

EVALUATION OF IMMUNOGENICITY AND TOLERANCE OF SINGLE DOSE HAEMOPHILUS INFLUENZAE TYPE B (PRP-T) VACCINE

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ABSTRACT

Objective : Evaluation of immunogenicity and acceptability of PRP-T vaccine among the Indian children.

Design: Multicentric, open, parallel group, comparative study of Haemophilus influenzae type B vaccine, given as single (Group I) or associated (Group II) with DPT vaccine.

Setting: Five different vaccination clinics.

Subjects: 125 children between the age group of 18-24 months.

Parameters : Measurement of (i) pre and post vaccination antibody titres of Haemophilus influenzae type B specific antibody; (ii) Adverse events; and (iii) Tolerance as graded by the physician.

Results : Prevaccination antibody levels were >0.15 mcg/ml in 56.3% in Group I and 35.7% in Group II. Post-seroconversion was seen in 97% in Group II receiving single and all in Group II ($P>0.05$). The vaccine was well tolerated.

Before the introduction of effective vaccines, *Haemophilus influenzae* type B was a leading cause of invasive bacterial disease in children less than 5 years of age in the United States(1,2). Poly ribitol phosphate (PRP), a capsular antigen, confers virulence, and helps Hib to develop in blood and cause invasive infection(3). PRP antigen is also responsible for the immunological characteristics of Hib, which evokes response by producing anti PRP antibodies in humans(4). Although, newborns are protected by maternal antibodies, the protection is short lived and disappears by the age of 2 months(5). With naturally acquired infection, protective levels of antibodies usually become significant between 3 to 5 years, whereas the peak incidence of infection is between 6 to 11 months of age(6).

In most of the developing countries Hib epidemiology is unknown but irrespective of the regions, the incidence of

Conclusions : The probability of subclinical infection or cross immunity is high in India. ACTHIB vaccine has a good immunogenicity and tolerance and association with DPT does not modify the immunogenicity of ACTHIB vaccine.

Key words: PRP-T vaccine, Immunogenicity, Haemophilus influenzae b vaccine.

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meningitis is highest between 6 to 11 months. Hib is a communicable disease and all children <5 years are at risk, but contact of a case has higher risk which is 10 times greater than meningococcal meningitis(7). It is, therefore, imperative to prevent the disease by vaccination, which is cost effective and would also lower the number of nasopharyngeal carriers. HIB immunization is routinely practiced in western countries and is a part of primary immunization program along with DPT and OPV. However, there is a paucity of data regarding the immunogenicity and tolerance of Hib vaccine in the Indian population.

Keeping in mind the utility of the Hib vaccine in the country, this study was planned to evaluate the immunogenicity and acceptability of a PRP-T vaccine (a tetanus conjugated *Haemophilus influenzae* b capsular protein vaccine manufactured by Pasteur Merieux Serums and Vaccines) in children of 18-24 months of age, given alone or in association with DPT.

Subjects and Methods

A multicentric, open, controlled, pre-registration trial was planned at five centres in 125 children. There were two centres from Ahmedabad and one centre each from Delhi, Baroda and Pune. The children who had already received the first booster dose of DPT were given only PRP-T (ACTHIB) (*Group I*). The children due for their first booster dose of DPT were also vaccinated for Hib simultaneously (*Group II*). They were healthy babies between the age group of 18 to 24 months, allocated in two groups. There were 90 babies in Group I receiving a single injection of ACTHIB (age

19.14 ± 0.36 weight 10.05 ± 0.20 kg; 53 males and 37 females). There were 35 children who received ACTHIB vaccination in association with DPT (18 males and 17 females; age 18.23 ± 0.49 mo; weight 9.85 ± 0.02 kg). Informed consent was obtained from parents of the children. It was ensured that all the children would be available for follow up and subsequent blood collection.

ACTHIB is a capsular polysaccharide, covalently conjugated to tetanus protein (20 meg). The 0.5 ml dose of reconstituted vaccine corresponds to 10 meg of polysaccharide. Ready to use vaccine in a prefilled syringe was obtained from the manufacturer. A single dose of 0.5 ml PRP-T was administered intramuscularly, on the lateral regions of the thigh to babies in Group I while babies in Group II received ACTHIB in one thigh and DPT booster on the other thigh. Three ml of venous blood was collected prior to vaccination and 4 weeks after the event. A variation of up to 7 days was accepted if the child was unable to come on the stipulated date. Sera were separated by centrifugation and were carefully stored at -20°C.

The sera samples were transported in frozen condition to Lyon, France for analysis. Anti PRP antibody titration was done by RIA technique using FARR assay method(8).

The investigator/physician observed every child for any immediate adverse events. The parents of the child were asked to observe the child carefully and to inform the investigator in case of any side effects. The child was brought to the clinic 24 and 48 hours after vaccination for the follow up. In case of fever,

antipyretic was administered. Investigators scored the local as well as systemic reactions on 0-3 scale ranging from nil to severe. Student's t-test was applied for comparison of pre and post vaccination PRP antibodies. Man-Whitney test was applied for analysis of adverse events and global evaluation of vaccine.

The usual reported seroconversion rate after single ACTHIB immunization is 85%. However, the sample size of the current investigation permits evaluation of a seroconversion rate of at least 15% with 80% power and 5% level of significance.

Results

Out of 125 children, 123 were available for follow up and paired sera were obtained from 119 babies for analysis. In 31 children receiving only single dose of PRP-T and 12 children receiving vaccination in association with DPT the pre vaccination protective titers were higher than 0.15 mcg/ml. Eight babies had pre vaccination titers above 1 mcg/ml.

Table I shows the mean anti PRP antibody titers in these 2 groups. There was no difference in the pre vaccination antibody titers between the groups. A sig-

nificant increase in antibody levels was observed after vaccination in both the groups ($p < 0.001$). However the post vaccination titers in both the groups were comparable. Two children in Group I did not achieve the post vaccination antibody titer > 1 mcg/ml and 1 of them had titer < 0.15 mcg/ml.

The vaccine was very well accepted and tolerated. All the local adverse reactions like pain at the site of injection, erythema and inflammation were mild and did not necessitate any treatment. The reactions observed at the ACTHIB site in both the groups, were of milder degree than those at the DPT site in Group II (Table II). Fever was the most common symptom amongst the systemic adverse reactions observed. The incidence of fever was highest at 12 hours [30 in single vaccination group and 21 in the associated group ($p > 0.05$)]. The acceptability was also assessed on a scale for clinical global evaluation. It was good to excellent in 97.7% of children in Group I against 68.6% in Group II.

Discussion

Mean antibody titres > 0.15 mcg/ml

TABLE I—Mean Anti PRP Antibody Titers (mcg/ml + SD) and Seroconversion Rate (%)

Parameter	Before vaccination		After vaccination	
	Single	Associated	Single	Associated
N	88	35	84	35
GMT	0.167	0.153	14.060*	17.140*
SD	± 2.679	± 2.275	± 3.573	± 2.541
Sero Conversion (> 4 fold)			97.7	100

* p value < 0.001 .

TABLE II—Local Reactions (%)

Reaction	Hour	Single	Incidence (%)	
			ACTHIB	Associated DPT
Pain	0.25	40.9	57.1	80.0
	12	30.7	42.9	74.3
	24	5.7	11.4	40.0
	48	1.1	8.6	20.0
Erythema	0.25	9.1	11.4	34.3
	12	3.4	8.6	34.3
	24	0.0	0.0	22.9
	48	0.0	0.0	11.4
Inflammation	0.25	0.0	0.0	0.0
	12	1.1	5.7	31.4
	24	0.0	0.0	14.3
	48	0.0	0.0	5.7

are considered protective and a serum concentration of 1 mcg/ml is believed to correlate with long term protection(9,10). In our study, 56.3% of children in Group I and 35.7% in Group II had pre vaccination antibody level >0.15 mcg/ml. This may be related to subclinical infection or cross resistance.

There was a significant increase in the anti PRP antibody titers after vaccination in both the groups and giving PRP-T in association with DPT did not modify the immunogenicity of PRP-T vaccine. In fact, even extemporaneously mixed PRP-T vaccine with DPT in the same syringe at the time of injection, did not affect immunogenic response of different antigens(11,12).

In this study, no adverse effects were

reported following PRP-T administration in both the groups. The incidence of local adverse effects at the site of DPT vaccination was significantly higher. A higher number of children having fever in Group II could be attributed to the simultaneous administration of DPT.

Although, in India, a clear picture of the incidence of Hib related disease is not known, some of the studies report its presence in about 14-35% of cases of pneumonia and meningitis(13,14). *S. Pneumoniae* and *H. influenzae* type B continued to be the most common infecting bacterial pathogens in both severe and very severe pneumonia(15).

Increasing resistance to beta lactamases has posed a major problem in treatment of Hib infections. A large

population is at a risk of contracting Hib infection during early life. Vaccination in early infancy offers protection to the babies at the most vulnerable age and seems logical and a promising approach towards prevention considering the highly infectious nature of Hib.

The newer vaccines have been developed by conjugating a protein carrier with the capsular antigen PRP. This conjugation renders PRP with a good immunogenic response and memory(16,18). The combined vaccination of PRP-T with DPT can be easily accommodated in the primary immunization schedule. For a long term benefit, vaccination with PRP-T in the primary immunization schedule along with DPT and also as a single booster dose needs consideration. It is concluded that the probability of subclinical infection or cross immunity is high in India. ACTHIB vaccine has a good immunogenicity and tolerance and association with DPT does not modify the immunogenicity.

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