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The Effect of Inflammatory Reactions on Antibody Unresponsiveness to Hepatitis B Vaccine in Hemodialysis Patients

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Background and Aims: **In this study, the effect of infections and inflammation developed during hepatitis B vaccination program on antibody response in hemodialysis (HD) patients was evaluated.**

Methods: **In total, 94 patients who had hepatitis B surface antigen (HBsAg) (-), antibody to hepatitis B surface (antiHBs) (-), antibody against hepatitis B core immunoglobulin G (antiHBcIgG) (-) (Group A) and who were previously vaccinated but having antibody titer levels lower than 10 mIU/mL (Group B), on maintenance HD program were included in this study. In group A, 40 μg intramuscular vaccine on 0, 1, 2 and 6 months and in group B, 40 μg of intramuscular booster dose vaccine were administered. Antibody titer of 10 mIU/mL was considered as positive. Group A was then divided into two subgroups with respect to antibody response (Group A1 and Group A2).**

Results: **Eighty-one patients completed the study (Group A; n=64, mean age=42.3±11.4 years; Group B: n=17, mean age=53.6±10.6 years). In Group A, antibody response was positive in 82.8% (Group A1), negative in 17.2% (Group A2) and it was positive in 100% of Group B. Inflammatory parameters, nutritional and demographic features were found similarly in all groups. Throughout the study, infections developed most frequently in Group A.**

Conclusions: **We concluded that acute infections and inflammations developed in patients vaccinated according to vaccination schedule recommended for HBV prophylaxis during HD treatment does not affect antibody response and acute phase reactants are not indicators for negative antibody response.**

Keywords: **Hemodialysis, Hepatitis-B Vaccine, Inflammation, Antibody Response**

Introduction

Hepatitis B virus (HBV) infection still preserve its importance for both HD (hemodialysis) patients and HD staff and has high risk for this population [(1)]. Among HD patients, probably because of depression of immune system, HBV infection becomes chronic at a rate of 60-80% [(1-4)]. For this reason, in cases of end stage renal disease (ESRD), not only treatment or prophylaxis for HBV infection but also removing the factors affecting the prophylaxis is needed [(3,4)].

HBV incidence in HD patients is 0.1% in western countries [(5)]; however, it is, according to Turkish Society of Nephrology (TSN) data, 4.8% [(6)]. In patients who will be taken on regular HD program, serologic markers of HBV should be analyzed and cases with negative hepatitis-B surface antigen (HBsAg) and anti-HBs antibody, should be vaccinated. In addition to the universal precautions to protect HD patients from HBV infections and to prevent new hepatitis cases, double doses (40 μg/ml) of recombinant hepatitis-B vaccine on 0, 1, 2 and 6th months are recommended by "Centers for Disease Control and prevention" (CDC) [(5)]. HBV vaccination program is an efficient precaution for HD patients, however antibody response to the vaccine differs from person to person and provides protection only in 50-60% of patients [(2, 7-9)]. The

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factors such as older age, suppressed immune system, and inflammation may negatively affect antibody response in HD patients.

In this study we aimed to evaluate the effects of infections developed during hepatitis-B vaccination program and consequent pro-inflammatory cytokines [interleukin 1-beta (IL-1β), interleukin 6 (IL-6), tumor necrosis factor-alpha (TNFα)] and C-reactive protein (CRP) on antibody response in HD patients.

Materials and Methods

Patients having sufficiency for HD, receiving rHuEPO (recombinant human Erythropoietin) therapy and with HBsAg(-), antiHBs(-) and antiHBcIgG(-) and patients previously vaccinated but having antibody titer levels lower than 10 mIU/mL, totally 94 patients on chronic conventional bicarbonated HD treatment at Hemodialysis Center of Dicle University School of Medicine were enrolled to the study. Among these patients, cases who were transferred to another center, underwent transplantation or died were excluded from the study. 81 patients completed the study. Patients were divided into two groups with respect to vaccination status. Group A: patients having HBsAg(-), antiHBs(-), and having no history of vaccination against hepatitis B, received 40 µg vaccine (Hepavax-Gene-inj(R) recombinant 20 µg/ml, Green Cross Vaccine Corp. Korea) on 0, 1, 2 and 6th months, Group B: patients previously vaccinated but having antibody titer levels lower than 10 mIU/mL, received a 40 µg intramuscular booster dose. Then Group A was divided into two subgroups as positive responders (Group A1) and negative responders to hepatitis-B vaccine (Group A2).

Patients' demographic features, age, sex, HD time, URR (urea reduction rate) and Kt/V were recorded at the beginning of the study. Basal hemoglobin (Hb), hematocrit (Hct) levels were measured on Cell-dyn 3700 (Abbott Diagnostics, USA), serum levels of albumin and total cholesterol (TC) were measured by using routine biochemical procedures on Aeroset/C8000 autoanalyzer (Abbott Diagnostics, USA), and bicarbonate (HCO_3) levels were assessed in heparinized arterial blood on radiometer blood gas analyzer ABL-735 (Belgium). IL-1β, IL-6, and TNF-α levels were performed by chemoluminescence method on Immulite 1000, and CRP levels were established by electrochemilüminescence method on Roche Elecsys 2010 immunoassay analyzer.

rHuEPO was administered to all patients in a dosage of 80-120/kg/week. Hb and Hct analyses were restudied at the end of the study. Pro-inflammatory cytokines like IL-1β, IL-6, TNFα, CRP were detected at the beginning of the study and 1 month after the last dose. Infections developed during the study were recorded. Anti-HBs titers were recorded before the vaccination program (antibody titers before vaccination) and 1 month after the fourth dose (last antibody titers), and anti-HBs titers above 10 mIU/mL were considered as positive response.

Kt/V values were calculated using computer program on Braun mark HD device, URR was calculated using [1-(postdialysisBUN/Pedialysis BUN)] Formula.

Statistical analyses were done by student's t test, one-way Anova and Wilcoxon Signed Ranks Test on SPSS-13 program. $P<0.05$ was considered as significant. Data were shown ±SD and prevalence rates were shown as percent (%).

Results

In the beginning of the study, 94 patients (52 males and 42 females) were included in the study. 13 patients were excluded from the study because of several reasons [transfer to other centers (n=8), death (n=4), transplantation (n=1)]. 81 patients (43 males and 38 female) completed the study, Group A: 64 patients, and Group B: 17. Demographics, urea kinetic models, hematologic features and nutritional status of patients are shown in table 1. The antibody response of groups is shown in table 2. Positive antibody response was found in 82.8 % (group A1, n=53) and negative antibody response was found in 17.2 % (group A2, n=11) in Group A patients. On the other hand positive antibody response was found 100 % in patients of group B whose booster dose was administered. The mean postvaccination antibody titers were found as 363.0 ± 418.5 in Group A1, and 70.8 ± 55.3 in Group B. At the beginning of the vaccination program, apart from a minimal, statistically not significant, lowness in Hb and Htc levels in Group A when comparing with Group B, no marked difference was determined between two groups' nutritional parameters. After completing vaccination program, again no statistically significant difference was found in the same parameters (table 1).

Inflammatory markers of groups are summarized in table 3. There were not any significant differences in inflammatory markers between all groups. During the study, several infections were observed in

Table 1. The demographic, hematologic, and nutritional parameters of groups

Parameters		GROUP A		GROUP B	p
		Group A1 n:53	Group A2 n:11	n:17	
Sex(F/M)		22/25	9/8	7/10	0.055
Age(years)		40.1 ± 11.3	48.3 ± 7.9	53.6 ± 10.6	0.017
Dialysis duration (months)		15.8 ± 14.3	20.8 ± 15.3	22.2 ± 26.8	0.459
URR (%)	Pre-vaccination	69.0± 8.6	68.1 ± 8.7	68.2 ± 8.1	0.958
	Post-vaccination	66.4± 7.8	67.3± 7.9	67.5± 8.6	0.873
Kt/V	Pre-vaccination	1.48 ± 0.50	1.39 ± 0.51	1.41 ± 0.34	0.721
	Post-vaccination	1.4 ± 0.66	1.42± 0.47	1.43 ± 0.53	0.779
Hct (%)	Pre-vaccination	26.8 ± 4.8	31.1 ± 3.7	30.0 ± 7.1	0.199
	Post-vaccination	31.4 ± 3.2	30.1 ± 3.3	33.0 ± 3.7	0.438
rHuEPO(IU/w)	Pre-vaccination	4500 ± 2150	4000 ± 2200	3500 ± 1150	0.264
	Post-vaccination	4250 ± 2550	4500 ± 2350	4650 ± 1100	0.341
HCO_3(mmol/L)	Pre-vaccination	25.2 ± 2.4	24.0 ± 3.4	24.2 ± 3.0	0.669
	Post-vaccination	23.7 ± 3.2	22.1 ± 3.4	25.2 ± 2.4	0.445
Albumin (g/dL)	Pre-vaccination	3.6 ± 0.5	3.5 ± 0.5	3.4 ± 0.5	0.229
	Post-vaccination	3.7 ± 0.4	3.5 ± 0.7	3.5± 0.2	0.323
Cholesterol(mg/dL)	Pre-vaccination	138.5± 29.8	133.1 ± 41.6	135.5 ± 23.3	0.933
	Post-vaccination	148.5± 24.7	141.2 ± 31.9	145.8 ± 20.6	0.948

Table 2. Anti-HBs response of groups

Anti-HBs(mIU/mL)	Group A1 (n:53)	Grpup A2 (n:11)	Grpup B (n:17)	p
Pre-vaccination titers	2.0 ± 0.0	2.1 ± 0.0	8.9 ± 0.5	0.319
Post-vaccination titers	363.0 ± 418.5	4.5 ± 3.8	70.8 ± 55.3	<0.0001

patients from all groups. However, infections were mostly determined in Group A1. Upper respiratory tract infections and urinary system infections were the most frequent ones; several other infections like periodontitis, arthritis, AV fistula infection and pyoderma were diagnosed. Among patients having positive antibody response (group A1), 23 patients had upper respiratory tract infection [10] urinary system infection [5], periodontitis [4], AV fistula infection [2], arthritis [1], pyoderma [1] while among patients having negative antibody response (group A2), 9 patients had upper respiratory tract infection [4], urinary infection [2], AV fistula infection [2] and periodontitis [1] and in group B, 6 patients had upper respiratory tract infection [2] urinary system infection [1], periodontitis [1], AV fistula infection [2].

Discussion

HD patients, because of being a high risk group for HBV infection, are recommended to be vaccinated before being infected with HBV. The protective antibody level (10 mIU/mL), is obtained by 95-99 % with three doses of recombinant hepatitis-B vaccine in healthy population. However, in cases older than 40 years and having immune suppression disorder like ESRD, the immunization rate is lower than 70% [10]. To obtain better antibody responses in this population, CDC recommends double doses of IM vaccine (40 µg/ml) on 0, 1, 2 and 6th months [5]. In this study, we obtained a positive antibody response in 82.8 % of patients in whom we administered IM 40 µg/ml recombinant hepatitis-B vaccine for 4 times. This result was found in accordance with positive antibody percentages that Anandth *et al.* [12] and Peces *et al.* [11] obtained in their studies.

It is suggested that the factors such as age [13], HD duration and adequacy [14], using bio-compatible membrane [15, 16], existence of anemia and administration of rHuEpo [17], nutritional status [18], and infections and inflammation affect antibody response to HBV vaccination in HD population.

Population younger than 40 years develops better antibody response [12]. In this study, in Group A1 who had higher antibody titers, the mean age was about 40 years, and in Group A2 who had lower antibody titers, the mean age was above 40 years (40.1±11.3 vs 48.3±7.9, p=0.017), and this results were similar to data of the literature. However, the result that although mean age was high, antibody titers were within acceptable borders constitutes a contradiction with the data in literature. But group B patients, since they were vaccinated before uremic

Table 3. Inflammatory parameters of groups

Parameters		GROUP A		GROUP B	P
		Group A1 n:53	Group A2 n:11	n:17	
IL-1β (pg/mL)	Pre-vaccination	5.0 ± 0.0	5.0 ± 0.0	7.0 ± 0.0	0.933
	Post-vaccination	5.1± 0.5	4.9 ± 0.2	5.0 ± 0.0	0.442
IL-6 (pg/mL	Pre-vaccination	5.1 ± 0.7	6.3 ± 3.2	9.6 ± 9.5	0.145
	Post-vaccination	2.2 ± 0.7	3.8 ± 4.3	7.6 ± 13.4	0.241
TNFα (pg/mL)	Pre-vaccination	15.0 ± 15.9	9.8 ± 5.8	19.5 ± 15.4	0.456
	Post-vaccination	12.2 ± 13.6	8.6 ± 3.9	12.2 ± 12.2	0.811
CRP (mg/L)	Pre-vaccination	6.0 ± 4.7	6.5 ± 5.5	25.8 ± 16.2	0.061
	Post-vaccination	9.8 ± 7.9	20.8 ± 27.9	18.9 ± 23.9	0.346

syndrome and occurrence of weakness in immunity, and of course with the contribution of advanced age have decreased anti-HBs titers lower than preventive levels in years. Thus, anamnestic antibody response produced with a booster dose is expected in these patients [10-14].

When evaluating the effect of chronic HD duration and adequacy on antibody response, no significant difference was determined between groups with respect to the time of HD therapy (p 0.05). These results were similar to those Peces *et al.* [11] determined. The groups fulfilled the targets proposed NKF/DOQI guide [13] with respect to HD adequacy. When analyzing effect of HD adequacy on antibody response, no statistically significant difference was found between groups (p 0.05). However, positive antibody response obtained after hepatitis-B vaccine was better than the expected value in this population and HD sufficiency can be said to have contribution on this result. When comparing with our results, among studies on the effect of HD adequacy on antibody response, Elwell *et al.* [9] reported different and Kovacic *et al.* [19] reported similar findings.

When comparing groups with respect to hematological parameters, although Hb/Htc value in the group of negative antibody response was within target levels and that in the group of positive antibody response was under target levels, positive antibody response group reached target Hb/Htc levels at the end of vaccination program. This result, in addition to the fact that positive antibody titer, being 82.8 % , was found higher than the expected rate, as in many studies in the literature [20-22] was linked to the fact that all patients included in the study received rHuEPO therapy.

We determined no association between nutritional parameters such as serum albumin, cholesterol and HCO_3 levels and antibody response to hepatitis-B vaccine. This finding was different from the result reported in a study by Fernandez *et al.* although, serum albumin level of the positive antibody response group is less than the desired levels, in the literature it has been shown that factors such as younger age (< 40 years), HD adequacy, bio-compatible membr-ane and rHuEPO medication may have effects on formation of antibody response. In recent study we found a relation between age and antibody response, although no association with other parameters, and this might be due to low number of non-responders.

During hepatitis-B vaccination program, 23 patients in positive antibody response group developed acute infections (most frequently upper respiratory tract infection in 10 cases), whereas 9 patients developed acute infections (most frequently upper respiratory tract infection in cases) in negative responders group. When considering inflammatory states of all patients studied during vaccination program, no significant difference was seen between serum levels of pro-inflammatory cytokines and antibody response in groups. On the other hand, level of CRP which is an important indicator of acute phase response to inflammation was determined as statistically insignificantly higher in negative antibody response group than in the other group. (p>0.05). Therefore, it was concluded that antibody response developed against hepatitis-B vaccine is independent of pro-inflammatory cytokines. Although in recent study the number of non-responder group is lower and this low number limited the strength of study, our findings are controversial when compared with the literature [23-29].

In conclusion, when starting HD treatment of ESRD patients who are at high risk for hepatitis-B infection, serologic markers should be analyzed, and patients having HBsAg (-) and anti-HBs (-) should be vaccinated and in patients whose antiHBs antibody titer decreased, booster dose vaccine should be administered to form anamnestic response. It is thought that acute infection and inflammation developed during this period do not affect antibody response negatively and markers of

acute phase response are not indicators for negative antibody response.

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