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Brief report

Antibody persistence of two pentavalent DTwP–HB–Hib vaccines to the age of 15–18 months, and response to the booster dose of quadrivalent DTwP–Hib vaccine

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ABSTRACT

Objectives: Antibody persistence in children following three doses of primary vaccination with diphtheria, tetanus, whole-cell-pertussis (DTwP), hepatitis B, and *Haemophilus influenzae* type b (Hib) vaccines (SIIL Pentavac vaccine *vs.* Easyfive® of Panacea Biotec), and response to the booster dose of DTwP–Hib (Quadrovax®) vaccine.
Methods: Children who completed their primary immunization were assessed for antibodies at 15–18 months of age, and then given a booster dose of DTwP–Hib vaccine. Reactogenicity and safety of the booster dose was evaluated.
Results: Both pentavalent vaccines demonstrated a good immune response at 15–18 months. Following the booster dose, all vaccinated subjects achieved protective titers against diphtheria, tetanus and Hib, whereas the response to pertussis antigen was ~78%. Fever and irritability was noted in 24%, local pain in 51%, and swelling in 36% of the children following booster dose.
Conclusions: Primary immunization with either pentavalent vaccine induced an excellent immunity lasting till the second year of life. A booster dose with DTwP–Hib (Quadrovax®) vaccine effectuated a good anamnestic response to all vaccine components, being specially strong for Hib in children previously vaccinated with SIIL liquid pentavalent vaccine (Pentavac®). Also, the safety profile of SIIL quadrivalent vaccine (Quadrovax®) administered as booster dose was acceptable.

1. Introduction

In addition to the traditional trivalent diphtheria–tetanus and pertussis (DTwP) vaccine, the World Health Organization (WHO) recommends incorporation of newer vaccines in the Expanded Programme of Immunization (EPI) program. Among those are vaccines against hepatitis B (HBV) and *Haemophilus influenzae* type b (Hib) diseases [1,2]. The Hib component is already included in several combination vaccines [3–8], and has led to a substantial reduction in the incidence of Hib infections [9–11]. Serum Institute of India Ltd. (SIIL), Pune, India, has manufactured Hib conjugate through joint development and technology transfer from the Netherlands Vaccine Institute (NVI), The Netherlands.

In most industrialized countries, a Hib-containing booster dose is recommended at 12–18 months of age; whereas in developing countries, the need for and the timing of booster have not yet been clearly defined [1]. WHO and Indian Academy of Pediatrics (IAP) recommend a booster dose of DTP and Hib vaccines at 15–18 months of age.

This study was planned to assess the long-term persistence of antibodies in children who had received liquid pentavalent vaccine manufactured either by SIIL or Panacea Biotec Ltd., India, during their primary immunization series. In addition, safety and immunogenicity of a booster dose of quadrivalent DTwP–Hib vaccine (SIIL) was also investigated.

2. Subjects and methods

2.1. Study design

The aim of this post-licensure, open-label study was to assess the long-term persistence of the diphtheria, tetanus, pertussis,

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HBV, and Hib antibodies in children at 15–18 months of age who had completed their three-dose primary immunization schedule at 6–10–14 weeks of age, with one of the pentavalent vaccines [12]. In addition, we studied the performance of the quadrivalent DTwP–Hib booster. The study was approved by the Ethical Committees of the participating institutions; Principles of Declaration of Helsinki were followed and the study was conducted adhering to Good Clinical Practice guidelines.

2.2. Vaccinees, vaccines and vaccination

In all, 229 children were enrolled in the study. Children who suffered from any disease, or had been immunized with any vaccine, except oral polio (OPV) or the measles–mumps–rubella (MMR) vaccine were excluded.

The composition and description of the vaccines administered during the primary immunization vaccination is given in an earlier publication [12]. DTwP–Hib vaccine of SIIL (Quadrovax®) was given to all vaccines as a booster dose. Each dose of the booster vaccine (batch number: 120S9005; date of manufacture: June 2009; date of expiry: May 2011) contained diphtheria toxoid ≥20 Lf to ≤30 Lf, tetanus toxoid ≥5 Lf to ≤25 Lf, *Bordetella pertussis* (whole cell) ≥4 IU, purified capsular Hib polysaccharide (PRP) conjugated equivalent to 10 μg, tetanus toxoid (career protein) 19–33 μg, ≤0.01% thiomersal as preservative, and the Al^{3+} content as aluminum phosphate equivalent to ≤1.25 mg.

The vaccine was injected in a dose of 0.5 ml intramuscularly, in the antero-lateral aspect of the upper thigh using a 24 G′ needle.

2.3. Follow up

For any immediate adverse events following the booster, each child was closely observed for 30 min. Solicited adverse events within 24–48 h included local (pain, redness, swelling and nodule at the injection site) and systemic reactions (fever, persistent crying, seizure, drowsiness, irritability, loss of appetite, vomiting and diarrhea). All potential delayed events were recorded on diary cards by the parents of the subject. This observation period was for 1 month, postvaccination. During follow up visit, post 1 month after the booster vaccination, the data from the diary cards was transcribed on the case record forms (CRF).

2.4. Serology

Two venous blood samples were obtained from each child, one pre-booster, and the other at 1 month post-booster. Sera was separated and the coded samples were stored at −20 °C. IgG antibody levels of the vaccine components, were measured using ELISA kits (EN ISO 9001:2000 certified and licensed by the National Regulatory Authority, India).

The cut-off value for seroprotection against diphtheria and tetanus was ≥0.1 IU/ml [13,14]. A lower antibody concentration (≥0.01 IU/ml) was also used. For Hib, two concentrations, ≥0.15 μg/ml (suggesting short-term protection) and ≥1.0 μg/ml (long-term protection) were used [15]. For HBV, the cut-off level suggesting protection from disease was any antibody titer of ≥10 mIU/ml measured by ELISA [2]. Pertussis serology is problematic. The Pertussis organism comprises a mixture of crude pertussis antigens including LPS. Even though whole cell pertussis vaccine has been used successfully for several decades, there still is no reliable measure of immunity to pertussis. Although for pertussis, no serological correlate of protection is recognized, an IgG level >22 U/ml was suggested by the kit's instructions and literature [16,17]. The same was used in this study.

All serological testing was carried out at Manipal Acunova Ltd., a College of American Pathologists (CAP) certified and National Accreditation Board of Laboratories (NABL) accredited laboratory at Bangalore, Karnataka, India. The laboratory personnel were masked on the identity of the source of coded samples.

Table 1
Adverse events profile.

Adverse event	Total (n = 229)	% of AE	95% CI
Pain[a]	**116**	**50.6**	**42.0, 60.5**
Grade 1	22	9.6	6.44, 14.12
Grade 2	84	36.6	30.70, 43.10
Grade 3	10	4.3	2.41, 7.85
Redness (cm)	**35**	**15.2**	**10.8, 21.0**
Up to 2.5	25	10.9	7.51, 15.62
2.5–5.0	10	4.3	2.41, 7.85
Swelling (cm)	**75**	**32.7**	**26.0, 40.8**
Up to 2.5	51	22.2	17.37, 28.10
2.5–5.0	21	9.1	6.09, 13.61
>5.0	3	1.3	0.47, 3.76
Nodule (cm)	**2**	**0.8**	**0.15, 2.89**
<0.5	1	0.4	0.10, 2.39
0.5–<1.0	1	0.4	0.10, 2.39
Fever (°C)	**68**	**29.6**	**23.2, 37.4**
<38.0	19	8.3	5.39, 12.59
38.0–38.4	28	12.2	8.60, 17.11
38.5–38.9	12	5.2	3.04, 8.93
39.0–39.4	9	3.9	2.10, 7.29
Irritability	**54**	**23.5**	**17.9, 30.5**
Drowsiness	**2**	**0.8**	**0.15, 2.89**
Loss of appetite	**14**	**6.1**	**3.5, 10.0**
Vomiting	**5**	**2.1**	**0.80, 4.84**
Diarrhea	**3**	**1.3**	**0.33, 3.57**

[a] Grade 1: present, but leg movement not affected; grade 2: discomfort, interferes with or limits leg movement; and grade 3: disabling, unable to move leg.

2.5. Statistical analysis

The vaccine reactogenicity and immunogenicity were assessed on the basis of intention-to-treat (ITT) and per-protocol (PP) analyses. Immunogenicity analysis was carried out in terms of response to each vaccine component measured at pre- and post-booster. Antibody seroprotection/response rates against DPT, HBV, and Hib were calculated. Geometric mean concentration (GMC), and 95% confidence intervals were calculated for each component. For individual vaccine groups, changes between pre- and post-vaccination GMCs were assessed using paired t-test. Changes in the post-vaccination GMCs between the two groups were compared using unpaired t-test. Adverse events were analyzed by chi-square or Fisher's exact test.

3. Results

Out of 462 children who completed the primary vaccination study, only 304 were available for the long term antibody persistence assessment as most of them had shifted their residence or had already taken a booster dose. 200 children were found to be adequate to demonstrate non-inferiority between the two vaccine groups. 229 children (121 and 108 children had previously been vaccinated with SIIL Pentavac® or Easyfive®, respectively), were enrolled in this study. This sample was obtained from two of the three sites participated in the primary study [12]. Both these sites had an independent principal investigator (PI) conducting the study. The PIs were responsible for assessing the causality of the adverse events. As five children were dropped out from the 1 month follow up following booster dose, 224 children (118/SIIL Pentavac®, 106/Easyfive®) were analyzed for immunogenicity. In SIIL Pentavac® group, the mean age was 18.7 months and mean weight 9.4 kg compared with 18.2 months and 9.5 kg respectively, in Easyfive® vaccine group. No difference between the two groups