CLINICAL SCIENCE

Antibody response following Hepatitis B vaccination in peritoneal dialysis patients: does normalized urea clearance matter?

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OBJECTIVES: Data on the factors that contribute to the antibody response to hepatitis B virus vaccination in peritoneal dialysis patients are scarce. The current study was conducted on a group of peritoneal dialysis patients to learn how the response to hepatitis B virus vaccination varies according to the patient's clearance of urea normalized to total body water (Kt/V).

METHODS: A convenience sample of 33 peritoneal dialysis patients (13 women and 20 men, with a mean age of 49 ± 12 years) was administered double doses (20 μg IM in each deltoid muscle) of recombinant hepatitis B vaccine at 0, 1, 2, and 6 months. Response to immunization was measured at one to three months after the final dose of vaccine. The subjects were divided into groups according to the level of antibodies to hepatitis B surface antigen (anti-HBs), including non-responders (<10 IU/L), weak responders (10-100 IU/L), and good responders (>100 IU/L).

RESULTS: Among non-responders, weak responders, and good responders, significant differences were found in age (54 ± 12 *vs.* 56 ± 9 *vs.* 45 ± 12 years, respectively; $p = 0.049$) and recombinant human erythropoietin use (20 *vs.* 29 *vs.* 76%, respectively; $p = 0.016$). No significant differences in weekly total Kt/V ($p = 0.704$), weekly peritoneal Kt/V ($p = 0.064$) and residual glomerular filtration rate ($p = 0.355$) were found across the three groups.

CONCLUSIONS: Delivered clearance measured by weekly peritoneal Kt/V and total clearance measured by weekly total Kt/V did not predict the response to hepatitis B virus vaccination in patients on peritoneal dialysis.

KEYWORDS: Continuous ambulatory peritoneal dialysis; Hepatitis B virus; Vaccination; Dialysis adequacy; Kt/V.

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INTRODUCTION

Control of a hepatitis B virus (HBV) infection has been a continuous challenge in the management of patients with end-stage renal disease (ESRD). Patients undergoing hemodialysis (HD) are particularly at risk for HBV infection.[1] In contrast to HD patients, patients on peritoneal dialysis (PD) appear to be at low risk for HBV infection. Nevertheless, all susceptible ESRD patients, including those on PD, are encouraged to be vaccinated because it is likely that they will eventually need HD when PD becomes unfeasible, either temporarily or permanently.[2] Furthermore, the peritoneal dialysate of patients positive for the hepatitis B surface antigen (HBsAg) contains sufficient infectious particles to cause hepatitis outbreaks in dialysis units.[3,4]

Uremic patients elicit weak response to vaccination with recombinant HBV vaccine.[5] To improve the immunogenicity of HBV vaccination for dialysis-dependent patients, the vaccine should be administered at a dose of 40 μg, in contrast to the 20 μg dose for healthy adults, through the intramuscular route.[6] Moreover, instead of a three-dose schedule (i.e., 0, 1, and 6 months), a four-dose schedule (i.e., 0, 1, 2, and 6 months) is recommended.[6] Blood antibody levels of hepatitis B antigen (anti-HBs) ≥10 IU/L are considered protective in healthy individuals; however, some authors believe that higher levels (>100 IU/L) are desirable in patients on chronic HD.[7,8]

Factors associated with a favorable response to HBV vaccination in HD patients include young age, good nutritional status, and effective dialysis.[1,5,9] Dialysis adequacy is defined as the weekly clearance of urea normalized to total body water (Kt/V). To date, only four studies have described the effects of dialysis adequacy on HBV vaccination response in PD patients. The results of these studies, however, have been inconclusive.[10-13] We conducted this study on a group of PD patients undergoing PD to demonstrate how the response to HBV vaccination varies with weekly Kt/V.

METHODS AND MATERIALS

The Faculty of Medicine Ethics Committee of Kocaeli University School of Medicine approved this prospective observational study protocol, and all of the patients provided written informed consent before entry into the study. Both incident and prevalent PD patients over the age of 18 years with ESRD at our university hospital clinic were screened for HBV markers. Patients with negative HBsAg, negative antibodies to hepatitis B surface antigen (anti-HBs), and negative antibodies to hepatitis B core antigen (anti-HBc) were included in the study. Patients using immunosuppressive agents or patients with a history of malignancy, human immunodeficiency virus (HIV) infection, alcoholic liver disease, HBV vaccination, abnormal liver function results during the six months prior to recruitment, or positivity for HBsAg, anti-HBs, or anti-HBc at any time in the past were excluded. Peritoneal dialysis patients were on a standard continuous ambulatory peritoneal dialysis (CAPD) program, with four or five administrations daily of 2000 mL each.

Between January 2009 and May 2010, all participants were administered double doses (20 μg IM in each deltoid muscle) of recombinant hepatitis B vaccine (Euvax B, LG Life Sciences, Jeonbuk-do, Korea) at 0, 1, 2, and 6 months. The antibody response was determined by measuring the HBsAg antibody 1 to 3 months after the last dose of vaccine. Anti-HBs titers were measured using a commercial enzyme immunoassay kit (Cobas, Roche Diagnostics GmBH, Mannheim, Germany).

Demographic and clinical data, including age, gender, body mass index (weight/height^2), primary renal disease, recombinant human erythropoietin (rHuEpo) use, and time on dialysis, were recorded at study entry. Hemoglobin, urea, creatinine, albumin, and high sensitivity C-reactive protein (hs-CRP) were measured in venous blood samples.

Patients who had anti-HBs antibody levels <10 IU/L after the fourth dose of vaccine were considered non-responders. Responders were divided into weak responders (an anti-HBs level after the last vaccine dose of 10-100 IU/L) and good responders (an anti-HBs level after the last vaccine dose >100 IU/L).[5]

All participants underwent assessments of peritoneal membrane function and residual renal function during the vaccination process. Peritoneal membrane function was evaluated by the standard peritoneal equilibrium test (PET) using a 2.5% glucose PD solution.[14] Adequacy was calculated as weekly total Kt/V (weekly clearance of urea normalized to total body water). Peritoneal Kt/V was calculated by performing a 24-hour collection of dialysate effluent and measuring its urea content and taking a plasma sample during the PET. The dialysate-to-plasma creatinine (D/P Cr) concentration ratio at four hours of dwell was used to describe the peritoneal small solute transport rate. The protein equivalent of nitrogen appearance (PNA) was calculated using the Randerson[15] formula and normalized by the ideal body weight (nPNA). Residual renal function was evaluated by collecting all urine over the same 24-hour period as the dialysate collection. Residual glomerular filtration rate (GFR; mL/minute) was calculated as the mean of the renal urea and creatinine clearances.[16]

Statistical analysis

Statistical analyses were performed using Statistical Package for Social Sciences (SPSS) version 13.0 for Windows® (SPSS Inc., Chicago, USA). Data are expressed as the mean ± standard deviation (SD), unless otherwise stated. The normality of data distributions was determined using the Kolmogorov-Smirnov test. Comparisons between groups were made using Student's *t*-test and analysis of variance (ANOVA) for normally distributed variables, while the Mann-Whitney U test and Kruskall-Wallis variance analysis were used for parametric variables with non-normal distributions. The chi-square test was used to analyze categorical data. A *p*-value of less than 0.05 was considered statistically significant.

RESULTS

Thirty-five patients gave written informed consent to participate in the study. Two patients did not complete the vaccination program; one patient underwent transplantation, and one patient died. Therefore, 33 patients (13 women and 20 men, mean age 49 ± 12 years) were included in the final analysis. Only one patient was positive for antibodies to hepatitis C virus (anti-HCV). The demographic, clinic, and laboratory features of the patients are shown in Table 1.

After the fourth double dose of the vaccine, a response (anti-HBs titer ≥10 IU/L) was observed in 28 (84.8%) of the patients. No significant differences were observed for age, time on dialysis, body mass index, urea, creatinine, hemoglobin, serum albumin, nPNA, or hs-CRP between responders and non-responders (Table 2). Similarly, weekly total Kt/V ($p = 0.573$), weekly peritoneal Kt/V ($p = 0.385$) and residual GFR ($p = 0.404$) were not significantly different between responders and non-responders. The response rate to immunization was 86% among the 29 CAPD patients with a weekly Kt/V of 1.7 or greater. This rate was not significantly different as compared to the 75% response rate among the four CAPD patients with lower values of weekly Kt/V ($p = 0.558$).

Of the 28 patients responding to vaccination, seven patients (25%) were weak responders, and 21 patients (75%) were good responders. Time on dialysis, body mass index, urea, creatinine, hemoglobin, serum albumin, nPNA, and hs-CRP were not significantly different among non-responders, weak

Table 1 - Demographic, clinical, and laboratory characteristics of 33 CAPD patients at study onset. Values are expressed as the mean ± SD, unless otherwise noted.

Variables	n = 33
Age	49 ± 12
Gender (F/M)	13/20
Cause of ESRD (%)	
Hypertensive nephropathy	8 (24%)
Diabetic nephropathy	5 (15%)
Chronic glomerulonephritis	3 (9%)
Polycystic kidney disease	3 (9%)
Reflux nephropathy	2 (6%)
Amyloidosis	1 (3%)
Unknown	11 (33%)
Time on dialysis (months)	28 ± 23
Diabetes, n (%)	7(21)
Body mass index (kg/m^2)	28.1 ± 4.9
Urea (mg/dL)	105 ± 40
Creatinine (mg/dL)	6.7 ± 3.5
Serum albumin (g/dL)	3.8 ± 0.5
Hemoglobin (g/dL)	11.3 ± 1.9
Hs-CRP (mg/dL)	1.06 ± 1.61

SD, standard deviation; ESRD, end stage renal disease; hs-CRP, high sensitive C-reactive protein.

Table 2 - Responses to four-dose HBV vaccination schedule measured by anti-HBs titers and clinical, laboratory, dialysis adequacy, and residual renal function results in 33 peritoneal dialysis patients. Values are expressed as the mean ± SD, unless otherwise noted.

Variables	Non-responders <10 IU/L	Responders ≥10 IU/L	*p*-value
Subjects (n)	5	28	-
Age (years)	54±12	48±12	0.296
Time on dialysis (months)	18±17	29±23	0.379
Erythropoietin use, n (%)	1 (20)	18 (64)	0.065
Diabetes, n (%)	2 (40)	5 (18)	0.265
Body mass index (kg/m^2)	29.1±3.2	28.0±5.2	0.641
Urea (mg/dL)	89±28	108±41	0.348
Creatinine (mg/dL)	6.3±5.7	6.8±3.1	0.291
Hemoglobin (g/dL)	10.6±1.3	11.4±1.9	0.498
Serum albumin (g/dL)	3.5±0.7	3.8±0.4	0.138
hs-CRP (mg/dL)	1.8±1.4	0.9±1.6	0.079
Weekly total Kt/V	2.9±1.4	2.6±1.1	0.573
Peritoneal Kt/V	1.5±0.5	1.7±0.5	0.385
RRF (mL/min)	4.7±2.9	6.5±4.0	0.404
Patients with RRF, n (%)	4 (80%)	23 (82%)	0.909
D/P creatinine	0.75±0.18	0.73±0.35	0.300
nPNA, g/kg/day	1.1±0.5	1.2±0.4	0.979

hs-CRP, high sensitive C-reactive protein; Kt/V, clearance of urea normalized to total body water; RRF, residual renal function (patients with RRF had a daily diuresis over 200 mL); D/P, dialysate/plasma; nPNA, normalized protein equivalent of nitrogen appearance.

responders and good responders (Table 3). A statistically significant difference was observed across non-responders, weak responders and good responders in age (54±12 *vs.* 56±9 *vs.* 45±12 years, respectively; $p=0.049$) and rHuEpo use (20 vs. 29 vs. 76%, respectively; $p=0.016$). There were no statistically significant differences in weekly total Kt/V ($p=0.704$), weekly peritoneal Kt/V ($p=0.064$) and the residual glomerular filtration rate ($p=0.355$) across non-responders, weak responders and good responders.

DISCUSSION

In the present study, we found an overall response rate of 84.8%, which is comparable to the rates reported in the literature for peritoneal dialysis patients (53% to 93.3%).[2,10,11,17,18] Delivered clearance measured by weekly peritoneal Kt/V, total clearance measured by weekly total Kt/V, and residual GFR did not predict response to HBV vaccination in the study's patients treated with CAPD. Regarding the effect of weekly total Kt/V on HBV vaccination response, Dacko et al. examined the influence of residual renal function and dialysis adequacy on HBV vaccination response in 32 peritoneal dialysis (PD) patients. Consistent with our findings, they found that the incident residual renal function and the incident weekly Kt/V in well-dialyzed peritoneal dialysis patients did not predict responses to the hepatitis B vaccine.[10] Similarly, Chow et al. did not find a relationship between weekly total Kt/V and immune response for 52 patients with ESRD on PD.[12] More recently, this same group of researchers evaluated the factors associated with hepatitis B vaccine response in 87 PD patients and found that higher nPNA, but not weekly Kt/V, was significantly associated with better vaccination response.[13] In contrast to our findings, however, Svac et al. found a vaccination response rate of 78% for 40 PD patients with a weekly Kt/V greater than 1.7 (n=28) compared to a response rate of just 8% in patients with a weekly Kt/V below 1.7 ($p=0.0003$).[11] The limitations of their study, however, included a lack of information on the vaccination schedule and dose; in addition, the overall seroconversion in their patients was only 53%. The proportion of patients receiving inadequate dialysis in their study (30%) was larger than in our study (12%), making comparisons difficult.

Based on our data and those of other reliable studies, total clearance in peritoneal dialysis as measured via weekly total Kt/V is not predictive of responses to HBV vaccination in PD patients.

In ESRD patients, the inadequate antibody response following HBV vaccination is multifactorial.[1] In addition

Table 3 - Responses to four-dose HBV vaccination schedule measured by anti-HBs titers and clinical, laboratory, dialysis adequacy, and residual renal function values in 33 peritoneal dialysis patients. Values are expressed as the mean ± SD, unless otherwise noted. Statistically significant values are underlined.

Variables	Non-responders <10 IU/L	Weak responders 10-100 IU/L	Good Responders >100 IU/L	*p*-value
Subjects (n)	5	7	21	-
Age (years)	54±12	56±9	45±12	<u>0.049</u>
Time on dialysis (months)	18±17	32±22	29±24	0.554
Erythropoietin use, n (%)	1 (20)	2 (29)	16 (76)	<u>0.016</u>
Diabetes, n (%)	2 (40)	0 (0)	5 (24)	0.220
Body mass index (kg/m^2)	29.1±3.2	27.9±3.8	28.0±5.6	0.897
Urea (mg/dL)	89±28	96±42	112±41	0.437
Creatinine (mg/dL)	6.3±5.7	6.9±4.0	6.8±2.8	0.551
Hemoglobin (g/dL)	10.6±1.3	11.6±1.8	11.4±2.1	0.690
Serum albumin (g/dL)	3.5±0.7	3.9±0.5	3.8±0.4	0.337
hs-CRP (mg/dL)	1.8±1.4	0.8±0.7	1.0±1.9	0.212
Weekly total Kt/V	2.9±1.4	2.8±1.4	2.5±0.9	0.704
Peritoneal Kt/V	1.5±0.5	2.0±0.7	1.6±0.4	0.064
RRF (mL/min)	4.7±2.9	8.3±3.8	6.0±4.0	0.355
Patients with RRF, n (%)	4 (80%)	5 (71%)	18 (86%)	0.693
D/P creatinine	0.75±0.18	0.82±0.61	0.70±0.28	0.317
nPNA, g/kg/day	1.1±0.5	1.4±0.6	1.2±0.4	0.798

hs-CRP, high sensitive C-reactive protein; Kt/V, clearance of urea normalized to total body water; RRF, residual renal function (patients with RRF had a daily diuresis over 200 mL); D/P, dialysate/plasma; nPNA, normalized protein equivalent of nitrogen appearance.

to age, many variables such as albumin levels,[9] hepatitis C infection,[7] and erythropoietin deficiency[1] may influence the response of a patient to hepatitis B vaccine. In addition, cell-mediated immunity factors such as impairment of monocyte function, reduced T cell proliferation, and decreased production of interleukin 2 are related to poor antibody response.[19,20] In the present study, we found that younger PD patients had significantly better responses to HBV vaccination. Similarly, a meta-analysis of 17 clinical trials showed an increased response to hepatitis B virus vaccination among younger dialysis patients;[21] these findings may be due to age-associated changes in immune status. Our study also showed that rHuEpo use in PD patients was associated with better response to hepatitis B virus vaccination, a finding that suggests an immunomodulating effect of rHuEpo.[22] As found in other studies,[7,10,23] the duration of dialysis therapy as well as the levels of hemoglobin and serum albumin failed to predict responses to the hepatitis B vaccine in PD patients in this study. Fernandez et al.[9] and Kara et al.[24], however, found that malnutrition in HD patients, which is defined by low serum albumin levels, negatively influenced responses to HBV vaccination. We could not determine this association in our study because we lacked a subgroup of malnourished patients. Only one patient in our study had antibodies to hepatitis C virus, making it difficult to determine the effects of HCV positivity on hepatitis B vaccination responses.

The positive effects of dialysis adequacy, which are characterized by single-pool Kt/V, on the response to HBV in HD patients have been well documented.[5] However, given the results of both the present study and previous research,[10,12,13] this association may not be present in PD patients. Clearance delivered continuously, as in PD, is more efficient than the same amount of clearance delivered intermittently, as in hemodialysis; thus, the Kt/V values are not comparable.[25] A limitation of our study is that the small number of patients (n = 33) resulted in a small number of patients in each vaccination response subgroup, which precluded reliable statistical analysis of any differences. In addition, the persistence of protective antibodies 12 months after completion of the fourth dose was not measured.

CONCLUSIONS

We demonstrated that delivered clearance (measured by weekly peritoneal Kt/V), total clearance (measured by weekly total Kt/V), and residual GFR did not predict responses to HBV vaccination in patients on CAPD. The influence of dialysis adequacy on responses to hepatitis B vaccination would be best studied in a larger group of PD patients with wide variations in Kt/V and residual renal function.

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