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Original Article

Effectiveness of oral levamisole as an adjuvant to hepatitis B vaccination in healthcare workers non-responsive to previous vaccination: A randomized controlled trial

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ABSTRACT

Background: Healthcare workers are at risk for HBV infection through percutaneous or mucosal contact with infected blood, body secretions, or blood products or via sharps injury. Hepatitis B vaccination, despite immunogenicity, may not induce a proper immune response in 5–10% of the general adult population. Increased immune response in healthcare providers that do not respond properly to conventional hepatitis B vaccination is an important health challenge. Therefore, the aim of the present study was to evaluate the effectiveness of hepatitis B vaccination plus oral levamisole as adjuvant in healthcare providers non-responsive to routine vaccination.

Materials and methods: The healthcare workers that were non-responsive to previous hepatitis B vaccination were enrolled in a double-blind randomized placebo-controlled clinical trial. The participants were then randomized to two groups including hepatitis B vaccination (as a three-dose series on a 0, 1, and 2-month schedule in the deltoid muscle) plus levamisole (levamisole group) and hepatitis B vaccination plus placebo (placebo group) at a 1:1 ratio. The outcome measure was the HBs antibody titer one month after receiving each dose as well as the seroprotection ratio. The side effects were also evaluated in all participants.

Results: In total, 22 subjects finished the trial (11 individual in per group). The median antibody titer one month after receiving the first and third doses increased more in the levamisole group compared to the placebo group but the difference was not significant ($p = 0.34$, $p = 0.66$, respectively).The seroprotection ratio after three doses was similarly high in both groups (90.9% in per group). Furthermore, the seroprotection ratio and median antibody titer had no significant correlation with age, sex, BMI, and history of smoking in intervention and control groups ($p>0.05$). No serious side effects were noted in both groups.

Conclusions: Re-vaccination can boost the immune response in healthcare professionals that were non-responsive to previous vaccination although the mean antibody titer was higher in the levamisole group.

1. Introduction

Hepatitis B is a liver infection caused by hepatitis B virus (HBV) that can be prevented through vaccination. It is one of the leading causes of mortality and morbidity in the world [1]. According to the WHO, about 257 million people are infected with HBV across the word resulting in the death of 887,000 patients due to hepatic carcinoma and cirrhosis every year [2,3]. Elimination of viral hepatitis by 2030 as one of the International Sustainable Development Goals puts hepatitis B vaccination at the front line [4]. In fact, global vaccination is the most economic and safest option for reducing HBV infection [5].

Although every person that is not immune against HBV is susceptible to this infection, some groups are at a higher risk of infection due to risk factors such as a compromised immune system or occupational exposure [6]. Healthcare workers are at risk for HBV infection through percutaneous or mucosal contact (eye, oral mucosa, skin) with infected blood, body secretions, or blood products or via sharps injury [7,8]. Recombinant hepatitis B vaccines, despite high immunogenicity, may not induce

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an appropriate immune response in 5–10% of the general adult population [9]. Moreover, there are reports of the ineffectiveness of routine hepatitis B vaccination in some patients with renal insufficiency, especially hemodialysis patients, and HIV patients. Healthcare workers that are non-responsive to routine hepatitis B vaccination are at risk of infection. It has been reported that about two million healthcare workers are exposed to HBV across the world every year [10]. Different methods have been proposed to improve responsiveness to hepatitis B vaccination, including repeat vaccination, administration of a double dose, co-administration with Granulocyte-macrophage colony-stimulating factor (GM-CSF), co-administration with hepatitis A vaccine, and use of cellular immunity boosters [11,12]. Levamisole, a synthetic imidazothiazole derivative, is an anthelminthic drug and an immune modulator [13]. It is believed that this adjuvant increases the activity of T cells through increasing the production of IL-1, IL-2, IL-12, and IL-18 [14]. In addition, levamisole has shown beneficial effects in different inflammatory and autoimmune diseases like rheumatoid arthritis, systemic lupus erythematosus, and vitiligo through enhancing cellular immunity [15]. Therefore, the aim of the present study was to evaluate the effectiveness and safety of oral levamisole as adjuvant for hepatitis B vaccination in healthcare workers non-responsive to previous hepatitis B vaccination compared to placebo.

2. Materials and methods

2.1. Study design

A double-blind randomized placebo-controlled clinical trial was conducted on healthcare workers working in Imam Reza Hospital and Imam Khomeini Hospital affiliated with Kermanshah University of Medical Sciences, Kermanshah, Iran.

2.2. Participants

First, the occupational health records of all healthcare workers that were exposed to patients' blood and body secretions and had a work experience of at least three months in the hospital were reviewed. Then, those who were non-responsive to previous hepatitis B vaccination were enrolled in the study according to the following criteria:

1 A negative history of infection or contact with HBV as indicated by negative HBsAg and HBcAb results
2 Non-responsiveness to a three-dose schedule of hepatitis B vaccination as shown by HBsAb titer below 10 mIU/ml [23].

The exclusion criteria were a history of allergy to hepatitis B vaccination, malignancy, compromised immune system, use of immunosuppressants like corticosteroids, history of hepatitis C infection screened by a positive HCV antibody result, HIV infection screened by a positive HIV antibody result, pregnancy, and lactation.

2.3. Sample size

Sample size was estimated based on the previous study [25], for estimating the proportion (P) of serum anti-HBs antibody level >100 (37.8% in control group vs. 90.8% in levamisole group), with considering the confidence level of 95% and power of 80%, the required sample size was 12 subjects per group.

2.4. Randomization and concealment

All participants were randomized to levamisole and placebo groups at a 1:1 ratio. After applying the inclusion and exclusion criteria, twenty-six subjects were randomly allocated to levamisole and placebo groups using the block randomization method. For this sample, eight block sizes of four was generated for two groups named A (placebo) and B (levamisole).Then, the generated random sequence (n = 8) was written on a separate card and placed in non-transparent envelope. For example, the block number 1 with sequence ABAB assigned randomly four participants in the study groups. Non of the participants outcome evaluators were aware of the allocation groups.

2.5. Intervention

The participants received 20 μg of the hepatitis B Recombinant vaccine (Euvax B recombinant HBsAg®) intramuscularly in the deltoid muscle on a 0, 1, and 2-month schedule plus either 50 mg levamisole (Rouz Darou Co., Iran) (levamisole group) or placebo (placebo group) administered orally twice a day for 12 days (6 days before and 6 days after vaccination).

2.6. Laboratory evaluation

Before the intervention, demographic characteristics including age, sex, height, weight, smoking, comorbid diseases, and lab tests results such as HBsAg, HBsAb, and HBcAb (ELISA kits produced by Pishtazteb Iran) were extracted from the participants' health records. Blood samples (2 ml) were collected from each participant at the beginning of the trial and one month after administering each dose. After separating the serum, the HBsAb titer was measured using an ELISA kit (DIA PRO Diagnostic Bioprobes, Srl., Italy).

2.7. Outcomes

The primary outcome was the median HBsAb titer one month after administering the first and third doses and the seroprotection ratio. An antibody titer above 20 mIU/mL was considered as protective and lower titers were regarded as non-protective [23].

2.8. Side effects

The vaccine-related side-effects up to one month after administration of each dose were collected using self-reports. These side effects included general reactions such as headache, fever, fatigue, muscle pain, cough, nausea, vomiting, diarrhea, skin rash, local pain or inflammation as well as serious side-effects like allergic shock, vascular edema, nervous system reaction, etc.

2.9. Statistical analysis

The data were analyzed using the SPSS software. Quantitative variables are presented as median with IQR and qualitative variables are reported as frequency and percentage. BMI was calculated by dividing the measured body weight in kg by the square of the measured height in meters. T-test and chi-square test were used to compare the immune response between the two groups and to determine the relationship between independent variables and the immune response. A non-parametric tests (Mann-Whitney test) was used if the data had a non-normal distribution. Finally, multivariate regression analysis was used to adjust for the effect of possible confounding factors. The level of significance was set at 0.05.

3. Ethical approval

To observe research ethics principles, informed consent was obtained from the participants. The objectives of the study were explained to the participants. Moreover, they were informed that participation was voluntary, they could leave the study at any time during the study, the results were confidential, and they could have access to the results if they were interested. The protocol of the study was registered in the Iranian Registry of Clinical Trials (IRCT2014012110417N3).

4. Results

In total, 197 subjects were screened of whom 28 were eligible for inclusion. Two eligible subjects were not willing to join the study since they believed they did not need further vaccination. Of the remaining 26 subjects, 2 were excluded due to vaccine side effects and 2 were excluded due to not completing the vaccination protocol. Finally, the data of 22 subjects, including 15 men and 7 women, were analyzed (Fig. 1).

The participants' mean age, BMI, vaccination series, and administered doses before the study was 39.9 years and 24.15 kg/m^2, 1.7, and 5, respectively. Only 4 participants were smokers. The median (IQR) HBsAb titer was 6 (6) and 6 (7) in the placebo and levamisole groups before the intervention, respectively. There was no significant difference in age, sex, smoking, BMI, number of vaccinations, time from the last vaccine dose, and baseline HBsAb titer between placebo and levamisole groups ($p>0.05$) (Table 1).

The median HBsAb titer one month after receiving the first dose was 151 (IQR 453.2) in the placebo and 225 (IQR 418) in the levamisole group. Although the mean antibody titer was higher in the levamisole group, the difference was not significant ($p = 0.34$). Similarly, one month after receiving the third dose, the mean HBsAb titer was non-significantly higher in the levamisole group ($p = 0.66$). In terms of final response to vaccination, 10 out of 11 participants in both groups (90/9%) responded to vaccination. A summary of the results is presented in Table 2.

Table 1
Baseline characteristics of the study population.

	Levamisole group (n = 11)	Placebo group (n = 11)	P-value
Age (years)	36 (18)	45 (19)	0.307
Male, n(%)	7 (63.64)	8 (72.73)	1.00
BMI, kg/m^2	23 (6.2)	23.8 (3)	0.532
Smoking, n(%)	2 (18.18)	2 (18.18)	1.00
Hepatitis B vaccination,	1 (1)	2 (1)	0.667
Time from last dose to blood collection	4 (5)	4 (5)	0.569
Antibody titer in day 0	6 (7)	6 (6)	0.572

*Quantitative variables are shown as median and interquartile range.

Table 2
Seroprotection rates and titers of anti-HBs after the first and third vaccination.

	Levamisole group	Placebo group	P-value
HBs-Ab titer one month after the first vaccination, median(IQR)	225 (418)	151 (453.2)	0.340
HBs-Ab titer one month after the third vaccination, median(IQR)	250 (185)	245 (289.2)	0.669
Seroprotection[a] rate, n (%)	10 (90.9)	10 (90.9)	1.00

[a] HBS Ab titer <20 mIU/ml.

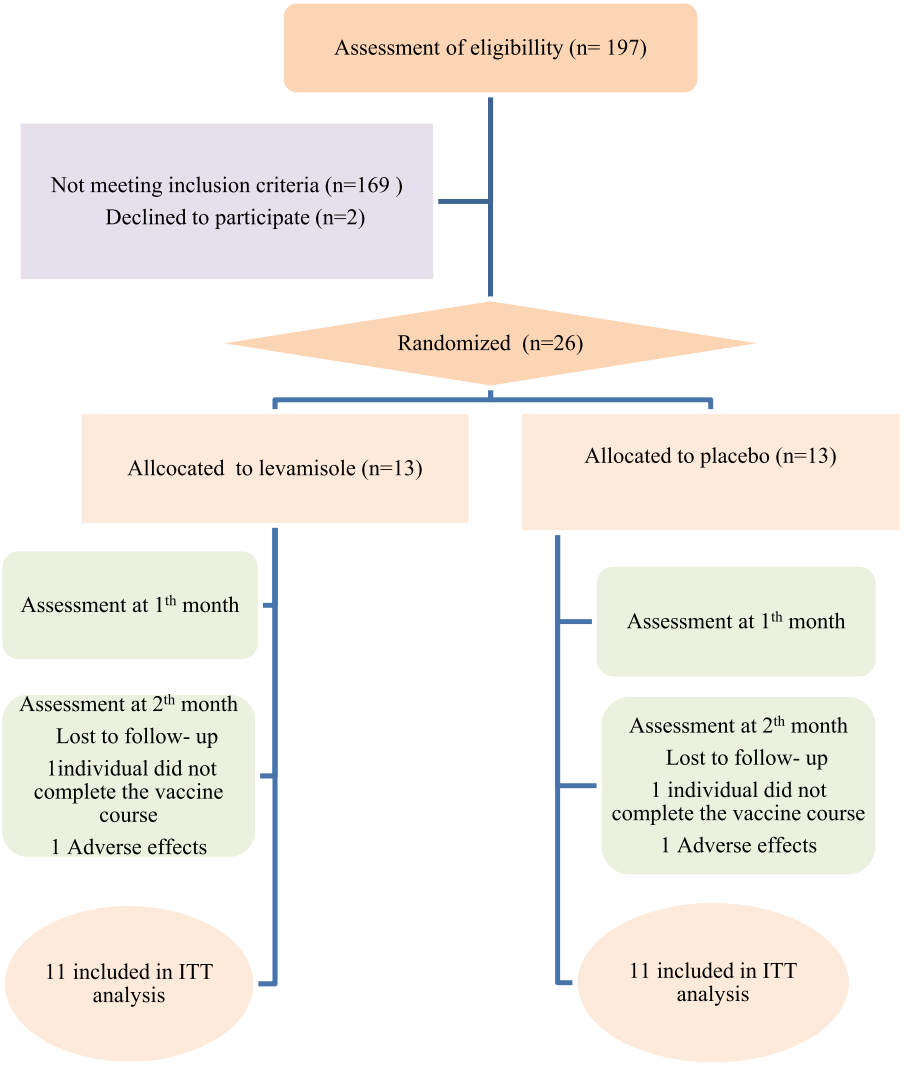

Fig. 1. Flowchart of study selection.

Side effects were reported by four subjects in the levamisole group and one subject in the placebo group. Nausea, vomiting, and skin rash were the most common side effects. There was no significant difference in side effects between the two groups ($p = 0.31$). No serious side effects were reported. All side effects were mild to moderate and resolved spontaneously within 24 h.

Based on the median, the antibody titer was divided into two groups (<200 and ≥ 200). Logistic regression model showed that the odds of higher antibody titer after first dose vaccination in females is 1.72 times higher than males. Inversely, the odds of higher antibody titer after third dose in females is 13% lower than males. The odds of higher antibody titer after both first and third doses vaccination in non-smokers is two times higher than smokers. The odds of higher antibody titer after both first and third doses vaccination and decreased with increasing BMI. However, no significant correlation was found between confounding factors (BMI, smoking, sex) and the antibody titer one month after administering the first and third doses ($p>0.05$)(Table 3).

Table 3
Relationship between confounding factors (BMI, smoking, gender) and antibody titer one month after administering the first and third doses using logistic regression.

	Antibody titer after first does		Antibody titer after third does	
	OR (95% CI)	P-value	OR (95% CI)	P-value
Sex				
Male	Ref	-	Ref	-
Female	1.72 (0.17, 17.1)	0.643	0.87 (0.09, 8.4)	0.911
Smoking				
Yes	Ref	-	Ref	-
No	2.31 (0.18, 30.2)	0.523	1.67 (0.11, 24.8)	0.710
BMI	0.82 (0.58, 1.15)	0.249	0.87 (0.61, 1.23)	0.429

*Abbreviation: OR: odds ratio, CI: confidence interval.
Model adjusted for baseline antibody titer.

5. Discussion

This study was the first double-blind controlled randomized clinical trial of the healthy non-responsive subjects using levamisole as adjuvant plus revaccination.

Levamisole is a synthetic phenylimidothiazole that was introduced as a strong anthelminthic drug in 1966. Its immune enhancing properties were discovered later in 1972 [16]. In 1976, a study was conducted to evaluate the effects of levamisole on healthy chickens. The results showed that levamisole could enhance cellular and humoral immunity through activation of T lymphocytes [17]. Levamisole stimulates T lymphocytes and macrophages and improves cellular immunity [18]. Moreover, it has positive effects on the response rate of hemodialysis patients to tetanus vaccination [19].

Several studies evaluated the effects of levamisole on increasing response to hepatitis B vaccination. Most of these studies were conducted on HIV positive and dialysis patients. Although a number of studies found that levamisole had no effects on increasing the serum levels of anti-HBV antibodies in dialysis patients [16,20], the majority of the studies reported the positive effects of this drug on increasing the immune response [21–28].

The first study in this regard was conducted by Kayatas in Turkey in 2002 [21]. In this study, the subjects were divided to four groups and seroprotection was evaluated in each group. The results of the study showed that levamisole, through modulating the host cellular immunity response, increased the serum response to hepatitis B vaccination, especially in the first vaccination. Argani and Akhtarishojaie [22] conducted a study on a similar group of patients in Iran in 2006. In this study, the antibody titer was measured 1 and 6 months after the last vaccine dose. The results showed a high seroconversion rate following the administration of 20 μg HBV vaccine with more than 95% purity plus 100 mg levamisole injected subcutaneously, indicating the positive effect of levamisole on increasing serum response to hepatitis B vaccination. Ayli et al. [23] evaluated the effect of levamisole in dialysis patients and found that a combination of levamisole and hepatitis B vaccination increased the serum response in these patients.

In a study in 2012, Sayad et al. [24] evaluated the effect of levamisole on increasing the serum response to hepatitis B vaccination in HIV patients. Patients with a negative history of hepatitis B infection or vaccination were included in this study, and their immune response was evaluated through measuring the HBsAb titer concurrently with the second and third vaccine doses as well as one and three months after the end of the vaccination program. The immune response and mean antibody titer following vaccination increased more in the levamisole group compared to the control group. The results indicated the effect of levamisole on increased responsiveness to hepatitis B vaccination in immunocompromised patients. Somi et al. [25] investigated the role of different adjuvants in patients with chronic renal disease with a history of vaccination in 2012. In this study, the serum response and anti HBsAb titer were higher in patients receiving levamisole although the difference was not significant.

Hosseini et al. [26] conducted a study on the healthy family members of chronic hepatitis B patients to evaluate the role of oral levamisole as an adjuvant to hepatitis B vaccination and its effect on the serum response (seroprotection). Hepatitis B surface antibodies were measured one month after the last dose in levamisole and placebo groups. The results showed significantly higher antibody titers in the levamisole group. The authors concluded that oral levamisole as an adjuvant to hepatitis B vaccine increased the HBsAb tier in healthy vaccinated individuals. In line with most reports on the immune enhancing effects of levamisole, a meta-analysis by Fabrizi et al. [27] and Alavian and Tabatabaei [28] in indicated the effect of levamisole on increasing the serum response to hepatitis B vaccination in dialysis patients.

The results of the present study showed although the median antibody titer one month after receiving the first and third doses increased more in the levamisole group compared to the placebo group, the difference was not significant. However, most of the previous studies evaluated the effects of levamisole on the rate of antibody production and serum response in hemodialysis and HIV positive patients. The present study investigated the effect of levamisole on antibody production in non-responsive healthcare workers with an intact immune system. Sex, BMI, and smoking had no significant effects as confounding variables on the serum response and antibody titer. Since these variables were distributed equally in levamisole and placebo groups, they had no effects on the final antibody titer in each group. As for the effect of sex on the response rate, the findings of the present study and a study by Al Saran et al. [29] and were in contrast to the centers for disease control and prevention (CDC) data that introduced male sex as a risk factor for lack of adequate response [30].

Most of the previous studies evaluated the effects of levamisole on the rate of antibody production and serum response in hemodialysis and HIV positive patients. The present study investigated the effect of levamisole on antibody production in non-responsive healthcare workers with an intact immune system.

According to previous studies, the inability of Th cells in cytokine production (IL-2, IL-10, and IFN-γ) [31] and inadequate production of Antigen-specific B cells HBsAg [32] are the main reasons for lack of antibody production in immunocompromised patients. Therefore, the known effect of levamisole, which is promoting cellular immunity through reinforcing the effect of T lymphocytes, and its effect on antibody production by the same lymphocytes can explain increased responsiveness to hepatitis B vaccination in HIV-positive and CKD patients. On the other hand, it seems that levamisole has no effect on the serum response to hepatitis B vaccination in non-responsive healthcare workers with an intact cellar immunity.

The results of this study showed a response rate of 91% to hepatitis B vaccination in levamisole and placebo groups. Although levamisole had

no significant effects on response to hepatitis B vaccination (no significant difference was found between levamisole and placebo groups), the high response rate of the participants to revaccination regardless of levamisole administration was an interesting finding. Therefore, revaccination and repeat vaccination can be offered to healthcare workers not responding to primary vaccination.

However, the study also had several limitations like small sample size and not assessing many fatty liver biochemical markers such as oxidative stress, serum lipid profile and serum and liver iron levels, a short follow-up period, and lack of evaluation of the number of levamisole tablets received by the participants.

Availability, high tolerability, and low price make levamisole a good adjuvant to hepatitis B vaccination, especially in immunocompromised patients. This study found that instead of using levamisole as an adjuvant, revaccination could be offered to increase the immune response in healthcare workers not responding to primary hepatitis B vaccination although the antibody titer might be higher with levamisole. The effect of levamisole may be significant in a larger sample size. Therefore, similar studies should be conducted in larger samples of non-responsive patients to obtain more conclusive results.

Credit author statement

BS: designed the study, NS: designed the study, EH: designed the study, RK: designed the study, NS collected the dataFKS: conducted the statistical analyses, FN: conducted the statistical analyses, AB: drafted the first version of the paper, ZR: drafted the first version of the paper, BD: drafted the first version of the paper, All co-authors made a substantial contribution to interpreting data, critically revised the article, and approved the final version, including the authorship list.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Abbreviations

WHO	World Health Organization
HBV	Hepatitis B virus
BMI	Body Mass Index
HIV	Human Immunodeficiency Virus
ELISA	Enzyme-linked Immuno_sorbent Assay
GM-CSF	Granulocyte-Macrophage Colony-Stimulating Factor
IL-1	Interleukin-1
IL-2	Interleukin-2
IL-12	Interleukin-12
IL-18	Interleukin-18
HBsAg	Hepatitis B Surface Antigen
HBcAb	Hepatitis B Core Antibody
HBsAb	Hepatitis B Surface Antibody
mIU/ml	milli-international units per milliliter
HCV	hepatitis C virus
CDC	Centers for Disease Control and Prevention
IFN-γ:	Interferon gamma
CKD	Chronic kidney disease

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