate promotion of immune responses.[23] For example, Naveen Gara *et al.* documented that 94% of their healthy adult subjects receiving booster HBV vaccine developed antibody level >12 mIU/mL.[23] This antibody titer, however, was not perfectly favorable when the efficient protective level of HBV antibody was 100 IU/L.[21,23]

HBV revaccination in hemodialysis patients too was neither durable nor successful. For instance, only 44% of the weak responders whose antibody titers varied from 10.0 IU/L to 99.9 IU/L, developed persistent and protective antibody level for 12 months.[24] The persistency of antibody responses failed while different approaches were exploited. In fact, neither Td vaccine nor the high application of HBV vaccine significantly promoted durable immune responses. Of the subjects, 14.6% simultaneously vaccinated with both Td and HBV vaccine were not allocated as persistent responders, whereas 18.9% of the patients treated with four doses of HBV vaccine did not have a protective level of HBS antibody.[12,21]

From the viewpoint of the antibody difference, in comparison with the control group, our study documented that the level of antibody in the intervention group significantly increased. In addition, antibody titer of the intervention group was significantly higher than the control group. These findings were in compliance with some of the previous studies. Sönmez *et al.* showed that non-responders, who were found in both hemodialysis patients and healthy individuals and simultaneously vaccinated with tetanus toxoid (TT) and HBV recombinant vaccines (S2SRHB), produced more significant HBS antibody.[25] Furthermore, Ocak *et al.* showed that TT vaccine, which was administered 2 days before HBV vaccination, might be efficient for immune response stimulation.[26] Nevertheless, Shahidi *et al.* documented that HBsAb did not persist in those who were simultaneously vaccinated with Td and HBV.[12] It seems plausible that these patients did not produce durable HBsAb due to their uremia.[26] On the other hand, adult immunization schedule in 2014, which was prepared by the centers for disease control and prevention in the USA, suggested Td as a booster dose of HBV vaccine for every 10 years.[27] Furthermore, tetanus vaccination and its immunomodulatory impact might affect the levels of immunoglobulin G subclasses, which could constitute a dominant subclass of HBsAb.[28-30] Altogether, this data showed that Td vaccination was a feasible opportunity to promote HBV antibody in healthy individuals.[12]

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Conflicts of interest

There are no conflicts of interest.

AUTHOR'S CONTRIBUTIONS

MS contributed in the conception and design of the work, definition of the intellectual content, literature research,

clinical and experimental studies, data acquisition as well as manuscript preparation, editing, and review. AH contributed in the conception and design of the work, definition of the intellectual content, literature research, clinical and experimental studies, data acquisition as well as manuscript preparation, editing, and review. HS contributed in the conception and design of the work, definition of the intellectual content, literature research, clinical and experimental studies, data acquisition as well as manuscript preparation, editing, and review. RT contributed in the conception and design of the work, definition of the intellectual content, literature research, clinical and experimental studies, data and statistical analyses as well as manuscript preparation, editing, and review. MS contributed in the conception and design of the work, definition of the intellectual content, literature research, clinical and experimental studies, data acquisition as well as manuscript preparation, editing, and review. NK contributed in the conception and design of the work, definition of the intellectual content, literature research, clinical and experimental studies, data acquisition as well as manuscript preparation, editing, and review. MM contributed in the conception and design of the work, definition of the intellectual content, literature research, clinical and experimental studies, data and statistical analyses as well as manuscript preparation, editing, and review. MMS contributed in the conception and design of the work, definition of the intellectual content, literature research, clinical and experimental studies, data acquisition as well as manuscript preparation, editing, and review.

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