Original Articles

HAEMOPHILUS INFLUENZAE TYPE B VACCINE IN INDIA: NEED AND TIMING, IMMUNOGENECITY AND TOLERANCE

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Objective: *(i) To assess the natural immunity and susceptibility to Haemophilus influenzae type b (Hib) infections in children in India, (ii) To study the immunogenecity and tolerance of Hib vaccine (ACTHIB) in young infants.* ***Designs:*** *(i) Cross sectional study, (ii) Prospective trial.* ***Setting:*** *Well baby and immunization clinics.* ***Methods:*** *(i) PRP antibody titers against Hib estimated in 172 healthy infants and children aged 1 month to 10 years, (ii) Antibody titres estimated before and after ACTHIB vaccine given with primary immunization (age group 6 to 8 weeks) in 50 babies.* ***Results:*** *(i) Naturally protective levels of Hib antibodies found in less than 20% of infants under one year, but in over 80% above 4 years, (ii) Seroconversion after ACTHIB vaccination was 100% with very high protective levels. There were no significant adverse reactions.* ***Conclusions:*** *ACTHIB vaccine proved to be safe and highly immunogenic. As susceptibility to Hib is highest in the first year of life, the vaccine should be recommended in the primary immunization schedule (combined with DPT). The very high titers achieved suggest the possibility of decreasing the number of doses or the amount of antigen to reduce the prevalent high cost.*

Key words: *Haemophilus influenzae infection, Immunization.*

Haemophilus influenzae type 'b' (Hib) is a common cause of invasive bacterial infections in children aged 3 months to 5 years, causing a spectrum of serious illnesses such as meningitis, epiglottitis and pneumonia(l,2). Mortality and morbidity of these conditions is high, especially if treatment is delayed(3). Emerging resistant strains pose further problems in successful treatment^). High titers of anti Poly Ribosyl Phosphate (PRP) antibodies in convalescent sera led to the development of conjugate vaccines, which since 1988, are in regular use in developed countries(5-7). Routine vaccination in these countries has led to a remarkable decline in the incidence of Hib infections(8, 9). In Finland, **for** example, the incidence in children under 5 years has fallen from 52/100,000 in pre-vaccination era to virtually nil since 1992(7).

The exact incidence of Hib, related disease in children in India is largely unknown. The few reported studies quoting 8 to 14% of meningitis(10-12) and 7 to 15% of

lobar pneumonias are likely to be underestimates because of poor bacterial culture facilities in our laboratories(13). Though effective, Hib conjugate vaccines are expensive and not yet available for routine use in India. A preliminary multicentric trial of the vaccine in 125 Indian children between the ages of 18 to 24 months (1st booster age group) has given encouraging results(14). But before recommending routine immunization against Hib in our country the questions that need to be answered are: (i) What is the natural prevalence of Hib in children in India? Is the vaccine really needed in our country?; *(ii)* What is the critical period of susceptibility to the disease and therefore what is the optimum timing of the vaccine?; and *(iii)* What is the immunogenecity and tolerance of the ACTHIB vaccine in combination with DPT in young infants? This study was specifically planned to address the aforementioned issues.

Subjects and Methods

These studies were conducted by the Department of Pediatrics, KEM Hospital, Pune, over a period of one year. The protocol was reviewed and approved by the Ethics Committee of the hospital.

Subjects

Study I: Study of Cross Sectional Survey of Anti-PRP Antibodies.

One hundred and seventy two healthy children between the ages of one month to 10 years attending the Well Baby Clinic or Immunization Clinic were randomly selected in their respective age groups. Children with acute infections and those suffering from chronic debilitating illnesses, were excluded.

As anticipated population prevalence in the country is unknown, it was assumed to be 50%. The estimated prevalence on 170 children will fall within 7.5 percentage points of the true prevalence with 95% confidence.

Study II: Immunogenecity and Tolerance Study

Fifty infants of age 6 to 8 weeks and requiring primary schedule of DPT and polio vaccination under Universal Immunization Programme (UIP) were recruited. Babies suffering from any infection, neurologic disorders, immunocompromized babies, or those undergoing steroid therapy were excluded. Informed consent was obtained from parents of the babies after giving full description of the vaccine and schedule of blood collection.

Vaccine

ACTHIB (Pasteur Merieux) is a capsular polysaccharide covalently conjugated to tetanus protein (PRP-T). The 0.5ml dose of reconstituted vaccine corresponds to 10 meg of polysaccharide. DPT vaccine and Polio Vaccine (OPV) were supplied through UIP programme. Vaccines were maintained in cold chain conditions.

Vaccination Schedule

Study II (a): The babies recruited were randomly allocated to Groups A or B. Babies enrolled in Group A were given 0.5 ml ACTHIB intramuscularly (lateral region of thigh), in association with DPT, *i.e.,* they received the DPT at a different site. Babies enrolled in Group B received combined vaccination with DPT, *i.e.,* ACTHIB and DPT were mixed extemporiously in the same syringe and administered intramuscularly. The vaccination was carried out at approximately 2,3, and 4 months of age. At the same time, they also received the OPV as per the primary vaccination schedule.

Blood Collection

In Study I, 3 ml of blood was collected from all the enrolled children by venepuncture at one time. In Study II, 3 ml of

blood was collected prior to vaccination and four weeks after the last dose of vaccination from all enrolled babies. Sera were separated by centrifugation and coded.

Adverse Reactions

The babies who received vaccination were observed for any immediate adverse reactions upto 1 hour after vaccination. Parents were instructed to record and report local as well as systemic reactions such as fever, irritability, persistent crying, anorexia, vomiting, rash or convulsions.

Serological Analysis

All the separated sera were carefully stored at — 20° C and were dispatched in frozen state to Lyon, France. Serum anti-PRP antibody was measured with a FARR type of FJA using intrinsically labelled PRP that used 1125 labelled polysaccharide(15).

Titers above 0.15 mcg/ml were considered as seroconversion (natural protection threshold) and whereas, levels above 1 mcg/ml taken to indicate long term vaccine protection threshold(16,17).

Statistics

Postvaccination geometric mean antibody titers (GMT) between the two study groups were analyzed by unpaired 't' test. Pre and post vaccination titers within each group were analyzed using paired 't' test.

From our data and sample size, the power of the study for estimating the immunogenecity of the vaccine given combined or associated with DPT vaccine exceeds 0.9. Power calculations have been made considering two tailed distribution and 95% level of significance.

Results

Study I: In the cross-sectional survey a total of 172 samples were collected. Two samples could not be analyzed due to insufficient quantity. The analysis of 170 samples shows age related increase in anti-PRP antibodies *(Table I).* Irrespective of age group,

TABLE I– *Cross-sectional Survey of Anti PRP Antibodies (Against Hib) in 170 Healthy Infants and Children.*

Age group	n (n males)	n (%) with Anti PRP level >0.15 mcg/ml	Mean anti PRP (± SE)
Group I (1-12 mo)	27 (21)	5 (19)	0.30 (0.08)
Group II (12-24 mo)	25 (14)	9 (36)	0.21 (0.03)
Group III (24-36 mo)	23 (18)	8 (35)	0.30 (0.07)
Group IV (36-48 mo)	9 (6)	6 (67)	0.55 (0.35)
Group V (48-60 mo)	16 (10)	13 (81)	0.94 (0.50)
Group VI (>60 mo)	70 (36)	60 (86)	1.18 (0.31)
Total	170	101 (59.4)	

levels above 0.15 mcg/ml (natural protection) were observed in 101 babies of which 16 had titers above 1 mcg/ml (long term protection). Two one month old babies had titers of 0.57 and 1.3 mcg/ml, possibly due to transplacental transfer. However, 22 (81%) babies below the age of 12 months had titers below 0.15 mcg/ml, *i.e.*, they were susceptible to Hib infections. The susceptibility reduced with increasing age so that less than 30% of children over the age of 3 years had titers below 0.15 mcg/ml.

Study II: All 50 babies completed the primary schedule of vaccination and the subsequent follow up. Paired sera were available from 48 babies as two refused postvaccination blood sampling. There was no significant difference in the mean weight and age at initiation of study in the two groups.

Table II shows immunogenic response of ACTHIB in primary immunization schedule. The pre vaccination anti PRP antibodies were higher than 0.15 mcg/ml (natural protection) in only three babies in Group A and seven babies in Group B. The post vaccination titers were significantly higher in both the groups with 100% seroconversion in all ($p = 0.0001$). There was no significant difference in the seroconversion rate of babies with prevac titers below or above 0.15 µg/ml. All but one baby achieved post vaccination titers of more than 1 mcg/ml (long term vaccine induced protection). The post vaccination titers in Group B *(i.e.,* DPT and ACTHIB combined in same syringe) were significantly higher than in Group A (ACTHIB and DPT at different sites) ($p = 0.003$).

Adverse Reactions

There were no serious adverse effects in the form of vomiting, convulsions or hypotonia. A total of 11 babies had mild fever lasting for 48 hours. Local pain and erythema were seen in 2 babies and one baby developed induration, which subsided on its own without any surgical intervention. There were no differences in the groups receiving ACTHIB and DPT concurrently or in combination.

Discussion

Diseases known to be related to Hib infections such as meningitis and pneumonias are not uncommon in India(10-13). Mortality and morbidity of these conditions is high especially in infants and children under 3 years(18,19). Our cross-sectional survey for Hib antibodies con-

TABLE II–*Anti PRP Antibody Titers (µg/ml) in 48 children given with Primary DPT.*

Anti PRP antibodies (µg/ml)	Group A (24)		Group B (24)	
	Associated	Injections	Combined	Injections
	Prevac	Postvac	Prevac	Postvac
n (%) < 0.15	21 (88)	0 (0)	17 (71)	0 (0)
n (%) > 0.15	3 (12)	24 (100)	7 (29)	24 (100)
n (%) > 1.0	0 (0)	23 (96)	0 (0)	24 (100)
GMT	0.17	11.97	0.21	31.48
(SEM)	(1.09)	(1.3)	(1.13)	(1.2)

Prevac and postvac GMT in Group A – $p < 0.0005$
Prevac and postvac GMT in Group B – $p < 0.0005$
Postvac GMT in Group A and Group B – $p < 0.003$

ducted in 170 healthy children in various age groups (1 month to 10 years) demonstrates a high prevalence of subclinical Hib infections, and least natural protection under the age of 3 years, especially under one year.

More than 70% of children above the age of 3 years had natural protective antibody titers above 0.15 mcg/ml indicating subclinical infections. However, 70% of infants and children under age 3 years had titers below 0.15 mcg/ml and were hence, susceptible to Hib infections. This susceptibility was highest (81%) under the age of 1 year. This study therefore, emphasizes the need for Hib vaccination in our country and that too at an earlier age, namely, in the primary immunization age group rather than at booster age of 18 months as given in our recently reported multicentric study(14).

The vaccine ACTHIB (Pasteur Merieux) proved highly successful with 100% seroconversion with antibody titers well above 1 mcg/ml in all but one baby. Titers of more than 1 mcg/ml are generally correlated with long term protection(20). Infact, the post vaccination titers with 3 doses in our study, were two to three times greater than generally reported in western countries(21-23). Similar strikingly high post immunization response is also reported in Venezuelian children(24). The high antibody response in developing countries could well be due to subclinical infections or racial variations.

The enhanced serological conversion in our babies suggest the possibility of administering fewer doses of vaccine or smaller amounts of antigen with great potential for saving public health resources. This hypothesis needs to be corroborated with further studies of immune response following each dose to formalise the optimum schedule in our country.

Immunogenicity and safety of PRP-T (ACTHIB) given in combination with DPT has been assessed in several studies besides ours. The antibody response of other antigen has not been affected with ACTHIB(25,26). In our study, the antibody response in babies receiving PRP-T vaccination in combination with DPT was infact better than when given alone or at different sites.

The vaccine was remarkably well tolerated as seen in our earlier study (booster age group)(14). There were no significant differences in the local of systemic reactions in the two study groups. The safety and efficacy of Hib vaccine has been confirmed by other studies earlier(22).

In conclusion, the high susceptibility of our infants and young children to Hib infections emphasizes the urgent need for a protective vaccine in India. The vaccine must be introduced early in life, preferably in a conjugate preparation combining DPT with Hib along with OPV. However, prospective studies of suspected infections are necessary to determine the exact incidence of Hib in India.

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