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Immunogenicity and safety of a liquid Pentavalent (DTwP-Hb-Hib) combination vaccine manufactured by Human Biologicals Institute in 6–8 weeks old healthy infants: A phase III, randomized, single blind, non-inferiority study

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ABSTRACT

Background: A liquid Pentavalent (DTwP-Hb-Hib) combination vaccine, developed by Human Biologicals Institute, underwent a Phase III clinical study in India. In this randomized, single blind, non-inferiority study, the immunogenicity and safety of this Investigational vaccine was compared with Pentavac SD® vaccine in 6–8 weeks old healthy infants.

Methods: A total of 405 healthy infants aged 6–8 weeks old were randomized in 2:1 ratio to receive three doses of either the Investigational liquid Pentavalent (DTwP-Hb-Hib) combination vaccine or Pentavac SD® vaccine at four to six weeks interval. Immunogenicity was compared by estimation of antibody titers before the first dose and 4–6 weeks after the third dose of vaccination. Safety of each vaccine was assessed and compared by collection of data on solicited and unsolicited adverse events throughout the study period.

Results: Out of a total of 405 enrolled subjects, 387 subjects completed the study. The seroconversion rates, seroprotection rates and geometric mean titres of the Investigational liquid Pentavalent (DTwP-Hb-Hib) combination vaccine group were found to be comparable and non-inferior to the Pentavac SD® vaccine group at 4–6 weeks after the third dose of vaccination. Pain, erythema and swelling at the site of injection were found to be the most common local adverse events whereas fever, irritability and unusual crying were found to be the most common systemic adverse events in both the vaccine groups. No vaccine related serious adverse event was reported. In this study, both the Investigational vaccine as well as the Comparator vaccine were found to be immunogenic and well tolerated.

Abbreviations: AE, adverse event; CI, confidence interval; GMT, geometric mean titer; HBI, Human biologicals institute; LAR, Legally acceptable representative; Lf, Limit of flocculation; PPP, Per protocol population; q.s, quantum satis; SCR, seroconversion rate; SD, standard deviation; NTAGI, National Technical Advisory Group of India; EPI, Extended Program on Immunization; UIP, Universal Immunization Program.

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Conclusion: After assessment of the results of the study it was concluded that the Investigational liquid Pentavalent (DTwP-Hb-Hib) combination vaccine developed by Human Biologicals Institute was immunogenic and safe when administered to infants aged 6–8 weeks and was non-inferior in immunogenicity and safety to Pentavac SD® vaccine.

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1. Introduction

The practice of combining vaccines against more than one disease in a single shot began with combining of individual vaccines diphtheria, tetanus and pertussis into a single DTP vaccine. This type of combined vaccine was first used in infants and children in 1940 [1]. The approach was successful and created interest in formulating more combination vaccines. The combination of multiple antigens into a single vaccine has many benefits for the vaccinee (reduced number of injections, ease of compliance), the parents (increased acceptability), the medical staff (simplified, manageable immunization schedule, time saving, reduced logistics requirement) and the society (direct and indirect delivery logistics, improved disease control) [2–4]. The Scientific Advisory Group of Experts (SAGE) constituted by the World Health Organization's (WHO) global program on vaccination recognized the importance of combination vaccines as the future of immunization. A combination pentavalent vaccine was subsequently formulated by adding Hepatitis B and *Haemophilus influenzae* type b (Hib) antigens to DTP vaccine. Such pentavalent vaccines were proved to be safe and efficacious after clinical studies and extensive use [5]. The National Vaccine Policy (2011) of India advocated the use of Hib containing combination vaccines for Universal Immunization Programme (UIP) [6].

The five diseases targeted by the combination pentavalent vaccine contribute significantly to morbidity and mortality in India. As per WHO records, 6094 cases of diphtheria, 46,706 cases of pertussis and 5017 cases of tetanus were reported in 2014 in India [7]. More than 257 million people in the world were estimated to be persistently infected with Hepatitis B virus (HBV) and 887,220 people died with complications of HBV like Hepatocellular carcinoma, Cirrhosis and Acute hepatitis in 2015 [8]. *Haemophilus influenzae* type b or Hib, was estimated to be responsible for approximately 3 million serious illnesses and 386,000 deaths globally through meningitis and pneumonia [9]. According to a study published in 2009, there were estimated 72,000 deaths due to Hib in India in children less than 5 years of age in the year 2000 [10]. According to Hospital based studies it was observed that 40–50% of all meningitis and 25–30% of all pneumonia among children were contributed by Hib. The overall case-fatality rate for Hib meningitis and pneumonia were 10–30%, and nearly 25–30% of surviving children suffered from major disabilities [11–13]. As per recommendations by National Technical Advisory Group of India (NTAGI) subcommittee and The Indian Academy of Pediatrics (IAP), Hepatitis B (Hep B) and *Haemophilus influenzae* type b (Hib) vaccines were introduced in India's National Immunization Programme (NIP) and offered to all children [14,15]. The use of pentavalent combination vaccine as part of government sponsored routine immunization in India started in 2011 initially in two states and by 2015 all the states were covered [16]. Immunization against diphtheria, tetanus, pertussis, hepatitis B and *Haemophilus influenzae* type b with the administration of pentavalent combination vaccine has been associated with a striking decrease in the incidence of morbidity and mortality from these diseases [17,18].

Whole cell pertussis vaccines are considered to result in better protection than acellular pertussis vaccines as immunity after vaccination with acellular pertussis vaccines has been found to wane faster [19,20]. A liquid formulation of combination vaccine containing diphtheria, tetanus, whole-cell pertussis, hepatitis B and *Haemophilus influenzae* type b antigens (DTwP-Hb-Hib), was developed by Human Biologicals Institute (HBI). A phase III study sponsored by HBI was conducted in India to compare the immunogenicity and safety of this Investigational liquid Pentavalent (DTwP-Hb-Hib) combination vaccine with Pentavac SD® vaccine, manufactured by Serum Institute of India (commercially available in India). The primary objective of the study was to determine the immunogenicity by assessing the humoral immune response of individual diphtheria, tetanus, pertussis, hepatitis B and Hib components after vaccination with Investigational liquid Pentavalent (DTwP-Hb-Hib) combination vaccine developed by HBI (Test Vaccine). Secondary objectives of the study were to determine whether the immunogenicity of Test vaccine was non-inferior to that of Pentavac SD® vaccine (Comparator vaccine) and to compare the adverse events among the vaccine groups during the study period.

2. Methods

2.1. Study design

This was a Phase III, randomized, single blind, multicentric, non-inferiority study to evaluate the immunogenicity and safety of Investigational liquid Pentavalent (DTwP-Hb-Hib) combination vaccine as compared to Pentavac SD® vaccine, which is a commercially available pentavalent vaccine in India. This study was conducted in 405 healthy subjects by independent investigators at 10 Indian cities between January 2016 and October 2016. The study was approved by the Drugs Controller General (India), New Delhi and the respective Institutional Ethics Committees of each site. The study was conducted in accordance with the Declaration of Helsinki, International Conference on Harmonisation - Good Clinical Practice, Schedule Y (of Drugs and Cosmetics Rules, 1945) guidelines of India and all applicable laws and regulations. Written informed consent was obtained from parents or legally acceptable representatives before any study related procedures were performed.

2.2. Study population

The study participants included healthy infants of 6–8 weeks of age and with a history of being born after normal gestational period (36–42 weeks) with a birth weight $\geq$ 2.5 kg and having received birth dose of Hepatitis B vaccine. Main exclusion criteria included recent participation or plan to participate in any clinical trial during the study period; history of immunization with any vaccine other than birth doses of Polio, BCG and Hepatitis B vaccines; planned receipt of any other vaccine within the period from 7 days before to 7 days after any dose of vaccination during the trial period except OPV; history of allergic disease or allergy to any component of the study vaccine; evidence of previous infection with diphtheria, tetanus, pertussis, hepatitis B and *H. influenzae*; the subject being immunocompromised or having received immunotherapy or immunosuppressive therapy; history of fever

in the last three days before the vaccination; past or current receipt of any immunoglobulin, blood or blood-derived product or planned receipt during the trial period; any history or evidence of thrombocytopenia or a bleeding disorder or receipt of anticoagulants. Subjects were also excluded if they met the following criteria while receiving second and third dose of vaccination: Temperature >40 °C (>104 °F) within 48 h of receiving the previous dose unexplained by another cause, collapse or shock like state, persistent or inconsolable crying lasting 3 h or more occurring within 48 h and convulsions with or without fever occurring within three days.

2.3. Blinding and randomization

This was a single blind study and only the subjects were unaware of the treatment group they were assigned to whereas the investigators and study site personnel were aware of the group. Block randomization was used to randomize all the enrolled participants to get either the Test vaccine or the Comparator Pentavac SD® vaccine in a ratio of 2:1. In each study site, the subjects were assigned to the treatment arm in a sequence as per the randomization code provided in a closed envelope by the sponsor. The Investigator/the delegated personnel administered the vaccine as per the randomization code. The adverse events were assessed by the Principal Investigators/Co-Investigators of each site independently. The blood samples were analyzed at the central laboratory in a blinded manner as per the assigned sample number. All the data from the sites and the central laboratory were directly sent to the data management team. All the study assessments were carried out independent of intervention of the sponsor.

2.4. Vaccines and vaccination schedule

Each 0.5 ml dose of the investigational liquid pentavalent (DTwP-Hb-Hib) combination vaccine developed by Human Biologicals Institute contained: diphtheria toxoid $\geq$ 30 IU ($\geq$20 Lf to $\leq$30 Lf), tetanus toxoid$\geq$ 60 IU ($\geq$5 Lf to $\leq$10 Lf), inactivated whole cell Bordetella pertussis $\geq$ 4 IU, rDNA hepatitis B surface antigen $\geq$ 10 μg, Hib polysaccharide covalently bound to tetanus toxoid (PRP-TT) $\geq$ 10 μg, Al^{+++} content (as $AlPO_4$ gel) $\leq$ 1.25 mg, thiomersal (as preservative) $\leq$ 0.01% w/v, normal saline q.s. Batch numbers 15GPEN001 & 15GPEN002 with both having expiry date of August 2017 were used in the study.

Each 0.5 ml dose of the comparator vaccine Pentavac SD® manufactured by Serum Institute of India Limited contained: diphtheria toxoid $\geq$ 20 Lf to $\leq$30 Lf, tetanus toxoid $\geq$ 2.5 Lf to $\leq$10 Lf, B. pertussis (whole cell) $\geq$ 4 IU, HBsAg (rDNA) $\geq$ 10 mcg, purified capsular Hib polysaccharide (PRP) Conjugated to tetanus toxoid (carrier protein) 10 mcg, adsorbed on aluminum phosphate, Al^{+++} - $\leq$ 1.25 mg, thiomersal (as preservative) 0.005%. Batch number 137L5002A with expiry date December 2016 and batch number 137L5033C with expiry date June 2017 were used in the study.

The subjects received three doses of 0.5 ml of either the Investigational Liquid Pentavalent (DTwP-Hb-Hib) combination vaccine or the Pentavac SD® vaccine as per the randomization, at four to six weeks interval between the doses following the 6, 10 and 14 weeks regimen. The vaccine was administered at the antero-lateral aspect of the thigh by intramuscular route.

3. Immunogenicity

Blood samples for the antibody titre estimation were collected before the first dose (pre vaccination titre) and four to six weeks after third dose of vaccination (post vaccination titre). The serum samples (both pre and post vaccination) were analyzed for antibodies by SRL Limited diagnostic laboratory, located at Mumbai, India (Central laboratory). Anti-diphtheria, anti-tetanus and anti-pertussis antibody levels were estimated by IBL EIA kits (manufactured by IBL International GmbH, Hamburg, Germany). Anti PRP antibody titre (Hib vaccine) binding site kit (manufactured by The Binding Site Group Ltd., Edgbaston, Birmingham, UK) was used to detect IgG antibodies to *H. influenzae* type b. The anti-HBs IgG levels were determined by using enzyme-linked immunosorbent assay (ELISA) kit Anti HBS Abs/AUSAB (manufactured by Abbot Ireland, Diagnostics Division, Sligo, Ireland). Geometric mean titre, seronversion rate and seroprotection rate for each component of the vaccine were calculated and compared between the vaccine groups. Seroconversion was defined as any rise in titre post vaccination in comparison with pre vaccination.

4. Safety

After each vaccination, subjects were observed at the study hospital for 30–60 min for any possible adverse events. A diary card was provided to the parents or legally acceptable representatives of the subjects and they were trained to record any adverse events observed during the study period. Solicited local adverse events included local pain, swelling, erythema and induration and solicited systemic adverse events included fever, irritability, anorexia, drowsiness, vomiting and persistent or unusual crying. Both solicited and unsolicited local and systemic adverse events were recorded on the diary card till the next visit. All subjects were followed up for local and systemic adverse events up to four to six weeks after the last dose of vaccination. All the reported adverse events were assessed for their causal relationship with the vaccine and for their seriousness and severity.

5. Statistical analysis

The sample size calculation was based on probability of determination of non-inferiority of the Investigational Liquid Pentavalent (DTwP-Hb-Hib) combination vaccine compared to Pentavac SD® vaccine in terms of the primary efficacy parameter (immunogenicity) to the vaccine antigens. The number of evaluable subjects required was calculated to be 369 (to be in a 2:1 ratio of Test vaccine group to Comparator vaccine group: 246:123). Allowing for a 10% loss to follow-up, the total number of subjects recruited was 405; 270 in Test vaccine group and 135 subjects in the Comparator vaccine group.

All statistical analyses were performed using Statistical Analysis Software SAS® version 9.4 (SAS institute, Cary, NC, USA). For continuous variables, descriptive statistics (minimum, maximum, range, mean and standard deviation) were used and counts with percentages were used for discrete variables. Comparative analysis of immunogenicity was done by comparing the Seroconversion rate, Seroprotection rate and the Geometric mean of antibody titres against each vaccine component between the vaccine groups. Comparisons of the proportion of subjects with seroconversion between the groups were made with the use of 'z' test. Geometric mean titres (GMT) and 95% confidence intervals were calculated for each vaccine component. Within vaccine group, changes between pre and post vaccination GMTs for each component were assessed using paired two-sample Student's *t*-test on log- transformed titres. Differences in the post vaccination GMTs for each component between two vaccine groups were made using an unpaired two-sample Student's *t*-test on log-transformed titres. Immunogenicity analysis was based on the Per Protocol Population (PPP). The two sided 95% confidence interval (CI) of the difference (Test vaccine group vs Comparator vaccine group) in seroconversion rate after 4–6 weeks of administration of third dose of vaccine was computed. The Test vaccine would be concluded to be

non-inferior to the Comparator vaccine if the lower bound of the one sided 95% CI of difference in seroconversion between the groups was $\geq -10\%$.

Safety was analyzed by summarization of reported solicited and unsolicited adverse events by using frequencies and percentages, by event severity and event relationship to the study vaccines during the study duration. All adverse events were listed. Frequency and percentage of subjects with at least one adverse event after each dose of vaccination and during the study period were calculated. The adverse events and their rates were compared between the study vaccine groups. Adverse events were compared between groups using chi-square test. Safety analysis was based on 'Intention to Treat' population (ITT) that included all subjects who were given at least one dose of the vaccine.

6. Results

6.1. Demographics

In all, 405 subjects were enrolled and randomized; 270 subjects into the Test vaccine group and 135 subjects in the comparator vaccine group. Out of the 405 enrolled subjects, 387 subjects completed the study, 257 in Test vaccine group and 130 in the comparator vaccine group. The reason for withdrawal of the 18 subjects were loss to follow up for 15 subjects and consent withdrawal for 3 subjects. No subject in the study was withdrawn due to adverse event (Fig. 1). After analysis, the baseline characteristics were found to be comparable between the vaccine groups (Table 1).

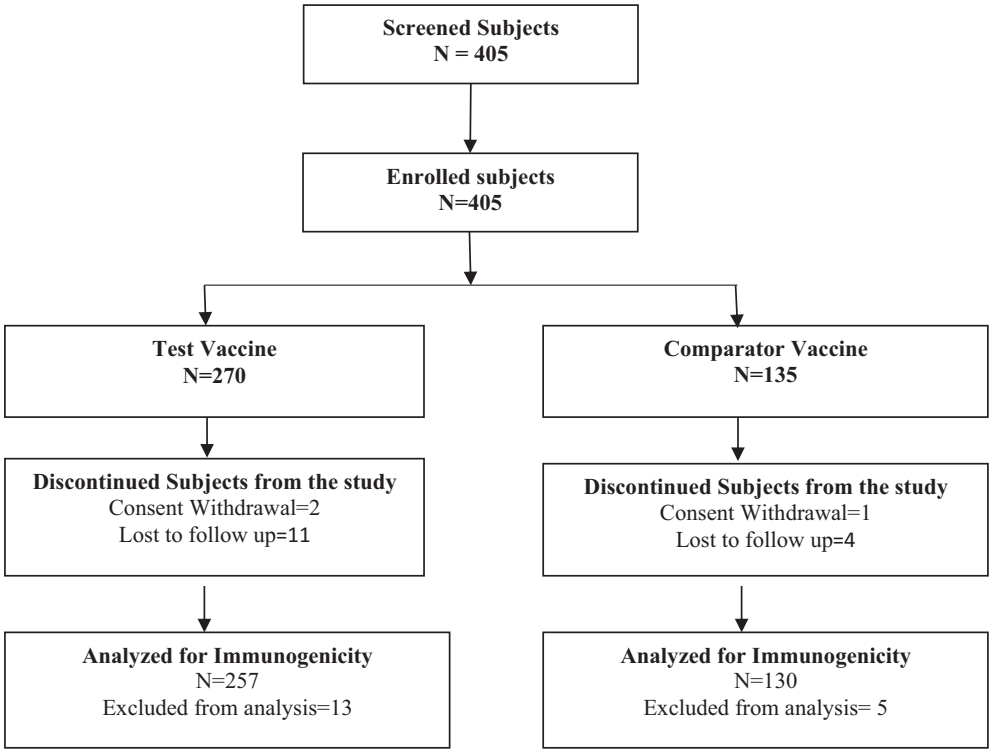

Fig. 1. Disposition of subjects.

Table 1
Demographics and baseline characteristics.

Parameter	Statistical Parameter	Test Vaccine n = 270	Comparator n = 135
Age (in Days)	Mean†	47.84	47.56
	SD	3.53	3.36
Gender	Male, n (%)††	146 (54.07%)	74 (54.81%)
	Female, n (%)††	124 (45.93%)	61 (45.19%)
Height (in cms)	Mean†	54.49	54.66
	SD	3.00	2.57
Weight (in Kgs)	Mean†	4.33	4.37
	SD	0.55	0.57
Head Circumference (cms)	Mean†	36.39	36.42
	SD	2.25	1.97

Abbreviations: SD = Standard deviation.
† Mean for the respective treatment group.
†† Percentage (%) for the respective treatment group.

6.2. Immunogenicity

Immunogenicity of each of the vaccine component were compared individually between the vaccine groups based on the change in the individual antibody titre values from pre vaccination to post vaccination. The seroconversion rates were found to be high for diphtheria, pertussis, hepatitis B and Hib components for both the Test vaccine as well as the Comparator vaccine group (Table 2). The percentage of seroconversion for the Test vaccine group for diphtheria, pertussis, hepatitis B and Hib components were 98.44%, 92.61%, 99.22% and 95.72% respectively. The corresponding values for the Comparator vaccine group were 90.0%, 89.23%, 100% and 90.77% respectively. The seroconversion rate for tetanus component for Test vaccine group was 50.97% where as for the Comparator vaccine group it was 39.23%, though there was good seroprotection, both pre and post vaccination. After analysis of the results post 3 doses of vaccination, the Test vaccine was found to be non-inferior as the lower bound of 95% confidence interval of the difference (for each component) between the vaccine groups was greater than −10%.

In this study, good seroprotection rate was observed for all the components in both the Test vaccine and the Comparator vaccine groups and was comparable between the two groups. There was a significant rise in geometric mean titres post vaccination in comparison to pre-vaccination titres for all the components of the vaccine in both the groups (Table 3). In Test vaccine group, the post

Table 2
Comparison of seroconversion between Vaccine Groups for Diphtheria, Tetanus, Pertussis, Hepatitis B and Hib components.

Components	Statistical Parameter	Test vaccine (n = 257)	Comparator vaccine (n = 130)
Diphtheria	n (%)	253 (98.44)	117 (90.00)
	95% CI	(0.97–1.00)	(0.85–0.95)
	95% CI of difference[¥]	(0.03, 0.14)	
	p-value[*]	0.00012	
Tetanus	n (%)	131 (50.97)	51 (39.23)
	95% CI	(0.45–0.57)	(0.31–048)
	95% CI of difference[¥]	(0.01, 0.22)	
	p-value[*]	0.02852	
Pertussis	n (%)	238 (92.61)	116 (89.23)
	95% CI	(0.89–0.96)	(0.84–0.95)
	95% CI of difference[¥]	(−0.03, 0.10)	
	p-value[*]	0.26272	
Hepatitis B	n (%)	255 (99.22)	130 (100.00)
	95% CI	(0.98–1.00)	(1.00–1.00)
	95% CI of difference[¥]	(−0.02, 0.00)	
	p-value[*]	0.3125	
Hib	n (%)	246 (95.72)	118 (90.77)
	95% CI	(0.93–0.98)	(0.86–0.96)
	95% CI of difference[¥]	(−0.01, 0.11)	
	p-value[*]	0.05118	

[¥] 95% CI of difference between Test vaccine and Comparator vaccine groups.
[*] p-value obtained using z – test for rates (between the treatment group, two tailed, $\alpha = 0.05$).

Table 3
Comparison of Geometric Mean titre levels at pre-dose to post-dose between Vaccine Groups for Diphtheria, Tetanus, Pertussis, Hepatitis B and Hib components.

Component	Statistical Parameter	Test (n = 257)		Comparator (n = 130)	
		Visit1 (Pre-dose)	Visit 4 (Post-dose)	Visit1 (Pre-dose)	Visit 4 (Post-dose)
Diphtheria	Geometric mean (IU/mL)	0.15	0.53	0.23	0.44
	95% CI	(0.13–0.18)	(0.47–0.59)	(0.17–0.30)	(0.37–0.52)
	p-value[*]	–	<0.0001		0.0141
	p-value[**]	–	–	0.0195	0.1472
Tetanus	Geometric mean (IU/mL)	1.06	1.31	1.16	0.77
	95% CI	(0.96–1.18)	(1.17–1.46)	(0.98–1.39)	(0.66–0.91)
	p-value[*]	–	0.0048	–	0.0003
	p-value[**]	–	–	0.2685	<0.0001
Pertussis	Geometric mean (U/mL)	3.69	9.06	4.18	8.56
	95% CI	(3.33–4.08)	(8.09–10.16)	(3.71–4.72)	(7.55–9.71)
	p-value[*]	–	<0.0001	–	<0.0001
	p-value[**]	–	–	0.1690	0.2865
Hepatitis B	Geometric mean (mIU/mL)	25.05	614.65	37.03	251.66
	95% CI	(18.67–33.63)	(492.10–767.72)	(19.14–71.66)	(195.54–323.88)
	p-value[*]	–	<0.0001	–	<0.0001
	p-value[**]	–	–	0.1961	0.1035
Hib	Geometric mean (μg/mL)	0.32	4.79	0.32	1.72
	95% CI	(0.29–0.36)	(4.01–5.72)	(0.28–0.37)	(1.39–2.14)
	p-value[*]	–	<0.0001	–	<0.0001
	p-value[**]	–	–	0.8321	<0.0001

[*] Comparison between visit 4 and visit 1 (within the treatment group). p-value obtained using paired *t* test (two tailed, $\alpha = 0.05$).
[**] Comparison between Test vaccine and Comparator vaccine groups. p-value obtained using unpaired *t* test (two tailed, $\alpha = 0.05$).

vaccination geometric mean titres were 0.53 IU/mL, 1.31 IU/mL, 9.06 U/mL, 614.65 mIU/mL and 4.79 μg/mL for diphtheria, tetanus, pertussis, hepatitis B and Hib components respectively. The corresponding values for the Comparator vaccine group were 0.44 IU/mL, 0.77 IU/mL, 8.56 U/mL, 251.66 mIU/mL and 1.72 μg/mL. There was statistically significant difference between the values of pre and post vaccination titres for each component (for all components: $p < 0.05$).

6.3. Safety

Safety of the vaccines were compared by the percentage of adverse events reported immediately following each dose of vaccination (during the 30–60 min observation period at the study site) and during the follow up period. During the observation period at the study hospital, immediately following vaccination, a total of 108 (13.65%) solicited adverse events were recorded in Test vaccine group which included local pain, local swelling, local erythema, irritability and persistent or unusual crying. A total of 57 (14.21%) solicited adverse events were recorded in Comparator vaccine group which included local pain, local erythema, and persistent or unusual crying. No unsolicited adverse event was observed up to 30 to 60 min after vaccination in either vaccine group. Thus all the adverse events recorded within 30–60 min after vaccination were assessed to be related to the use of vaccines (Table 4).

Among the local adverse events, pain (34.26% in Test vaccine group and 37.66% in Comparator vaccine group) was the commonest adverse event followed by swelling, erythema and induration at the injection site. Fever, irritability and persistent crying were the common systemic events in both groups (Table 5). Other solicited systemic events were anorexia, drowsiness and vomiting. The number and percentage of the events were comparable between the two groups. The unsolicited adverse events like cold, cough, infantile colic, upper respiratory tract infection, acute watery diarrhea, respiratory tract infection, and sub-occipital lymphnode abscess in test vaccine group and abscess in the neck region, constipation, cough and diarrhea in the comparator vaccine group were reported. All the subjects who reported above events recovered with or without symptomatic treatment. The investigators assessed 15 adverse events in the Test vaccine group and 4 in the Comparator vaccine group as unrelated and the rest all as related to the vaccines. Only 1 (0.37%) serious adverse event was reported in this study. It included hospitalization due to 'acute watery diarrhoea' reported 23 days after the 3rd dose of the vaccination with the test vaccine. The subject recovered fully after management with supportive care including Oral Rehydration Solution (ORS) and oral zinc drops. All parameters of routine baseline investigations were found to be clinically insignificant. The subject was discharged on the third day after being symptom free, active and alert. This event was assessed by the investigator as 'unrelated' to the administration of the Test vaccine.

Table 4
Solicited adverse events: up to 30 to 60 min after vaccination with all doses (Safety/ITT Population) - Event count.

Adverse Event	Test vaccine	Comparator vaccine
No. of Solicited AE: n (%)	108 (13.65)	57 (14.21)
No. of doses	791	401
Local Events	**n (%)**	**n (%)**
Local Pain	89 (11.25)	48 (11.97)
Local Swelling	3 (0.38)	0
Local Erythema	13 (1.64)	7 (1.75)
Local Induration	0	0
Systemic Events	**n (%)**	**n (%)**
Fever	0	0
Irritability	1(0.13)	0
Anorexia	0	0
Drowsiness	0	0
Vomiting	0	0
Persistent or unusual crying	2 (0.25)	2 (0.50)

Abbreviations: AE = Adverse event.
Events mentioned are the number of times an event was reported during the study period in the respective treatment group.
Percentage of each event was calculated from the total number of doses administered in the respective treatment group.

Table 5
Adverse events between visits with all doses (Safety/ITT Population) - Event Count.

Reactions	Test	Comparator
No. of AE between the visits	1049	583
No of doses	791	401
Solicited AE between visits: n (%)		
Local Pain	271 (34.26)	151 (37.66)
Swelling	104 (13.15)	60 (14.96)
Erythema	55 (6.95)	31 (7.73)
Induration	49 (6.19)	24 (5.99)
Fever	162 (20.48)	92 (22.94)
Irritability	144 (18.20)	89 (22.19)
Anorexia	53 (6.70)	27 (6.73)
Drowsiness	51 (6.44)	26 (6.48)
Vomiting	14 (1.77)	5 (1.25)
Persistent or unusual crying	129 (16.30)	74 (18.45)
Unsolicited AE between visits: n (%)		
Abscess in the neck region	0	1 (0.25)
Acute watery diarrhea	1 (0.13)	0
Cold	6 (0.76)	0
Constipation	0	1 (0.25)
Cough	4 (0.51)	1 (0.25)
Diarrhoea	0	1 (0.25)
Infantile Colic	2 (0.25)	0
Respiratory Tract Infection	1 (0.13)	0
Suboccipital lymphnode abscess	1 (0.13)	0
Upper respiratory tract infection	2 (0.25)	0

Abbreviations: AE = Adverse event.
Events mentioned are the number of times an event was reported during the study period in the respective treatment group.
Percentage of each event was calculated from the total number of doses administered in the respective treatment group.

7. Discussion

This study explored comparative immunogenicity and safety of Investigational Liquid Pentavalent (DTwP-Hb-Hib) combination vaccine in comparison to the Pentavac SD® vaccine (commercially available in the market) in infants of 6–8 weeks of age. Seroconversion was taken as the parameter for comparison of non-inferiority and the non-inferiority margin was fixed at within −10%. The seroconversion rates for diphtheria, pertussis, hepatitis B and Hib components in both the Test vaccine group as well as the Comparator vaccine group were good and found to be comparable between the two vaccines. But low seroconversion rates were observed for the Tetanus component in both the vaccine groups. A literature search on the subject was conducted and it was found that earlier studies also observed low antibody titre against the tetanus component. In a study published in1992 (on antibody response in infants after one month of vaccination with 3 doses of DTP and Hib conjugate vaccine, concurrently but at separate sites, at the age of 3, 5 and 9 months), Robert Booy et al. reported significantly lower antibody against tetanus toxoid and pertussis antigens in infants in whom pre-immunization (maternally derived) antibody concentrations were high [21]. Adarsh Eregowda et al. reported (in 2013) highly significant rise in antibody titre for all components except tetanus, after one month of vaccination with 3 doses (administered at 6, 10 and 14 weeks of age) of a pentavalent DTwP–HepB–Hib vaccine in Indian infants, which they attributed to presence of maternal anti-

bodies [22]. Christine Jones et al reported (in 2014) an inverse correlation between infant antibody concentration at birth and fold-increase in antibody concentration post-immunization for tetanus [23]. Merryn Voysey et al. observed in their meta-analysis that 2 fold higher maternal antibody (pre-vaccination) was associated with lower post-vaccination antibody against pertussis, tetanus and other antigens [24]. Stefan Niewiesk explained in a review article (in 2014) that such lower responses are due to interference by the maternal IgG [25]. Kusnandi Rusmil et al. in a study in 6–11 weeks old children in Indonesia, reported (in 2015) lower antibody titre against tetanus component after vaccination with 3 doses of a pentavalent DTP-HB-Hib vaccine [26]. Such interference is more likely in countries like India (as reported by other investigators as well) [22], where pregnant women are commonly vaccinated with tetanus toxoid to prevent neonatal tetanus. So, the lower response to tetanus and pertussis component was attributed to interference of the maternal antibodies though information on maternal vaccination status was not collected in this phase III study. The seroconversion rates for all the components of the Investigational Liquid Pentavalent (DTwP-Hb-Hib) combination vaccine were found to be comparable and non-inferior to the Pentavac SD® vaccine.

The study demonstrated good seroprotection rate for all the components in both the vaccine groups. There was a significant rise in Geometric mean titres post-vaccination in comparison to pre-vaccination titres for all the components of the vaccine in both the groups. Overall, the immunogenicity was found to be comparable between the vaccine groups.

The safety of both the vaccines were compared on the basis of the observed adverse events following administration of the vaccine doses. Both the Test vaccine as well as the Comparator vaccine were found to be well tolerated. Among the local adverse events, pain, erythema and swelling at the site of injection were common whereas among the systemic adverse events, fever, irritability and unusual crying were common in both the vaccine groups. Most of the adverse events were of mild or moderate nature and all of them resolved with or without concomitant medications in both the vaccine groups. There was one serious adverse event which was acute watery diarrhoea that happened 23 days after the third dose of vaccination, which the investigator assessed as unrelated to the Test vaccine. Overall, the safety profile was found to be comparable among the vaccine groups.

The safety and immunogenicity results of this study were comparable to the results of previously published studies using pentavalent vaccines in India and other Asian countries (e.g. studies published by Eregowda et al., Rusmil et al., Bavdekar et al., Chatterjee et al. and Dalvi et al.) [22,26,27,28,29].

Strengths of the study include randomization, single blinding, multicentric and non-inferiority design. As the compliance to protocol was good, protocol deviations were minimal, and a very few subjects were withdrawn, the strength of the study is considered to be good.

Limitations: This study was conducted only in one country.

8. Conclusion

It was concluded that the Test vaccine (Investigational Liquid Pentavalent (DTwP-Hb-Hib) combination vaccine) is immunogenic and safe when administered to infants and is non-inferior to the comparator vaccine (Pentavac SD® Vaccine).

Declaration of Competing Interest

The study site investigators (MG, MM, RD, SB, RKD, RKR, RBT, RMD, KMB, SJ) had no financial interest in the vaccine or related to the sponsor in any way. But they received research grants from Human Biologicals Institute (a division of Indian Immunologicals Limited) for conducting the study at their respective sites. SKS, DPS, SG and SM are employees of the sponsor, Human Biologicals Institute (a division of Indian Immunologicals Ltd.) and were involved in the planning, analysis and interpretation of the study.

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