RESEARCH PAPER

Immunogenicity and Safety of a Liquid Hexavalent Vaccine in Indian Infants

JUGESH CHHATWAL, #SANJAY LALWANI AND *EMMANUEL VIDOR

*From Pediatrics Department, CMC and Hospital, Brown Road, Ludhiana, Punjab; #Pediatrics Department, Bharati Vidyapeeth University Medical College, Pune; India; and *Sanofi Pasteur, Lyon, France.*

Correspondence to: Emmanuel Vidor, Sanofi Pasteur, 2 avenue Pont Pasteur, 69367 Lyon, France.

Emmanuel.Vidor@sanofipasteur.com

Received: February 16, 2016; Initial review: March 28, 2016; Accepted: September 03, 2016.

Objective: To evaluate the immunogenicity and safety of a fully liquid, hexavalent diphtheria-tetanus-acellular pertussis–inactivated poliovirus–hepatitis B–*Haemophilus influenzae* type b (DTaP-IPV-HB-PRP~T) vaccine in Indian infants.

Design: Phase III, single-arm study.

Setting: Two tertiary-care hospitals.

Participants: 177 healthy, 6-week-old infants.

Intervention: All participants received hepatitis B vaccine and Oral polio vaccine (OPV) at birth and DTaP-IPV-HB-PRP~T at 6, 10, 14 weeks of age.

Main outcome measures: Serum was analyzed for immune responses to all antigens 1 month post-3rd dose; safety was assessed for 30 minutes post-vaccination, and for 7 days (solicited reactions) and 30 days (unsolicited events).

Results: Seroprotection rates were 100% for anti-HB (≥10 mIU/mL), anti-PRP (≥0.15 µg/mL), anti-T (≥0.01 IU/mL), anti-polio 1, 2, and 3 (≥8 [1/dil]), and 99.3% for diphtheria (≥0.01 IU/mL). For the pertussis antigens, vaccine response rate was 93.8% for anti-PT and 99.3% for anti-FHA. 37.9% and 54.6% of participants experienced at least one solicited injection site and systemic reaction, respectively, and 20.3% of participants experienced at least one unsolicited event (none of which was related to the vaccination). Four serious adverse events (including one death) were reported, but none was related to the vaccination.

Conclusion: The fully liquid DTaP-IPV-HB-PRP~T vaccine is highly immunogenic in infants in India when administered in a 6, 10, 14 week schedule along with HB and OPV administered at birth, and was well tolerated.

Keywords: *Diphtheria-Pertussis-Tetanus vaccine, Haemophilus influenzae, Hepatitis B, Immunization, Poliovirus, Vaccination program.*

WHO Universal Trial Number: U1111-1127-6936.

Published online: November 05, 2016. PII:S097475591600026

The improved safety of acellular pertussis (aP)-combination vaccines compared to whole cell pertussis (wP) vaccines [1], the need to deploy routine *Haemophilus influenzae* type b (Hib) and hepatitis B (HB) vaccination [2,3], and the need for inactivated poliovirus vaccine (IPV) in the global polio eradication strategy [4] are current drivers for a wide expansion of the use of hexavalent combination vaccines. Such vaccines are increasingly pivotal to national immunization programs [5]. A fully liquid hexavalent DTaP-IPV-HB-PRP~T vaccine was developed based on a pentavalent vaccine (Pentaxim/Pentavac) that has a well-documented safety, immunogenicity, and effectiveness profile, based on extensive clinical experience [6]. This hexavalent vaccine incorporates a new HB antigen, has proven immunogenicity and safety [7-10], and was approved by the European Medicines Agency (EMA) *via* the Centralized Procedure and licensed in 104 countries as of August 2016. The 6, 10, 14 weeks-of-age schedule is recommended in many countries. Many similar combination vaccines have been documented using this regimen [11-14], which is used in many national immunization programs (NIPs). The present study was undertaken after the initial licensure of the DTaP-IPV-HB-PRP~T vaccine to evaluate its performance in Indian infants, when administered in the 6, 10, 14 week infant primary series schedule, with HB vaccine and OPV administered at birth as per the Indian NIP recommendations [15].

Accompanying Editorial: Pages 11-13.

METHODS

This was a Phase III, open-label, multi-center study conducted in India. The study was conducted between February 2014 and October 2014 in two hospitals, (Christian Medical College and Hospital, Ludhiana, Punjab; and Bharatiya Vidya Peeth Hospital, Pune). Independent ethics committees of both institutes approved the study protocol. Informed consent was obtained from the parent(s) or legally acceptable representative(s) of each participant. Healthy infants aged

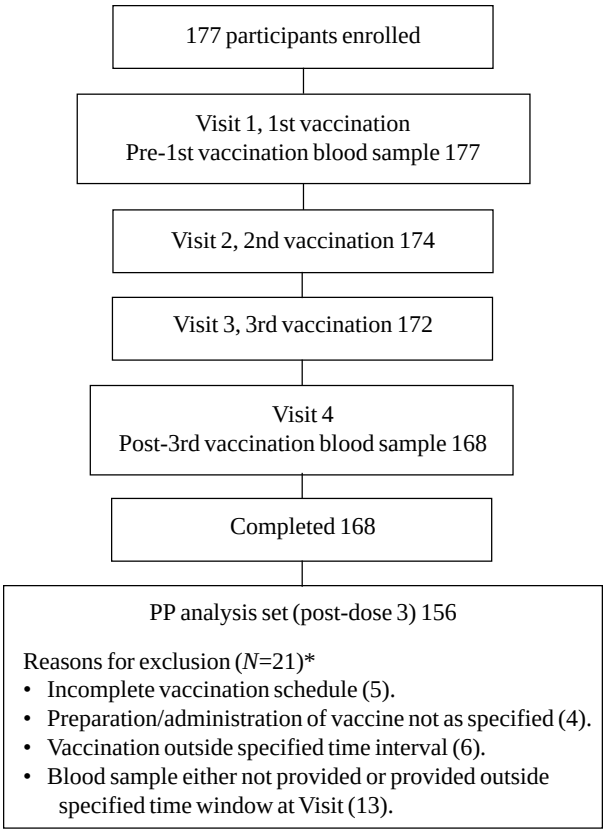
between 42-56 days (6-8 weeks), born at full term (≥37 weeks) to HBsAg seronegative mothers, with birth weight ≥2.5 kg and who had received one dose of HB vaccine and OPV at birth per Indian NIP were eligible for inclusion. The main exclusion criteria were: recent (in the 4 weeks prior to the first vaccination), current, or planned participation in another clinical study or non-study vaccination (except rotavirus) during or in the 4 weeks prior to the study; any prior vaccination against diphtheria, tetanus, pertussis, poliomyelitis, *Haemophilus influenzae* type b (Hib), or any history of these infections; receipt of blood products or immune suppressants for more than two consecutive weeks since birth; personal/maternal history of human immunodeficiency virus; known hypersensitivity to any vaccine component; history of seizures or encephalopathy; bleeding disorder; chronic illness that could interfere with study conduct/completion; or acute illness at enrolment.

The investigational vaccine, DTaP-IPV-HB-PRP~T (batch number S4370) was manufactured by Sanofi Pasteur and was presented as a fully liquid suspension for injection in single dose (0.5 mL) pre-filled syringes and stored at + 2 to + 8°C. Each pre-filled syringe contained ≥20 IU (30 limit of flocculation [Lf]) D-toxoid; ≥40 IU (10 Lf) T-toxoid; 25 µg PT; 25 µg FHA; 40, 8 and 32 D antigen units of IPV type 1, 2 and 3, respectively; 10 µg HBsAg; 12 µg Hib polysaccharide conjugated to 22-36 µg tetanus protein (PRP~T); and 0.6 mg aluminum hydroxide.

Enrolled participants received DTaP-IPV-HB-PRP~T at 6, 10 and 14 weeks of age, administered into the right thigh. Blood samples were taken prior to the first vaccination (Day 0) and 1 month after the third vaccination (Day 90, approximately 18 weeks of age). Anti-HB, anti-diphtheria, anti-PT, and anti-FHA antibodies were determined using the Day 0 samples, and all antibodies were determined using the Day 90 samples. Assays were performed at a centralized laboratory under the responsibility of the Sponsor's Global Clinical Immunology laboratory (Swiftwater, PA, USA). Anti-diphtheria (IU/mL) antibody concentrations and anti-polio 1, 2, 3 (1/dil) antibody titers were assayed by neutralization assay, anti-tetanus (IU/mL), anti-PT (EU/mL) and anti-FHA (EU/mL) concentrations by enzyme linked immunosorbent assays (ELISA), anti-HBsAg concentrations (mIU/mL) by a commercially available chemiluminescence assay (VITROS ECi/ECiQ), and anti-PRP-T concentrations (µg/mL) by radio-immunoassay.

Immediate adverse reactions (ARs) were monitored for 30 minutes after each vaccination (the term AR being used to define an adverse event [AE] that was considered to be related to the vaccination, with all immediate AEs being classified as ARs). For 7 days after each vaccination, parent(s)/legal representative(s) used diary cards to record the duration and intensity of pre-defined (solicited) injection site (tenderness, redness, and swelling) and systemic (temperature, vomiting, crying abnormal, drowsiness, appetite lost, irritability) reactions (also considered by definition to be related to the vaccination). For temperature measurement, the preferred route was axillary, and parent(s)/legal guardian(s) were to record the route used and the classification for intensity was made at the time of the statistical analysis. Unsolicited AEs were recorded using diary cards for 30 days after each vaccination: unsolicited injection site AEs were automatically considered to be related to the vaccination but for each unsolicited systemic AE, the investigators assessed the relationship to the vaccination. Serious adverse events (SAEs) were collected throughout the study and until 1 month after the last vaccination, and the investigators assessed their relationship to the vaccination.

Statistical analysis: All analyses were descriptive. The 95% confidence intervals (CIs) were calculated using the exact binomial method (Clopper-Pearson method) for single proportions and using the normal approximation of the $\log_{10}$ titers, followed by a back transformation for geometric mean concentrations (GMCs) and geometric mean titers (GMTs). Immunogenicity criteria were described for all valid serological results from all available sera obtained before the first dose and 1 month after the third dose. The antibody thresholds and criteria used to compute seroprotective (SP) and vaccine response (VR) rates for the various antigens are presented in ***Table* I** and ***Table* II.** Geometric mean concentrations (GMCs) (anti-D, anti-T, anti-PT, anti-FHA, anti-HB and anti-PRP), geometric mean titers (GMTs) (anti-polio 1, 2, 3), and GMC and GMT ratios (post-dose 3/pre-dose 1) were also calculated. Assuming seroprotection/seroconversion rates of ≥94% for any given vaccine antigen, a sample size of 150 evaluable participants ensured 95% CI limits within a range less than 8.3 percentage points for all antibody responses. For safety, this sample size allowed, with 95% probability, the observation of any given AE occurring with a true frequency of 2% or more, using the rule of three. Assuming an attrition rate of approximately 15%, 177 participants were to be included in the study. Data from the per protocol (PP) population (participants with no protocol violation that could have interfered with the primary evaluation criteria) are presented for all



** Some participants had more than one reason for exclusion.*

FIG. 1 *Flow of study participants.*

immunogenicity assessments; the evaluation of safety was done using the full analysis set (FAS) (participants who received at least one vaccination). All statistical analyses were done using SAS software, at least Version 9.2 (SAS Institute, Cary, NC, USA).

RESULTS

A total of 177 participants were enrolled and received at least one vaccination. Of these, 168 participants completed the study and 156 participants were included in the PP analysis set. The participant flow and disposition is presented in ***Fig.* 1**.

***Table* I** presents the post-dose 3 SP and VR rates for the investigational vaccine. ***Table* II** presents the post-dose 3 immunogenicity data for all thresholds assessed, GMCs and GMTs post-dose 3, and geometric mean ratios, where applicable.

No immediate AR (in the 30 minutes after vaccination) was reported after any vaccination. The frequency of solicited injection site and systemic reactions is summarized in ***Table* III**. A total of 60 unsolicited AEs were reported by 20.3% of participants.

TABLE I SEROPROTECTION RATES AND VACCINE RESPONSE RATES 1 MONTH POST-DOSE 3

Antibody	*Threshold*	*% participants (95% CI)[#] (N=156)*
Anti-HB	≥10 mIU/mL	100.0 (97.6; 100)
Anti-PRP	≥0.15 µg/mL	100.0 (97.7; 100)
Anti-D	>0.01 IU/mL	99.3 (95.9; 100)
Anti-T	>0.01 IU/mL	100.0 (97.3; 100)
Anti-polio type 1	≥8 (1/dil)	100.0 (97.5; 100)
Anti-polio type 2	≥8 (1/dil)	100.0 (97.5; 100)
Anti-polio type 3	≥8 (1/dil)	100.0 (97.5; 100)
Anti-PT	VR*	93.8 (88.6; 97.1)
Anti-FHA	VR*	99.3 (96.3; 100)

*[#]Data are seroprotection rate for anti-HB, anti-PRP, anti-D, anti-T, anti-polio 1, 2, 3, and vaccine response rate for anti-PT and anti-FHA; *%participants with post-dose 3 concentration ≥4 ×LLOQ of the assay (2 IU/mL) if pre-vaccination concentration was <4 × LLOQ or with post-dose 3 concentration ≥pre-vaccination concentrations if pre-vaccination concentrations ≥4 × LLOQ.*

The most frequently reported unsolicited AEs were upper respiratory tract infection (24 AEs reported by 11.9% of participants); no other unsolicited AE was reported by >10% of participants. Four SAEs were reported in three participants (1.7%): none was considered to be related to the vaccination. These included the death of a girl 27 days after the second vaccination: no autopsy was performed, and this participant had experienced 3-4 days of diarrhea with no fever or vomiting prior to death. This death was assessed as not related to the vaccination. The other SAEs were: an episode of severe sepsis following the first dose with hypovolemic shock accompanied with viral lower respiratory tract infection in the same girl who died after the second dose, an episode of bronchopneumonia, and one episode of infantile epilepsy.

DISCUSSION

Routine vaccination against diphtheria, tetanus, pertussis, poliomyelitis and HB diseases using monovalent (standalone) or combined vaccines at 6, 10 and 14 weeks of age, followed by a booster dose at 15-18 months of age, is recommended in India. It is also recommended to administer a first dose of HB vaccine and OPV at birth and to administer three subsequent doses of any HB-containing vaccine at 6, 10 and 14 weeks of age, possibly concomitantly with DTP vaccinations [15]. Participants in this infant primary series study therefore received a total of four HB doses, *i.e.* at birth and 6, 10, 14 weeks of age, and three doses of DTaP-IPV-HB~PRP-T (at 6, 10, and 14 weeks of age). We documented high immunogenicity of this hexavalent vaccine (DTaP-IPV-

TABLE II IMMUNOGENICITY RESULTS PRE-DOSE 1 AND POST-DOSE 3 (N=156)

Antibody	*Criteria*	*% participants (95% CI)[#](N=156)*	
		Day 0	Day 90
Anti-HB	≥10 mIU/mL	13.2 (8.23;19.6)	100 (97.6;100)
	GMC (mIU/mL)	3.78 (3.23;4.43)	2491 (2073;2995)
Anti-PRP	≥0.15 µg/mL	ND	100 (97.7;100)
	≥1.0 µg/mL	ND	93.6 (88.5;96.9)
	GMC (µg/mL)	ND	7.86 (6.35; 9.73)
Anti-D	≥0.01 IU/mL	67.1 (59.0;74.5)	99.3 (95.9;100)
	≥0.1 IU/mL	15.8 (10.4;22.6)	49.6 (40.9;58.4)
	GMC (IU/mL)	0.019 (0.015;0.025)	0.120 (0.099;0.146)
Anti-T	≥0.01 IU/mL	ND	100.0 (97.3;100)
	GMC (IU/mL)	ND	1.95 (1.75; 2.17)
Anti-polio type 1	≥1:8 (1/dil)	ND	100 (97.5;100)
	GMT ([1/dil))	ND	1124 (861;1468)
Anti-polio type 2	≥1:8 (1/dil)	ND	100 (97.5;100)
	GMT ([1/dil))	ND	1401 (1108;1771)
Anti-polio type 3	≥1:8 (1/dil)	ND	100 (97.5;100)
	GMT ([1/dil))	ND	2019 (1672;2437)
Anti-PT	GMC (EU/mL)	3.84 (3.00;4.91)	191 (173;210)
	VR*		93.8 (88.6;97.1)
Anti-FHA	GMC (EU/mL)	6.17 (5.10;7.48)	226 (208;247)
	VR*		99.3 (96.3;100)

*[#]Except for GMCs and GMTs; *% participants with post-dose 3 concentration ≥4 x LLOQ (2 IU/mL) if pre-vaccination concentration was <4 × LLOQ or with post-dose 3 concentration ≥pre-vaccination concentrations if pre-vaccination concentrations ≥4 x LLOQ; ND=not determined.*

HB-PRP~T) in Indian infants. The 4-dose HB administration regimen, with 1 dose given at birth (leading to a '1+3' regimen) was no more reactogenic than the 3-dose administration schedules ('0+2+1' or '0+3+1') that have previously been assessed in numerous studies conducted with DTaP-IPV-HB-PRP~T combined vaccines [13,16-19]. Overall, the DTaP-IPV-HB-PRP~T vaccine was well tolerated and there was no safety concern. Although one death occurred 27 days after the second vaccination, this was not considered to be related to the vaccination.

The interpretation of anti-PT and anti-FHA responses in this study is constrained by the lack of serological correlates of protection for aP vaccines and by the existence of several additional factors that drive the long-term effectiveness of these vaccines. A further limitation of the study is that, although a last dose of HB after 24 weeks of age is recommended by the Indian Academy of Pediatrics, no HB dose was administered after 14 weeks of age in the present study. However, previous studies with the same vaccine have shown that the administration of HB at birth followed by three consecutive administrations of the DTaP-IPV-HB-PRP~T vaccine, followed then by an additional administration of an HB vaccine (in the form of the DTaP-IPV-HB-PRP~T vaccine) is extremely immunogenic [20-22]. No control vaccine was used in this study as this was not required for a licensing study in India, and since numerous randomized controlled clinical studies had already been performed outside India during the clinical development of the study vaccine.

These safety and immunogenicity results are consistent with those from previous studies using the same vaccine and conducted outside India in a range of primary series schedules [17,18,20,21,23,24], especially when administered in the same primary series schedule [18]. In particular, the incidence of solicited injection site (37.9%) and systemic (54.6%) reactions was lower than that in an earlier study in which the DTaP-IPV-HB-PRP~T vaccine was administered in the same 6, 10, 14 week schedule with HB being given at birth (incidences of 92.6% and 94.1%, respectively) [18].

In conclusion, this study demonstrated the

WHAT IS ALREADY KNOWN?

- The hexavalent (DTaP-IPV-HB-PRP~T) vaccine has been shown to be well tolerated and immunogenic in many countries.

WHAT THIS STUDY ADDS?

- Primary vaccination with DTaP-IPV-HB-PRP~T vaccine is immunogenic and safe when used in the EPI schedule (6, 10, 14 weeks) in Indian infants.

TABLE III PARTICIPANTS EXPERIENCING SOLICITED INJECTION SITE AND SYSTEMIC ADVERSE REACTIONS OCCURRING IN THE 7 DAYS AFTER ANY DOSE

	*Grade**	*% participants (95% CI)*	
*Any injection site reaction***	Any	37.9	(30.7; 45.6)
Pain/Tenderness	Any	30.5	(23.7; 37.9)
Erythema	Any	7.5	(4.0; 12.4)
Swelling	Any	14.9	(10.0; 21.1)
Any systemic reaction	Any	54.6	(46.9; 62.1)
	Grade 3	2.3	(0.6;5.8)
Fever	Any	19.0	(13.4; 25.6)
	Grade 3	0	(0; 0.2)
Vomiting	Any	14.9	(10.0; 21.1)
	Grade 3	0	(0; 0.2)
Crying abnormal	Any	24.1	(18.0; 31.2)
	Grade 3	1.1	(0.1; 4.1)
Drowsiness	Any	13.2	(8.6; 19.2)
	Grade 3	1.1	(0.1; 4.1)
Appetite lost	Any	10.9	(6.7; 16.5)
	Grade 3	0	(0; 0.2)
Irritability	Any	36.2	(29.1; 43.8)
	Grade 3	0.6	(0; 3.2)

**Grade 3 fever was defined as temperature >39.5°C. Other Grade 3 systemic symptoms were defined as: vomiting (≥6 episodes/day or requiring parenteral hydration), crying abnormal (>3 hours), drowsiness, (sleepy most of the time or difficult to wake up), appetite lost (missed ≥3 meals) and irritability (inconsolable); **Grade 3 data were not calculated for injection site reactions.*

immunogenicity of DTaP-IPV-HB-PRP~T vaccine for use in the 6, 10, 14 week, 3-dose primary series in Indian infants who received standalone HB vaccine and OPV at birth per the Indian NIP recommendations. As such, this vaccine can be considered as an efficacious combined vaccine that can be used for primary immunization in Indian infants.

Acknowledgement: This manuscript was formatted with the assistance of a professional medical writer, Dr Andrew Lane (Lane Medical Writing, France), who provided assistance in its finalization in accordance with the European Medical Writers Association guidelines and Good Publication Practice.

Contributors: JC and L were Principal investigators for the study, and were responsible for its clinical conduct, data acquisition and data interpretation; EV acted as the Sponsor's clinical lead and was responsible for study design, data interpretation, and manuscript writing. All authors reviewed and approved this manuscript.

Funding: Sanofi Pasteur, Lyon, France.

Competing interests: No clinical investigator involved in this study received any direct payment from Sanofi Pasteur in this regard, but received expenses for conference attendance for the presentation of data from this study. EV is an employee of Sanofi Pasteur.

REFERENCES

1. WHO. Pertussis vaccines: WHO position paper - September 2015. Wkly Epidemiol Rec. 2015;90:433-58.
2. WHO. Hepatitis B vaccines. Wkly Epidemiol Rec. 2009;84:405-19.
3. WHO. Haemophilus influenzae type b (Hib) Vaccination Position Paper - July 2013. Wkly Epidemiol Rec. 2013;88:413-26.
4. WHO. Polio vaccines: WHO position paper, January 2014. Wkly Epidemiol Rec. 2014;89:73-92.
5. Decker MD, Edwards KM., Bogaerts, HH. Combination vaccines. *In*: Plotkin SA, Orenstein, W.A, Offit, P.A, editor. Vaccines. 6th ed. PA, USA: Saunders Co.; 2013. p. 981-1007.
6. Plotkin SA, Liese J, Madhi SA, Ortiz E. A DTaP-IPV// PRP~T vaccine (Pentaxim): a review of 16 years' clinical experience. Expert Rev Vaccines. 2011;10:981-1005.
7. Tregnaghi MW, Voelker R, Santos-Lima E, Zambrano B. Immunogenicity and safety of a novel yeast Hansenula polymorpha-derived recombinant hepatitis B candidate vaccine in healthy adolescents and adults aged 10-45 years. Vaccine. 2010;28:3595-601.
8. Lyseng-Williamson KM, PL. DTaP-IPV-Hep B-Hib vaccine (Hexyon®/Haxacima®): A guide to its use in the primary and booster vaccination of infants and toddlers in Europe. Drug Ther Perspect. 2013;29:329-35.
9. McCormack PL. DTaP-IPV-Hep B-Hib vaccine (Hexaxim®) : A review of its use in primary and booster vaccination. Paediatr Drugs. 2013;15:59-70.
10. Santos-Lima E, B'Chir S, Lane A. Combined immunogenicity data for a new DTaP-IPV-Hep B-PRP-T vaccine (Hexaxim) following primary series administration at 2, 4, 6 months of age in Latin America. Vaccine. 2013;31:1255-8.
11. Capeding MR, Cadorna-Carlos J, Book-Montellano M,

Ortiz E. Immunogenicity and safety of a DTaP-IPV//PRP approximately T combination vaccine given with hepatitis B vaccine: A randomized open-label trial. Bull World Health Organ. 2008;86:443-51.
12. Dutta AK, Verghese VP, Pemde HK, Mathew LG, Ortiz E. Immunogenicity and safety of a pentavalent diphtheria, tetanus, acellular pertussis, inactivated poliovirus, Haemophilus influenzae type B conjugate combination vaccine (Pentaxim) with hepatitis B vaccine. Indian Pediatr. 2009;46:975-82.
13. Gylca R, Gylca V, Benes O, Melnic A, Chicu V, Weisbecker C, *et al*. A new DTPa-HBV-IPV vaccine co-administered with Hib, compared to a commercially available DTPw-IPV/Hib vaccine co-administered with HBV, given at 6, 10 and 14 weeks following HBV at birth. Vaccine. 2000;19:825-33.
14. Madhi SA, Cutland C, Jones S, Groome M, Ortiz E. Immunogenicity and safety of an acellular pertussis, diphtheria, tetanus, inactivated poliovirus, Hib-conjugate combined vaccine (Pentaxim) and monovalent hepatitis B vaccine at 6, 10 and 14 weeks of age in infants in South Africa. S Afr Med J. 2011;101:126-31.
15. Indian Academy of Pediatrics. IAP recommended immuni-zation schedule 2013 for children aged 0-18 years (with range) 2013. Available from: *http://www.iapindia.org/files/IMM%20Schedule.pdf*. Accessed December 05, 2016.
16. del Canho R, Grosheide PM, Voogd M, Huisman WM, Heijtink RA, Schalm SW. Immunogenicity of 20 micrograms of recombinant DNA hepatitis B vaccine in healthy neonates: a comparison of three different vaccination schemes. J Med Virol. 1993;41:30-4.
17. Kosalaraksa P, Thisyakorn U, Benjaponpitak S, Chokephaibulkit K, Santos-Lima E. Immunogenicity and safety study of a new DTaP-IPV-Hep B-PRP-T combined vaccine compared to a licensed DTaP-IPV-Hep B//PRP-T comparator, both concomitantly administered with a 7-valent pneumococcal conjugate vaccine at 2, 4, and 6 months of age in Thai infants. Int J Infect Dis. 2011;15:e249-56.
18. Madhi SA, Mitha I, Cutland C, Groome M, Santos-Lima E. Immunogenicity and safety of an investigational fully liquid hexavalent combination vaccine versus licensed combination vaccines at 6, 10, and 14 weeks of age in healthy South African infants. Pediatr Infect Dis J. 2011;30:e68-74.
19. Pichichero ME, Blatter MM, Reisinger KS, Harrison CJ, Johnson CE, Steinhoff MC, *et al*. Impact of a birth dose of hepatitis B vaccine on the reactogenicity and immunogenicity of diphtheria-tetanus-acellular pertussis-hepatitis B-inactivated poliovirus-Haemophilus influenzae type b combination vaccination. Pediatr Infect Dis J. 2002;21:854-9.
20. Aquino AG, Brito MG, Doniz CE, Herrera JF, Macias M, Zambrano B, *et al.* A fully liquid DTaP-IPV-Hep B-PRP-T hexavalent vaccine for primary and booster vaccination of healthy Mexican children. Vaccine. 2012;30:6492-500.
21. Ceyhan M and Santos-Lima E. Immunogenicity and safety of an investigational hexavalent fully liquid DTaP-IPV-Hep B-PRP-T vaccine given at 2, 3, 4 months of age with a booster at 15-18 months compared to licensed vaccines in Turkish infants. 5th Asian Congress of Pediatric Infectious Diseases; 23-26 September 2010, Tapiei, Taiwan [Abstract].
22. Madhi SA, Koen A, Cutland C, Groome M, Santos-Lima E. Antibody persistence and booster vaccination of a fully liquid hexavalent vaccine coadministered with measles/mumps/rubella and varicella vaccines at 15-18 months of age in healthy South African infants. Pediatr Infect Dis J. 2013;32:889-97.
23. Macias M, Lanata CF, Zambrano B, Gil AI, Amemiya I, Mispireta M, *et al.* Safety and immunogenicity of an investigational fully liquid hexavalent DTaP-IPV-Hep B-PRP-T vaccine at two, four and six months of age compared with licensed vaccines in Latin America. Pediatr Infect Dis J. 2012;31:e126-32.
24. Tregnaghi MW, Zambrano B, Santos-Lima E. Immunogenicity and safety of an investigational hexavalent diphtheria - tetanus - acellular pertussis - inactivated polio-virus-hepatitis B-Haemophilus influenzae B conjugate combined vaccine in healthy 2-, 4-, and 6-month-old Argentinean infants. Pediatr Infect Dis J. 2011;30:e88-96.