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Original Article

Effect of levamisole supplementation on hepatitis B virus vaccination response in hemodialysis patients

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SUMMARY:

Aim: As an immune-modulating agent, levamisole has been reported to stimulate depressed T-cell activity, enhance B lymphocyte function and restore delayed hypersensitivity reactions in immune-depressed patients. There are a number of recent studies claiming that levamisole can improve response rate to hepatitis B virus (HBV) vaccination in haemodialysis patients. The present study has examined this hypothesis amongst some Iranian patients, using double-blind randomized clinical trial.

Methods: During a 12 month period, 70 patients on maintenance haemodialysis with negative anti-hepatitis B surface antibody (HBsAb) and HBV core antibody (HBcAb), from four different dialysis centres were enrolled into the study. The patients were randomized to two groups. The first group (levamisole group) received 40 μg doses of recombinant HBV vaccine i.m. at 0, 1 and 6 months, plus 100 mg levamisole p.o., after each haemodialysis session. The second group (placebo group) received the same vaccination protocol, except for the placebo instead of levamisole. The patients were followed on serum HBsAb level. Those with an HBsAb level of above 10 mIU/mL, 1 month after the third dose of vaccination, were considered as responders.

Results: The levamisole group was comprised of 38 patients and the placebo group of 32 patients. Thirty-one patients (81.6%) of levamisole group and 26 patients (81.3%) of placebo group responded to vaccination. The difference between two groups was not significant.

Conclusion: This study indicated that in a haemodialysis population with high response to HBV vaccination, levamisole might have no significant effect in enhancing the response. Further studies with higher power can give more accurate results.

KEY WORDS: **clinical trial, haemodialysis, hepatitis B vaccination, levamisole.**

Patients with end-stage renal diseases (ESRD) undergoing maintenance haemodialysis are highly exposed to being infected with blood born viruses, such as hepatitis B virus (HBV). Primary prevention with vaccination is the most effective strategy to reduce morbidity caused by HBV infection and homodialysis patients are strongly recommended to be vaccinated against HBV.[1–3]

It is well known that the immune system is compromised in uraemic patients.[4] Overall impaired immune response in these patients has resulted in poor responsiveness to HBV vaccination.[2,5,6] HBV vaccination response rate in healthy and immune-competent individuals has been 90–100%,[7] but the patients with ESRD have been reported to achieve only 50–60% sufficient antibody response after HBV vaccination.[8–11] Furthermore, antibody levels in these patients decrease rapidly compared to healthy subjects.[10] Therefore, various interventions have been suggested in recent years to improve immune system function and increase immune response to HBV vaccination in haemodialysis patients. However, the majority of these interventions are experimental and expensive. Thus, it cannot be recommended as a routine protocol as yet.

One of the exceptionally cheap and available interventions in this regard is using a supplementation of oral levamisole. Levamisole, a synthetic low molecular weight compound, is the first member of a new class of drugs which can increase the functions of cellular immunity in normal, healthy laboratory animals. Levamisole can act, either as an immune-stimulant agent or as an immunosuppressive agent,

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Table 1 Basic characteristics of patients in two study groups

	Levamisole group ($n = 38$)	Placebo group ($n = 32$)	P-value
Gender			0.27
Male	20 (52.6%)	21 (65.6%)	
Female	18 (47.4%)	11 (34.4%)	
Age (years)†	42.9 ± 2.1	46.6 ± 2.3	0.24
Weight (kg)†	61.5 ± 2.0	63.6 ± 2.2	0.50
Hemodialysis duration (months)†	13.2 ± 3.0	17.0 ± 3.3	0.41
History of HBV vaccination‡			0.86
Yes	21 (55.3%)	17 (53.1%)	
No	17 (44.7%)	15 (46.9%)	

†Mean ± standard error. ‡History of hepatitis B virus (HBV) vaccination was considered as yes if the patient received a complete course of HBV vaccination within the last 5 years but hepatitis B virus surface antibody titre was ≤10 mg/dL at the time of this study.

mostly depending upon the dose administered and the timing of its administration. At lower dosages and on intermittent administration, it can act as immune-stimulant agent.[12,13] Levamisole is metabolized by the liver and it is then secreted from the kidney. This drug does not uptake by haemodialysis or by peritoneal dialysis. In addition, it is generally well tolerated with rare limited side-effects.[14]

The present study was conducted to evaluate the effectiveness of levamisole supplementation in responsiveness to HBV vaccination in a population undergoing haemodialysis.

METHODS

A double-blind randomized clinical trial was carried out to evaluate the effectiveness of oral levamisole in responsiveness to HBV vaccination in haemodialysis patients. During a 12 month period from August 2005 to August 2006, all ESRD patients undergoing maintenance haemodialysis and receiving three dialysis sessions/week in four dialysis centres in Tehran (i.e. Labbafinejad Hospital, Chamran Hospital, Baqyatallah-el-Azam Hospital and Milad Hospital), were nominated to enrol in the study. Exclusion criteria were: age older than 50 years, positive history of malignancy, consuming immunosuppressive medicines at least 2 months before enrolment to the study, abnormal liver function tests including alanine aminotransferase (ALT; >30 U/L in men and >25 U/L in women), platelet counts (<200 000/μL) and serum albumin (<3.1 g/dL), positive for HIV antibody (HIVAb), positive for hepatitis C virus antibody (HCVAb), positive for hepatitis B virus surface antigen (HBsAg), positive for hepatitis B virus core antibody (HBcAb) and hepatitis B virus surface antibody (HBsAb) with a titre higher than 10 mIU/mL.

The patients enrolled into the study were randomized to two groups by applying a table of random numbers. The first group (levamisole group) received HBV vaccine plus 100 mg levamisole p.o. after each haemodialysis session. The second group (placebo group) received HBV vaccine plus placebo, instead of levamisole. HBV vaccination protocol was the same in both groups. HBV vaccine was administered in the deltoid muscle at a dose of 40 μg (recombinant HBV vaccine; Recombivax HB; Merck, White House Station, NJ, USA) at 0, 1 and 6 months. Levamisole was also administered for 6 months. The patients' serum HBsAb levels were followed and those with levels higher than 10 mIU/mL 1 month after completing a full vaccination schedule were considered responders. Serum HBsAb levels were checked using commercially-available enzyme-linked immunosorbent assay (ELISA) kits (Radim anti HBsAb; Radim, Roma, Italy). The patients were initially assessed 4–2 weeks before vaccination and then monthly during the vaccination course. Complete physical examination was performed and cell blood count was checked during every visit session.

Informed written consent was taken from each patient involved. The patients were permitted to quit the study whenever they wished. The study protocol was approved by the ethical committee at Shahid Beheshti University of Medical Sciences. The patients received the vaccine free of charge. The Ministry of Health and Medical Education was responsible for providing the vaccines, management and supervision of the study process.

Results are expressed as mean ± standard deviation (SD) for continuous variables. Comparison between groups was made using the Student's t-test for continuous variables and χ^2 test or Fisher's exact test, when appropriate, for categorical variables. $P < 0.05$ was considered statistically significant. All statistical analyses were performed using SPSS software for Windows (ver. 13.1; SPSS Software, Chicago, IL, USA).

RESULTS

A total of 70 patients comprised of 41 males and 39 females were enrolled into the study. The patients were randomized to the levamisole group (38 patients) and placebo group (32 patients). The demographics and basic characteristics of the patients are summarized in Table 1. There was no significant difference between the two groups regarding these characteristics.

Fifty-five patients (81.4%) had sufficient response to vaccination overall. Comparing the vaccination response in the levamisole and placebo groups, no significant difference was found between the two groups in this regard (Table 2). In addition, the vaccination response was compared in four groups of the study population disaggregated by levamisole/placebo and history of HBV vaccination yes/no. No significant difference was found among the groups in this regard either (Table 2).

Levamisole was well tolerated by the patients and none of them experienced any adverse events suspected to be due to levamisole.

DISCUSSIONS

There have been various trials conducted in the use of immune-modulating agents for improving both humeral and

Table 2 Comparison of HBV vaccination response between Levamisole group and placebo group

	Levamisole group		Placebo group		*P*-value
Sufficient response to vaccination	31 (81.6%)		26 (81.3%)		0.97
Insufficient response to vaccination	7 (18.4%)		6 (18.8%)		
	With a hx. of vaccination	With no hx. of vaccination	With a hx. of vaccination	With no hx. of vaccination	
Sufficient response to vaccination	17 (81.0%)	13 (76.5%)	14 (82.4%)	13 (86.7%)	0.90
Insufficient response to vaccination	4 (19.0%)	4 (23.5%)	3 (17.6%)	2 (13.3%)	

hx., history.

cellular immune responses to increase the HBV vaccination response rate in patients on haemodialysis; for example, adjutants such as γ-interferon,[15] interleukin (IL)-2,[16] zinc,[17] thymopentin[18] and granulocyte macrophage colony-stimulating factor (GM-CSF).[19] Recently, usage of levamisole as a supplementation in addition to the vaccine has also been suggested. Levamisole, used first for parasitic infections, has been reported to stimulate depressed T-cell activity,[20,21] enhance B lymphocyte function[21] and restore delayed hypersensitivity reactions in immune-depressed patients.[22] Moreover, some studies have showed some association between using levamisole and increasing serum levels of IL-2 receptors in patients with cancer[20] and increasing IL-1 production in mice models.[23] Another study, evaluating specifically the effect of levamisole on the immune system of patients on haemodialysis, revealed that it could improve granulocyte chemotaxis in these patients.[24] Considering such evidences, as well as availability and low cost of levamisole, the strategy of levamisole supplementation to HBV vaccine has been interesting to researchers in recent years. There is no high-grade evidence for this strategy, as yet and only few small clinical trials with limited sample sizes have been conducted in this regard so far. Ayli *et al.* found that levamisole supplementation increased immune response after HBV vaccination from 53.3% in the control group to 73.3%.[25] In another study by Kayatas, response rate in patients receiving levamisole in addition to vaccine was 82% compared to 57% in the control group. Moreover, this study showed that, in patients who had a past history of HBV vaccination, levamisole could increase the response rate from 15% in the control group to 77%.[26]

The authors' findings in the current trial are not in line with the above studies. It was observed that no significant difference existed between levamisole and placebo groups regarding immune response rate. A very noticeable point in the current study was the high immune response rate in haemodialysis patients who received routine HBV vaccine. In the placebo group, 81.3% of the patients achieved sufficient immune response while most of the studies reported a response rate of approximately 50–60% in patients on haemodialysis.[2,7–9,25,26] In addition, a response rate of higher than 81% was found in patients with a past history of HBV vaccination. It is possible these patients had not received a double dose of vaccine during the first course. Searching for available databases, no comprehensive study with a large sample size in Iran was found to report HBV vaccination response rate in haemodialysis patients. However, most of the studies conducted in this regard, reported a response rate higher than 80%.[27–29] Studies by Ayli *et al.* and Katays showed that levamisole increased vaccination response rate from 53% to 73% and from 57% to 82%, respectively,[25,26] whereas according to the indicated Iranian studies, including current study, immune response to routine vaccination was higher than 80% even in patients with a past history of HBV vaccination.

Another recent study from Iran by Argani *et al.* evaluated the effectiveness of levamisole in immune responsiveness to HBV vaccination in chronic haemodialysis patients.[30] This study indicated that adding levamisole to routine i.m. HBV vaccination enhanced response rate from 60% to 90% 1 month after vaccination completion and from 20% to 80% 6 months after vaccination completion. The interesting finding in this study was the response rate to routine vaccination (60%). This study reported a response rate to routine vaccination in Iranian haemodialysis patients comparable with international studies. Small sample size (11 cases in each group) was observed in this study like the other studies in this regard. Upon closer examination of the findings of the current study and the study by Argani *et al.*,[30] it was found that approximately 82% of patients in the current study among the levamisole group achieved immune response 1 month after vaccination completion. This is comparable with the study by Argani *et al.* in which the rate was 90%. The main difference is the response rate in the group on routine vaccination protocol. This rate was approximately 81% in the current study versus 21% in the study by Argani *et al.*[30] Therefore, it seems that levamisole may enhance immune response only in cases of low vaccination response rate.

One of the main shortcomings of the current study is sample size, resulting in low power and increasing type two error. This is also the main shortcoming observed in other clinical trials in this regard.[25,26,30] Having a greater sample size in further studies could lead to more reliable evidences.

In short, the current study indicates that, in a haemodialysis population who had a high response to HBV vaccination, levamisole may have no significant effect in enhancing response. Hemodialysis patients in Iran seem to have a higher response rate to HBV vaccination in comparison with patients in other countries. Further studies of higher power could provide more accurate results.

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