

FACTORS FOR PREDICTING SUCCESSFUL IMMUNE RESPONSE TO HEPATITIS B VACCINATION IN HIV-1 INFECTED PATIENTS

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Abstract. This study aimed to determine the predicting factors for successful hepatitis B vaccination among HIV-1 infected patients. A prospective study was conducted among HIV-1 infected patients who had negative HBV serologies. Anti-HBs antibody was evaluated one month after completing a 3-injection course of hepatitis B vaccine. Patients who had an anti-HBs antibody level >10 mIU/ml were defined as responders. There were 65 patients with a mean age of 39±8.5 years, 68% were females. Fifty-seven (88%) patients had received antiretroviral therapy for a mean (SD) duration of 26.1 (22.3) months and 75% of these had an HIV-1 RNA count <50 copies/ml. The mean (SD) CD4 cell count and percentage at the time of vaccination were 345 (194) cells/mm³ and 16 (7) %, respectively. Thirty patients (46%) were responders. Compared to non-responders, responders had a higher mean CD4 cell count ($p = 0.047$) and a trend toward a younger age ($p = 0.052$). On multivariate analysis, younger age ($p = 0.049$) and higher CD4 cell count ($p = 0.048$) were predictors for successful response to hepatitis B vaccination. Determination of antibody levels after vaccination in HIV-infected patients is warranted.

INTRODUCTION

Human immunodeficiency virus (HIV-1) and hepatitis B virus (HBV) share many similar risk factors and routes of transmission, both viruses are common in Asia. The prevalence of previous HBV infection among HIV-infected patients in Asia has been reported as high as 34-98% (Heng *et al*, 1995; Rathi *et al*, 1997; Sud *et al*, 2001) and the prevalence of chronic HBV carriers in this population has been reported as 8-16% (Heng *et al*, 1995; Rathi *et al*, 1997; Sud *et al*, 2001; Sungkanuparph *et al*, 2004; Devi *et al*, 2005).

HIV-1 infection can modify the natural history of HBV infection. HIV-1 infected patients have been found to have higher HBV replication rates than non-HIV infected patients (Perillo *et al*, 1986; McDonald *et al*, 1987; Bodsworth *et al*, 1989; Koblin *et al*, 1992; Gilson *et al*, 1997; Thio *et al*, 2004) and are more likely to become chronic carriers and develop complications such as hepatitis, cirrhosis, and hepatocellular carcinoma (Di Martino *et al*, 2002; Núñez, 2003). These lead to higher morbidity and more mortality rates in HIV-1/HBV co-infected patients. HBV co-infection with HIV-1 can cause treatment to be less effective and may lead to the rapid development of hepatitis B viral resistance compared to HBV mono-infected patients (Di Martino *et al*, 2002; Núñez, 2003).

Immunization with HBV vaccine is necessary to protect HIV-1 infected patients from

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future HBV infection and reduce morbidity and mortality (Masur *et al*, 2002; Aberg *et al*, 2004; Mast *et al*, 2006). A poor response to hepatitis B vaccination in HIV-1 infected patients has been reported in Caucasians (Collier *et al*, 1988; Odaka *et al*, 1988; Bruguera *et al*, 1992; Tayal *et al*, 1994; Overton *et al*, 2005), while a study of Asians has not been previously reported. The purpose of this study was to determine the predicting factors for successful hepatitis B vaccination among HIV-1 infected patients in a Thai population.

MATERIALS AND METHODS

Study design and subjects

A prospective observational study was conducted among HIV-1 infected patients at an infectious disease clinic in a medical-school hospital. Inclusion criteria were as follows: 1) age greater than 18 years, 2) negative serology for anti-HBs antibody, anti-HBc antibody, and HBs antigen, and 3) willingness to participate in the study. The study was approved by the institutional review board.

The sex and age of each eligible patient were recorded, then the patients were interviewed to obtain the possible route of HIV-1 transmission, history of intravenous drug use (IVDU), history of blood transfusion, complete medical history including whether receiving antiretroviral therapy (ART) or not and the antiretroviral regimen used. A physical examination was performed and a blood sample was collected to evaluate anti-HCV antibody, CD4 cell count, and HIV-1 RNA. Hepatitis B vaccine (using 20 µg of recombinant DNA hepatitis B vaccine, Engerix-B®, SmithKline Biologicals) was administered by intramuscular injection in the deltoid muscle at 0, 1, and 6 months. An anti-HBs antibody levels was measured one month after the third injection. A patient who had an anti-HBs antibody level greater than 10 mIU/ml was defined as a responder. Those who did not were defined as non-responders.

Laboratory methods

Testing for anti-HIV-1 antibody was performed by ELISA chemiluminescence (Architect i2000SR®, Ortho-Clinical Diagnostics, Johnson & Johnson) and confirmatory testing by Vitros ECiQ® (Abbott laboratories, IL) and Serodia® test (Fujibio, Japan). Testing for HBV and HCV serologies were done using ELISA chemiluminescence (Architect i2000SR®, Ortho-Clinical Diagnostics, Johnson & Johnson, NJ). The CD4 cell count was tested using CD4 flow cytometry (FACSCalibur®, Becton Dickinson, NJ) and HIV-1 RNA was tested using HIV-1 viral load-UltraSense AMPLICOR test (COBAS AmpliPrep/COBAS AMPLICOR HIV-1 monitor test, version 1.5, Roche, IN) which as a limit of quantitation of 50 copies/ml ($\log_{10} = 1.7$) to 750,000 copies/ml ($\log_{10} = 5.9$).

Statistical analysis

Data were described using mean \pm standard deviation (SD) (or median and range where appropriate) and frequency (%) for continuous and categorical variables, respectively. The chi-square test was used to assess the association between categorical variables and anti-HBs antibody results (responder and non-responder group). The Student's *t*-test was used to compare means and the Mann-Whitney *U* test was used to compare medians between groups for continuous variables. Univariate analysis and multivariate analysis of potential predicting factors were performed to evaluate the association with anti-HBs antibody results. Pearson correlation was used to estimate the correlation between CD4 cell count at vaccination and anti-HBs antibody level after vaccination. All analyses were performed using SPSS version 13. A *p*-value less than 0.05 was considered to be statistically significant.

RESULTS

Sixty-five patients were enrolled in the study. The mean (SD) age was 39 ± 8.5 years,

Table 1
Clinical characteristics of 65 HIV-1 infected patients who received hepatitis B vaccine.

Characteristics	Responders (N=30)	Non-responders (N=35)	p-value
Age, years, mean \pm SD	36.6 \pm 8.8	40.7 \pm 7.8	0.052
Gender, number (%)			0.870
Male	10 (33)	11 (31)	
Female	20 (67)	24 (69)	
Risk of HIV-1 acquisition, number (%)			0.462
Sexual transmission	29 (97)	35 (100)	
IVDU	0 (0)	0 (0)	
Sexual transmission and IVDU	1 (3)	0 (0)	
Positive anti-HCV antibody, number (%)	2 (7)	0 (0)	0.121
Received ART at time of vaccination, number (%)	27 (90)	30 (86)	0.600
ART regimen, number (%)			0.472
NNRTI-based	22/27 (82)	28/30 (93)	
PI-based	4/27 (15)	2/30 (7)	
Others	1/27 (3)	0 (0)	
Duration of ART prior to vaccine, months, mean \pm SD	29.4 \pm 25.4	23.2 \pm 19.1	0.377
CD4 cell count, cells/mm ³ , mean \pm SD	397 \pm 207	301 \pm 172	0.047
Percent CD4, %, mean \pm SD	17.3 \pm 7.2	14.4 \pm 6.2	0.079
HIV-1 RNA, number (%)			0.168
Undetectable (< 50 copies/ml)	25 (83)	24 (69)	
Detectable (\geq 50 copies/ml)	5 (17)	11 (31)	
Time from 1 st to 2 nd vaccination, months, mean \pm SD	1.3 \pm 0.6	1.1 \pm 0.4	0.270
Time from 1 st to 3 rd vaccination, months, mean \pm SD	6.4 \pm 1.1	6.3 \pm 0.9	0.802
Time from 3 rd vaccination to test of anti-HBs antibody, months, mean \pm SD	1.4 \pm 0.9	1.7 \pm 1.8	0.658

44 (68%) patients were female. Of the 65 patients, 3% had positive serology for anti-HCV antibody. At the time of vaccination, 57 (88%) patients were receiving antiretroviral therapy and the mean (SD) duration of ART prior to vaccination was 26.1 (22.3) months. An NNRTI-based regimen was the most commonly used regimen (50 patients, 88%) in these patients. The mean (SD) CD4 cell count was 345 (194) cells/mm³. In all, 51 patients (79%) had CD4 cell counts greater than 200 cells/mm³. The median HIV-1 RNA count was <50 copies/ml [49 patients (75%) had undetectable HIV-1 RNA (<50 copies/ml)]. The mean times from the first to the second injection, first to third injection, and third injection

to test of anti-HBs antibody were 1.2, 6.4, and 1.6 months, respectively. The clinical characteristics of the responders and non-responders are summarized in Table 1.

In all, 30 patients (46%) developed anti-HBs immunity (anti-HBs antibody > 10 mIU/ml after vaccination, responders). On univariate analysis, a higher CD4 cell counts ($p = 0.047$) was found to be a predictor of successful vaccination. There were trends toward significance in age ($p = 0.052$) and CD4 percentage ($p = 0.079$). There were no other significant differences between responders and non-responders. On multivariate analysis using a logistic regression entry model, age and CD4 cell count were independent predicting

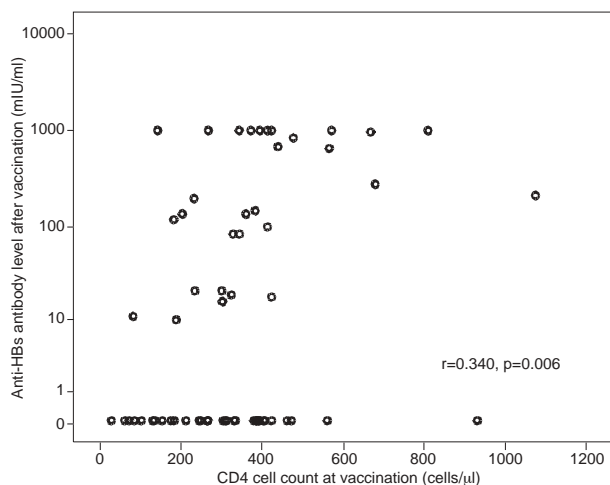


Fig 1—Correlation of CD4 cell count at vaccination and anti-HBs antibody level after vaccination.

factors for responders [adjusted odds ratio (OR) 0.937, 95% confidence interval (CI) 0.872-1.000, $p = 0.049$ and OR 1.003, 95% CI 1.000-1.006, $p = 0.048$, respectively]. There was a correlation between CD4 cell count at vaccination and anti-HBs antibody level after vaccination ($r = 0.340$, $p = 0.006$). The patients who had a higher CD4 cell count at vaccination achieved higher levels of anti-HBs antibodies after vaccination (Fig 1).

DISCUSSION

The results from the present study demonstrate younger age and higher CD4 cell counts at vaccination were predicting factors for successful HBV vaccination among Thai HIV-1 infected patients. In addition, we also found that there was a significant correlation between CD4 cell count at vaccination and anti-HBs antibody level after vaccination. Human aging is associated with a decline in immune function. T cell dependent processes play a pivotal role in the primary immune response to protein antigens not previously encountered, which decrease in both quantity and quality with age (Jackola *et al*, 1994; De

Paoli *et al*, 1988). These changes impair humoral and cellular immune functions which result in less effective vaccinations (Ginaldi *et al*, 1999; Wick *et al*, 1997). Previous studies of hepatitis B vaccines in healthy individuals found that older age was associated with poorer results with hepatitis B vaccination (Shaw *et al*, 1989; Fisman *et al*, 2002). For hepatitis B vaccination in an HIV-1 infected population, we found no studies describing age as a predictive factor. The present study demonstrated age as a predictive factor for successful hepatitis B vaccination in HIV-infected adults. A previous study of hepatitis B vaccination in Thai HIV-infected children found a high failure rate (Siriaksorn *et al*, 2006).

CD4 cell counts were taken to evaluate the primary immune response. Previous studies among the HIV-infected population have shown that higher CD4 cell counts are associated with successful hepatitis B vaccination (Bruguera *et al*, 1992; Tayal *et al*, 1994; Veiga *et al*, 2006) which agree with our results. Although the CD4 cell count can be influenced by aging, We demonstrated it as an independent factor on multivariate analysis.

We found neither ART nor HIV-1 RNA counts were associated with successful vaccination, while a previous study demonstrated these were predicting factors (Overton *et al*, 2005). This may be explained by the fact that most of our study patients received ART and had undetectable HIV-1 RNA counts. There was a trend toward a higher proportion of patients with undetectable HIV-1 RNA numbers in responders (83% vs 69%, $p = 0.168$). Forty-six percent of our study patients had a successful hepatitis B vaccination. This rate is higher than many previous studies that included a lower proportion of patients with ART (Odaka *et al*, 1988; Bruguera *et al*, 1992; Tayal *et al*, 1994). Combining our results with those of previous studies, we recommend hepatitis B vaccination in patients with a high CD4 count and undetectable HIV-1 RNA levels af-

ter receiving ART.

There were limitations in our study. The first is the small sample size. Our results showed a marginal significance for both predicting factors. A larger sample size would give more powerful results, which could clarify statistical significance. Second, we used a recombinant DNA hepatitis B vaccine in our study. The results may not apply to other hepatitis B vaccines in the HIV-infected population. However, comparative studies of different hepatitis B vaccines in healthy individuals show no significant differences in efficacy (Eyigun *et al*, 1998; Baldy *et al*, 2004).

In conclusion, the predicting factors for successful hepatitis B vaccination were younger age and higher CD4 cell counts. These results may help determine which patients and when to administer the hepatitis B vaccine. In the standard immunization program for HIV-1 infected patients, the practitioners should be aware of suboptimal protective immunity. A test of antibody response after vaccination is necessary to confirm the response.

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