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Original Article

Antibody Responses of Three *Haemophilus Influenzae* Type b (Hib) Conjugate Vaccines After a Primary Vaccination Series in Filipino Infants

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ABSTRACT

Serum antibody responses to three *Haemophilus influenzae* type b (Hib) capsular polysaccharide-protein conjugate vaccines (PRP-OMP, PRP-T, and HbOC) were evaluated in 174 Philippine infants after a primary vaccination series. Children were randomized to receive one of the Hib vaccines (Hib groups) or into a control group. Vaccination was carried out at 6, 10 and 14 weeks of age based on the local Expanded Program of Immunization schedule. Sera were collected at six weeks of age for the Hib groups and one month after the third dose for all subjects. Anti-Hib concentrations were determined by the Farr-type radioimmunoassay. There were no significant differences in the prevaccination anti-Hib geometric mean concentration (GMC) among the three Hib groups. Differences in the GMC after the primary series of three doses were significant; GMC was highest for PRP-T (6.62 μg/ml), followed by HbOC (1.9 μg/ml), and the lowest for the control group (0.11 μg/ml). Hib conjugate vaccines are generally well tolerated. Both local and systemic reactions observed in the infants resolved within 24 hours from onset. We conclude that all three Hib conjugate vaccines (PRP-T, HbOC, and PRP-OMP) were immunogenic after the primary doses among Filipino infants.

INTRODUCTION

Invasive diseases mainly acute respiratory infection (ARI) and meningitis caused by *Streptococcus pneumoniae* (Pnc) and *Haemophilus influenzae* type b (Hib) are leading infectious disease health problems especially in young infants in developing countries. In the Philippines, no population-based epidemiologic information on Hib diseases is yet available. The limited data are from hospital patient population. Etiology studies on ARI since 1985 have reported that *H. influenzae* and *S. pneumoniae* are the predominant bacteria isolated from blood cultures in Filipino children under 5 years of age. [1,2,3] In a six-year review of 88 bacterial meningitis cases among children at the Research Institute for Tropical Medicine (RITM) *S. pneumoniae* and *H. influenzae* were the most frequent causes.[4] Majority of these isolates were from children less than one year of age.

A new generation of vaccines consisting of polysaccharide-protein conjugates have been developed to prevent pneumococcal and Hib diseases. Hib conjugate vaccines have proven to be highly immunogenic and protective in infants and young children in both industrialized[5,6] and developing countries.[7,8] These vaccines have been included in infant immunization programmes in many industrialized countries and in some developing countries such as Chile, Uruguay and Vanuatu. In developing countries with high risk population for Hib disease, the immunogenicity of Hib vaccines should be ensured if they are planned to be used in large population and vaccination should be carried out based on the national EPI schedule. Therefore, this study aimed to ascertain the immunogenicity of three Hib conjugate vaccines (PRP-OMP, PRP-T, and HbOC) in Filipino infants when given at 6, 10 and 14 weeks of age and to evaluate its reactogenicity when given together with EPI vaccines.

SUBJECTS AND METHODS

This study was a comparative, controlled, randomized, one-way, and blinded. It was carried out from November 1992 to December 1993 at Barangay Mamatid in the municipality of Cabuyao, in the province of Laguna, the Philippines. The study

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site is a stable, semi-urban, middle-to-lower class community chosen because of its accessibility from the town proper and the minimal mobility of its residents. Most heads of households were fishermen, farmers, and/or orchard tenders, and a small number were factory workers. The majority of mothers were homemakers. The barangay had a health station staffed by a midwife.

Subjects

Study subjects were infants brought to the local health center and who fulfilled the entry criteria: who are 6 (+ 2) weeks old, are to start their DPT vaccination, live in the barangays of Mamatid or Banlic and whose parents or guardian had given their informed consent.

Nutritional status

The nutritional status was determined using the weight for age by local nutritional standard.[9]

Vaccines

Three of four widely used Hib conjugate vaccines were used in this study. The PRP-OMP (Pedvax-Hib), polysaccharide (PS) coupled to an outer membrane protein of Neisseria meningitidis group B, was produced by Merck Sharp and Dohme (West Point, Pa); the PRP-T (Act-Hib), the PS coupled to tetanus toxoid, was manufactured by Pasteur Merieux (Lyon, France); and the HbOC (Hibtiter), PS coupled to nontoxic variant diphtheria toxin CRM 197, was produced by Lederle Praxis Biologicals, Inc. (Rochester, NY). The regular EPI vaccines used were BCG, OPV, DPT and Hepatitis B vaccines.

Vaccination and sampling schedule

The Hib vaccination was carried out simultaneously with the local EPI schedule. Infants in the Hib groups were given one of the study vaccines in a three-dose series at 6, 10 and 14 weeks. These vaccines were administered intramuscularly in the right anterolateral thigh concurrently with the DPT in the opposite thigh and hepatitis B vaccine in the deltoid area of the left arm. Oral polio vaccine was given simultaneously. The control group received only the EPI vaccines. At the end of the study, Hib vaccines were given to these infants at 10 and 12-15 months of age as recommended by the Ethical Review Board of the Research Institute for Tropical Medicine (RITM). Each child was observed for 15 minutes after vaccination for adverse reactions. Parents were asked to monitor the child for local and systemic reactions during the first 24 hour post-vaccination. During the study, 1-3 ml of venous blood was drawn before each of the three Hib doses and one month after the third vaccination. In the control group, serum samples were collected before and one month after the DPT/OPV dose. The vaccination and sampling schedule is shown in Table 1.

Table 1. Vaccination and sampling schedule

Age: (schedule) (mean age)	6-8 wks (6.6)	10-12 wks (11.0)	14-16 wks (15.5)	18-20 wks (19.9)
Vaccines: (Hib group)				
BCG	OPV/DPT/HBV Hib	OPV/DPT/HBV Hib	OPV/DPT/HBV Hib	
Samples: (Hib group)				
	serum	serum	serum	serum

Hib = *Haemophilus influenzae* type b; OPV = oral polio vaccine;
DPT = diphtheria pertussis tetanus; HBV = hepatitis B vaccine

Antibody assay

Anti-Hib polysaccharide (anti-Hib) antibody concentration (μg/ml) was determined using the Farr-type radioimmunoassay, the classic method of quantitating anti-Hib polysaccharide antibodies, using reference serum from the U.S. Food and Drug Administration (Bethesda, MD) containing 90 μg/ml of anti-Hib polysaccharide/ml. The detection threshold of the assay was 0.06 μg/ml.[10] This assay was performed at the National Public Health Institute (Helsinki, Finland).

Statistical analysis

The geometric mean concentrations (GMCs) of anti-Hib antibody were calculated for each of the four study groups. Comparisons were made before and after the three-dose immunizations. Analysis of variance was used to determine the significance of the variation of the log-transformed antibody titers among the test groups. The significance level was 5%.

Ethical approval

The study was approved by the Institutional and Ethical Review Board of the Research Institute for Tropical Medicine.

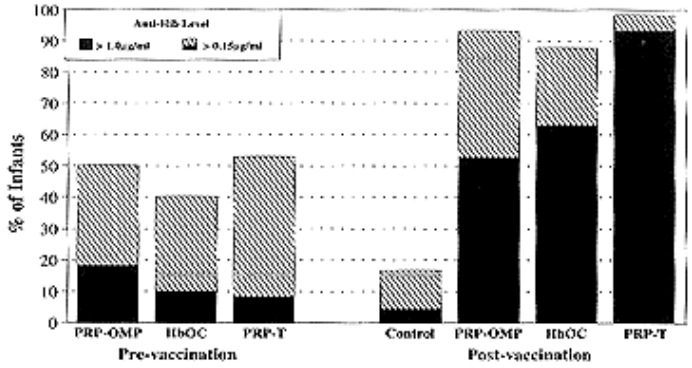
RESULTS

A total of 200 infants were enrolled in the study, 50 infants for each study group (three vaccine groups and a control group). One hundred ninety-seven subjects completed the primary series of three doses. Of these infants, 130 provided pre- and post-vaccination serum samples including 44, 44, and 42 infants who received the three doses of PRP-OMP, PRP-T, and HbOC; in addition, pre- and post-vaccination serum was obtained according to the protocol from 44 infants of the control group. Seven infants were withdrawn from the study, five because of transfer of residence and two because their parents or guardians refused blood extraction. Nineteen subjects who had incomplete serum samples were excluded from the analysis.

There were 79 (65%) boys and 42 (35%) girls. Majority (80%) of the infants were of normal nutritional status (> 2 SD above mean FNRI reference), 13% mildly malnourished (< 1 SD below mean) and 7% moderately malnourished (< 2 SD below mean).

The serum concentrations of anti-Hib antibody in the study infants prior to and after vaccination with the Hib conjugate vaccines are shown in Figure 1. Forty-eight infants had no detectable anti-Hib level (< 0.06 µg/ml) prior to vaccination. After the primary series, one hundred twenty-one (93%) of the vaccinated infants had an antibody concentration ≥ 0.15 µg/ml and 90 (69%) a concentration ≥ 1.0 µg/ml, whereas in the control group only 27% and 5% had anti-Hib serum concentrations ≥ 0.15 µg/ml and ≥ 1.0 µg/ml, respectively. After the primary series, 98% of the PRP-T group had an anti-Hib level of ≥ 0.15 µg/ml, followed by PRP-OMP (93%) then HbOC (88%). Ninety three percent (93%) of the PRP-T group, 62 % HbOC and 52% of the PRP-OMP group had anti-Hib level of ≥ 1.0 µ/ml.

Fig. 1 Anti-PRP-antibody serum concentrations before and after three primary doses of Hib conjugate vaccines



There were no significant differences in the pre-vaccination anti-Hib GMC among the three vaccine groups. Following the first dose, there was no significant increase in the GMC with PRP-T and HbOC whereas with the PRP-OMP there was a minor but not significant increase. After two doses, PRP-T and PRP-OMP elicited a significant increase in the GMC (p. < 0.05) however, there was no significant increase in GMCs with HbOC. The third dose of PRP-T and PRP-OMP caused a further significant increase in the GMCs. A response was seen already with HbOC which produced significantly higher mean concentration than PRP-OMP but the GMC remained significantly lower than after three doses of PRP-T. (Table 2).

Table 2. Pre- and post-vaccination anti-Hib geometric mean concentration (GMC) of the vaccine groups

Vaccine Group	Pre-vaccination N	Pre-1st dose GMC (µg/ml)	Post-vaccination N	1st dose GMC (µg/ml)	N	2nd dose GMC (µg/ml)	N	3rd dose GMC (µg/ml)
PRP-OMP	35	0.369	38	0.645	53	1.122*	31	1.408*
HbOC	34	0.265	42	0.336	39	0.493	34	2.321*
PRP-T	35	0.329	38	0.386	38	1.651*	31	5.006*

*Post second dose, statistically significant relative to the HbOC group ($p < 0.05$).
*Post third dose, statistically significant between vaccine groups (pairwise $p < 0.05$).

No serious adverse reactions occurred. The incidence of local reactions such as redness, swelling, and pain in the three vaccine groups was less than the reactions associated with the DPT vaccine (20%) given in the other thigh and almost the same as with the hepatitis B vaccine (8%) given in the left arm of the same subjects. Fever (defined as a temperature ≥ 38°C) was noted in 26%, 24%, and 30% in the PRP-OMP, PRP-T, and HbOC groups, respectively. In the control group, fever and irritability were observed in 22% and 22% of the children, respectively.

Table 3. Local and systemic reactions among study patients given Hib conjugate vaccines*

	PRP-OMP (n = 148 doses)		PRP-T (n = 147 doses)		HbOC (n = 150 doses)	
	No.	%	No.	%	No.	%
Redness	17	11	8	5	5	3
Swelling	10	7	8	5	1	0.6
Pain	16	11	19	13	11	7
Irritability	39	26	40	27	32	21
Fever (≥ 38°C)	39	26	36	24	45	30

*Hib = *Haemophilus influenzae* type b conjugate vaccine. Reactions were reported as aggregate of the three doses of PRP-OMP, PRP-T, and HbOC.

DISCUSSION

The three Hib conjugate vaccines have proven to be highly immunogenic after primary vaccination series of three doses in Filipino infants. This was the first immunogenicity study of Hib conjugate vaccines done in an Asian country.

The capsular polysaccharide (PS) is the essential virulence factor of Hib and antibodies to this polysaccharide confer protection from disease. The Hib capsular PS vaccine while safe

and immunogenic in older children and adults is poorly immunogenic in infants and young children, the age group at highest risk of the disease. This finding stimulated the development of a Hib PS conjugate vaccine in which the PS is coupled to a protein carrier capable of stimulating antibodies in early infancy and are able to evoke immunological memory.

The low pre-vaccination anti-Hib GMC in the subjects and the very low anti-Hib antibody serum concentration at 18 weeks of age in the control group indicate a need for a vaccine that would protect infants and young children from invasive Hib diseases. The level of anti-Hib required for protection against invasive disease caused by *H. influenzae* type b is not precisely known. However, previous studies on Hib polysaccharide had suggested that a serum antibody concentration ≥ 0.15 µg/ml is necessary for immediate protection and a concentration ≥ 1.0 µg/ml is associated with long-term protection.[11]

PRP-OMP was the only vaccine which induced an immune response after the first dose. After the second dose a remarkable antibody response was obtained, however, after the third dose the antibody increase was only marginal. Previous studies in Alaskan natives[12] and Gambians have shown similar results.[8] The use of this vaccine should be considered in population with high attack rate of Hib disease in early infancy and where vaccination coverage decreases with age. The third dose did not boost antibody response. A schedule consisting of two doses of PRP-OMP for the primary series is of practical advantage especially in developing countries with financial constraints, however, the duration of antibody response after primary series or its response to booster dose should be addressed to.

Among the three vaccines, PRP-T elicited the highest response. With the normal decrease with time of elicited antibody levels, the higher GMC can be expected to afford protection for Filipino infants especially during the first two years, the age at risk for invasive Hib infections. Nonetheless, conjugate vaccines evoke immunological memory, therefore, even lower anti-Hib concentration elicited by the other vaccine groups can be predicted to be enough for protection. Our results confirm findings of vaccine studies which showed that PRP-T was as good or better than the other Hib vaccines after the 3 doses.[14]

In this study, HbOC was found to be immunogenic in a smaller proportion of infants (88% had anti-Hib levels ≥ 0.15 µg/ml and 62% had levels ≥ 1.0 µg/ml) as compared with previous studies. In a comparative immunogenicity study in Alaska native infants, measuring anti-Hib at seven months of age after three doses, HbOC had the highest anti-Hib concentration among the four Hib vaccines studied.[12] The low antibody response may be due to the very young age at which this vaccine was administered. Studies in Finland have shown that antibody levels induced after two doses of HbOC at four and six months of age were similar to those induced by three doses given at two, four, and six months of age.[13]

The Hib conjugate vaccines are generally well-tolerated. In this study, in which DPT and hepatitis B vaccine were administered with Hib vaccine, however, a higher incidence of fever was noted in the three vaccine groups as compared with previous studies,[8,13] in which only DPT was added to the Hib vaccine. Nevertheless, it is to be noted that the Hib conjugate vaccines were administered simultaneously with other EPI vaccines, such as DPT and hepatitis B vaccines; therefore, it is not possible to attribute the systemic reactions to any individual vaccine used in the study. Both local and systemic reactions observed in the infants resolved within 24 hours from onset.

In summary, all three Hib conjugate vaccines (PRP-OMP, PRP-T, and HbOC) were immunogenic and safe after a primary immunization series with three doses in Filipino infants, in accordance with results reported from both industrialized and developing countries.

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