AUTHOR QUERY FORM

ELSEVIER	**Journal:** JVAC **Article Number:** 11613	**Please e-mail or fax your responses and any corrections to:** **E-mail:** corrections.eseo@elsevier.thomsondigital.com **Fax:** +353 6170 9272

Dear Author,

Please check your proof carefully and mark all corrections at the appropriate place in the proof (e.g., by using on-screen annotation in the PDF file) or compile them in a separate list. To ensure fast publication of your paper please return your corrections within 48 hours.

For correction or revision of any artwork, please consult http://www.elsevier.com/artworkinstructions.

No queries have arisen during the processing of your article.

Thank you for your assistance.

Vaccine xxx (2011) xxx–xxx

Contents lists available at ScienceDirect

Vaccine

journal homepage: www.elsevier.com/locate/vaccine

Comparison of hepatitis B surface antibody decay rates after vaccination between hemodialysis and peritoneal dialysis patients

Shih-Yi Lin[a], Jiung-Hsiun Liu[a], Chung-Chih Lin[a], Su-Ming Wang[a], Chen-An Tsai[b], Che-Yi Chou[a], Huey-Liang Kuo[a], J.-Kuan Wang[a], Yao-Lung Liu[a,c,*], Hsin-Hung Lin[a], Chiu-Ching Huang[a]

[a] China Medical University Hospital, Division of Nephrology, Department of Internal Medicine, Taichung, Taiwan, ROC
[b] Statistic Center, Graduate Institute of Biostatics, China Medical University, Taiwan, ROC
[c] China Medical University Hospital, Taiwan, ROC

ARTICLE INFO

Article history:
Received 14 January 2011
Received in revised form 17 March 2011
Accepted 17 March 2011
Available online xxx

Keywords:
HBV vaccination
Hemodialysis
Peritoneal dialysis
Waning immunity

ABSTRACT

Background and objectives: The available information about maintaining effective immunity after hepatitis B virus (HBV) vaccination in dialysis patients is limited. The aim of this study was to determine whether a difference exists in the persistence of immunity between hemodialysis (HD) and peritoneal dialysis (PD) patients. We compared the decay rate of hepatitis B surface antibody (anti-HBs) titers after HBV vaccination between HD and PD patients.
Design, setting, participants, and measures: A total of 103 HD and 53 PD patients who were completely vaccinated were enrolled. We examined their anti-HBs titers at the 1st month after vaccination then annually thereafter. Changes in the anti-HBs titers were assessed by comparing annual geometric mean titers (GMTs).
Results: The slopes of the anti-HBs titer decay rates plotted on a logarithmic scale for the HD and PD groups were −23.41 and −31.48, respectively. The decay rate of the PD group was significantly faster than that of the HD group ($P = 0.0053$).
Conclusion: The decay rate of anti-HBs titers in the PD group was faster than that in the HD group. Hepatitis B vaccination could not offer long-term protection in HD or PD patients. Post-vaccination testing every 6–12 months is necessary and revaccination may be protective in dialysis patients, especially in hyper-endemic areas of hepatitis B infection.

1. Introduction

Hepatitis B virus (HBV) infection, the leading cause of cirrhosis and hepatocellular carcinoma, is a global health concern [1]. It is important and protective to recommend that dialysis patients receive hepatitis B vaccination and then undergo regular check-ups for hepatitis B surface antibody (anti-HBs) titers [2]. Compared with healthy individuals, dialysis patients show a unique immune response to HBV vaccination. Among the general population, 90–100% of vaccinated subjects acquire protective immunity and maintain protection against HBV 10–15 years later, even after vaccination in infancy [3]. Despite adopting a higher frequency immunization schedule and higher vaccine doses, the vaccination failure rate remains up to 30–60% among dialysis patients [4]. Increasing age, malnutrition, and depression are currently recognized as negative determinants of immune response among the dialysis population [5–8]. In our previous study, we demonstrated that dialysis modality has no effect on response to HBV vaccination [9]. In addition, we observed rapidly declining proportions of seroconversion in hemodialysis (HD) as well as peritoneal dialysis (PD) patients at 2-year follow up. While the unfavorable initial effect of the HBV vaccine in the dialysis groups is documented, few studies have determined the kinetics of the anti-HBs titer waning pattern and the factors affecting immune persistence in the dialysis populations. We aimed to study trends in the decay rates of anti-HBs titers in the dialysis population and compare the decay rates between PD and HD groups. We also attempted to plan an efficient screening and boosting schedule based on the observed trends.

2. Methods

We initiated data collection on dialysis patients at our dialysis center from March 2002 to March 2008, and followed-up these patients until March 2009. We enrolled dialysis subjects who tested negative for HBV surface antigen (HBs Ag) and HBV

Abbreviations: Anti-HBs, hepatitis B surface antibody; HD, hemodialysis; PD, peritoneal dialysis; GMT, geometric mean titer; HBs Ag, HBV surface antigen.

* Corresponding author at: China Medical University Hospital, Taiwan, ROC. Tel.: +886 04 22052121 ext. 2902; fax: +886 04 22076863.
E-mail address: liuyaolung@yahoo.com.tw (Y.-L. Liu).

0264-410X/$ – see front matter © 2011 Published by Elsevier Ltd.
doi:10.1016/j.vaccine.2011.03.049

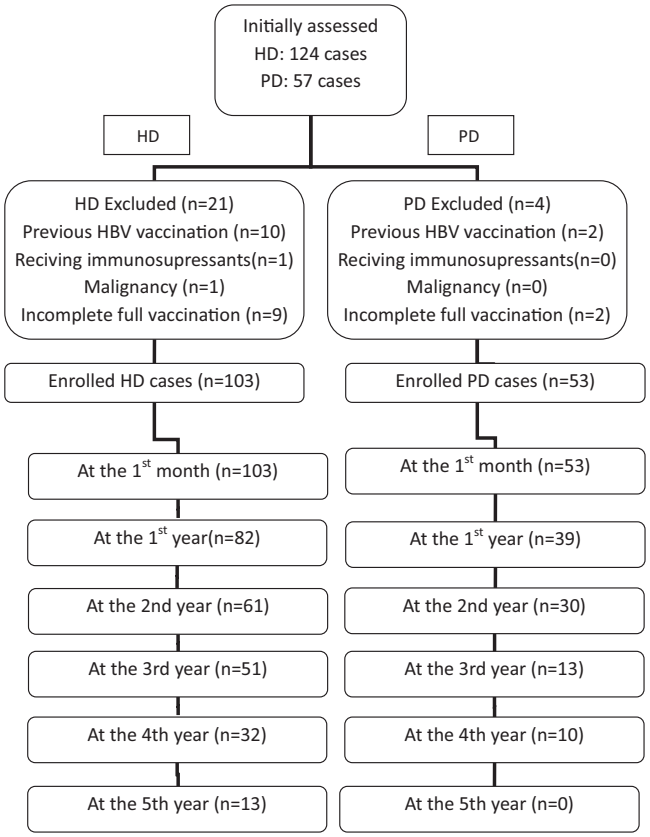

Fig. 1. The flow chart of patients enrolled in out study.

surface antibody (anti-HBs). We excluded patients who were receiving immunosuppressive agents, had malignancies, had previous HBV vaccination, or those who were unable to complete all 4 vaccinations. All patients were vaccinated with a 40 μg recommended hepatitis B vaccine (Engerix-B, GlaxoSmithKline Biologicals) injected into the deltoid muscle at 0, 1, 2, and 6 months.

Initially, there were 124 HD patients and 57 PD patients assessed. We excluded patients who were receiving immunosuppressive agents (1 HD case of frequent acute exacerbation of chronic obstructive lung disease), had malignancies (1 HD case of lung cancer), had previous HBV vaccination (10 HD cases and 2 PD cases, respectively), or those who were unable to complete all 4 vaccinations (9 HD cases and 2 PD cases, who transferred to other dialysis units) (Fig. 1). A total of 156 patients (64 male and 92 female) were enrolled. Serial blood samples were obtained from each available subject at the first month after the final vaccination (time t_0), the first year (time t_1), and second year (time t_2) after the final vaccination, and annually thereafter (time t_n). Anti-HBs titers were checked with an ELISA kit (AUSAB-EIA, Abbot Labs, USA). A total of 156 subjects were enrolled; 156 t_0 samples, 121 t_1 samples, 91 t_2 samples, 64 t_3 samples, 42 t_4 samples, and 13 t_5 samples were obtained from these patients. Anti-HBs titers ≥ 10 mIU/ml were regarded as protective in both HD and PD patients. The lower and upper limits of detection using this quantitative method were 0.1 mIU/ml and 1000 mIU/ml, respectively. In the case of undetectable titers, values of 0.05 mIU/ml and 1000 mIU/ml were assigned.

We calculated the geometric mean titers (GMTs) of anti-HBs from the available patients to determine the central tendency of the anti-HBs titers given the skewed distribution of the anti-HBs levels. We traced the trends in the decay rates of anti-HBs titers among the dialysis subjects. We also compared the decay rates of anti-HBs titers between the HD and PD groups.

Table 1
Patient characteristics.

Characteristics	HD	PD	*P* value
Number	103	53	
Age	60.89 ± 11.52	51.74 ± 15.10	<0.001
Gender (M:F)	45:58	19:34	0.393
Duration of dialysis	41.25 ± 41.39	35.64 ± 28.0	0.522
DM	37	13	0.205
Hemoglobin (g/dl)	9.71 ± 1.40	9.19 ± 1.32	0.041
Albumin (g/dl)	3.50 ± 0.37	3.48 ± 0.40	0.958
Responders (proportion)	71 (68.63%)	38 (71.69%)	0.854

Note: Data are shown as the number (%) or mean ± S.D. as appropriate. *Abbreviation*: DM, diabetes mellitus.

Comparisons of categorical data were performed by means of the Chi-square test. Continuous data were compared by the non-parametric Wilcoxon rank-sum test. The annual decay pattern of the log anti-HBs level in both HD and PD patients is assessed using the repeated measures ANOVA with the first-order auto-regressive variance-covariance structure. We also examined the association between anti-HBs titers and post-vaccination time lapse. A P value < 0.05 was considered statistically significant. The GMT for the anti-HBs levels was computed by the following model: GMT at time t_n = GMT at time t_0 + (β_1 log[time]), where 10 is the base of the natural logarithmic function and β_1 is the coefficient of this logarithmic function. We examined the differences between the slopes of the logarithmic functions of the HD and PD groups. To take into account the fact that many factors such as gender, age, albumin, and hematocrit levels might influence immune persistence, we used the regression function "titer at time t_n = titer at time t_0 + (β_1 log[time])" to identify the slope for each patient. A multiple linear regression model was then adapted to examine the effects of age, albumin, gender, modality, and hemoglobin on the slope.

3. Results

3.1. Patient characteristics and response rate

A total of 156 participants (103 HD and 53 PD individuals) were included in our study. Patient characteristics are summarized in Table 1. The GMT of the 156 vaccines checked at the first month after the fourth dose was 42.52 mIU/ml. Among all dialysis patients vaccinated, there were 110 responders and the overall seroconversion rate after primary vaccination was 70.5%. Among patients younger than 40 years of age, 93.7% responded compared with 83.3% of those aged 41–50 years, and 66.1% of those older than 50 years.

3.2. Anti-HBs GMT during 5-year follow-up

The log anti-HBs level is significantly different over time in both HD and PD groups (P = 0.0011 and P < 0.0001, respectively).

The GMT among both dialysis groups was 43.30 mIU/ml at time t_0, 13.9 mIU/ml at time t_1, 11.3 mIU/ml at time t_2, 5.8 mIU/ml at time t_3, 4.2 mIU/ml at time t_4, and 0.7 mIU/ml at time$_5$. During annual follow-up after the final injection, the PD group had a higher anti-HBs GMT than the HD group in the first 2 years (Table 2).

Table 2
Serial geometric mean times from 5-year follow-up of hemodialysis and peritoneal analysis patients.

	GMTs					
	1st month	1st year	2nd year	3rd year	4th year	5th year
HD	43.3	13.9	11.3	5.8	4.2	0.7
PD	55.2	16.7	12.7	3.07	1.5	

GMT, geometric mean titers.

Please cite this article in press as: Lin S-Y, et al. Comparison of hepatitis B surface antibody decay rates after vaccination between hemodialysis and peritoneal dialysis patients. Vaccine (2011), doi:10.1016/j.vaccine.2011.03.049

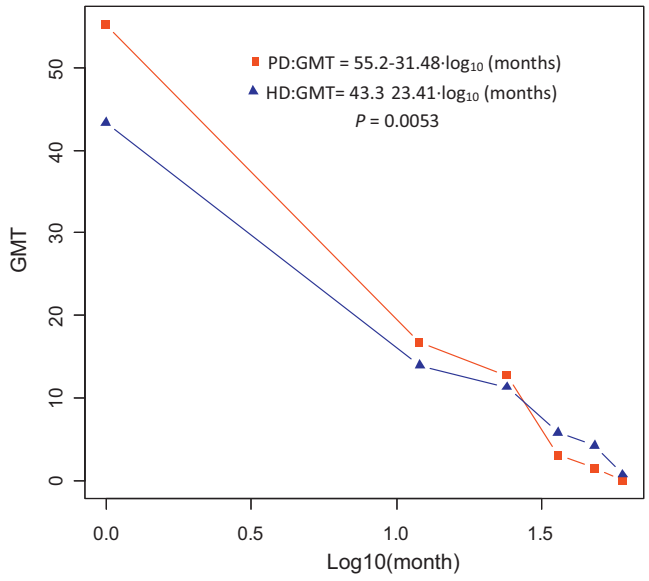

Fig. 2. Comparison of the slopes of the 2 regression lines showing a significant difference between the two dialysis modalities with a *P* value of 0.0053.

The Pearson correlation coefficients for serial anti-HBs GMTs and time in the HD and PD groups were −0.994 and −0.995, respectively ($P = 0.01$ for both). At the 1st year after vaccination, 25% of PD patients had lost their protection against HBV (anti-HBs levels < 10 mIU/ml), compared with 19.51% of HD patients who lost their protection against HBV at the same time point.

3.3. Anti-HBs decay rates after vaccination for each dialysis modality

The kinetics of anti-HBs antibody production and persistence based on the serum samples obtained annually after the final injection were analyzed. Both the HD and PD groups were examined. With the logarithmic model, the slope of the anti-HBs GMT change between the log 1st month and log 60th month in the HD and PD groups was −23.41 and −31.48, respectively. The anti-HBs GMT of each dialysis modality group in each follow-up year is plotted on this logarithmic scale (Fig. 2). We compared the slopes of the 2 regression lines from the 2 groups, and found that the anti-HBs titer decay rate was significantly faster in the PD group than in the HD group ($P = 0.0053$).

3.4. Factors influencing trends in the decay rates of anti-HBs titers in dialysis patients

We further analyzed the factors that might affect immune persistence after HBV vaccination. We found that the anti-HBs titer decay rate slope was influenced by modality and albumin ($P = 0.024$ and $P = 0.026$, respectively). There was no significant effect of age, gender, or hematocrit on the decay rates of the dialysis patients ($P > 0.05$).

4. Discussion

Our study demonstrated several novel findings among HBV-vaccinated dialysis patients: the non-persistence of immunity against HBV in both the HD and PD groups, trends in the anti-HBs titer decay rates in both groups, and the unique characteristic of an apparent rapid drop in anti-HBs titers among the PD population.

Despite low seroconversion against HBV among dialysis patients, available information relating to waning immunity after HBV vaccination is limited, and is almost solely focused on the HD population [5,10–12]. Pasko et al. and others, have shown that about 18.7–100% of HD patients lose their protective immunity in the 1–3 years after HBV vaccination [5,10–12]. Our results provide further support for these observations among the HD population, but we have additionally noted that PD patients also lack consistent immunity against HBV over the 5-year period following HBV vaccination.

Factors influencing the persistence of immunity against HBV among dialysis patients have been investigated in 2 previous studies [10,11] and our results confirm their findings of no impact of age, hematocrit, or gender on the status of effective immunity against HBV after HBV vaccination.

However, although the above studies did follow up on the effective immune status for at least 2 years, they did not determine factors influencing the dynamics of anti-HBs titer decay. In addition, their data were limited to HD patients, so their results relating to the persistence of immunity are not necessarily relevant for PD patients. We demonstrated that the anti-HBs titer decay rate was significantly faster in the PD group than in the HD group. Thus, dialysis modality might be the major factor affecting anti-HBs titer decay in the dialysis population.

Our findings could imply that PD patients cannot maintain effective immunity after HBV vaccination, that is, effective immunoglobulin G (IgG) levels in serum. Reduced IgG synthesis, peritoneal loss, or increased catabolism should all be considered when explaining rapidly reducing serum IgG levels.

Specialized memory B cells or plasma cells produce IgGs after class switching in lymphoid tissues. This lack of immunological memory, conferring protection from viruses such as HBV, requires synergistic actions among memory B cells, memory T cells, and antigen-antibody complexes [12,13]. Bouts et al. revealed a significant correlation between $CD27^+$B cell numbers and serum IgG level, implying that low levels of memory B cells contribute to low IgG levels in children with chronic renal failure [14]. In addition to declining levels of memory B cells in dialysis patients, amnestic responses to antigen exposure are also impaired. Peces et al. observed that only 50% of dialysis patients with low anti-HBs titers respond adequately to a booster dose [15]. We suggest that both decreasing memory B cell counts and a poor amnestic response account for the difficulty maintaining protective anti-HBs titers in dialysis patients.

The above hypothesis, although it might elucidate the defective immune memory of dialysis patients, cannot explain the discrepancy in the decay rate between the PD and HD groups. We suggest that rapidly declining anti-HBs titers might result from better middle molecule clearance in PD, considering that none of our HD study subjects received hemodiafiltration. In addition, hypogammaglobulinemia has been demonstrated along with low serum IgG, IgG1, IgG2, and IgG4 levels in PD children, and low serum IgG2 and IgG4 levels in PD adults [16–19]. Clinical findings reported by Barbara et al. revealed similar abnormalities in humoral immunity with regard to active immunization against *Hemophilus influenza* type b in PD children [20]. Thus, we propose that diminished capacity for immune memory and hypogammaglobulinemia concomitantly contributes to the significantly more rapid decay of anti-HBs titers in PD than in HD patients.

Hypoalbunemia was the 2nd influencing waning immunity against HBV in our study. Albumin, exclusively synthesized in the liver, is utilized as a clinical indicator of protein malnutrition. Although transperitoneal albumin losses are inevitable, PD patients are able to increase albumin synthesis to replace such losses [21]. Thus, malnutrition as well as a reduced albumin synthesis rate, resulting from an original decreased protein intake, inflammatory response, or both, would be the chief cause of hypoalbuminemia in either PD or HD patients [22]. Currently, malnutrition is recognized

Please cite this article in press as: Lin S-Y, et al. Comparison of hepatitis B surface antibody decay rates after vaccination between hemodialysis and peritoneal dialysis patients. Vaccine (2011), doi:10.1016/j.vaccine.2011.03.049

as a major cause of secondary immunodeficiencies, and it adversely affects both innate and adaptive immunity [23]. Fernández et al. reported that malnutrition negatively influenced the response to the hepatitis B virus vaccine among HD patients [24]. In our study, hypoalbuminemia accelerated the decay rate of anti-HBs titers in dialysis patients. The results of Fernández et al. in combination with our data suggest that malnutrition might not only affect the initial response to HBV vaccine, but also impair long-term immune memory in dialysis patients [24]. In an animal model of malnutrition, Tetsuji et al. demonstrated that mice with protein-energy malnutrition had significantly lower anti-HBs titers at the 4- and 6-week follow-ups, decreasing the stimulating capacity of spleen dendritic cells, and decreasing the levels of interferon-γ [25]. Thus, hypoalbuminemia weakened humoral immunity through several aspects of the immune pathways, inducing lack of immune memory after vaccination. In addition to the negative impact of old age and malnutrition on protective immunity after HBV vaccination, depression is closely related to antibody response following HBV vaccine in dialysis patients [8]. In our study, we did not examine the influence of depression or health-related quality of life on the consistency of immunity after HBV vaccination in dialysis patients and further study of these factors would be useful.

We attempted to utilize a logarithmic function to re-evaluate the necessity and period of follow-up after HBV vaccination in dialysis patients. David et al. have shown that 39% of HD-vaccinated patients had lost protective anti-HBs titers (≥10 mIU/ml) by 6 months. They suggested that yearly screening may not ensure protection against HBV in dialysis patients [26]. Consistent with their findings, about 60–70% of dialysis patients in our study had lost protective immunity by the 1st year after HBV vaccination. In view of high risk of exposure to HBV, poor amnestic immune response after HBV vaccination, and potential risks of transmitting HBV among dialysis patients, intense and frequent monitoring of anti-HBs titers is protective and necessary [27]. At least 6-monthly checks of anti-HBs titers after HBV vaccination would be appropriate based on the present study.

5. Conclusion

This study revealed that the anti-HBs titer decay rate was significantly faster in the in PD than in the HD group. Thus, dialysis modality and malnutrition are major determinants of anti-HBs titer decay rates. Hepatitis B vaccination could not offer long-term protection to either HD or PD patients. Post-vaccination testing at least every 6 months is thus necessary and revaccination may be protective in dialysis patients, especially in hyper-endemic areas of HBV infection.

References

[1] Dienstag JL. Hepatitis B virus infection. N Engl J Med 2008;14:1486–500.
[2] CDC. A comprehensive immunization strategy to eliminate transmission of hepatitis B virus infection in the United States. Recommendations of the Advisory Committee on Immunization Practices (ACIP). Part II: Immunization of adults. Appendix B. MMWR 2005;54:27–30.
[3] McMahon BJ, Bruden DL, Petersen KM, Bulkow LR, Parkinson AJ, Nainan O, et al. Antibody levels and protection after Hepatitis B vaccination: results of a 15-year follow-up. Ann Intern Med 2005;5:333–41.
[4] Stevens CE, Alter HJ, Taylor PE, Zang EA, Harley EJ, Szmuness W. Hepatitis B vaccine in patients receiving hemodialysis. N Engl J Med 1984;8:496–501.
[5] Pasko MT, Bartholomew WR, Beam TR, Amsterdam D, Cunningham EE. Long-term evaluation of the hepatitis B vaccine (Heptavax-B) in hemodialysis patients. Am J Kidney Dis 1988;4:326–31.
[6] Ramón P, Miguel de la T, Roberto A, José MU. Prospective analysis of the factors influencing the antibody response to hepatitis b vaccine in hemodialysis patients. Am J Kidney Dis 1997;2:239–45.
[7] Fabrizi F, Martin P, Dixit V, Bunnapradist S, Dulai G. Meta-analysis: the effect of age on immunological response to hepatitis B vaccine in end-stage renal disease. Aliment Pharmacol Ther 2004;10:1053–62.
[8] Baris Afsara B, Elsurer R, Eyileten T, Yilmaza MI, Caglar K. Antibody response following hepatitis B vaccination in dialysis patients: does depression and life quality matter? Vaccine 2009;27:5865–9.
[9] Liu YL, Kao MT, Huang CC. A comparison of responsiveness to hepatitis B vaccination in patients on hemodialysis and peritoneal dialysis. Vaccine 2005;30:3957–60.
[10] Ramezani A, Velayati AA, Eslamifar A, Banifazl M, Ahmadi F, Maziar S, et al. Persistence of Hepatitis B vaccine immunity in hemodialysis patients. Ther Apher Dial 2008;2:143–6.
[11] Elwell R, Neumann M, Bailie GR. Factors associated with long-term antibody production induced by hepatitis B vaccine in patients undergoing hemodialysis: a retrospective cohort study. Pharmacotherapy 2003;23:1558–63.
[12] Buti M, Viladomiu L, Jardi R, Olmos A, Rodriguez JA, Bartolome J, et al. Long-term immunogenicity and efficacy of hepatitis B vaccine in hemodialysis patients. Am J Nephrol 1992;3:144–7.
[13] Siegrist C-A. Neonatal and early life vaccinology. Vaccine 2001;25–26:3331–46.
[14] Bouts AHM, Davin JC, Krediet RT, Monnens LAH, Nauta J, Schroder CH, et al. Children with chronic renal failure have reduced numbers of memory B cells. Clin Exp Immunol 2004;3:589–94.
[15] Peces R, Laurés AS. Persistence of immunologic memory in long-term hemodialysis patients and healthcare workers given Hepatitis B vaccine: role of a booster dose on antibody response. Nephron 2001;2:172–6.
[16] Krediet RT, Zuyderhoudt FMJ, Boeschoten EW, Arisz L. Peritoneal permeability to proteins in diabetic and non-diabetic continuous ambulatory peritoneal dialysis patients. Nephron 1986;2:133–40.
[17] Fivush BA, Case B, May MW, Lederman HM. Hypogammaglobulinemia in children undergoing continuous ambulatory peritoneal dialysis. Pediatr Nephrol 1989;2:186–8.
[18] Krediet R, Koomen G, Vlug A, Struijk D, Buis B, van Olden R, et al. IgG subclasses in CAPD patients. Perit Dial Int 1996;3:288–94.
[19] Bouts AHM, Davin JC, Krediet RT, Van Der Weel MB, Schroder CH, Monnens L, et al. Immunoglobulins in chronic renal failure of childhood: effects of dialysis modalities. Kidney Int 2000;2:629–37.
[20] Fivush BA, Case B, Warady BA, Lederman H. Defective antibody response to Hemophilus influenzae type b immunization in children receiving peritoneal dialysis. Pediatr Nephrol 1993;5:548–50.
[21] Kaysen GA, Schoenfeld PY. Albumin homeostasis in patients undergoing continuous ambulatory peritoneal dialysis. Kidney Int 1984;1:107–14.
[22] Kaysen GA. Biological basis of hypoalbuminemia in ESRD. J Am Soc Nephrol 1998;12:2368–76.
[23] Javier C, William TS. Secondary immunodeficiencies, including HIV infection. J Allergy Clin Immunol 2010;2:S195–203.
[24] Fernández E, Betriu MA, Gómez R, Montoliu J. Response to the hepatitis B virus vaccine in haemodialysis patients: influence of malnutrition and its importance as a risk factor for morbidity and mortality. Nephrol Dial Transplant 1996;8:1559–63.
[25] Niiya T, Akbar SMF, Yoshida O, Miyake T, Matsuura B, Murakami H, et al. Impaired dendritic cell function resulting from chronic undernutrition disrupts the antigen-specific immune response in Mice. J Nutr 2007;3:671–5.
[26] Spiegel D, Martin G, Bispham B, Smith M, Jensen K, Chonchol M. Rapid loss of hepatitis B immunity in hemodialysis patients. Dial Transplant 2004;3:122–5.
[27] Tsouchnikas I, Dounousi E, Xanthopoulou K, Papakonstantinou S, Thomoglou V, Tsakiris D. Loss of hepatitis B immunity in hemodialysis patients acquired either naturally or after vaccination. Clin Nephrol 2007;4:228–34.

Please cite this article in press as: Lin S-Y, et al. Comparison of hepatitis B surface antibody decay rates after vaccination between hemodialysis and peritoneal dialysis patients. Vaccine (2011), doi:10.1016/j.vaccine.2011.03.049