

Original Article

**The evaluation of Tetanus-diphtheria (Td) vaccine impacts  
on immune response to hepatitis B (HB) vaccine in  
non-responder dialysis patients\***

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**Abstract**

**BACKGROUND:** The Hepatitis B (HB) vaccine response in hemodialysis patients is less than healthy individuals. Different strategies have been taken into account to improve the response rate. This study aimed to evaluate the effect of tetanus and diphtheria (Td) vaccine as an adjuvant therapy to HB vaccination.

**METHODS:** Sixty three end-stage renal disease patients were recruited on dialysis that were older than 18 years and had passed at least 3 doses of HB vaccination schedule, and had HBS antibody (Ab) with titer less than 10 IU/L. The patients were divided into two groups; A (30 patients) and B (33 patients). Both of the groups received a 3-dose HB vaccination schedule of 40 µg intramuscularly in the left deltoid muscle at 0, 1 and 6 months. Group A also received Td vaccine intramuscularly simultaneous with the first dose of HB vaccine. HBS Ab was measured in periods of 1 and 6 months after completion of the vaccination.

**RESULTS:** One month after completion of the vaccination, group A had better but not significant response rate (96%) than group B (83.9%) ( $p > 0.05$ ); in addition, after 6 month there was no difference between the two groups (87.5% vs. 83.3%) ( $p > 0.05$ ). Patients with HCV infection had lower response rate than patients who did not have HCV infection (33.3% vs. 92.5%) ( $p < 0.05$ ). Age had negative effect on immune response to HB vaccination ( $r = -0.339$ ;  $p = 0.005$ ).

**CONCLUSIONS:** The use of Td vaccine concurrent with HB vaccination may increase the response rate in non-responder individuals; however, it seems it does not have any role in the persistence of immune response. Age and HCV infection negatively affected the response to HB vaccination in dialysis patients.

**KEYWORDS:** Hemodialysis, Peritoneal Dialysis, Hepatitis B Vaccine, Tetanus-Diphtheria Vaccine.

**JRMS 2011; 16(5): 598-604**

**A**ntibody production in patients with end-stage renal disease impairs due to impairment of T-cell activation, reduction in number of B Lymphocytes, and dendritic cells which are the major antigen presenting cells.<sup>1-3</sup> Therefore, that infection is the second biggest cause of mortality in this population.<sup>4</sup>

One of the infections is hepatitis B virus. Not to consider immune disorder, these pa-

tients are exposed to an increased risk of acquiring HBV infection due to transfusion of blood products and contamination of dialysis equipments.<sup>5,6</sup> That is why early prophylaxis with HB vaccine is recommended in the course of renal diseases.<sup>4</sup>

However, HB vaccine is certainly effective on hemodialysis patients, but immunity persistence in this population is largely unknown.<sup>6</sup> The response rate to HB vaccine in hemodialy-

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sis patients is variable in different studies (30-80%),<sup>3,5,7-11</sup> while the seroconversion rate in healthy neonates, children, and adults varies from 80% to 100%.<sup>12</sup>

In dialysis patients, except for the immune deficiency, other factors such as dialysis adequacy, HCV infection, age, diabetes mellitus and nutritional status are effective on response rate.<sup>4,5,9,13-15</sup> That is why different methods of treatment including adding one extra dose of vaccine to make a 4-vaccine series, doubling the dose of vaccine up to 40 $\mu$ g/dose, injection of repeated booster dose, intra-dermal injection instead of intramuscular one and adding different adjuvant such as Levamizole, tetanus toxoid, AM3, GM-CSF, Polio vaccine, Pertusis vaccine and hemophilus influenza vaccine have been attempted to improve seroconversion which led to different results.<sup>3,6,12-16</sup>

There is no decisive method to improve response rate to HB vaccine in patients on dialysis, so that we decided to evaluate Td vaccine effectiveness as an adjuvant on seroconversion rate. We also investigated the impacts of different factors on response rate to HB vaccine.

## Methods

This was a clinical-trial study conducted from March 2009 to August 2010 (RCT No.: 138812093454N1). We evaluated 561 hemodialysis patients (2-3 dialysis sessions per week and 4 hrs for a session with KT/V = 1-1.2) in 5 hemodialysis centers and 161 dialysis patients in two peritoneal centers in Isfahan. Inclusion criteria were: 1) age equal or more than 18 years, 2) HBS Ag negative, 3) received at least a 3-dose HB vaccination schedule at 0, 1, and 6 months intervals (initial vaccination course) but with HBS Ab titer less than 10 IU/L.

We tried to match groups from different aspects including age, gender, diabetes mellitus, type of dialysis, and immunosuppressive drugs consumption.

Exclusion criteria were: 1) death, 2) transplantation, 3) noncooperation to the study. Formal consent and satisfaction, which had been accepted in local ethics committee, was

obtained from all the patients for their participation.

The patients were divided into two groups of A and B, included 30 (11 females and 19 males) and 33 patients (13 females and 20 males) respectively. All of them received a 3-dose HB vaccination schedule of 40 $\mu$ g injection at 0, 1, and 6 months intramuscularly in left deltoid muscle; in addition, group A received Td vaccine of 40 IU injection intramuscularly in right deltoid muscle also with the first dose of HB vaccine. (All vaccines had been made in Pasteur Institute of Iran)

During the vaccination, 6 patients (9.5%) (4 in A, and the rest in B) died and one patient in group A did not call on for vaccination (Figure 1). The HBS Ab levels were tested by ELISA method, and Dia Plus kit, 1-2 and 6 months after receiving the last dose. HBS Ab level  $\geq 10$  was accepted as protective level.<sup>4</sup>

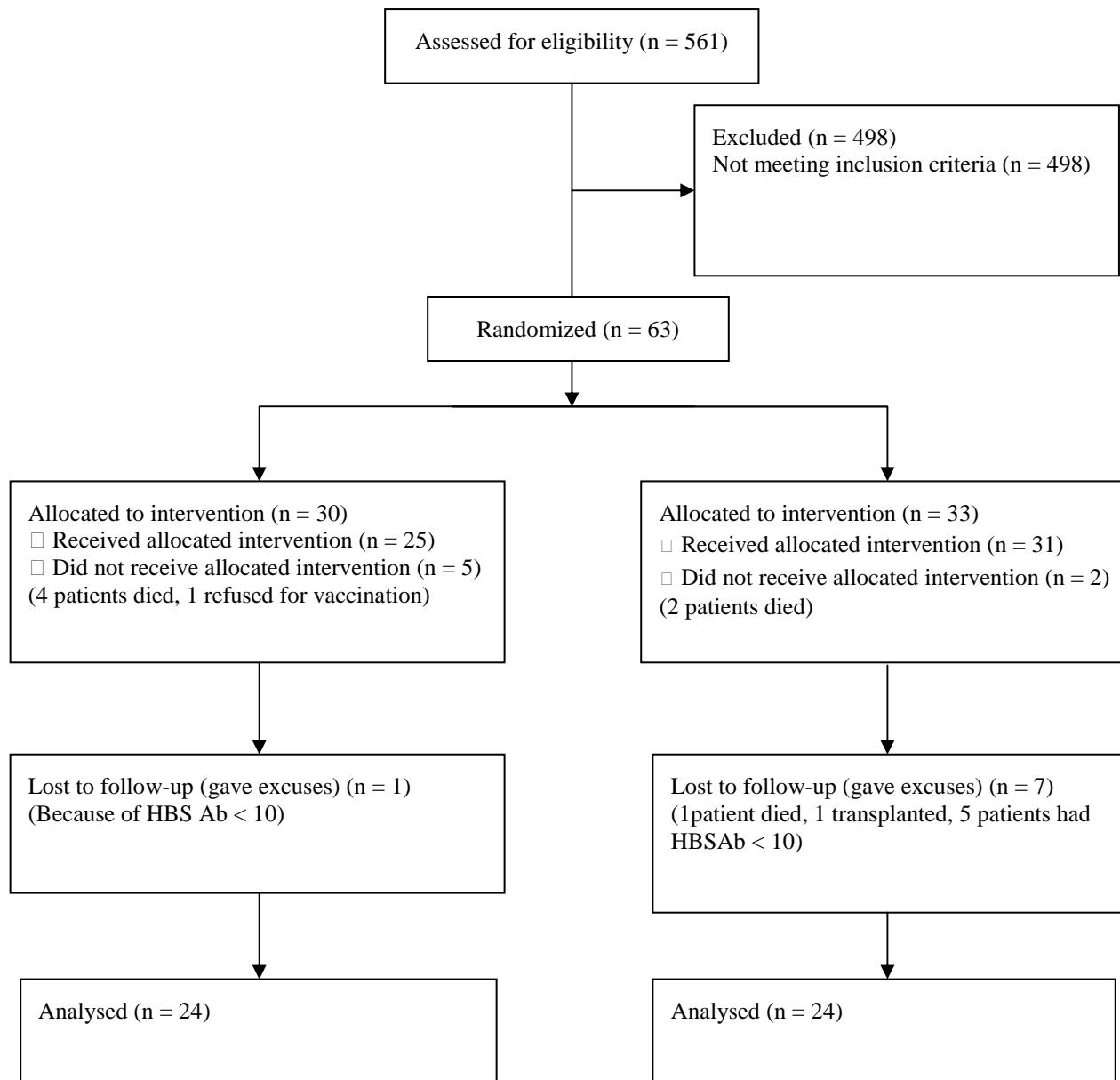
The statistical analysis (through SPSS Software version18.0, Chicago, Ill, USA) was done by independent t-test, chi square (if necessary), and Fisher's exact test and Pearson correlation test. P values less than 0.05 were considered to be significant. Values are expressed as mean  $\pm$  SD or when indicated as an absolute number and percentage.

## Results

The mean age of patients in groups A and B was  $60.46 \pm 11.6$  years (29-78 years) and  $57.7 \pm 14.3$  years (24-88 years) respectively. The characteristics of the patients of each group are shown in Table 1.

Three patients (4.8%) were infected by hepatitis C virus (HCV) infection, six (9.5%) had a history of immunosuppressive drug consumption and ten (15.9%) had two periods of vaccination schedule. Twenty patients (31.7%) were on peritoneal dialysis while 43 patients (68.3%) underwent hemodialysis. Thirty one out of 63 (49.2%) had had diabetes mellitus (DM) and the rest (50.8%) had diverse etiology of ESRD (Figure 2).

One month after vaccination completion, 24 patients (96%) in group A and 26 individuals

**Figure 1.** Flow chart of all the patients eligible for the study

(83.9%) in group B had HBS Ab titer  $\geq 10$  IU/L. This difference was not statistically significant ( $p > 0.05$ ) (Table 2).

Among 6 patients (10.7%) who had HBS Ab titer less than 10 IU/L, 5 (83.3%) were over 60 years, from whom 5 were men and 5 in group B.

After measuring of the first HBS Ab titer, 6 patients (1 in group A and 5 in group B) referred for revaccination; because HBS Ab titer was less than 10 IU/L. During the follow-up

duration, one patient in group B died and one underwent transplantation.

Therefore, the total number of patients declined to 48 at the end of the study. There was no difference between the persistence of immune response to HBV vaccine between group A and group B after 6 months. (87.5% vs. 83.3%) ( $p > 0.05$ ) (Table 2).

There was no significant correlation between HBS Ab level and gender, DM, type of dialysis and consumption of immunosuppressive drugs ( $p > 0.05$ ) (Table 3).

**Table 1.** Characteristics of the patients in groups A and B

Variables	*Group A	**Group B	P value
Number of the Patients	30	33	
Age (year)	60.46 ± 11.61	57.75 ± 14.34	0.2
Gender			
Male	19 (63.3%)	20 (60.6%)	
Female	11 (36.7%)	13 (39.4%)	0.82
Dialysis Duration (month)	33 ± 24.2	30.33 ± 19.63	0.316
Diabetes Mellitus	15 (50%)	17 (51.5%)	
***HCV infection	1 (3.3%)	2 (6.1%)	0.54
Dialysis			
Hemodialysis	19 (63.3%)	24 (72.72%)	
Peritoneal Dialysis	11 (36.7%)	9 (27.28%)	0.42

\*Group A: received Tetanus-diphtheria (Td) vaccine

\*\*Group B: not received Tetanus-diphtheria (Td) vaccine

\*\*\*HCV :Hepatitis C virus

**Table 2.** Response rate to hepatitis B (HB) vaccine in group A and B

Groups	1-2 months after the vaccination			6 months after the vaccination		
	n	*HBS Ab ≥10 IU/L	P value	n	*HBS Ab ≥10 IU/L	P value
A	25	24 (96%)	0.07	24	21 (87.5%)	0.34
B	31	26 (83.9%)		24	20 (83.3%)	
Total	56	50 (89.3%)		48	41 (85.4%)	

HBS AB: Hepatitis B Surface Antibody

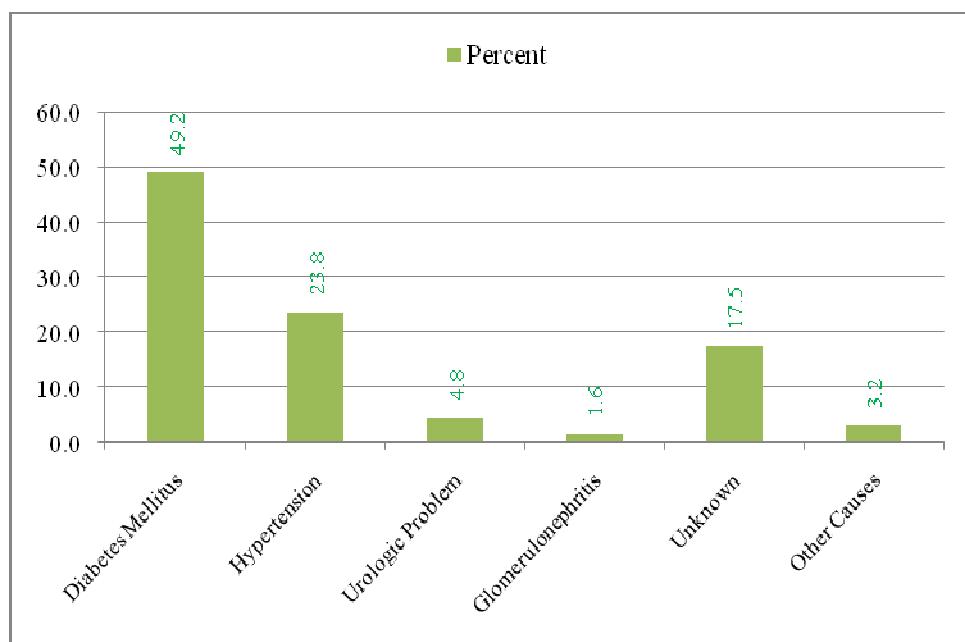
**Table 3.** Effects of age, type of dialysis, \*DM and immunosuppressive drugs on response rate to \*\*HB vaccine

Variables	***HBS Ab (1-2 months) IU/L	P-Value	***HBS Ab (6 months) IU/L	P-Value
Gender				
Female	189.05 ± 259.85	0.48	214.31 ± 265.48	0.443
Male	192.45 ± 225.08		225.42 ± 259.93	
DM(Diabetes mellitus)				
Yes	216.48 ± 233.48	0.223	295.7 ± 307.98	0.045
No	165.86 ± 258.63		141.7 ± 177.5	
Kind of Dialysis				
Hemodialysis	181.8 ± 184.96	0.079	116.75 ± 128.49	0.257
Peritoneal Dialysis	235.48 ± 289.5		219.67 ± 275.37	
Immunosuppressive Drugs				
Yes	295.2 ± 451.7	0.161	78.33 ± 119.3	0.17
No	179.9 ± 221.3		228.06 ± 265.4	

\*DM, Diabetes mellitus

\*\*HB vaccine, Hepatitis B vaccine

\*\*\*HBS AB, Hepatitis B Surface Antibody



**Figure 2.** Frequency of renal insufficiency causes

The response rate in younger patients was better than older ones ( $r = 0.339$  and  $p = 0.005$ ) and patients without HCV infection had better response than those with HCV infection (33.3% vs. 92.5%) ( $p = 0.02$ ).

## Discussion

Abnormality of phagocytosis, B and T lymphocytes causes humeral and cellular immunity alterations in dialysis patients.<sup>3,6</sup> Children with chronic renal failure significantly deal with lower numbers of memory type B cells in comparison to healthy controls,<sup>22</sup> that lessen Ig levels in the patients of this type. These factors may decrease response rate to HB vaccine.<sup>3</sup>

Because of lower and transient (rapid decline)<sup>3,6</sup> response to HB vaccination in dialysis patients, non-responders should receive repeated doses of HB vaccine to increase response rate. In this study, we used Td vaccine as an adjuvant to increase response rate and persistence of immune response.

From the view point of response persistence to the HB vaccine, our study demonstrated that 85.4% of patients had protective HBS Ab levels, 6 months after the vaccination, while in the study of Ramezani et al they showed that 81.1% of them had protective HBS Ab levels,

one year after administration of 4-dose schedule with 40 $\mu$ gr HB vaccine.<sup>6</sup>

In this study, 89.3% of non-responders had HBS Ab levels more than 10 after the administration of HB vaccine at 0, 1, and 6 months, just equal and the same as what Ocak et al had found in 89.7% of hemodialysis non-responders whom were induced 2-3 additional booster doses of 40 $\mu$ gr HB vaccine in 2008.<sup>3</sup> Protective HBS Ab levels of groups A and B were found to be 96% and 83.9%, respectively ( $p = 0.07$ ). However, these results might be statistically remarkable with an increase in the number of patients, but 6 months after vaccination, there was no difference in persistence of HBS Ab levels between groups (87.55% vs. 83.3%) ( $p > 0.05$ ).

Our results were different from those of Ocak and Sonmez et al. Ocak et al reported tetanus toxoid as an effective adjuvant to increase HBS Ab titer,<sup>3</sup> but in this study 18 months after repeated HB vaccine booster doses, only 5 patients did not have protective HBS Ab level, who received tetanus toxoid and HB vaccine. These patients had not been compared with control group. On the other hand, 4 months after the vaccination, one of them lost protective HBS Ab titer.<sup>3</sup>

Sonmez et al selected 76 subjects who received at least three shots of HB vaccine but remained negative for HBS Ab (non-responder). They divided them into three groups of healthy individuals ( $n = 40$ ), hemodialysis ( $n = 24$ ), and pregnant women ( $n = 12$ ).

Some patients of each group received HB vaccine with intervals of 1 month for three times simultaneous with tetanus toxoid, and the rest received 3 doses of HB vaccine.

The group who received tetanus toxoid had greater response rate and HBS Ab titer than another one ( $p > 0.05$ ).<sup>20</sup>

The difference between our study and Sonmez et al might be due to different sample size, population or effect of other factors on HBS Ab level.

In the present study, 6 months after vaccination, 7 patients (14.6%) with an effective response had lost HBS Ab. In these patients HBS Ab level was 10-100 IU/L at first month of vaccination. Therefore HBS Ab more than 100 may be essential for persistence of protective HBS Ab level after 6 months. Ramezani et al reported that HBS Ab titer above 100 IU/L guarantees efficacious protection.<sup>6</sup>

However, different studies mentioned many factors to be effective on the response rate to HB vaccine, but role of none of them were confirmed. In the current study, age, and concomitant infection with HCV significantly had a negative influence on response rate to HB vaccine. Patients with HCV infection and older patients had lower response rate ( $p < 0.05$ ).

Navarro et al reported that hemodialysis patients with HCV infection had a weaker response than those without it (23% vs. 62.7%) ( $p < 0.01$ ).<sup>9</sup>

There are different results about the effect of age on response rate. Navarro et al<sup>9</sup> and Ibrahim et al<sup>5</sup> reported that age has no negative effect on seroconversion, but these results were different from what Buti et al<sup>22</sup> and Jadoul et al<sup>13</sup> have reported.

Jadoul et al demonstrated that after 12 months, patients of ages less than 60 have better protective HBS Ab than those over 75 years (100% vs. 50%) ( $p = 0.048$ ).<sup>13</sup>

We came up to the same results of Navarro et al that gender and duration on dialysis had no effect on response rate ( $p > 0.05$ ).

In this study, there was no significant correlation between response rate and immunosuppressive drugs consumption ( $p > 0.05$ ). Patients who underwent hemodialysis had a weaker response than those treated with peritoneal dialysis, but it was not statistically significant (87.5% vs. 93.8%) ( $p > 0.05$ ). Having et al demonstrated that the response rate did not differ from patients of hemodialysis to those of peritoneal dialysis (78.7% vs. 77.3%) ( $p > 0.05$ ).<sup>10</sup>

In our study, diabetic patients had a better response rate than non-diabetics, that it was statistically significant ( $p < 0.05$ ) six months after the vaccination. It also was different from those of Ocak et al.<sup>3</sup>

In summary, it seems that Td vaccine simultaneous with HB vaccine may increase the response rate to vaccine, but it has no role in persistence of seroconversion; however, further and longer investigations on more population are recommended. Age and concomitant HCV infection have negative effect on response rate to HB vaccine. Perhaps these patients need close follow-up and/or repeated doses of HB vaccine.

### **Limitations of the study**

- 1) The deceased patients have not been evaluated for autopsy findings.
- 2) The history of immunosuppressive drug consumption was not obvious in our patients. In our hospitals there were no computerized medical records of the patients.

### **Acknowledgment**

We are pleased to thank the research council of Isfahan University of Medical Sciences for acceptance of the primary proposal and all necessary revisions needed.

Authors appreciated from all thoses who helped throughout the study, namely Mrs. Rayhani; nurse in Peritoneal Dialysis and Mr. Haydari head of Health Section of Al-Zahra Hospital. And special thank also goes to Research Division of Isfahan University of Medical Sciences, who helped us with financial budgets.

## Conflict of Interests

Authors have no conflict of interests.

## Authors' Contributions

All the authors carried out the study, participated in the design of the study and acquisition of data performed the statistical analysis and wrote the manuscript. All the authors read and approved the final manuscript.

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