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Immunogenicity of a *Haemophilus influenzae* type b–tetanus conjugate vaccine when administered separately or in combined vaccines for primary immunization in two consecutive national schedules in Turkey☆

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SUMMARY

Background: In Turkey, the *Haemophilus influenzae* type b–tetanus toxoid conjugate vaccine (Hib) was replaced by the combined diphtheria–tetanus–acellular pertussis and inactivated polio vaccine (DTaP–IPV/Hib) in 2008. This shift to the new schedule created different cohorts of vaccinated children as a consequence of the different schedules used. We evaluated the immunogenicity of the Hib vaccine in infants vaccinated with these different schedules.

Methods: Three groups of children were evaluated: group 1 comprised 145 infants vaccinated with diphtheria, tetanus, and whole cell pertussis (DTwP), oral polio vaccine (OPV), and Hib vaccines simultaneously at separate sites; group 2 comprised 204 infants vaccinated with the DTaP–IPV/Hib combined vaccine; group 3 comprised 100 infants vaccinated with a mixed schedule of DTwP, OPV, and Hib for the first one or two doses, followed by DTaP–IPV/Hib vaccine to complete the series.

Results: Anti-polyribosylribitol phosphate (anti-PRP) titers ≥0.15 μg/ml were similar in groups 1, 2, and 3. However, in group 1, who received all the vaccines at separate sites, ≥ l.0 μg/ml long-lasting antibody titers and anti-PRP geometric mean titers were higher ($p = 0.001$).

Conclusion: This study showed that even one dose administered in combination with other vaccines in a primary series decreased the level of anti-PRP.

1. Introduction

In 2007, the *Haemophilus influenzae* type b–tetanus toxoid vaccine (Hib; Act-Hib®) was added to the national vaccination schedule for infants in Turkey. Over a 1-year period, in addition to live oral poliovirus vaccine (OPV), infants were administered diphtheria, tetanus, and whole-cell pertussis (DTwP) and Hib vaccines as two simultaneous injections at separate sites as part of their primary vaccination course. In 2008 the combined diphtheria–tetanus–acellular pertussis, inactivated polio vaccine and conjugated Hib capsular polysaccharide (DTaP–IPV/Hib; Pentaxim®) vaccine became part of the national childhood immunization program.

In many countries, the incidence of invasive Hib disease among children aged <5 years declined markedly after the inclusion of Hib conjugate vaccines into the infant vaccination schedules.[1] However a reduction in anti-polyribosylribitol phosphate (anti-PRP) titers was observed when Hib conjugate vaccine was administered in a single injection with diphtheria–tetanus–acellular pertussis (DTaP)[2–5] or DTwP[6] when compared with the responses of infants who received these vaccines simultaneously but at separate sites. Another study showed that concurrent IPV seemed to interfere with the anti-PRP response to the DTaP/Hib vaccine.[7] In other studies, antibody titers to PRP in infants who had received the Hib and DTwP[8] or DTaP[9,10] vaccines separately or in combined vaccines were similar. The clinical significance of antibody levels is not yet known.

The change in routine Hib vaccination scheme from separate vaccine administration to the combined form created different cohorts of children in Turkey: (1) those who were vaccinated with the separate Hib vaccine in 2007; (2) those who had their initial

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vaccines in 2007 as separate vaccines, but who continued in 2008 with the combined vaccine, and (3) those born in 2008 who were vaccinated with the combined form for all primary doses. The immunogenicity of these schedules might differ from each other. Differences between the cohorts in relation to the risks of invasive Hib disease may be revealed by invasive Hib disease surveillance. To interpret the outcome of any surveillance, the initial immune responses of the combined or simultaneous vaccination schemes should be known. Therefore in this study we evaluated the immunogenicity of the Hib vaccine among these three cohorts of children vaccinated with the different schedules.

2. Materials and methods

2.1. Study population

The study was conducted between May 1, 2008 and April 30, 2009. All healthy infants aged 12 months who attended the Gazi University Faculty of Medicine Well Child Clinic within this period were enrolled in the study after obtaining written informed consent from the parents. All infant primary vaccinations had been completed at the same clinic 6 months before they were enrolled in the study. Those who had a chronic disease or who had been administered an immunosuppressive therapy were excluded.

One hundred and forty-five infants were vaccinated with DTwP, OPV, and Hib (Act-Hib) vaccines simultaneously at separate sites and formed group 1; 204 infants were vaccinated with the DTaP–IPV/Hib combined vaccine (Pentaxim) at 2, 4, and 6 months of age and formed group 2; 100 infants were vaccinated with a mixed schedule (first one or two doses administered as DTwP + OPV + Hib and the primary series completed with DTaP–IPV//Hib) and formed group 3. Hence, a total of 449 infants were included.

The study protocol was approved by the Ethics Committee of Gazi University Medical Faculty before the study was started, and procedures complied with the latest version of the Declaration of Helsinki.

2.2. Vaccines

In 2007, infants were vaccinated with DTwP vaccine (Triple Antigen; Serum Institute of India), the commercial Hib vaccine (Act-Hib; Pasteur Mérieux Serums et Vaccins, France), and OPV (Sabin Trivalent; Bio Farma, Indonesia). In 2008, the DTaP–IPV/Hib vaccine (Pentaxim; Pasteur Mérieux Serums et Vaccins) was applied. Parenteral vaccines were injected intramuscularly into the thigh, and OPV was administered by the oral route.

The DTwP vaccine contains diphtheria toxoid $\leq$25 Lf, tetanus toxoid $\geq$5 Lf, and pertussis toxoid $\geq$4 IU adsorbed on aluminum phosphate $\geq$1.5 mg, with thiomersal 0.01% as preservative. OPV contains poliovirus not less than 106.0 $CCID_{50}$ (50% cell culture infective dose) for type 1, 105.0 $CCID_{50}$ for type 2, and 105.8 $CCID_{50}$ for type 3. Hib vaccine contains 10 μg of polyribosylribitol phosphate covalently linked to 20 mg of tetanus toxoid (PRP–T) and was supplied in the lyophilized form in single-dose vials. Reconstitution was carried out at the time of injection with 0.5 ml of diluent (saline 0.4% w/v).

DTaP–IPV/Hib contains diphtheria toxoid $\geq$30 IU, tetanus toxoid $\geq$40 IU, adsorbed pertussis toxoid 25 μg, filamentous hemagglutinin 25 μg, inactivated poliovirus type 1 (40 DAgU), inactivated poliovirus type 2 (8 DAgU), and inactivated poliovirus type 3 (32 DAgU), and PRP–T 10 μg vaccine in lyophilized form, reconstituted at the time of injection.

2.3. Serology

Blood samples were drawn 6 months after the third dose, when all infants were 12 months of age. Serum was promptly prepared and stored at −20 °C until blinded serological analyses were performed. Concentrations of PRP antibodies were assayed using the micro enzyme immunoassay (EIA) method (VaccZYME Hib IgG enzyme immunoassay kit; The Binding Site Ltd, UK) and expressed in μg/ml. The assay cut-off was set at 0.15 μg/ml. Geometric mean antibody titers (GMT) were calculated for each group, with concentrations below the cut-off being attributed a value of one-half of the cut-off value.

2.4. Statistical analysis

The one-way analysis of variance (ANOVA) test was used for comparisons of the antibody levels between the three groups after logarithmic transformation. A p-value of <0.05 was considered to be statistically significant. Tukey's HSD test was used as a post-hoc test for multiple comparisons when the one-way ANOVA test revealed statistically significant differences between the three groups.

3. Results

The anti-PRP antibody responses of the 449 infants are shown for each group in Table 1. Six months after the third dose of vaccine, over 90% of children in each group attained seroprotective levels of PRP antibody ($\geq$0.15 μg/ml). The highest positivity rate was seen in group 1 (95.9%); in the mixed group it was lower compared to group 1 (93%), and the rate was lowest in the combined vaccine group (91.2%). These differences were not statistically significant ($p = 0.235$).

Two hundred and thirty-nine (53.2%) of the infants had antibody levels considered to confer long-term protection ($\geq$1.0 μg/ml). Group 1, who received all the vaccines separately, appeared to have long-lasting antibody levels higher than the other groups ($p = 0.001$).

The anti-PRP GMT values differed significantly between group 1 and the other groups. Group 1, who received all the vaccines separately, appeared to have antibody levels higher than group 2

Table 1
Anti-PRP levels 6 months after primary vaccination

Anti-PRP IgG (μg/ml)	DTwP + OPV + Hib[a] ($n = 145$), n (%)	DTaP–IPV/Hib[b] ($n = 204$), n (%)	Mixed[c] ($n = 100$), n (%)	p-Value
$\geq$0.15[d]	139 (95.9)	186 (91.2)	93 (93)	0.235
$\geq$1.0[e]	101 (69.7)	91 (44.6)	47 (47)	= 0.001
GMT (μg/ml)	1.87	0.90	0.93	= 0.0001[f]

Anti-PRP, anti-polyribosylribitol phosphate; GMT, geometric mean antibody titer.
[a] DTwP + OPV + Hib: diphtheria, tetanus and whole-cell pertussis and oral poliomyelitis and *Haemophilus influenzae* type b–tetanus toxoid vaccine.
[b] DTaP–IPV/Hib: diphtheria–tetanus–acellular pertussis and inactivated polio vaccine/*Haemophilus influenzae* type b–tetanus toxoid vaccine.
[c] Mixed group: DTwP + OPV + Hib or DTaP–IPV/Hib.
[d] Seroprotective level.
[e] Long-term protection level.
[f] Logarithmic transformation was applied to the antibody titer values for the statistical analysis.

($p = 0.0001$) and group 3 ($p = 0.001$). There was no statistically significant difference between the antibody levels in group 2 and group 3 ($p = 0.913$).

4. Discussion

Our study aimed to evaluate the immune response elicited by a conjugated Hib vaccine at 6 months after completion of the primary vaccination series, in infants who were vaccinated with different schedules over consecutive years, either associated or combined with other pediatric vaccines used routinely in our country (namely DTwP, DTaP, and IPV or OPV). As in many previous studies,[2–5] we also observed some differences with regard to the GMT values of PRP antibodies, with a tendency for a stronger response in the group who received DTP, OPV, and Hib vaccines separately.

An anti-PRP concentration $\geq$0.15 μg/ml has been suggested to be seroprotective.[11] Despite the observed differences in antibody levels 6 months after primary immunization, over 90% seroprotection rates were obtained for all groups.

Concentrations $\geq$1.0 μg/ml are reported to correlate with long-term protection.[11] In the present study, and in many others,[3–5,2] it was found that high antibody concentrations ($\geq$1.0 μg/ml) were obtained in the group who received all vaccines separately. This finding may indicate that the protection of Hib vaccine will be short when the vaccine is applied combined with other vaccines. This study revealed that both GMT values and antibody levels $\geq$1.0 μg/ml were decreased in the combined schedule, even if not all of the primary doses were administered in the combined form. Although the risky age for Hib infections in children is relatively short and long-term immunity may not be necessary, there is still insufficient information regarding the requirement for long-lasting antibody levels. Long-lasting antibody levels may be of particular importance for children who go on to develop secondary immune deficiencies.

The reduced anti-PRP antibody titers observed when the Hib conjugate vaccine was administered in a single injection with DTaP and IPV were reported not to be associated with an increased number of invasive Hib cases.[12] Greenberg et al. recently reported that incidence rates of invasive Hib disease among Canadian children aged <5 years declined markedly after the introduction of Hib conjugate vaccines, and the disease has remained under control with exclusive use of DTaP–IPV/Hib vaccine.[1] On the other hand McVernon et al.[13] retrospectively compared vaccine formulations given to fully vaccinated Hib cases with those administered to fully immunized age-matched controls using conditional logistic regression. More cases than controls had received all three doses of the infant primary course as DTaP–Hib, compared with two or three doses of Hib vaccine (conditional odds ratio 6.77, 95% confidence interval 3.26–14.07).

The factors responsible for the reappearance of invasive Hib disease cases in vaccinated children are not clear. Breukels et al. studied the anti-Hib antibody production both quantitatively and qualitatively in 12 patients who experienced Hib failure. Both anti-Hib antibody concentration and immunoglobulin-G_2 anti-Hib antibody avidity were significantly lower in patients who experienced Hib failure, at the onset of disease and after convalescence, when compared with controls. This finding suggests that the patients who developed invasive Hib disease, despite having received three or four Hib conjugate vaccinations, were inadequately primed by these vaccinations.[14] Lee et al. performed a retrospective study on 251 Hib vaccine failures and reported that children who experienced Hib disease despite vaccination appeared to have a defect in immunological priming, leading to a qualitative difference in Hib-specific memory B cells. Low anti-PRP antibody avidity decreases the functional activity of anti-PRP antibody in the serum of these children experiencing vaccine failure, leading to disease.[15] Eskola et al. also suggested that the lower antibody responses were not associated with impaired function of the antibodies induced, nor, and possibly more importantly, with the induction of immune memory against Hib.[16] Some studies have demonstrated the development of anti-PRP immune memory at an early age, after completion of a three-dose primary vaccination course of combined DTaP–IPV/Hib vaccine.[10,17] Many investigators believe that immune memory is most important, and even if the combined vaccines for primary immunization caused a decrease in anti-Hib antibody response after primary immunization, higher antibody responses to booster doses in children vaccinated with combined vaccine can be considered to be protective for invasive Hib disease. Nevertheless the duration of protection is not known accurately and continued surveillance of invasive Hib infections among children who have been vaccinated with different schedules can guide new vaccination strategies. Indeed, an 18-month-old infant vaccinated with combined vaccine was reported to have invasive Hib infection although he had no immune deficiency.[18]

It is well documented that Hib vaccine provides higher levels of PRP antibodies when administered as a separate antigen. Previous studies have compared separate vaccination with combined vaccination for all primary series. Our study also included a group consisting of children who received both separate and combined vaccination for the primary series. The results indicate that even one dose of combined vaccine lowers the level of antibody against PRP–T.

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Conflict of interest: No conflict of interest to declare.

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