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## Original Articles

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### IMMUNOGENICITY STUDY OF *HAEMOPHILUS INFLUENZAE* TYPE B CONJUGATE VACCINE IN INDIAN INFANTS

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Manuscript received: November 6, 1996; Initial review completed: December 31, 1996;

Revision accepted: March 14, 1997

**Objective:** To assess the immunogenicity in Indian infants to *Haemophilus influenzae* b oligosaccharide conjugate vaccine (HbOC). **Design:** Prospective multicenter study. **Setting:** Pediatric Out Patient Department of general hospitals in Pune and Mumbai. **Subjects:** 124 full term healthy infants brought for routine DPT/OPV immunization. **Methods:** Infants were administered 3 doses of 0.5 ml of HbOC, on the same day as their DPT/OPV immunization, injected intramuscularly on the limb opposite to that where DPT vaccine was administered. Data on local reactions and general symptoms was collected for three days after every dose. The children had their blood collected for assay of anti PRP (polyribosil ribitol phosphate) antibody titers, along with the first injection and one month after the third injection. One hundred and three infants completed the study protocol with two blood collections. **Results:** The initial geometric mean titers (GMT) of 0.124 mcg/ml rose by 37 times to 4.552 mcg/ml. Ninety eight children (95.1%) had a final titer of  $\geq 0.15$  mcg/ml, the minimum level associated with protection, and 77 children (74.8%) had a final level of  $\geq 1.0$  mcg/ml, a level associated with long term protection. **Conclusion:** HbOC is immunogenic in Indian infants when used as per the locally recommended DPT/OPV immunization schedule.

**Key words:** *Haemophilus influenzae* type b, Immunogenicity, Oligosaccharide conjugate vaccine.

OF the six capsular serotypes of *Haemophilus influenzae* (a to f), type 'b' is the cause of invasive disease in majority of the cases(1). The annual incidence of *Haemophilus influenzae* type b (Hib) disease in the first 5 years of life is estimated to range from 0.002-0.5%. The mortality of treated Hib meningitis varies from about 5% in developed countries to 50% or greater in many developing countries. About 25-

40% of surviving children have permanent neurological sequelae, the most serious being hearing loss and mental retardation(2). Type b capsular polysaccharide is a polymer of ribosylribitol phosphate (PRP). Immunity is correlated with the presence of antibody against PRP. However, PRP does not stimulate memory T lymphocytes. Conjugating PRP with an immunogenic carrier overcomes this problem. *Haemophilus*

*influenzae* b oligosaccharide conjugate vaccine (HbOC) is a conjugate of PRP with Diphtheria CRM 197 protein(1). The first Hib conjugate vaccine was licensed in USA in 1987 for toddler use, and in 1990 for use in infants. Since then it has been licensed in more than 40 countries worldwide. This vaccine elicits a production of more than 1.0 mcg of antibody per ml of serum in at least 75% of those vaccinated (1). In 61080 infants, the efficacy of this vaccine (3) following the three dose regimen was 100% (95% CI lower limit 68%).

In India, among 157 children with culture proven pyogenic meningitis admitted between 1987-1991 at Vellore, 40 had *H. influenzae* type b meningitis and 17 of these were multi antimicrobial resistant(4). Multiresistant strains of Hib have also been identified at other centers (5,6). *H. influenzae* b has also been identified as the causative organism in 12 of 44 cases of meningitis (7) and in 27 of 147 blood cultures in patients of pneumonia (8). Thus although not commonly identified and documented, *H. influenzae* b disease, both meningitis and respiratory infections, is prevalent in India. This study was designed to evaluate the immunogenicity of HbOC in Indian infants when used as per the recommended DPT/OPV immunization schedule.

## Subjects and Methods

One hundred and twenty four full term healthy normal infants of either sex between the ages of 5 to 16 weeks were included in this prospective study which was conducted in the Pediatric Out Patient Departments of B.J. Medical College, Pune and Lokamanya Tilak Medical College, Sion, Mumbai. After obtaining an informed consent from their parents or guardian, infants were administered three doses of HbOC (supplied by Cynamid India Limited) along with their routine DPT/OPV immunization. Blood was collected for analysis along with first dose and one month after the third dose. Each infant was thus to be followed up for at least four months. The pertinent demographic data of these 124 subjects is summarized in *Table I*. Children with known or suspected history of pertussis, diphtheria, tetanus or disease due to *Haemophilus influenzae* type b (Hib), those who had already received Hib vaccine, any other vaccine or gammaglobulin in the past four weeks, or those on immuno suppressant drugs were not included. Also excluded were children with history of seizures, major congenital malformations, known chromosomal abnormalities like Down syndrome, or chronic systemic disease or severe malnutrition. Since the

**TABLE I**— *Pertinent Demographic Data for 124 subjects*

	Consolidated	Center 1	Center 2
Total number of children enrolled	124	60	64
Males	66	32	34
Length (cm)	53.0 ±2.2	53.3 ±1.4	52.6 ±2.7
Weight (g)	3396 ±761	2912 ±408	3850 ±733
Age (weeks)	7.8 ± 1.9	8.4 ±2.20	7.2 ±1.4
No. of patients completing 3 doses & two blood collections	103	49	54

Values are expressed either as mean ± SD or numbers. None of the differences between the two centers were significant.

vaccine was to be administered in three doses along with DPT/OPV, there was a provision to withdraw the patient from the study if high fever, encephalopathy, convulsions or allergic reactions were noted, after any dose. Immunization was postponed if the child had a temporary acute and severe illness.

To the selected cases HbOC was administered 0.5 ml intramuscularly in the left thigh or buttock and DTP was administered on the right side. HbOC was used from a single dose vial containing 10 mcg of Hib capsular oligosaccharide covalently linked with CRM 197 mutant diphtheria toxin. Standard release DTP manufactured by the Government of India and used regularly in the hospital was used.

Blood (5 ml) was collected on the same day prior to immunization for measurement of anti polyribosyl ribitol phosphate (PRP) antibodies, and stored at minus 20 degree C. The next two doses were given along with DTP at an interval of 4 to 8 weeks. The child was called for blood collection 4 to 6 weeks after the third dose. Following administration of the vaccine, the child was observed for 20 to 30 minutes for any signs and symptoms of untoward events. Local and general reactions were observed for upto three days and the parents were instructed to bring the child to the hospital any time in case of emergency. Serum was sent to the laboratories of Wyeth-Lederle Vaccine and Pediatrics, Pearl River, USA, for analysis by ELISA.

Since there was no statistically significant difference between centers either in the patient characteristics or in the results, the data was pooled for analysis. Chi square and 't' tests were used as appropriate. From our data and sample size, the power of the study for estimating the immunogenicity of the vaccine, based on a two tailed distribution and 95% level of

significance exceeds 0.9.

## Results

Although the initially envisaged inclusion criteria was children between 7 to 12 weeks old, the minimum age of evaluated subjects was 5 weeks and maximum 16 weeks. We had 1 case 5 weeks old and one 16 weeks old. The maximum deviation from initially envisaged protocol was 31 children aged 6 weeks. These cases were later included for analysis because they had come for immunization for the first time. It would have been unethical to postpone immunization only to adhere to the protocol. Furthermore, earlier studies (9,10) had also included infants less than 5 weeks.

Out of 124 cases in whom initial blood samples were collected, 103 cases completed the study protocol along with the second blood collection. The immunogenicity data is summarized in *Table II*. Due to a wide scatter of data, geometric mean titers (GMT) were used. A pre-vaccination GMT of 0.124 mcg/ml rose to 4.552 mcg/ml, a rise of around 37 times ( $p < 0.001$ ). At the end of the study 95.1% (98 patients) achieved a titre of  $\geq 0.15$  mcg/ml and 74.8% (77 patients) had achieved a titre of  $\geq 1.0$  mcg/ml. The vaccines were also very well tolerated. The safety data along with

**TABLE II**-Summary of Immunogenicity Data.

Parameter	Pre-vaccination	Post-vaccination
No. of Subjects	103	103
Geometric mean titer (mcg/ml)	0.124	4.552*
Standard error	0.30	0.87
% $\geq 0.15$ mcg/ml (number of patients)	32.0% (33)	95.1% (98)
% $\geq 1.0$ mcg/ml (number of patients)	5.8% (6)	74.8% (77)

\*  $p < 0.001$  (paired t-test)

an additional cohort from another center has been reported elsewhere. The common side effects were mild fever (56%), irritability (18.4%) mild redness, (2.3%) and induration at the injection site (10.7%). No patient had to be withdrawn from the study due to side effects.

### Discussion

The current study documented the immunogenicity of HbOC in Indian infants. Ninety eight patients (95.1%) had a final titer of  $\geq 0.15$  mcg/ml, a level associated with protection against *H. influenzae* b (Hib) disease and 77 patients (74.8%) developed titers  $\geq 1.0$  mcg/ml, a level associated with long term protection. Only 33 children (32.0%) had a basal titer of  $\geq 0.15$  mcg/ml and 6 (5.8%) had a level  $\geq 1.0$  mcg/ml. Since all our subjects were breastfed, this could be due to passive immunity passed from the mother to the baby (11). An earlier report (12) also noted that 39% subjects had a basal level of  $\geq 0.15$  mcg/ml. This could also be explained by Hib disease in the mother as prenatal immunization of the mother gives high titers in the infants due to transplacental transfer (13).

The changes in anti PRP antibody titers are in consonance with an earlier study (3) in which 99% children developed titers  $\geq 0.15$  mcg/ml. In comparison to reports(3,9,10) of nearly 97% children having titers  $\geq 1.0$  mcg/ml, our value of about 75% appears low. However, some other studies have also shown similar percentage (73-80%) of children achieving titers  $\geq 1$  mcg/ml(14-16). The immunization schedule abroad is 2,4 and 6 months resulting in a gap of 5 months between two blood collections. As against this in India, the immunization schedule is monthly thus shortening the period between two blood collections to 3 months. An older age at vaccination, yields higher titers and also the duration

between the third dose and subsequent blood collection affects the titers(13).

Norman Begg (1990-1991) at Communicable Disease Surveillance, UK, (data on file, Wyeth Lederle Vaccines and Pediatrics) conducted a study wherein the doses of vaccine were given like in India, at 2,3 and 4 months. Seventy seven (96%) out of 80 children achieved a final titer of  $\geq 0.15$  mcg/ml and only 55 (69%) achieved level of  $\geq 1.0$  mcg/ml, a result very similar to ours. The level of 1.0 mcg/ml for long term protection was based on early trials with Hib polysaccharide vaccine to which a cell mediated immunity was not developed. With the use of conjugate vaccines, which help develop a cell mediated immunity, these cut off levels may not represent the ideal. The immunized infants are able to respond with a rapid and high antibody response after exposure to the organism. Hence, even a level of  $\geq 0.15$  mcg/ml should be adequate for protection (17).

An earlier Hib conjugate vaccine study from India documented a satisfactory immunogenic response in children between 18-24 months, who were administered only one dose (18). Another recent study/with a three dose schedule also revealed a good immunogenic response in infants (19).

It is concluded that HbOC is immunogenic in Indian infants when used as per the recommended DPT/OPV immunization schedule.

### Acknowledgement

We are grateful to M/s Cyanamid (India) Limited for providing the vaccine samples and financial grant for conducting the study.

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