Immunization of Saudi children against hemophilus influenza type b at the age of 6 weeks

Mohamed Khalil, MD, Yagob Al-Mazrou, FRCPG, Mohamed Al-Jeffri, MSc, Mansour Al-Howasi, MRCP

ABSTRACT

Objective: To study the immunogenicity of 3 doses of HbOC in 6 week old Saudi infants who were given either WHO DTP or FDA DTP formula. Methods: Six week old infants were randomized into three groups to receive 3 doses of HbOC and FDA DTP formula, HbOC and WHO DTP formula or in a control group to receive usual vaccines without HbOC at 6 weeks, 3 months and 5 months. Anti body levels for PRP, tetanus, diphtheria and pertussis were measured. Only the result of the Hib antibody will be presented. Results: After 3 doses no difference was found between anti-PRP either when given with FDA DTP or WHO DTP formula. Ninety two to ninety four percent of the vaccinated children in our sample acquired the protective level after the third dose. Thirty percent of the unvaccinated children do not have anti-Hib maternal antibody at the age of 6 months. No negative interactions with other vaccines were observed after the third dose. Conclusion: HbOC is highly immunogenic in Saudi children when the vaccine is started at the age of 6 weeks. The use of the vaccine in the extended program of immunization is expected to confer protection to a high proportion of Saudi children.

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Hemophilus influenzae type b is the leading cause of meningitis in Saudi children <5 years.1 In published studies, 52-60% of the positive cerebrospinal fluid (CSF) cultures were Hib,2,3 52-70% of the positive cultures occurred in under one year and 90% occurred before the age of two. The portion of disease occurring in Saudi children under 2 years of age is higher than the incidence in the USA, 81% and markedly higher when compared to Finland, 59%. This means that the pattern of susceptibility is more similar to the developing and native population.2 Although it is known that the peak incidence occurs in the second 6 months of the first year, detailed analysis of the data in Saudi Arabia shows that the peak incidence is in the second four months of the first year.4 Mortality due to Hib meningitis is 5.7%, while complication of diseases is 45%.5 The proportion of meningitis cases with positive culture is very low in relation to the number of suspected cases which lead to underestimation of the problem. From the experience in other countries, the use of conjugate Hib vaccine will not only prevent infection in the immunized children but will decrease the colonization of Hib in the pharynx, therefore, it blocks the transmission to other unimmunized children.6 However, even though reduced colonization may not persist with time, vaccinated children appear to respond with elevated and protective antibody titers upon acquisition.7,8

Four types of conjugate Hib vaccines are available on the market. They differ not only in the type of protein carrier but also in the length of polysaccharide chain, the ratio of the polysaccharide to protein and the type of covalent linkage. This difference is reflected on the pattern and persistence of post-vaccination antibodies which differ from one vaccine to another.9 Furthermore, simultaneous or prior vaccination with diphtheria or tetanus toxoid may modify anti-capsular antibody response to Hib conjugated with tetanus or diphtheria protein.10 The aim of this paper is to determine the immunogenicity of hemophilus influenzae type b oligosaccharide-CRM197 Conjugate (HbOC) when given at 6 weeks, 3 months and 5 months according to the established immunization schedule for diphtheria, tetanus and pertussis (DTP). This base data on immunogenicity is important for the implementation and evaluation of any vaccination program.

Methodology. Study design: The study was conducted in Saudi Arabia in three health centers. Two hundred and ten healthy infants were recruited for this study during their routine well-baby visit at 6 weeks. The sample size was calculated to end with 50 infants in three groups. After obtaining informed parental consent and normal physical examination, infants at the age of 6 weeks were allocated at random using the envelope method into three groups. The first group received HbOC (HibTITER, Lederle-Praxis in addition to DTP (Berna) and oral polio (SKF) which comply with vaccine specifications established by WHO and which are routinely used in Saudi

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Arabia. The second group received HibITITER in addition to DTP (TRI-IMMUNOL, Lederle and oral polio (ORIMUNE, Lederle). The latter two vaccines meet the release specifications required in the USA. The third group of children were not given Hib vaccine, but the usual vaccine (DTP Bema and OPV SKF). HibITITER vaccine was given concurrently with DTP, but in separate limbs, according to the routine immunization schedule in Saudi Arabia, 6 weeks, 3 months and 5 months of age. Two types of DTP and oral polio vaccines were used to study the differences in immunogenicity and possible effect, if any, on Hib antibody. Early studies carried out in USA with the Hib vaccine and the FDA formula of DTP and oral polio differs from the WHO formula used in the rest of the world. Only the serology results of Hib will be presented in this paper.

**Vaccines:** Hemophilus influenzae type b obiligosaccharide-CRM197 conjugate (HbOC). The HbOC (HibITITER) was developed and manufactured by Lederle-Praxis Biologicals, Sanford, NC. Two lots of vaccines were used in the study (M180HP and M150JC). Each 0.5 ml dose contained 10 μg of H influenzae type b capsular saccharide and 25 μg of CRM197. CRM197 is nontoxic diphtheria toxoid variant.

**Serology:** A minimum blood sample of 3 cc was collected one month after the third dose of vaccine from the children. Sera were identified by bar code and quantitative anti-PRP antibody determination was performed on each coded sample in Lederle-Praxis Laboratories in Rochester, NY by the method described by Phipps et al. Results were expressed in μg/mL based on the US FDA standard at 70 μg/mL. Sub-sample of the coded serum specimens were analyzed in the CDC, Diseases Immunology Section (George M Carlone, PhD). A correlation coefficient between Lederle-Praxis and CDC results was 0.97.

**Statistical analysis:** T-test and analysis of variance were performed on the logarithmic transformation of antibody levels and data was presented using geometric mean of the titer (GMT). Chi-square test was used to compare proportion of children with the protective titers in the three groups. One μg/mL level of antibody was used as the post-vaccination protective level while 0.15 μg/mL was used as a positive indication for maternal antibody in the control group.

**Results.** Table 1 shows the number of children who completed the study and the number of blood samples collected after the third dose. The dropout (almost 30%) was mainly due to blood extraction. It shows also, anti-PRP post vaccination titer compared to the control group.

In the control group, 30% (18-42%, 95% CI.) of children do not have the protective maternal antibody level (< 0.15 μg/ml) at the age of 5 months. No statistical difference was found in the post-3rd dose GMT of HbOC when given with FDA DTP (14.48 μg/ml) or with WHO DTP (13.3 μg/ml).

**Discussion.** This study shows that 30% of Saudi infants at the age of 6 months in our sample are lacking the natural maternal protecting levels (0.15 μg/ml) against Hib infection. The 95% confidence interval is 18-24% which is strong evidence for the need of immunization. In our sample, 92% in the first group and 94% in the second group acquired a level of 1 μg/ml or more after the third dose of vaccination which is recognized as the protective level after vaccination. The immunogenicity of HbOC after 3 doses, 14.4 μg/ml, appears higher than recent data reported from USA, 4.42 μg/ml, but comparable to figures which have been reported previously, 16.8 μg/ml. This phenomenon has been observed recently for all Hib vaccines in countries using the vaccine on a wide scale. This may be attributed to the overall decrease in the exposure of infants to Hib infection and accordingly to a reduction in both natural priming and boosting of the vaccine. This should be taken into consideration when we compare immunogenicity between different stages of vaccine implementation in different countries. Different degrees of natural exposure to Hib alone may not explain the difference in the immunogenic response in different populations. Other geographic or genetic factors may contribute to the response of Hib vaccine. This supports the importance of a base line immunogenicity study before starting the vaccine in any country. This will also affect the persistence of antibodies and the time of booster dose.

From our study and based on the excellent and comparable immunogenicity to other populations following two and three doses of HbOC, protection is expected to be conferred. Previous experience with the vaccine showed that vaccine provides better than expected protection against Hib than immunogenicity data, probably by reducing transmission to unvaccinated children.

In our study, the formula or immunogenicity of DTP did not affect the GMT of anti-PRP. Priming within a certain interval is more likely than co-administration of the carrier protein vaccine to enhance the response to PRP. The effect of priming with different DTP formula can be studied to evaluate the enhancement on PRP response if any. The study setting should always be taken into consideration during the interpretation. Incidence of Hib infection is very difficult to obtain in developing countries. Other epidemiological parameters of the disease like proportion of Hib meningitis ≤2 years, ratio of epiglottitis to meningitis and other risk factors for infection can be used to reflect the size of the problem by comparing these data to other countries. HbOC is highly immunogenic in Saudi children and comparing our results to other countries it is expected to be highly effective. Inclusion of Hib vaccine in the extended program is needed where almost one third of children are susceptible to Hib infection at 5 months of age.

**Table 1 - Antibody against Hib polysaccharide in different groups after the third dose of vaccine.**

<table>
<thead>
<tr>
<th></th>
<th>Hib-fDA</th>
<th>Hib-WHO</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of infants</td>
<td>50</td>
<td>47</td>
<td>53</td>
</tr>
<tr>
<td>GMT μg/ml</td>
<td>14.48</td>
<td>13.3</td>
<td>0.2</td>
</tr>
<tr>
<td>95% CI</td>
<td>9.1-23.03</td>
<td>7.9-22.28</td>
<td>0.15-0.25</td>
</tr>
<tr>
<td>%≥0.15 μg/ml</td>
<td>100%</td>
<td>100%</td>
<td>70%</td>
</tr>
<tr>
<td>%≥1.0 μg/ml</td>
<td>92%</td>
<td>94%</td>
<td>4%</td>
</tr>
</tbody>
</table>

T-Test unpaired
(Hib-fDA) Vs (Hib-WHO) 0.807
(Hib-fDA) Vs CONTROL 0.0001
(Hib-WHO) Vs CONTROL 0.0001
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References