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Original Article

Safety and Preliminary Immunogenicity of Recombinant Hepatitis B (Bio Farma) Vaccine in Adults and Children: A Phase 1 Clinical Trial

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Abstract

Background: In order to fulfill the requirements of the national immunization program and sustain the production capacity of the monovalent Hepatitis B vaccine, this study aimed to assess the safety and immunogenicity of the recombinant hepatitis B vaccine (Bio Farma) using the new hepatitis B bulk.

Methods: This study was an experimental, randomized, double-blinded, and controlled Phase I clinical trial, with 100 healthy subjects divided into 50 adults and 50 adolescents. Subjects were randomly assigned to receive either the Bio Farma registered recombinant hepatitis B vaccine (group A) or a new source of hepatitis B bulk (group B). Subjects received one or three vaccine doses, depending on the baseline anti-Hbs titer. Subjects were given diary cards to record solicited and unsolicited adverse events for 28 days following vaccination. Vaccine immunogenicity was assessed by measuring the level of HBsAg antibody titer elevation.

Results: No serious adverse events were reported during the clinical trials. The frequencies of adverse events were not significantly different between the two vaccine-randomized groups. The most immediately observed local reaction was local pain, reported by 35.7–42.8% of adults and 24.0–26.3% of adolescents, without any systemic reactions. Seroconversion in adults in group B reached 100% and 78.5% in group A. Meanwhile, seroconversion in adolescent subjects in both groups reached 100%. A substantial increase in geometric mean titer (GMT) was observed in the majority of subjects after immunization.

Conclusion: Recombinant hepatitis B vaccine with a new source of HBsAg B bulk is safe, well tolerated, and highly immunogenic.

Keywords: Adolescents, adults, immunogenicity, recombinant hepatitis B vaccine, safety

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Introduction

The world population showing serological evidence of current or past HBV infection is 30%.[1] Virus eradication is needed because humans are a major reservoir of hepatitis B virus (HBV). Therefore, a comprehensive control strategy through HBV vaccination can lead to eradication of the virus.[2] Eliminating the hepatitis B virus epidemic is crucial with immunization and will reduce new hepatitis B infections as well as chronic diseases and death rates.[3] Immunization against the hepatitis B virus is safe and has been accepted worldwide as a part of routine immunization programs. At least three doses of hepatitis B vaccine should be the standard for an immunization programs.[4] For those who are immunocompromised, including those who have HIV, are on dialysis, or have cirrhosis, the use of a multiple dose vaccine has been shown to increase the percentage of patients who achieve protective antibody titers.[5] Three doses are required to achieve a minimal acceptable immune response and demonstrate an anti-HBs antibody titer of ≥10

IU/L expected 1–2 months after administering the last dose of accine.[6]

A previous study on hepatitis B vaccination in adults was carried out by evaluating two different accelerated schedules, namely 0, 7, 21 day group (group I) and the 0, 1, 2 month group (group II). There was no significant difference between the two groups regarding seroprotection at day 365 (before booster vaccination).[7] Another study was also conducted in healthy late adolescents aged 15–18 years who had not previously received the hepatitis B vaccine to assess protectiveness and safety of the recombinant hepatitis B vaccine after 3 doses. The most frequent local and systemic reactions were pain 8.5% and fever 5.7%. No serious adverse events occurred, and all vaccinations were well tolerated.[8]

Bio Farma has now produced a recombinant hepatitis B vaccine using the developed active ingredients HBsAg (bulk HBsAg). As a newly developed vaccine, it is necessary to conduct phase 1 clinical trials to prevent HBV infection. In order to fulfill the requirements of the national immunization program and sustain the production capacity of the monovalent Hepatitis B vaccine, this study aimed to assess the safety and immunogenicity of the recombinant hepatitis B vaccine (Bio Farma) using the new hepatitis B bulk.

Methods

This was an experimental, randomized, double-blinded, controlled phase 1 clinical trial conducted at the Public Health Center Garuda, Bandung, Indonesia from November 2019 to May 2020. In this clinical trial, 100 subjects, consisting of 50 adults aged 18–40 years and 50 adolescents aged 10–17 years, recruited in sequential age de-escalation. Adult subjects gave written informed consent, meanwhile adolescent subjects provided informed consent from their parents.

Subjects were screened at V0 (3 days prior to first vaccination/visit 1). The subjects enrolled were healthy adults and adolescents based on medical history, physical examination, thorax radiology, electrocardiogram (ECG) results for adults subjects, and laboratory results. Subjects exclusion criteria were evolving mild, moderate, or severe illness, particularly infectious diseases or fever (axillary temperature ≥37.5°C) within the 48 hours following enrollment. Other criteria encompassed a history of allergy to any component of the vaccines (based on the anamnesis), a history of immunodeficiency disorders, uncontrolled coagulopathy or blood disorders, receiving medications that might alter the immune response within 4 weeks before participating in the study, the presence of abnormalities or chronic diseases, pregnancy and lactation, received a vaccination within 4 weeks before participating in the study, or planned to receive other vaccines within 4 weeks after immunization. Subjects who tested positive for HBsAg or had a history of hepatitis B infection were also excluded from the study.

Blood samples were drawn on all subjects at V0 (3 days prior to vaccination) for HbsAg testing and serological screening before recruitment. Blood samples V1a (7 days following first vaccination), V2, or /and V4 were used to evaluate post-immunization antibody titers (Anti-HBs test).

Subjects were randomized on day 0 (V1) into two arms. Group A received the registered Bio Farma hepatitis B vaccine (Vaccine A), whereas group B received the recombinant hepatitis B vaccine with the new bulk HbsAg (Vaccine B). Subjects with protective titers of anti-HBs (≥ 10mIU/ml) at baseline received one dose of study vaccine meanwhile subjects with non-protective titers of anti-HBs (<10mIU/ml) received three doses of study vaccine at 1-month intervals for each vaccination at V1, V2, and V3.

This study was approved by the Research Ethics Committee of Universitas Padjadjaran (ethical approval no.: 105/UN6.KEP/EC/2022), Bio Farma Quality Assurance Division, and the Indonesian Regulatory Authorities. The clinical trial has been registered at clinicaltrials.gov with entry number NCT04188223 and registered with registration number INA-DPS3T9B.

Seroconversion was defined as an increase in antibody titer of ≥4 times after receiving one dose of vaccination in subjects with protective anti-HBs (≥10mIU/ml), whereas in subjects with non-protective anti-HBs (<10mIU/ml) before immunization, then transitioned into protective anti-HBs (≥10mIU/ml) after three doses of vaccination was considered seroconverted.

For safety analysis, subjects were given a diary card at each follow-up visit to record solicited and unsolicited adverse events. The intensity, duration, and association of each adverse event to the trial vaccine were evaluated at 30 minutes, 72 hours, and 28 days after injection by interviewing subjects during post-surveillance visits namely V1, V2, V3, and V4.

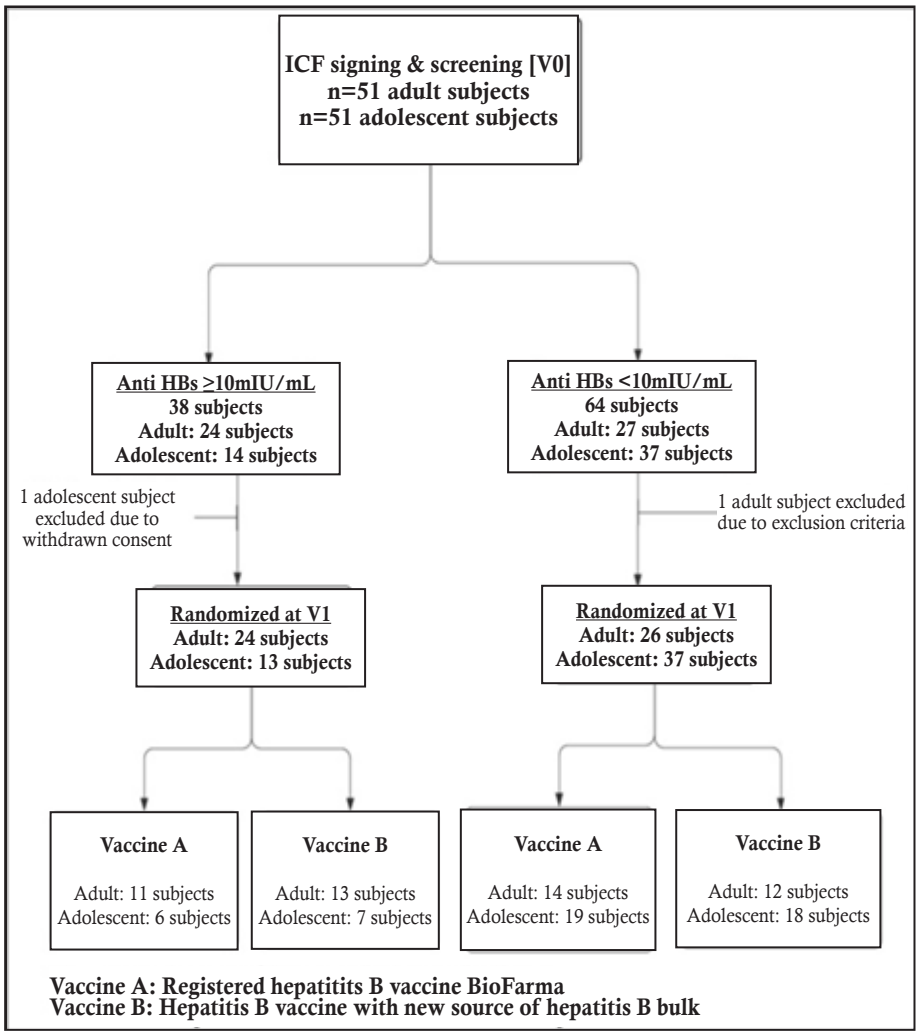

Figure 1 Disposition of Adult and Adolescent Subjects

Vaccine immunogenicity was determined by measuring serum levels of anti-HBs antibodies using chemiluminescent microparticle immunoassay (CMIA) Architect ausab reagent kit on architect i 1000sr. It was intended for quantitative measurement of antibody responses after hepatitis B virus (HBV) vaccination, determination of HBV immune status, and laboratory diagnosis of HBV disease associated with HBV infection when used with other laboratory results and clinical information as well.

Data was analyzed using the IBM SPSS Statistics version 24 for Windows. Fisher's exact test was performed to analyze data on the incidence of local and systemic events, and measure seroconversion rates. The correlation between the two groups was tested with an independent t-test.

The following parameters presented for immunogenicity analysis were seroprotection and/or seroconversion rates at 28 days after one dose (V2) or three doses (V4) of the study vaccine with 95% confidence intervals computed using exact binomial probabilities.

Results

All randomized subjects completed the study per protocol and were analyzed for safety and immunogenicity criteria, as shown in participant disposition in Figure 1.

Initially there were 51 adult subjects who approved and signed the informed consent, but one subject who was screened failed to meet the inclusion criteria due to abnormal laboratory results. Likewise with adolescent's subject, initially 51 adolescents were recruited but one of the adolescent subject's parents did not sign the informed consent. Thus, the

Table 1 Demographic Data on the Study Subjects

Description	Adult	Adolescent
Gender		
Female n (%)	29 (58.0)	23 (46.0)
Male n (%)	21 (42.0)	27 (54.0)
Age (years)		
Female		
Mean±SD	27.52 ± 5.673	13.13±2.242
Range	19–40	10–17
Male		
Mean±SD	30.00±4.648	12.67±2.148
Range	22–39	10–16

Note: SD= Standard deviation

final number of subjects was 50 healthy adult subjects and 50 healthy adolescent subjects who were then each divided into two groups (Group A and Group B) to receive one dose or three doses of vaccine based on baseline Anti-HBs levels (Figure 1).

Of the 50 adult subjects, 29 subject (58%) were female with a mean age of 27.52 years, while of the adolescent subjects, 27 of the 50 subjects (54%) were male with a mean age of 12.67 years (Table 1).The most frequently reported solicited adverse event, both in the adult and adolescent group, was local pain, which occurred after each injection, of mild intensity that resolved within two days. There was no immediate solicited systemic event occurred in adults after each immunization (Figure 2). Similar results were also found in the adolescent group (Figure 3). There was no solicited systemic reaction in Group B in both

Table 2 Serological Responses to Hepatitis B Vaccination Pre and Post One of Immunization in Adult and Adolescent Subjects with Anti-Hbs ≥10mIU/ml before Immunization

Adult Subjects with Antibody ≥10mIU/ml before Immunization						
Description	Pre-vaccination		p-value	Post-vaccination		p-value
	A (Control) n=11	B (IP*) n=13		A (Control) n=11	B (IP*) n=13	
Median	43.2	59.42	0.885	12412.67	34499.56	0.857
(Q1, Q3)	(15.02–947.31)	(14.10–382.68)		(5251.18–78650.34)	(8836.89–67395.63)	
GMT, mIU/ml	103.23	89.27		19994.01	22682.97	
(95% CI)	(25.46–418.50)	(29.01–274.73)		(6063.17–65947.75)	(8617.87–59717.28)	
Increasing antibody titer ≥ 4 times n (% SC)				11 (100.00)	13 (100.00)	
Diff (95 % CI)				0 (-25.88–22.81)		
Adolescent Subjects with antibody ≥10mIU/ml before immunization						
Description	Pre-vaccination		p-value	Post-vaccination		p-value
	A (control) n=6	B (IP*) n=7		A (control) n=6	B (IP*) n=7	
Median	42.69	13.49	0.082	12614.78	10621.96	0.574
(Q1, Q3)	(11.46–193.34)	(13.68–16.03)		(8744.28–26386.52)	(5182.43–21811.04)	
GMT, mIU/ml	41.70	14.69		13430.74	10263.61	
(95% CI)	(9.26–187.72)	(12.15–17.76)		(5580.84–32322.13)	(4761.02–22125.85)	
Increasing antibody titer ≥ 4 times n (% SC)				6 (100.00)	7 (100.00)	
Diff (95 % CI)				0 (-39.03–35.43)		

Note: A= Registered recombinant hepatitis B (Bio Farma) vaccine (control), B= Recombinant hepatitis B (Bio Farma) vaccine with a different source of hepatitis B bulk (*investigational product), GMT= Geometric mean titer, % SC= Seroconversion rate, CI= Confidence interval

Table 3 Serological Responses to Hepatitis B Vaccination Pre and Post Three of Immunization in Adult and Adolescent Subjects with Anti-Hbs <10mIU/ml before Immunization

Adult Subjects with Antibody <10mIU/ml before Immunization						
Description	**Pre-vaccination**			**Post-vaccination**		
	A (Control) n= 14	**B (IP*) n= 12**	**p-value**	**A (Control) n= 14**	**B (IP*) n= 12**	**p-value**
Median	2.315	7.010		212.145	940.205	
(Q1, Q3)	(1.428–5.150)	(1.765–8.188)		(26.03–1416.852)	(116.61–1731.61)	
GMT, mIU/ml	2.322	3.692	0.265	150.522	568.853	0.109
(95% CI)	(1.413–3.812)	(1.712–7.962)		(38.896–582.640)	(208.978–1548.103)	
Increasing antibody titer ≥ 4 times n (% SC)				11 (78.57)	12 (100.00)	
Diff (95 % CI)				-21.43 (-47.59–6.50)		
Adolescent Subjects with Anti-HBs <10mIU/ml before immunization						
Description	**Pre-vaccination**			**Post-vaccination**		
	A (control) n=19	**B (IP*) n=18**	**p-value**	**A (control) n=19**	**B (IP*) n=18**	**p-value**
Median	3.18	4.275		1530.31	4045.90	
(Q1, Q3)	(1.23–5.74)	(2.477–6.99)		(432.99–3152.67)	(2711.58–15132.50)	
GMT, mIU/ml	2.394	3.390	0.0361	1438.47	5716.102	0.002
(95% CI)	(1.286–4.457)	(2.074–5.541)		(852.707–3184.93)	(3379.09–9669.41)	
Anti HBs titer ≥ 10 (IU/ml)						
n (%SP)				19 (100.00)	18 (100.00)	
Diff (95%CI)				0.0 (-16.82-17.59)		
Increasing antibody titer ≥4 times						
n (%SC)				19 (100.00)	18 (100.00)	
Diff (95%CI)				0.00 (-16.82-17.59)		

Note: A= Registered recombinant hepatitis B (Bio Farma) vaccine (control), B= Recombinant hepatitis B (Bio Farma) vaccine with a different source of hepatitis B bulk (*investigational product), GMT= Geometric mean titer, % SC= Seroconversion rate, CI= Confidence interval

adult and adolescent subjects. In contrast, fever occurred in Group A in both adult and adolescent subjects , which appeared to occur in adult subjects 24–72 hours after the first injection with severe intensity. Meanwhile, in adolescent subjects, fever occurred 24–72 hours after the third injection.

The mean value of routine biochemical and haematological profiles of adult subjects at pre-vaccination (V0) and post-vaccination (V1a) results were within the normal laboratory reference range for male and female subjects (Table 2 and Table 3). The majority of subjects had similar safety laboratory evaluation results between pre-vaccination and post-vaccination. The safety of the subjects' laboratory tests was investigated and all abnormal tests were considered not clinically significant and had no possible causal relationship to the study vaccine.

Adult and adolescent subjects with protective anti-Hbs before immunization experienced seroconversion after receiving one dose of vaccination. For subjects with protective anti-HBs (≥10 mIU/ml) before immunization, seroconversion was defined as a ≥4 times increase in antibody titer after receiving one vaccination dose.

Subjects with non-protective anti-HBs before immunization and then transitioned to protective anti-HBs after receiving three doses of vaccination were considered seroconverted. After receiving three doses of vaccine, there were two subjects in group A whose anti-HBs was still <10 mIU/ml. All adult subjects in group B had an increase in antibody titer ≥4 times after receiving three doses of vaccination and showed seroconversion in 85.7% of subjects. Meanwhile, in adult subjects in group A, a seroconversion rate was observed in 78.57% of subjects. Conversely, all adolescent subjects in both groups had seroconversion.

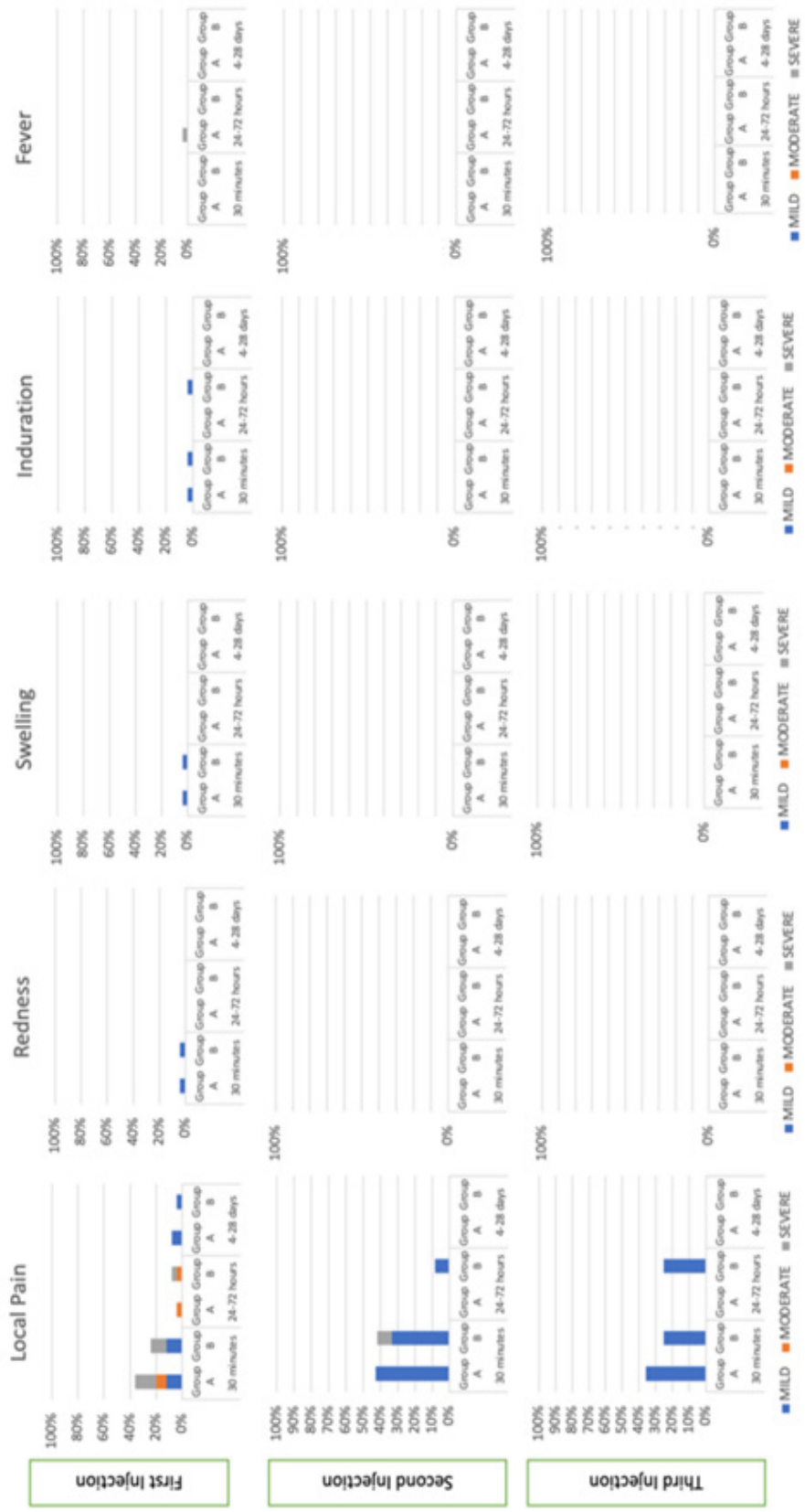

Figure 2 Percentage of Adult Subjects with Solicited Adverse Event

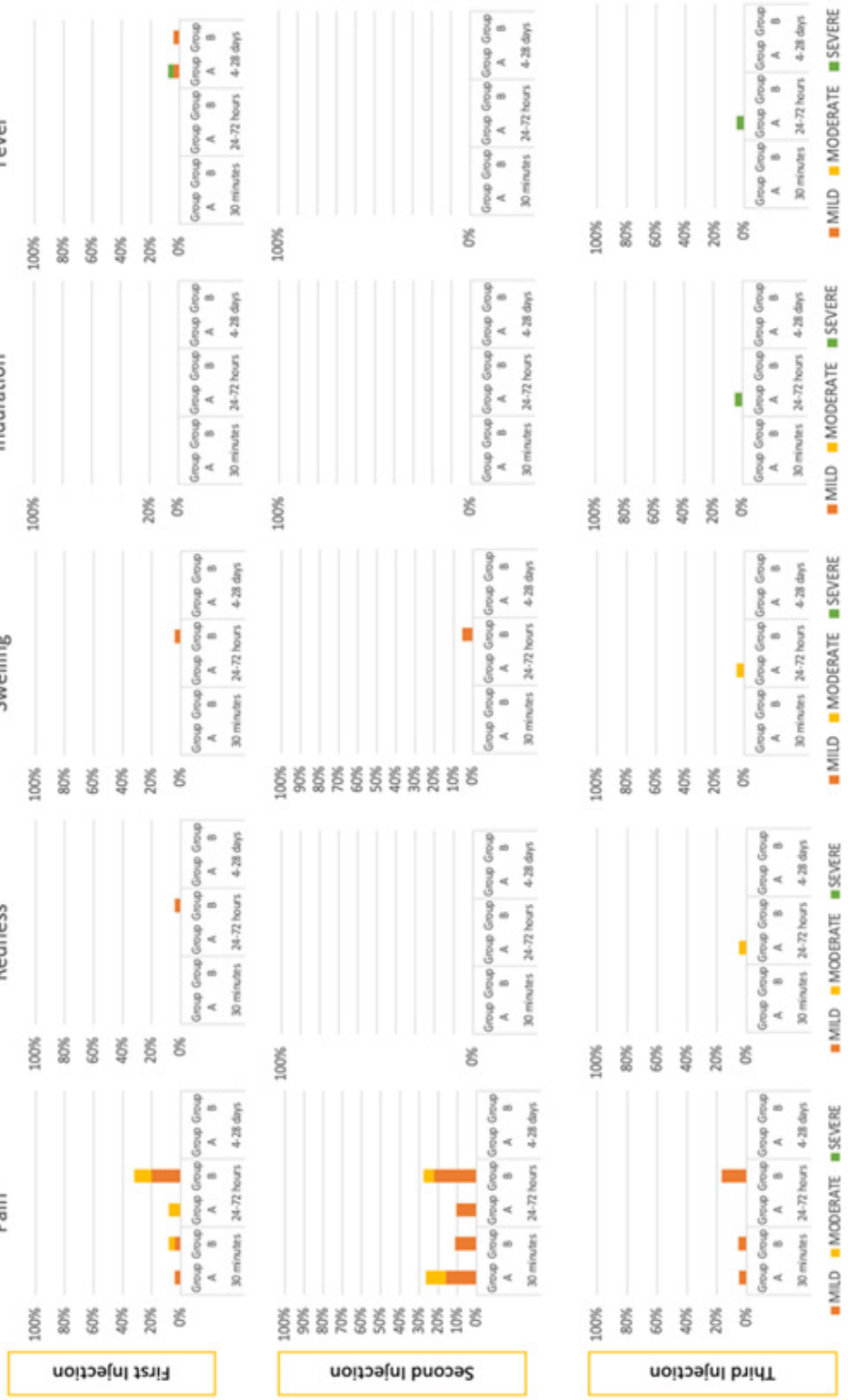

Figure 3 Percentage of Adolescent Subjects with Solicited Adverse Events

Discussion

In our study, the age of adult subjects was limited to 40 years, because other studies revealed that after age 40 years, the proportion of people who had a protective antibody response after vaccination decreased to 90% and 75% in those vaccinated over the age of 60 years.[10,11] The enrolled adult subjects (18–40 years old) showed that majority of adverse event reactions were mild and resolved within two days without any medication in both Group A and B. These results are in accordance with previous study carried out in Thailand which stated that the most common adverse event was pain at the injection site (42.4%).[12] Another study found a small incidence of both local and systemic reactions of mild intensity. Local reactions within 72 hours were local pain with a percentage of 10.7%, 8.0%, and 4.6% after each dose of vaccination 1, 2, and 3.[8] A study in India found pain at the injection site in 11.5% of the total subjects as the most common local reaction.[13] The majority of local pain in our study was reported to occur within 30 minutes after any dose. This result is different from another study which reported that local pain was more common 1-day after vaccination than 30 minutes after vaccination.[14]

There was no significant difference in solicited systemic reactions. Most systemic reactions were below 10% after any dose, and no serious adverse events were reported during the study. On the contrary, another study reported that most of the adverse events following immunization from 1,013 reports were general systemic reactions (47%) followed by local reactions (26%).[15]

There were two subjects in group A with anti-Hbs <10mIU/ml after three doses of the hepatitis B vaccine. Therefore, these subjects were administered a second series of hepatitis B vaccine, which increased their antibodies to protective levels (134.45 mIU/ml and 641.86 mIU/ml). This incidence is similar to a previous study which found that 5–15% of healthy subjects injected with monovalent plasma-derived or DNA recombinant hepatitis B vaccine were nonresponders (no antibody of anti-HBs) or hypo responders (low antibody levels of anti-Hbs).[16] Nonresponders were defined as people with anti-HBs levels of less than 10 mIU/ml after six or more doses of hepatitis B vaccine, in which case revaccination was indicated. Revaccination can be completed using one of two approaches, namely by administering a second complete hepatitis B vaccine series followed by anti-HBs testing one or two months later, or administering a single dose of hepatitis B vaccine followed by anti-HBs testing one to two months later.[17] Adult and adolescent subjects with protective anti-Hbs titer at baseline had higher increases in anti-Hbs titer after one dose of study vaccine, compared with antibody titer increases in subjects with nonprotective anti-Hbs titer at baseline who received three doses of study vaccine. It might be due to the presence of T cell memory in subjects with protective anti-Hbs titer at baseline.[18]

In a systemic review study, seroprotection rates defined as anti-HBs antibody levels ≥10 mIU/ml occurred between four weeks and eight months post-dose three following a 0-, 1-, and 6-month schedule.[19] Another study reported a post- three-dose seroprotection rate of >90.0%.[20] The high response may be due to the young adults population and three-quarters of the subjects being female, who have been documented to have a high response to HBV.[21] Another study reported seroprotection rates before complete vaccination of 20.0%, 61.0%, and 82.0% at 1, 2, and 3 months, respectively.[22]

This study has limitations. There was no complete documentation of whether subjects had received hepatitis B vaccination at an early age. More studies has been conducted documenting the history of hepatitis B immunization at a young age. All subjects enrolled in this study were HbsAg negative, with almost half of the adult subjects having protective anti-HBs titer levelsf. To the contrary, only 26% of adolescent subjects had protective anti-HBs titer levels. This shows that many subjects are still not protected from hepatitis B infection. Further research needs to be carried out to find out whether a booster dose of hepatitis B vaccine is required in adolescents aged ten years and over.

In conclusion, recombinant hepatitis B Vaccine with a new source of HBsAg bulk has a good safety and immunogenicity profile in adults and adolescents. The safety of this vaccine is comparable to the registered Bio Farma recombinant hepatitis B vaccine with higher immunogenicity. The results of this study can be used as a basis for further phase 2 or 3 studies.

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Declaration of Competing Interest

Rini Mulia Sari is the Head of the Surveillance and Clinical Research Division of PT. Bio Farma.

References

1. Trépo C, Chan HLY, Lok A. Hepatitis B virus infection. Lancet. 2014;384:9959
2. Das S, Ramakrishnan K, Behera SK, Ganesapandian M, Xavier AS, Selvarajan S. Hepatitis B vaccine and immunoglobulin: key concepts. J Clin Transl Hepatol. 2019;7(2):165–71.
3. World Health Organization. Global health sector strategy on viral hepatitis 2016–2021: towards ending viral hepatitis. Geneva: WHO; 2016.
4. World Health Organization. Hepatitis B vaccines: WHO position paper, July 2017–Recommendations. Vaccine. 2019;37(2):223–5.
5. Terrault NA, Lok ASF, McMahon BJ, Chang KM, Hwang JP, Jonas MM, et al. Update on prevention, diagnosis, and treatment of chronic hepatitis B: AASLD 2018 hepatitis B guidance. Hepatology. 2018;67(4):1560–99.
6. Koc ÖM, Damoiseaux J, van Loo IHM, Masquillier HIL, Oude Lashof AML. Case report of delayed seroprotection rather than non-response after primary three-dose hepatitis B vaccination. Vaccine. 2020;38(2):112–4.
7. Nency YM, Rahmadi FA, Anantyo DT, Farhanah N, Hapsari R, Farida H, Sadhana U, Djagat H, Kristina TN, Juniarto AZ, Puspita M. Protectivity and safety following recombinant hepatitis B vaccine with different source of bulk compared to hepatitis B (Bio Farma) vaccine in Indonesia. Clinical and Experimental Vaccine Research. 2022;11(1):43.
8. Fadlyana E, Rusmil K, Bachtiar NS. Kekebalan dan keamanan setelah mendapat imunisasi hepatitis B rekombinan pada anak remaja. Sari Pediatri. 2016;15(2):87–92.
9. World Health Organization. guidelines on clinical evaluation of vaccines: regulatory expectations, Annex 1, TRS No 924. Geneva: WHO; 2004.
10. Van Der Meeren O, Crasta P, Cheuvart B, De Ridder M. Characterization of an age-response relationship to GSK's recombinant hepatitis B vaccine in healthy adults: An integrated analysis. Hum Vaccin Immunother. 2015;11(7):1725–8.
11. Pattyn J, Hendrickx G, Vorsters A, Van Damme P. Hepatitis B vaccines. J Infect Dis. 2021;224(12 Suppl2):S343–51.
12. Chaiklang K, Wipasa J, Chaiwarith R, Praparattanapan J, Supparatpinyo K. Comparison of immunogenicity and safety of four doses and four double doses vs. Standard doses of hepatitis b vaccination in hiv-infected adults: a randomized, controlled trial. PLoS One. 2013;8(11):e80409.
13. Kalaivani M, Rastogi S, Kalaiselvan V, Singh GN. Adverse reactions after hepatitis B vaccination: a retrospective analysis using spontaneous reports. J Young Pharm. 2017;9(1):55–9.
14. Sundoro J, Rusmil K, Sitaresmi MN, Arhana, Djelantik IGG, Hadinegoro SR, et al. Profil Keamanan setelah pemberian dosis primer vaksin Pentabio® pada bayi di Indonesia. Majalah Kedokteran Bandung. 2017;49(2):86–93.
15. Rusmil K, Gunardi H, Fadlyana E, Soedjatmiko, Dhamayanti M, Sekartini R, et al. The immunogenicity, safety, and consistency of an Indonesia combined DTP-HB-Hib vaccine in expanded program on immunization schedule. BMC Pediatr. 2015;15(1):219.
16. Rusmil K, Fadlyana E, Bachtiar NS. Booster vaksinasi Hepatitis B terhadap anak yang non responder. Sari Pediatri. 2010;12(2):88–91.
17. Wilkins T, Sams R, Carpenter M. Hepatitis B: screening, prevention, diagnosis, and treatment. Am Fam Physician. 2019;99(5):314–23.
18. Huang Y, Yang Y, Wu T, Li Z, Xu H, Huang A, et al. Complementary presence of HBV humoral and T-cell response provides protective immunity after neonatal immunization. J Clin Transl Hepatol. 2022;10(4):660–8.
19. Van den Ende C, Marano C, van Ahee A, Bunge EM, de Moerlooze L. The immunogenicity and safety of GSK's recombinant hepatitis B vaccine in adults: a systematic review of 30 years of experience. Expert Rev Vaccines. 2017;16(8):811–32.
20. Zimmermann P, Curtis N. Factors that influence the immune response to vaccination. Clin Microbiol Rev.

2019;32(2):e00084.
21. Yang S, Tian G, Cui Y, Ding C, Deng M, Yu C, et al. Factors influencing immunologic response to hepatitis B vaccine in adults. Sci Rep. 2016;6:27251.
22. Koc ÖM, van Oorschot E, Brandts L, Oude Lashof A. Timing of primary three-dose hepatitis B vaccination and post vaccination serologic testing among a large cohort of healthy adults. J Med Virol. 2022;94(9):4433–9.