

The Difference of Anti-HBs Levels in Babies Vaccinated with Hepatitis B Vaccine Between at 2, 4, 6 Months with 2, 3, 4 Months Schedule in Denpasar

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Abstract: *Background:* Hepatitis B virus is one of the causes of the major health problem throughout the world. Hepatitis B vaccine is one way to control HBV infections effectively. The prevalence of HB infection shows a decreased effectivity of Hepatitis B vaccination. *Objective:* To discover the differences in anti-HBs levels between two schedules of vaccination recommended by the Indonesian Pediatric Society (at 2, 4, 6 months vs 2, 3, 4 months of age). *Methods:* This was an observational study on healthy babies comparing the effect of two treatment groups, each consisting of 30 subjects. Subjects were chosen by stratified random sampling. Blood samples were withdrawn three months after the subjects received their last dose of Hepatitis B vaccination, either in 2, 4, 6 months schedule (Group 1) or in 2, 3, 4 months schedule (Group 2). We used the independent t-test to assess the mean differences between the anti-HBs levels of the two groups. A p-value of <0.05 was considered statistically significant. *Results:* We found a significant difference in anti-HBs levels between Group 1 and Group 2 (820.06 mIU/ml vs 540.54 mIU/ml, respectively, $p=0.002$). *Conclusion:* Both vaccination schedules produced protective anti-HBs levels three months after the completed schedule. The anti-HBs level in group 1 produced higher anti-HBs level compared to Group 2.

Keywords: Children, Hepatitis B, Anti-HBs, Vaccination, Indonesia

1. Introduction

Hepatitis B is caused by the Hepatitis B virus (HBV). It is one of the world's health problem. It is estimated that one-third of the entire population in the world has been proven to be serologically infected with HBV [1]. The World Health Organization (WHO) estimates that more than 2 billion people in the world have been infected with HBV, of which 378 million or 4.8% are infected with chronic carriers with a mortality rate of 620,000 each year [2]. The highest prevalence of HBV infection is in Africa (8.83%) and followed by the Western Pacific region (5.26%). More than 4.5 million new cases of HBV infection occur each year, and ¼ of these cases develop into liver cirrhosis liver disease and primary hepatocellular carcinoma [3].

Immunisation is an effective way to control HBV infections. The prevalence of chronic HBV infection showed a significant decrease after HBV vaccination. A 1997 cohort study showed a decrease in the incidence of hepatocellular carcinoma in children aged 6-9 years after vaccination, from 0.52 to 0.13 per 100,000 [4]. HBV vaccination stimulates the formation of Hepatitis B surface antibodies (anti-HBs) [5]. Studies have shown that anti-HBs titers still provide protective effect at 2-4 years, even up to 10 years after primary vaccination [6-11].

Van Damme et al. assessed anti-HBs levels after five years of immunisation and compared different immunisation schedules, group A (at 0 and 6 months) and group B (0, 1, and 6 months) [12]. They found that the protective values of anti-HBs of 10 mIU/ml in group A were 79.5% compared to

group B (91.4%). Girisha et al. reported that immunisation schedules at 0, 1, and 6 months produced higher levels of anti-HBs compared to at 0, 1, and 2 months, but produced similar seroconversion [13]. Currently, the Indonesian Pediatric Society (IPS) recommended two immunisation schedules, at 2, 4, 6 months, and at 2, 3, 4 months.

The facts that the prevalence of HBV infection remains high even though a compulsory immunisation program has been enforced for years in Indonesia should be an interest of Indonesian paediatricians. This study aimed to study the differences in immune responses at the two scheduling of vaccines currently proposed by the IPS.

2. Methods

This was an observational study comparing the effect of two treatment groups. Subjects were obtained by the cluster random sampling method. Out of the eleven Public Health Centers (PHCs) available in Denpasar City, two PHCs were chosen randomly. The subjects in this study were infants who were given HBV vaccine at the 2, 4, 6 months schedule at one PHC (Southern Denpasar IV) and at the 2, 3, 4 months at another PHC (Western Denpasar I). Subjects were collected consecutively where all subjects who met the inclusion and exclusion criteria were recruited into the study until the minimum number of subjects were met. The study took place from January 2018 to December 2019. This study obtained ethical clearance from the Committee of Ethical Research of the Faculty of Medicine, Udayana University (2135/UN14.2.2.VII.14/LP/2018).

Inclusion criteria in this study include babies who born with bodyweight >2,000 g, gestational age upon delivery >37 weeks, received the first HBV vaccination as scheduled, and willing to participate in this study. We obtained written, informed consent from the parents or the legal guardians of the baby to be included in this study. Exclusion criteria include born to mother with known HIV/AIDS, the presence of major congenital diseases, subjects with hypersensitivity reactions to HBV vaccines, history of taking steroids within the past two weeks upon recruitment, and a known maternal history of HBV infection. Those who received did not meet the vaccination schedule, and those whose parents would like to resign from the study inclusion were classified as dropped-out subjects. A standard sample size formula obtained that the total subjects of the study should be at least 60 subjects.

After obtaining parental consent, we recorded the patient's data (name, address, date of birth, sex, weight and length at birth, gestational age at birth, history of exclusive breastfeeding, nutritional status, and history of atopic diseases. The babies who received the Pentabio® vaccine (Biopharma, Indonesia) at 2, 4, 6 months were assigned to Group 1, and those who received the similar vaccine at 2, 3,

4 months were assigned to Group 2. We collected the blood sample from each subject three months after the final schedule of the vaccination and examined it for anti-HBs blood levels.

We used the Kolmogorov-Smirnov test for normality test and the independent t-test or Mann Whitney test to see any significant differences in anti-HBs levels between the two groups. In this study, since the data were normally distributed, we conducted independent t-test. All analysis was conducted using the Statistical Packages for Social Sciences (SPSS) software (IBM Corp. Released 2015. IBM SPSS Statistics for Windows, Version 23.0. Armonk, NY: IBM Corp.) A p-value of ≤ 0.05 was considered significant.

Table 1. Characteristics of the study subjects.

Variables	Group 1	Group 2
Sex, n (%)		
Male	14 (46.7)	13 (43.3)
Female	16 (53.3)	17 (56.7)
Birthweights (g), mean±SD	2652.67±297.18	2895.67±330.22
Length at birth (cm), mean±SD	47±2.98	48.07±2.03
Mean of birth, n (%)		
Spontan delivery	17 (56.7)	18 (60)
Cesarean section	13 (43.3)	12 (40)
Prematurity, n (%)		
Yes	0 (0)	0 (0)
No	30 (100)	30 (100)
Nutritional status, n (%)		
Good	30 (100)	28 (93.3)
Poor	0 (0)	2 (6.7)
Breast milk only feeding, n (%)		
Yes	22 (73.3)	27 (90)
No	8 (26.7)	3 (10)
History of atopy, n (%)		
Yes	4 (13.3)	2 (6.7)
No	26 (86.6)	28 (93.3)
Origin of living, n (%)		
Denpasar city	22 (73.3)	28 (93.3)
Outside of Denpasar city	8 (26.7)	2 (6.7)

SD: standard deviation.

3. Results

This research was conducted at the South Denpasar IV PHC and West Denpasar I PHC from January 2018 until December 2019. We collected 60 subjects consisted of 30 children vaccinated at 2, 4, 6 months (Group 1), and 30 children vaccinated at 2, 3, 4 months (Group 2). Both groups contained a predominantly female population (53.3% vs 56.7%, respectively), as seen in Table 1. Both sets of vaccination schedule reached protective anti-HBs level of >10 mIU/mL. After a complete vaccination set, the anti-HBs value in group 1 was 862.06 mIU/mL, compared to 540.54 mIU/ml in Group 2 ($p=0.002$) as displayed in Table 2.

Table 2. Mean difference of Anti-HBs level after complete vaccination set.

Variable	Group 1	Group 2	Mean difference	CI95%	p
Anti-HBs (mIU/mL), mean±SD	820.06±379.48	540.54±392.22	279.52	122-520	0.002

IU: international unit; SD: standard deviation; CI: confidence interval.

4. Discussion

Antibodies to HBsAg (anti-HBs) will occur after natural infection or can be caused by immunisation. These antibodies begin to appear in the 3rd month after immunisation, thus encouraging researchers to examine anti-HBs levels after three months of HB vaccination schedule. Anti-HBs are formed in the immune clearance phase and are useful for providing long-term immunity. The time of occurrence can be faster or slower than a few weeks of HBsAg serum clearance. The hepatitis virus also has a window period, that is, when HBsAg has not been detected, but anti-HBs have not yet formed. Anti-HBs antibodies begin to be produced at 12-24 weeks, whereas HBsAg has not been before 20 weeks of age [14].

Our study found a significant relationship between groups of infants who received HB vaccinations given at 0, 2, 4, 6 months and on the 2, 3, 4 months. At present, referring to the immunisation schedule recommended by IPS, the first (monovalent) HB vaccine is best administered within 12 hours after birth and preceded by vitamin K injections at least 30 minutes before. The schedule for the monovalent HB vaccine is at 0, 1, and 6 months of age. If an HB combination vaccine is given with DTPw, the schedule is given at 2, 3, 4 months. If HB vaccine is combined with DTPa, then the schedule of administration is at age 2, 4, 6 months of age [12, 15].

The HB vaccine given at 2, 4, 6 months will trigger the body's immune response by starting the formation of anti-HBs levels which increases slowly starting at eight weeks. The next dose of vaccine will be recognised by the T cells and will later trigger the formation of the next level of anti-HBs, which will also maintain the child's immunity in the future.² The vaccination schedule at 2, 3, 4 months will also form anti-HBs levels; however, the anti-HBs is not optimally formed yet four weeks from the first vaccination, so the anti-HBs is not optimal enough for the next vaccination schedule. That may explain why this study found that the anti-HBs levels were higher in subjects with HB vaccination at 2, 4, 6 months compared to 2, 3, 4 months.

Reviews from other studies regarding the use of monovalent HB vaccines in infants show a higher level of seroprotection in administration with schedules 0, 1, 6 months compared to 0, 1, 2 months, but this has long been abandoned because HB vaccines are now widely combined with other vaccines. A study reported that the immunity to long-term HB is present in the human body when the anti-HBs concentration reaches at least 10 mIU/ml [16].

Zhang *et al.* reported the difference of anti-HBs level between children who were vaccinated at 0, 1, 6 months and 0, 1, 2 months. The study found significantly higher levels of anti-HBs in the group vaccinated at 0, 1, and 6 months. Previously, Van Damme *et al.* reported that vaccination schedules that are given in two doses show higher levels of anti-HBs compared to immunisations given by three doses [12].

After complete HB immunisation, the anti-HBs level will decrease rapidly in the first year and slower in subsequent

years [9]. Among children who had previously responded well (antibody levels >10 mIU/mL), 50% of them had antibody levels that were no longer detected in 5-15 years after vaccination [2]. As many as 94.1% of the vaccinated population will have an anti-HBs level of ≥ 10 mIU/mL for five years, and 91.2% of the same population will have for at least ten years [12]. The success of immunisation requires immunologic cell maturity. The formation of specific antibodies to particular antigens is still deficient in infants younger than two years, so repeat immunisation (booster) needs to be done at a specific period.

According to IPS recommendation, HB immunisation is given as early as possible after birth, bearing in mind that at least 3.9% of pregnant women have hepatitis, with a risk of maternal transmission of approximately 45%. The schedule for administration is based on maternal HBsAg status. Given the epidemiological pattern of HB in Indonesia, it can be concluded that a booster dose of the vaccine at the age of 5 years is not needed. However, at this age, an anti-HBs examination is ideally carried out [14].

The most common problems encountered in daily practice are immunisations is the on-time visits for vaccinations [15]. The provision of immunisations that are not on schedule or incomplete is not an obstacle to continuing immunisation. According to the IPS, an HB immunisation should ideally be given within 12 hours after birth, and the next dose should be given four weeks from the first dose [16]. The distance between the second and third dose should ideally at least two months apart, and is best after five months. If the child has never received HB immunisation in infancy, he may get a full series of immunisation at any time during a visit. This can be done without having to check for anti-HBs levels [17].

Limitation of this study is the limited subject size. A larger subject size with better control over the factors that influence anti-HBs levels, such as prematurity and low birthweights, is needed to provide us with better insight regarding this topic in the Indonesian population. This study also did not employ any investigations, such as genetic or chromosome examination, to see genetic or congenital abnormalities in the study sample that may influence the anti-HBs level.

5. Conclusion

There is a significant difference between the levels of Anti-HBs in the immunisation group 2, 4, 6 months with 2, 3, 4 months, where Anti-HBs levels are more protective in the vaccine group schedule 2, 4, 6 months, so expect to get more protective anti-HBs levels can be used HB vaccine schedule with a schedule of 2, 4, 6 months, even though schedule 2, 3, 4 months has also reached protective levels. Factors such as gender, birth weight, nutritional status, exclusive breastfeeding have no effect on anti-HBs levels.

Conflict of Interest

All the authors do not have any possible conflicts of interest.

References

- [1] Terrault NA, Bzowej NH, Chang KM, et al. AASLD guidelines for treatment of chronic hepatitis B. *Hepatology*. 2016; 63 (1): 261-283. doi: 10.1002/hep.28156.
- [2] World Health Organization. WHO manual for the establishment of national and other secondary standards for vaccines. Geneva: World Health Organization Publication; 2011. Available at: <https://apps.who.int/iris/handle/10665/70669>.
- [3] Chang MH, Chen CJ, Lai MS, Hsu HM, Wu TC, Kong MS, et al. Universal hepatitis B vaccination in Taiwan and the incidence of hepatocellular carcinoma in children. Taiwan Childhood Hepatoma Study Group. *N Engl J Med*. 1997; 336 (26): 1855-1859. doi: 10.1056/NEJM199706263362602.
- [4] Chen HL, Chang CJ, Kong MS, Huang FC, Lee HC, Lin CC, et al. Pediatric fulminant hepatic failure in endemic areas of hepatitis B infection: 15 years after universal hepatitis B vaccination. *Hepatology*. 2004; 39 (1): 58-63. doi: 10.1002/hep.20006.
- [5] Armstrong GL, Mast EE, Wojczynski M, Margolis HS. Childhood hepatitis B virus infections in the United States before hepatitis B immunisation. *Pediatrics*. 2001; 108 (5): 1123-1128. doi: 10.1542/peds.108.5.1123.
- [6] Chang MH, Shau WY, Chen CJ, Wu TC, Kong MS, Liang DC, et al. Hepatitis B vaccination and hepatocellular carcinoma rates in boys and girls. *JAMA*. 2000; 284 (23): 3040-3042. doi: 10.1001/jama.284.23.3040.
- [7] Chongsrisawat V, Theamboonlers A, Khwanjaipanich S, Owatanapanich S, Sinlaparatsamee S, Poovorawan Y. Humoral immune response following hepatitis B vaccine booster dose in children with and without prior immunisation. *Southeast Asian J Trop Med Public Health*. 2000; 31 (4): 623-626.
- [8] Lin YC, Chang MH, Ni YH, Hsu HY, Chen DS. Long-term immunogenicity and efficacy of universal hepatitis B virus vaccination in Taiwan. *J Infect Dis*. 2003; 187 (1): 134-138. doi: 10.1086/345871.
- [9] Petersen KM, Bulkow LR, McMahon BJ, Zanis C, Getty M, Peters H, et al. Duration of hepatitis B immunity in low risk children receiving hepatitis B vaccinations from birth. *Pediatr Infect Dis J*. 2004; 23 (7): 650-655. doi: 10.1097/01.inf.0000130952.96259.f0.
- [10] Banatvala JE, Van Damme P. Hepatitis B vaccine – do we need boosters? *Journal of Viral Hepatitis*. 2003; 10: 1-6. doi: 10.1046/j.1365-2893.2003.00400.x.
- [11] Jafarzadeh A, Montazerifar SJ. Persistence of anti-HBs antibody and immunological memory in children vaccinated with hepatitis B vaccine at birth. *J Ayub Med Coll Abbottabad*. 2006; 18 (4): 4-9.
- [12] Van Damme P, Moiseeva A, Marichev I, Kervyn AD, Booy R, Kuriyakose S, et al. Five years follow-up following two or three doses of a hepatitis B vaccine in adolescents aged 11-15 years: a randomised controlled study. *BMC Infect Dis*. 2010; 10: 357. doi: 10.1186/1471-2334-10-357.
- [13] Girisha KM, Kamat JR, Nataraj G. Immunological response to two hepatitis B vaccines administered in two different schedules. *Indian J Pediatr*. 2006; 73 (6): 489-491. doi: 10.1007/BF02759892.
- [14] IPS Immunisation Task Force. Jadwal imunisasi rekomendasi IDAI (*article in Indonesian*). *Sari Pediatri*. 2000; 2 (1): 43-47. doi: 10.14238/sp2.1.2000.43-7.
- [15] Gunardi H, Kartasasmita CB, Hadinegoro SRS, Satari HI, Oswari H, Puspongoro HD, et al. Jadwal Imunisasi Anak Usia 0–18 Tahun Rekomendasi Ikatan Dokter Anak Indonesia 2017 (*article in Indonesian*). *Sari Pediatri*. 2017; 18 (5): 417-22. doi: 10.14238/sp18.5.2017.417-22.
- [16] Zhang X, Wang J, Chen X, Yu M, Yu S, Sun Y, et al. Short-term immunogenicity of standard and accelerated hepatitis B virus vaccination schedules in healthy adults: a comparative field study in China. *Biosci Rep*. 2018; 38 (5): BSR20180846. doi: 10.1042/BSR20180846.
- [17] Rusmil K. Melengkapi/Mengejar Imunisasi (*article in Indonesian*). Indonesian Pediatric Society. Last updated: May 30, 2015. Last accessed: May 23, 2020. Available at: <https://www.idai.or.id/artikel/klinik/imunisasi/melengkapi-mengejar-imunisasi-bagian-i>.