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Efficacy of Hepatitis B Vaccination among Children in Special Region of Yogyakarta, Indonesia: Evaluation of Humoral and Cellular Immunity

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Hepatitis B remains a global burden, with estimated 15 to 40 percents of infected individuals eventually suffer from liver cirrhosis, liver failure, and hepatocellular carcinoma. Vaccination aims to form anti-HBs antibody with protective titer to prevent infection. CD4 T cell lymphocytes are known to play a major role in establishing immunity after vaccination. This study aimed to investigate protective titer rate among Indonesian children in Special Region of Yogyakarta following hepatitis B vaccination and correlation between anti-HBs titer and CD4 count. This is a cross-sectional study with 52 subjects between 8 months to 5 years of age in Bungas Community Health Service, Special Region of Yogyakarta, Indonesia. Anti-HBs titer was examined using enzyme immunoassay and CD4 count was examined using immunocytochemistry method. Of 52 subjects, median anti-HBs titer was 72.965 IU/L (interquartile range 360.98), mean CD4 count was 49.73% ± 29.75. Protective level of antibody was found in 73.1% of subjects. Correlation test was conducted and no correlation was found between anti-HBs titer and CD4 count (r=-0.104, *p*=0.464). Age was found to have a weak negative correlation with anti-HBs titer (r=-0.367, *p*=0.007). We found high rate of protective titer among children in Special Region of Yogyakarta who have completed hepatitis B vaccination series. No correlation was established between anti-HBs titer and CD4 count.

Globally, more than 2 billion people have been exposed to hepatitis B virus infection and approximately 378 million people suffer from chronic infection.[1] Of infected individuals, 15 to 40% will suffer from liver cirrhosis, liver failure, and hepatocellular carcinoma.[2] Newborns and infants that contract hepatitis B perinatal infection have 90% risk of developing chronic liver condition.[3] Vaccination given to newborns can prevent infection in 80% – 95% of all cases.[4] Data from various countries show significant decline in hepatitis B virus infection after introduction of vaccination. Taiwan used to have around 90% of the population aged 40 years estimated to have been infected with hepatitis B virus. Almost 30 years after its universal vaccination program commencement, the prevalence of HbsAg has decreased to 0.9%.[5] In the United States, the rate of new infection has declined by approximately 82% since its national implementation of vaccination in 1991.[6]

Following vaccination, antibody titer against hepatitis B surface antigen (anti-HBs) equal to or more than 10 IU/L is effective in disease prevention and generally considered as protective level against hepatitis B infection.[7] However, some healthy individuals fail to develop adequate antibody response to exposure of HbsAg. Previous studies found as many as 2 – 15% healthy individuals were considered hypo- or non-responders, mounting antibody titer of less than 10 IU/L.[8,9,10]

Production of anti-HBs antibody by B lymphocytes, which is the goal of vaccination, depends on stimulation of CD4 T cells. Previous studies found that factors related with mounted anti-HBs titer are age of vaccination, types of vaccine (plasma-derived or recombinant, single or combined with other vaccines), administration of vaccine (intramuscular or intradermal), dose and timing of vaccine, sex, body mass index, smoking habit, malnutrition, chronic kidney failure, immunosuppresion, immunodeficiency and genetic factor.[11, 12]

Poor response to HBV vaccination in HIV-1-infected persons has been associated with low CD4 T cell count.[13] Only 20 – 70% of the HIV-seropositive patients develop protective antibody titers, as opposed to 90 –

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95% of healthy individuals, mostly depending on CD4 T cell counts and HIV viral loads.[14] However, it is yet to be elucidated whether healthy individuals mounting low titer of anti-HBs also have disorder in T cell count and response.

The aim of this study is to investigate protective titer rate among Indonesian children in Special Region of Yogyakarta following hepatitis B vaccination and correlation between anti-HBs titer and CD4 count.

MATERIALS AND METHODS

Study Design and Population

This cross sectional study was conducted among children aged 8 months to 5 years attending Bungas Community Health Service, Jetis Subdistrict, Bantul Regency, Special Region of Yogyakarta, Indonesia. The study employed consecutive sampling, where every subject who fulfilled inclusion criteria was included in the study within certain period of time and until sample size has been achieved. Inclusion criteria are completion of hepatitis B vaccination proven by written medical record and written consent by parents. Children who had hepatitis, malignancy, kidney failure requiring hemodialysis, HIV/AIDS, chronic liver disease, malnutrition, or were undergoing therapy with steroid and/or immunosuppresive agents were excluded from the study. Demographic and clinical data were collected in standard medical report form. Nutritional status was assessed using World Health Organization guidelines.[15] Children whose weight-for-height Z score fell below -3 standard deviation or mid-upper arm circumference measured less than 115 mm were classified as malnourished. Those whose weight-for-height Z score fell between -3 and -2, -2 and +2, +2 and +3, and above +3 standard deviation were classified as undernourished, good, overweight, and obese nutritional status, respectively. This study was reviewed and approved by Ethics Committee of Faculty of Medicine Universitas Gadjah Mada.

Procedures

After informed consent was obtained from the parents, blood sample was withdrawn and HBV markers were checked for all participants by use of HBV surface antigen (Bioline HbsAg; Standard Diagnostics, Gyeonggi-Do, Republic of Korea) and HBV surface antibody (VIDAS ®, bioMérieux, Marcy-l'Étoile, France). No sample was positive for HbsAg and all samples were then examined for CD4 count. Immunocytochemistry method was preferred in the limited resources setting of developing countries such as Indonesia, due to its affordability and reproducibility. Furthermore, previous study revealed similar results in the determination of lymphocyte subsets characterized by CD3, CD4 and CD8 antigens using flow cytometry and immunocytochemistry.[16] A blood smear was made for each subject using 50 µL of sample. To prevent sample destruction, peroxidase blocking using 0.3% H_2O_2 in methanol was performed for 15 minutes at room temperature prior to primary antibody incubation. Phosphate-buffered saline (PBS) was used to rinse, followed by coating in background spike solution for 15 minutes. Remaining solution was discarded and the slides were incubated in CD4 monoclonal antibody (Concentrated and Prediluted Monoclonal Antibody; Biocare Medical LLC, California, United States of America) at temperature of 4^{o}C for 8 to 20 hours.

Detection step was then performed by rinsing with PBS and incubation in Streptavidin solution for 10 minutes at room temperature. After rinsing with PBS, the slides were coated in Diaminobenzidine solution for 8 minutes, stained with hematoxylin and eosin as counterstain solution and examined under the microscope.

In high-power fields, the number of CD4 T cells, which would appear as brown-stained nucleated lymphocyte, were then counted in every 100 lymphocytes in each sample slide and the result was expressed in percentage.

Statistical Analysis

Data analysis was performed using correlation test with SPSS version 17.0 to find correlation between anti-HBs titer and CD4 count. We used linear and logistic regression methods to assess the relationship between demographic and response to vaccination. Variables evaluated including age, sex and nutritional status.

RESULTS

Fifty-two children (26 male and 26 female) participated in this study. Baseline characteristics of subjects are presented in Table I.

Table I. Baseline characteristics of subjects

Characteristic	Number (n = 52)	Mean ± SD or %
Age (months)		33.35 ± 14.83
Sex		
Male	26	50
Nutritional status		
Good	40	77
Others	12	23
Anti-HBs titer (IU/L)		72.965* (interquartile range 360.98)
Anti-HBs titer category		
Protective (≥10 IU/L)	38	73.1
Non protective (<10 IU/L)	14	26.9
CD4 count (%)		49.73 ± 29.75

* expressed in median due to non-normal data distribution

Comparison between children who achieved protective titer and non protective titer is shown in Table II. Demography and baseline characteristics of these groups were analyzed using Pearson's chi-square test for sex and nutritional status, and Mann-Whitney U test for mean age and CD4 count.

Table II. Characteristics comparison between protective and non protective titer groups

Characteristic	Protective titer (n = 38)	Non protective titer (n = 14)	*p*
Mean age ± SD (months)	30.02 ± 14.27	42.36 ± 12.78	0.008
Sex (male/female)	17/21	9/5	0.211
Nutritional status (good/others)	30/8	10/4	0.712
Mean CD4 count (%)	50.25 ± 27.26	48.27 ± 36.79	0.812

Using Spearman correlation test, we examined correlation between anti-HBs titer and CD4 count. No correlation was found between anti-HBs titer and CD4 count (r=-0.104, p=0.464). We also performed correlation test to examine correlation between anti-HBs titer and age and found weak negative correlation with statistical significance (r=-0.367, p=0.007).

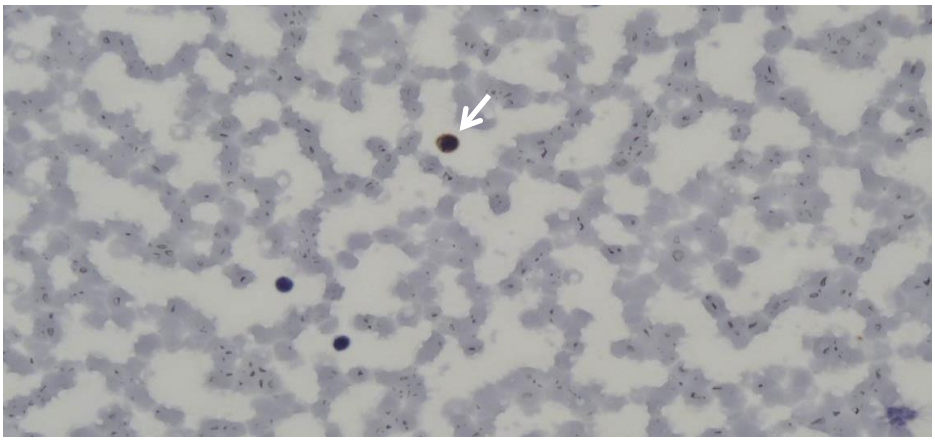

Figure 1. Specimen of subject showing cell membrane expression of CD4 (white arrow).

In multivariate analysis, only age was statistically significantly associated with lower titer of anti-HBs and thus multivariate linear regression analysis was not possible to be conducted.

Table III. Linear regression analysis result

Variable	Univariate linear regression	
	β (CI 95%)	*p*
Age	-6.045 (-15.640 – 3.550)	0.212
Sex	-6.209 (-292.478 – 280.060)	0.965
Nutritional status	-84.824 (-288.034 – 118.386)	0.406
CD4 count	-0.712 (-5.567 – 4.142)	0.769

Subsequently, using logistic regression method, relationship between demographic, CD4 count and protective titer of anti-HBs was assessed.

Table IV. Logistic regression analysis result

Variable	Univariate logistic regression		Multivariate logistic regression	
	β (CI 95%)	*p*	β (CI 95%)	*p*
Age	0.937 (0.891 – 0.986)	0.012	0.937 (0.891 – 0.986)	0.012
Sex	0.450 (0.127 – 1.596)	0.216	0.418 (0.105 – 1.662)	0.215
Nutritional status				
Good	Ref			
Underweight	2.000 (0.291 – 13.738)	0.481		
Obese	1.077 E9 (0.000 – ∞)	0.999		
Severely obese	0.333 (0.017 – 6.654)	0.472		
CD4 count	1.002 (0.982 – 1.023)	0.828		

Among the variables, age was significantly associated with protective anti-HBs titer of ≥ 10 IU/L.

DISCUSSION

This study found that 73.1% of subjects retain protective titer of anti-HBs (≥ 10 IU/L). This number is higher than previous finding in East Java, where only 23.6% of children aged 8 to 13 years had detectable titer of antibody.[17] There are several probable underlying reasons for this difference. Firstly, subjects with older age group may have waning antibody titer. Previous studies in Taiwan showed that the seropositivity rate of anti-HBs declined from 99% at 1 year to 83% at 5 years[18] and from 71.1% at 7 years to 37.4% at 12 years[19] after hepatitis B vaccination series at first year. Secondly, national health survey and study consistently identified higher vaccination rate in Special Region of Yogyakarta compared to other provinces in Indonesia. According to 2010 survey conducted by Ministry of Health,[20] national vaccination coverage was 53.8%, whereas in Special Region of Yogyakarta the rate was exceeding 91%.[21]

Furthermore, this study found no correlation between anti-HBs antibody titer and CD4 count. Various factors that influence antibody formation in response to hepatitis B vaccination can be classified as vaccine factor and host factor. Vaccine factor includes administration and temperature of vaccine storage. All subjects in this study received hepatitis B vaccination in Jetis II Primary Health Centre, Bantul Regency with uniform mode of vaccine type (Pentabio; PT Bio Farma, Bandung, Indonesia), storage, schedule and administration.

Host factor includes ability of an individual to generate specific antibody for exposed antigen during vaccination. Anti-HBs antibody titer is currently used to evaluate efficacy of vaccination. However, it is yet to be elucidated whether individuals mounting low titer of anti-HBs also have disorder in T cell response. To the best of our knowledge, this is the first study to examine correlation between anti-HBs titer and CD4 count in healthy pediatric population following complete Hepatitis B vaccination.

Production of anti-HBs antibody by B lymphocyte is the end goal of vaccination, and depends on the stimulation of CD4 T cell. Upon administration of vaccine, peptides derived from antigen is presented to T cell in class II human leukocyte antigen (HLA) and activate CD4 T cell. CD4 T cell proliferates and secretes Th2 cytokines that activate B cell differentiation to produce antibody.[22]

Non-response or hyporesponse to hepatitis B vaccination is influenced by activation of CD4 T cells specific for HBsAg. In individuals infected with human immunodeficiency virus (HIV), there is 3.33 times increase of risk for occurrence of non-response, with lower CD4 T cell count as independent predictor for non-response.[23]

T cell subset profile's role in evaluation of an individual's immunity status has been identified, especially in HIV infected population.[24] In healthy pediatric subjects aged 1 to 15 years in China, CD4 T cell percentage was

found to remain unchanged throughout childhood and adolescence.[25] Another study also shown that CD4 and CD8 T cell percentages did not undergo significant change during childhood.[26]

A study in subjects with HIV found that response to hepatitis B vaccination is related with CD4 T cell count.[27] However, other factors have been shown to determine T cell response to antigenic exposure in vaccination.[28, 29] These include the strength of the receptor signal received by T cell receptor, the cytokine milieu, and specific costimulatory pathways that will allow full activation of T cells. Costimulatory signals are mediated by the B7–CD28 pathway and the CD40–CD40 ligand (CD40L) interaction.[30]

The role of cytokine and ligand interaction in immune response following vaccination is strengthened by the findings of several studies. Lu et al. found correlation between presence of low production of representative T cell cytokines, IFN-γ and interleukin-5 specific for HBs antigen, and negative or low T cell response to vaccination.[31] Another study by Gonclaves et al. showed a diminished CD40L expression on Th cells in hepatitis B vaccine nonresponders, suggesting inability of Th cells to induce switching of immunoglobulins during maturation of B cell response as the cause of absence of humoral-specific response to HbsAg.[32]

The aforementioned studies evaluated T cell function in demonstrating response to hepatitis B vaccination by measuring cytokine production and ligand expression in vitro, while we measured quantity of T cells expressed in CD4 T cell percentages in our subjects. Upon our finding, we hypothesized that CD4 T cell count may not represent ability of T cell in eliciting response to exposure of HbsAg antigen. Examination on antigen-specific T cells to evaluate hepatitis B vaccine immunogenicity was also shown to be better performed by measuring several cytokines (IFN-γ, TNF-α, IL-2 and IL-4) rather than single cytokine.[33]

Aside from the limited resources to perform flowcytometry, another shortcoming in this study is inability to take into account genetic factor in antibody formation. Several studies found that anti-HBs production has been correlated with gene polymorphisms of interleukins involved in the Th1 system such as interleukin-18 and IFN-γ.[34-39] Previous studies also found involvement of both class I and class II HLA molecules in immune response to hepatitis B antigen. The diversity of HLA influences recognition of HbsAg, with less HLA molecules participate in poor responders, rendering disturbance in T cell activation and production of cytokines that play roles in antibody formation. Correlation between class I HLA and non-response was found in HLA B8 and B44.[40, 41] In class II HLA, non-response is related with HLA-DRB1*03, DRB1*04, DRB1*07, DRB1*1302, DRB1*14 and DQB1*02 alleles.[42, 43] Whether identified HLA related to non-response present in our subjects warrants further investigation.

This study did not find other variable influencing anti-HBs titer, as presented in multivariate analysis. Age was the only variable with statistically significant value and weak negative correlation with anti-HBs titer. This finding is consistent with previous study that shown protective antibody titer would wane in older subjects.[44]

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