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Original Article

The immunogenicity and safety of the new Indonesian DTwP-HB-Hib vaccine compared to the DTwP/HB vaccine given with the Hib vaccine

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Abstract

Background Haemophilus influenzae type b (Hib) causes infection with predominant manifestations of pneumonia, meningitis, and other invasive diseases, occurring primarily in children aged under 2 years, particularly in infants. The World Health Organization (WHO) and Indonesian Technical Advisory Group for Immunization recommend to include the Hib vaccine into the national immunization program. The newly developed DTwP-HB-Hib combination vaccine is anticipated to be the preferred choice for Hib vaccine introduction; it is efficient, simple, and has higher coverage.

Objective To evaluate the immunogenicity and safety of a new, combined Bio Farma DTwP-HB-Hib vaccine, compared to the registered Hib monovalent vaccine given simultaneously with the local DTwP-HB vaccine, when used as the primary vaccination of Indonesian infants.

Methods A prospective, randomized, open-label, phase II study was conducted on the DTwP-HB-Hib vaccine compared to the Hib (registered) vaccine given simultaneously with the DTwP-HB vaccine, in Bandung from July 2011 to January 2012. Infants were serially vaccinated at 6-11, 10-15, and 14-19 weeks. Serological assessments were done prior to the first vaccine dose and 28 days after the third dose. Safety was assessed from the time of first injection until 1 month after the last injection.

Results Of 220 healthy infants enrolled, 211 completed the study, with 105 receiving the combined vaccine and 106 the two separate vaccines. All vaccines were well tolerated. No differences in rates of local and systemic reactions were seen between the two methods of administration. No serious adverse events were considered to be related to the vaccines. In the DTwP-HB-Hib primary-vaccination group, at least 98% of the infants reached protective levels of antibodies (seropositivity) against the antigens employed in the vaccines while 96% in the control group.

Conclusion The DTwP-HB-Hib combined vaccine is immunogenic and safe, as well as comparable to the Hib vaccine given simultaneously with to the DTwP-HB vaccine.**[Paediatr Indones. 2017;57:129-37; doi: http://dx.doi.org/10.14238/pi57.3.2017.129-37]**.

Keywords: *DTwP-HB-Hib; Hib; immunogenicity; infants; safety; vaccine*

Before the vaccination era, *Haemophilus influenza* type b (Hib) caused infection with predominant manifestations of pneumonia, meningitis, and other invasive diseases occurring primarily in children aged under 2 years, particularly in infants.[1,2] Pneumonia was responsible for 19% of deaths in children below 5 years of age, of which more than 70% were in Sub-Saharan Africa and Southeast Asia.[2] In Asia, 23% of pneumonia cases were caused by Hib, while other causes were pneumococcus, staphylococcus, streptococcus, and viruses.[3] In Indonesia, pneumonia and meningitis

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caused an estimated 15.5% and 8.8% of all deaths recorded in under-five children, respectively.[4,5]

The WHO has recommended worldwide incorporation of Hib vaccination into all routine infant immunization programs, after 6 weeks of age. A diphtheria-tetanus-pertussis (DTP)-based combination, would be preferable, in order to allow for rapid integration into the existing DTP vaccination schedules.[2] A DTwP-HB vaccine was licensed in Indonesia in 2004 and has been routinely given to infants at 2, 3, and 4 months of age. Phase I of this study showed that the Hib monovalent vaccine was immunogenic and well-tolerated when administered either as a single injection in adults, or in combination (as the DTP-HB-HIB vaccine) in infants, with a one-month interval between doses.[6,7]

The objective of this study was to evaluate the immunogenicity and safety of a new, combined *Bio Farma* DTwP-HB-Hib vaccine, compared to the registered Hib monovalent vaccine given simultaneously with the local DTwP-HB vaccine, when used as the primary vaccination of Indonesian infants according to *Expanded Program on Immunization* (EPI) schedule at 6, 10, and 14 weeks of age, after a birth dose of hepatitis B vaccine, as recommended by the WHO.

Methods

This prospective, randomized, open-label, phase II study of the combined DTwP-HB-Hib vaccine was conducted at three primary health care centers in Bandung from July 2011 to January 2012 and was approved by the Institutional Review Board of Padjadjaran University. Subjects' parents provided written informed consent before enrollment. The study was conducted in accordance with the Declaration of Helsinki and Good Clinical Practice guidelines with approval of the Ethics Review Committee and the *National Regulatory Authority* (NRA).

The study population comprised of healthy infants who were 6-11 weeks of age at enrollment, born at 37-42 weeks of gestation, with a birth weight of 2,500-4,000 g, and had received a single dose of monovalent hepatitis B vaccine at 0-7 days after birth, as recorded in their vaccination documentation. Infants were excluded if they had a history of allergic reaction likely to be stimulated by any vaccine component, a history of congenital or acquired immunodeficiency, diphtheria, tetanus, pertussis, hepatitis B, *Haemophilus influenzae* type b infection, uncontrolled coagulopathy or blood disorders, chronic illness, immunosuppressive condition, were undergoing immunosuppressive therapy, had received immunoglobulin therapy or blood products prior to starting or during the study, acute febrile illness at the time of the vaccination, any previous vaccination other than oral polio, BCG vaccine, or HB at birth, or were participating in another clinical study.

We aimed to evaluate the immunogenicity and safety outcomes of the new *Bio Farma* DTwP-HB-Hib vaccine compared to the Hib monovalent vaccine given simultaneously with the DTwP-HB vaccine (DTwP-HB +Hib). At the time of enrollment, subjects were assigned to one of two vaccine groups using a randomized block permutation list.

The study vaccine was a new, liquid DTwP-HB-Hib (pentavalent) vaccine produced by Bio Farma. This vaccine contained 5 antigens. Each 0.5ml dose contained > 30 IU of purified diphtheria toxoid, > 60 IU of purified tetanus toxoid, > 4IU inactivated Bordetella pertussis, 10μg recombinant hepatitis B surface antigen (HBsAg), and 10μg Hib/polyribosyrsibitol phosphate (PRP) conjugated to tetanus toxoid. The DTwP-HB vaccine (*Bio Farma*) contained 4 antigens, with similar amount of antigens, except for hepatitis B (5 μg HBsAg) for each 0.5 mL dose). The Hib monovalent vaccine was imported and already registered in Indonesia. It also contained 10μg Hib/PRP conjugated to tetanus toxoid per dose. Vaccines were administered at 6, 10, and 14 weeks of age, with a 4-week interval between doses. One group received the new DTwP-HB-Hib combination vaccine, while the other group received the DTwP-HB and Hib (registered) vaccines simultaneously. The vaccines were given intramuscularly in the external anterolateral region of the thigh.

Subjects provided 4-mL blood specimens, collected before the first dose of vaccine and 28 days after the third dose, to evaluate antibody responses. Serum specimens were tested for antibodies against all vaccine antigens. Serology assays, except for anti-HBs, were conducted in the *Bio Farma Immunology Laboratory of the Clinical Trial Department*, by technicians who were blinded to group assignment.

Tests for anti-HBs were conducted in a commercial laboratory which had been approved by *Bio Farma's Quality Assurance*.

Antibodies to tetanus and diphtheria were measured by using an enzyme-linked immunosorbent assay (ELISA). An anti-diphtheria and anti-tetanus concentration of >0.01 IU/mL is generally accepted to be the minimum protective threshold, and a concentration of >0.1 IU/mL was regarded to be the standard protective threshold. Pertussis antibodies were measured using a microagglutination assay, with a cut-off dilution of 1/40. An adequate vaccine response was defined to be a post-vaccination antibody titer of four times more than the pre-vaccination titer. Antibodies to hepatitis B surface antigen (anti-HBs) were assayed using a chemiluminescent microparticle immunoassay (CMIA) by AUSAB, Abbott, with a 10 mIU/mL cut-off. Antibodies to PRP were measured by using Improved, Phipps ELISA, to assess serum antibody to Haemophilus influenzae type b. Anti-PRP concentration of ≥ 0.15 μg/mL was generally accepted to be the minimum protective threshold, and a concentration of ≥ 1.0μg/mL was regarded to be the long-term protective threshold.[8]

Safety assessments were conducted by parents and investigators. Study personnel monitored subjects for 30 minutes after each vaccination to detect immediate reactions. Parents were given thermometers and diary cards, and asked to record the occurrence and intensity (mild, moderate, or severe) of local (i.e., pain, redness, swelling, and induration at injection-site), and systemic (e.g., fever [≥38°C] and irritability) reactions, from day 0 through 28 days after each vaccination. For the analyses, adverse events were graded from 1 to 3 in intensity. For local reactions, grade 3 redness, swelling, or induration was defined as areas >5 cm in diameter and grade 3 pain was defined as cried when the leg was moved. For systemic reactions, grade 3 fever was defined as axillary temperature >39°C and grade 3 irritability was defined as inconsolable crying lasting more than three hours. For all other general adverse events, grade 3 was defined as preventing normal daily activities.

Parents of subjects were contacted by telephone three days after each vaccination to ensure completeness of reporting and to screen for adverse events (AEs) requiring medical evaluation or an office visit, an emergency department visit, or hospitalization. Serious adverse events (SAEs) were recorded throughout the study and evaluated by investigators for possible relationships to the study vaccines. At each subsequent visit, the investigator transcribed information from the diary cards onto the Case Report Form, and confirmed other adverse experiences that occurred after the period covered by the diary card.

The minimum required target sample size was established at 220 assessable infants for this study. A 10% dropout rate was anticipated. Data analyses were performed using *SPSS version 18.0* software. Demographic data were expressed as mean (SD) and range. The statistical significance of differences between the vaccine groups in demographic characteristics was assessed by Chi-square test. A P values <0.05 were considered to be an indicator of statistically significant differences between the vaccine groups.

The immunogenicity analyses were performed on the per-protocol population, defined as subjects who received the 3-dose primary series of the appropriately assigned study vaccines, had all blood samples obtained within the time intervals specified in the study protocol, and had a valid post-vaccination serology test result. Antibody seroprotection rates against diphtheria and tetanus toxoids, HBsAg, PRP, and vaccine response rate to pertussis were calculated with 95% confidence intervals (CI). Geometric mean antibody concentration (GMC) with 95% CI were calculated by taking the log-transformation of individual concentration and calculating the anti-log of the mean of these transformed values. Exploratory analyses were performed to compare GMCs and seroprotection rates between the vaccine groups using Mann-Whitney and Chi-square or Fisher's tests.

The safety analyses were based on the intention-to-treat population, defined as all subjects who received at least one dose of vaccine. Exploratory analyses were performed to compare incidences of solicited local and systemic adverse events (any grade intensity) between the vaccine groups using two-sided Fisher's exact test.

Results

Of the 220 infants recruited, 9 did not complete the study protocol due to voluntary withdrawal (3 infants), discontinuation by investigator (3 infants), and disparency with protocol for immunogenicity analyses (3 infants). Investigator excluded 3 infants which dead by severe respiratory failure and severe dehydration (1 infant), had febrile convulsion 3 days after vaccination (1 infant), and had inconsolable crying for more than 3 hours within 3 days (1 infant). The 3 subjects were excluded according to protocol for immunogenicity analyses: 1 due to non-compliance with vaccination procedure (received non-trial vaccine) and 2 due to protocol deviation from the inclusion criteria (different randomization), but these last 2 subjects were not excluded for safety analysis. Hence we had a total of 213 infants in safety analyses, but only 211 subjects in immunogenicity analysis.

The demographic characteristics of subjects are shown in **Table 1**. No clinically significant differences with respect to gender and age were observed between the two groups.

Table 1. Subjec's characteristics

Characteristics	DTwP-HB-Hib (n=105)	DTwP-HB+Hib (n=106)	P value
Gender, n(%)			
Male	47 (44.8)	50 (47.2)	0.832
Female	58 (55.2)	56 (52.8)	
Age, weeks			
Mean (SD)	8.2 (1.5)	8.1 (1.6	0.795
Min-max	6-11	6-11	

Seroprotection and vaccine response rates for each antigen in the study are summarized in **Table 2**. For seroprotection and vaccine response rates, no significant differences were observed between the two groups before and after vaccination with different cut-off values.

Geometric mean concentrations (GMCs) of antibody are presented in **Table 3**. The GMCs before immunization were not significantly different between the two groups for all antigens. After immunization, also not significantly different between the two groups except for anti-HBs. The DTwP-HB-Hib group had significantly higher anti-HBs GMC than the DTwP-HB+Hib group (441.54mIU/mL vs. 213.84 mIU/mL, respectively, P=0.001).

Table 2. Summary of seroprotection rates of antibody concentration

Antibody	Timing of blood collection	Criterion	DTwP-HB-Hib			DTwP-HB+Hib			P value
			N[a]	%SP[b]	95%CI	N	%SP	95%CI	
Diphtheria	Pre-dose 1	≥ 0.01 IU/mL	35	33.3	25.0 to 42.8	44	41.5	32.6 to 51.0	0.228
	Pre-dose 1	≥ 0.1 IU/mL	3	2.9	1.0 to 8.1	3	2.8	1.0 to 8.0	1.00
	Post-dose 3	≥ 0.01 IU/mL	105	100.0	96.5 to 100	106	100.0	96.5 to 100	1.00
	Post-dose 3	≥ 0.1 IU/mL	86	81.9	73.5 to 88.1	88	83.0	74.7 to 89.0	0.831
Tetanus	Pre-dose 1	≥ 0.01 IU/mL	105	100.0	96.5 to 100.0	105	99.1	94.8 to 99.8	1.00
	Pre-dose 1	≥ 0.1 IU/mL	99	94.3	88.1 to 97.4	102	96.2	90.7 to 98.5	0.538
	Post-dose 3	≥ 0.01 IU/mL	105	100.0	96.5 to 100.0	106	100.0	96.5 to 100.0	1.00
	Post-dose 3	≥ 0.1 IU/mL	101	96.2	90.6 to 98.5	104	98.1	93.4 to 99.5	0.445
Pertusis	Pre-dose 1	≥ 40 (1/dil)	7	6.7	3.3 to 13.1	6	5.7	2.6 to 11.8	0.761
	Pre-dose 1	≥ 80 (1/dil)	5	4.8	2.1 to 10.7	4	3.8	1.5 to 9.3	0.748
	Post-dose 3	≥ 40 (1/dil)	94	89.5	82.2-94.0	100	94.3	88.2 to 97.4	0.199
	Post-dose 3	≥ 80 (1/dil)	89	84.8	76.7 to 90.8	95	89.6	82.4 to 94.1	0.291
	Post-dose 3	VRR[c]	90	85.7	77.8 to 91.1	98	92.5	85.8 to 96.1	0.177
Hepatitis B	Pre-dose 1	≥ 10 mIU/mL	19	18.1	11.9 to 26.5	21	19.8	13.3 to 28.4	0.751
	Post-dose 3	≥ 10 mIU/mL	104	99.0	94.8 to 99.8	102	96.2	90.7 to 98.5	0.369
PRP (Hib)	Pre-dose 1	≥ 0.15 µg/mL	30	28.6	20.8 to 37.8	28	26.4	19.0 to 35.5	0.726
	Pre-dose 1	≥ 1.0 µg/mL	14	13.3	8.1 to21.1	17	16.0	10.3 to 24.2	0.579
	Post-dose 3	≥ 0.15 µg/mL	103	98.1	93.3 to 99.5	105	99.1	94.8 to 99.8	0.621
	Post-dose 3	≥ 1.0 µg/mL	101	96.2	90.6 to 98.5	101	95.3	89.4 to 98.0	1.00

[a] N= number of subjects with a valid serology result pre-dose 1 and post-dose 3

[b] %SP= seroprotection rate

[c] VRR (vaccine response rate) was defined as >4 times the pre-vaccination concentration

Table 3. Summary of geometric mean antibody concentration

Antibody	Timing of blood collection	DTwP-HB-Hib		DTwP-HB+Hib		P value
		GMC	95%CI	GMC	95%CI	
Diphtheria	Pre-dose 1	0.040	0.026 to 0.062	0.045	0.029 to 0.068	0.413
	Post-dose 3	0.259	0.209 to 0.321	0.289	0.233 to 0.359	0.543
Tetanus	Pre-dose 1	0.639	0.530 to 0.771	0.685	0.575 to 0.816	0.787
	Post-dose 3	1.147	0.916 to 1.147	1.137	0.894 to 1.446	0.818
Pertusis	Pre-dose 1	6.819	5.799 to 8.019	6.666	5.809 to 7.651	0.607
	Post-dose 3	219.63	160.36 to 300.88	332.81	257.69 to 429.83	0.067
Hepatitis B	Pre-dose 1	2.082	1.508 to 2.876	2.458	1.693 to 3.650	0.661
	Post-dose 3	441.57	347.70 to 560.79	213.84	156.64 to 292.01	0.001
PRP (Hib)	Pre-dose 1	0.988	0.925 to 1.055	1.075	0.9990 to 1.167	0.989
	Post-dose 3	12.612	9.689 to 16.421	11.663	8.962 to 15.81	0.876

GMC=geometric mean concentration

Table 4. Local reaction within 72 hours after each injection

Reactions	DTwP-HB-Hib (1)			DTwP-HB+Hib						P value	
				DTwP-HB site (2)			DTwP-HB site (2)				
	mEv	nSj	%Sj	mEv	nSj	%Sj	mEv	nSj	%Sj	1 vs 2	1 vs 3
After 1st injection											
Local reaction	36	16	14.9	27	19	17.3	14	9	8.2	0.779	0.177
Solicited reactions											
Pain	11	11	10.3	9	9	8.2	7	7	6.4	0.764	0.424
Redness	8	8	7.3	3	3	2.7	2	2	1.8	0.742	0.199
Swelling	10	10	9.3	7	7	6.4	3	3	2.7	0.572	0.077
Induration	7	7	6.5	8	8	7.3	2	2	1.8	0.956	0.098
After 2nd injection											
Local reaction	14	14	13.2	19	11	3.7	14	9	8.3	0.618	0.341
Solicited reactions											
Pain	8	8	7.5	8	8	7.4	6	6	5.6	0.840	0.742
Redness	3	3	2.8	3	3	2.8	3	3	2.8	1.0	1.0
Swelling	3	3	2.8	3	3	2.8	2	2	1.8	1.0	0.680
Induration	0	0	0	5	5	4.6	3	3	2.8	0.060	0.247
After 3rd injection											
Local reaction	7	6	5.7	13	13	12.0	11	11	10.2	0.168	0.341
Solicited reactions											
Pain	1	1	0.9	6	6	5.5	6	6	5.5	0.119	0.119
Redness	2	2	1.9	3	3	2.8	1	1	0.9	1.0	0.618
Swelling	3	3	2.8	2	2	1.8	3	3	2.8	0.680	1.0
Induratio	1	1	0.9	2	2	1.8	1	1	0.9	1.0	1.0

nEv=number of event, nSj=number of subject, %Sj=percentage of subject

Subject's local reactions within 72 hours are presented in **Table 4**, where 1 subject may experience more than 1 local reactions. After the first, second, and third injections, 57 forms of local reactions occurred in 36 subjects (11.3%) in the DTwP-HB-Hib group. The most frequent reaction was pain; other reactions were redness, swelling, and induration. In the DTwP-HB site, 59 local reactions were noted after the injections in 43 subjects (13.3%). The most frequent reaction was pain; other reactions were redness, induration, and swelling. In the Hib site, 39 local reactions were reported after the injections in 29 subjects (8.9%). The most frequent reaction was pain; other reactions were redness, swelling, and induration. In this study, 2 subjects in the DTwP-HB-Hib group site and 1 subject in the Hib site of the DTwP-HB+Hib group

Table 5. Systemic reactions within 72 hours after each injection

Systemic reaction	DTwP-HB-Hib		DTwP-HB+Hib		P value
	nSj	%Sj	nSj	%Sj	
After 1st injection					
Fever (≥ 38°C)	30	28.0	28	25.5	0.274
Irritability	3	2.8	4	3.6	0.121
Others	2	1.8	1	0.9	0.666
After 2nd injection					
Fever (≥ 38°C)	27	25.3	19	17.6	0.444
Irritability	1	0.9	3	2.8	0.500
Others	4	3.8	2	1.8	0.831
After 3rd injection					
Fever (≥ 38°C)	21	20.0	15	13.9	0.049*
Irritability	1	0.9	1	0.9	0.246
Others	5	4.8	2	1.8	0.691

nSj=number of subjects

presented with severe local reactions within 72 hours after each injection, (swelling and induration). The incidence and intensity of symptoms were comparable in both vaccine groups. There was no increase in reactogenicity with doses for local symptoms. Local reactions were low in both groups; most reactions were mild, and resolved spontaneously within the two-day follow-up period. No subjects presented with local reactions between 72 hours and 28 days after each injection.

Subject's systemic reactions within 72 hours are presented in **Table 5**. In the DTwP-HB-Hib group, fever was reported in 28.0%, 25.3%, and 20.0% of subjects after the 1st, 2nd, and 3rd injections, respectively. In the DTwP-HB+Hib group, fever was reported in 25.5%, 17.6%, and 13.9% of subjects after the 1st, 2nd, and 3rd injection, respectively. There were no significant differences between the DTwP-HB-Hib and DTwP-HB+Hib groups with regards to fever after each injection, except after the 3rd injection, with significantly fewer in the DTwP-HB+Hib group (P=0.049). No anaphylactic or other severe reactions were reported within 30 minutes after any dose of vaccines.

During the study, 11 cases of serious adverse events (SAEs) were reported. There was one death during the study due to respiratory failure and septicemia 20 days after the infant received the first combination vaccine dose. The investigators and the *Indonesian National Committee of Adverse Event Following Immunization* did not consider the death to be related either to the vaccination or study procedure. One subject suffered from complex febrile convulsion, classified as a vaccine reaction in field classification and probable in causality assessment, but the patient resolved spontaneously. The remaining 9 SAEs were mainly due to infectious diseases such as bronchopneumonia, diarrhea, and aspiration pneumonia. The children recovered after treatment and hospitalization. All SAE cases were audited by I*ndonesian National Committee of Adverse Event Following Immunization*.

Discussion

As of 2000, the WHO had achieved 90% coverage with the DTP vaccination in infants aged less than one year. In countries with endemic hepatitis B, early infant immunization is recommended. Since the coverage with hepatitis B immunization is much lower in Indonesia, combining it with DTP was thought to be the best way to increase hepatitis B immunization coverage. The first clinical trial of the DTwP-HB vaccine started in April 2002 in three centers, involving about 730 healthy infants from Bogor, Bandung, and Banjar Baru. The trial consisted of 5 groups of subjects, each with different doses of hepatitis B and different schedules of immunization. The immunogenicity and safety of the DTwP-HB vaccine were not significantly different to that of separate administrations of the DTwP and hepatitis B vaccines, which had been commonly used in the Immunization Programme up to that point.[9]

In 1998, the WHO recommended the Haemophilus influenzae type B (Hib) vaccine to be

included in routine infant immunization programs.[2] Due to limited national capacity, the Hib antigen was integrated as a DTP-based combination vaccine. Bio Farma had developed a new, pentavalent, combined diphtheria-tetanus-whole cell pertussis-hepatitis B/Hib (DTwP-HB-Hib) vaccine containing 10 μg of polyribosylribitol phosphate (PRP) conjugated to tetanus toxoid.

The first Hib vaccine was used in a phase I trial of 25 healthy adults, where 1 subject received 1 dose of Hib monovalent vaccine. No serious adverse events followed vaccination. However, pain occurred in 11 subjects and systemic reactions (myalgia) occurred in 5 subjects. Most reactions were mild and disappeared within 24 hours. All subjects (100%) reached protective levels of antibodies (seroprotectivity) against Hib. The GMT increased from 0.68 μg/mL to 30.16 μg/mL.[6] The first clinical trial of the DTwP-HB-Hib vaccine was conducted in April–June 2011, involving 30 pediatric subjects. Eighteen subjects (60%) reported fever within 3 days after the vaccination. Most cases of fever were mild in intensity and resolved within 3 days. Furthermore, no serious adverse events were reported. All subjects had seroprotective antibodies against tetanus, diphtheria, hepatitis B, and Hib.[7]

The main objective of this study was to compare the immunogenicity and safety of the new DTwP-HB-Hib pentavalent combination vaccine to separate injections of DTwP-HB and Hib (DTwP-HB+Hib) vaccines, in a group of infants who had received a dose of hepatitis B vaccine at birth. After the primary series, 100% of subjects in both vaccine groups achieved levels considered to be protective for diphtheria (>0.01U/mL) and tetanus (>0.01 IU/mL). Also, 99% of the DTwP-HB-Hib pentavalent group and 96.2% of the DTwP-HB+Hib group achieved protective levels of hepatitis B (>10 mIU/mL). For pertussis, 89.5% in the DTwP-HB-Hib pentavalent group and 94.3% in the DTwP-HB+Hib group achieved seroprotection of 40 (1/dil). We observed no differences in seroprotection rates between the two groups. We also noted that the Hib response in the DTwP-HB-Hib pentavalent combination group was not significantly different to that of the separately administered monovalent Hib registered vaccine. In our Bandung study of the primary-vaccination three-dose course, 98.1% of the infants in the DTwP-HB-Hib group and 99.1% of the DTwP-HB+Hib group had anti-PRP titers above the conservative threshold of protection (0.15 μg/mL). In addition, 96.2% of those in the DTwP-HB-Hib group and 95.3% of the DTwP-HB+Hib group had titers above 1.0 μg/mL.

A 2009-2010 Indian study in 661 infants aged 6 to 8 weeks, found 100% seroprotection to anti-PRP using pentavalent combination vaccines with a one month-interval between doses.[10] Another study also used pentavalent vaccines at one month-intervals in 608 infants aged 6 weeks and showed anti-PRP results similar to our study: 100% protection for short-term protection (> 0.15μg/mL) and 95% for long-term protection (> 1 μg/mL).[11] Furthermore, another Indian study in 165 infants at 6, 10, and 14 weeks of age found results similar to our study: at one month after the third vaccination, percentages of infants achieving predefined protective antibody levels were 99% diphtheria; 100% tetanus; 98% hepatitis B; 100% Hib short-term (≥ 0.15 μg/mL); 95% Hib long-term (≥ 1.0 μg/mL) protection; and 99% for pertussis (relevant immune response).[12] These three studies were conducted without control groups.

An Ankara, Turkey study in 2003-2004 was conducted in 303 infants 6 weeks of age. Infants received three doses at one month-intervals, of either a combination vaccine or a control DTP-Hib with separate hepatitis B vaccine. Seroconversion of all antigens were similar between the two groups.[13] A Latin American study used pentavalent vaccines in 1,000 infants. Statistical comparisons following the primary vaccination showed that, in terms of the antibody response to the PRP antigen, the combined DTP-HB-Hib vaccine was clinically non-inferior to the licensed DTP-HB and Hib vaccines. Other antigens also showed similar immune responses.[14] In addition, an Indian study of a new, pentavalent vaccine compared it to two other vaccines, the DTP-HB+Hib vaccine (separate injections) and another registered pentavalent vaccine. The authors found that 98.32% of subjects in the vaccine trial group had seroprotective anti-PRP-T IgG antibody concentrations (≥0.15 μg/mL) as compared to 100% and 98.94% of subjects in the DTP-HB+Hib and the other registered pentavalent vaccine groups, respectively. Seroprotective levels for anti-HBs (≥10 mIU/mL) were observed in 97.77%, 97.83%, and 98.94% of subjects in the vaccine trial group, DTP-

HB+Hib, and other registered pentavalent vaccine groups, respectively. Comparable immune responses were observed for the other three components (D, T, and P) in all groups.[15]

Compared to all studies noted above, we found that both our groups had similar results, in terms of immune response, except for anti-HBs. In the DTwP-HB-Hib group, the hepatitis B response reached 99.0% seroprotection after three doses of vaccine, with GMCs of 441.57 mIU/mL, compared to a 96.2% seroprotection rate, with GMCs of 213.84 mIU/mL in the DTwP-HB+Hib group. Although the seroprotection rate was not significantly different, the GMCs were (P= 0.001), perhaps because of differing doses. The HBsAg in DTwP-HB was only 5μg/dose (according to Indonesian immunization policy at that time for DTwP-HB vaccine), while the HBsAg in DTwP-HB-Hib was 10μg/dose, according to the international regulation for hepatitis B vaccines.

After the first, second, and third injections, local reactions were seen in 14.9%, 13.2%, and 5.7% of infants at the DTwP-HB-Hib site, 17.3%, 3.7%, 12.0% at the DTwP-HB site, and 8.2%, 8.3%, 10.2% at the Hib site, respectively. Local reactions classified as severe were seen in only two subjects from the DTwP-HB-Hib sites (swelling & induration) and one subject from the Hib site (swelling), after the first injection. Pain at the injection site was the most commonly reported local reaction. Both forms of administration produced comparable and acceptable rates of local reactions. Fever was the most frequent systemic event. In the DTwP-HB-Hib group, fever was reported in 28.0%, 25.3%, and 20.0% of subjects, and in the DTwP-HB+Hib group fever was reported in 25.5%, 17.6%, and 13.9% of subjects after the 1st, 2nd, and 3rd injection, respectively. There were no significant differences in rates of fever between vaccine groups, except after the 3rd injection (P=0.049). Most systemic events were mild in severity at all three doses. Another systemic event, irritability, was very rare, only 1 to 4 subjects in each group. One subject had a complex febrile convulsion, classified as a vaccine reaction in field classification and probable in causality assessment, but it resolved spontaneously.

An Indian study also found pain to be the most frequent local reaction, with 29%, 18%, and 10%, after the 1st, 2n^{d}, and 3rd injections, respectively. Severe local reaction occurred only as pain in 5%, 4%, and 5% of subjects, after the 1st, 2nd and 3rd injections, respectively. Fever was found in 21%, 18%, and 15%, after the 1st, 2nd and 3rd injections, respectively.[12] Rao *et al.* compared a new pentavalent vaccine to the DTP-HB+Hib vaccine (separate injection) and another registered pentavalent vaccine. They also found that pain was the most frequent local reaction in all groups, with 35.54%-36.26% of subjects. Severe local reactions were found in all groups for swelling, with 17.42%-20.21%. Fever was only found to be 5.68%-7.09%. The most frequent systemic event was crying, in 23.34%-25.89%.[15] Compared to other studies, our trial vaccine induced fewer local reactions, but for systemic events, a higher percentage of fever, and fewer other systemic events than other pentavalent vaccines. According to the WHO information sheets for DTP-based vaccines, fever > 38°C and irritability may occur 45-75% of vaccinees, much higher than our findings.[16]

In our study, one child had a complex febrile convulsion, classified as a vaccine reaction. According to WHO information sheets for DTP-based vaccines, febrile seizure may occur in 60 cases out of 100,000 doses. Barlow et al. reported that the risk of febrile seizure may be increased only on the day of the DTP-based immunization, with a relative risk of 5.7.[17] Likewise, Sun et al. reported that the risk of febrile seizure may be increased after DTaP immunization, with relative risk of 6.02 on the first day and decreasing to 3.94 on the second day.[18]

In conclusion, the DTwP-HB-Hib combined vaccine is immunogenic and safe, as well as comparable to the Hib vaccine given simultaneously with the DTwP-HB vaccine.

Conflict of Interest

None declared.

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