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Immunogenicity of two diphtheria-tetanus-whole cell pertussis-hepatitis B vaccines in infants

A comparative trial

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Aim: Because of the high mother-to-infant transmissibility of hepatitis B (HB) infection, neonatal vaccination is necessary, but the further doses of HB vaccines can be combined with conventional diphtheria-tetanus-whole cell pertussis (DTPw) vaccines. We compared immunogenicity and reactogenicity of two tetravalent vaccines in Indian children, who after neonatal HB immunization, were vaccinated thrice with one of these vaccines.

Results: Out of the 250 subjects who completed the study, 123 received the Indian and 127 the European vaccine. After 3 doses, the seroprotection/seropositivity rates were 99% and 100% for diphtheria, 98% and 95% for tetanus, 89% and 94% for pertussis, and 100% and 100% for hepatitis B, respectively. GMT of tetanus antibodies was significantly higher with the Indian vaccine. Low-grade reactogenicity was rather similar in the two vaccine groups, the most common events being local pain, redness, swelling, fever, irritability, unusual crying, drowsiness and non-specific gastrointestinal symptoms.

Methods: In this open-label randomized study, 287 infants received a dose of an Indian-(Q-Vac™) or European-made (Tritanrix-HB™) tetravalent vaccine at age 6, 10 and 14 weeks. The ELISA antibodies were measured prior to the first and one month after the third dose. Immunogenicity was determined by measuring the seroprotection/seropositivity rates and geometric mean titers (GMT), whereas vaccine reactogenicity was elucidated with diary cards for 7 d following each dose. The potential unsolicited events were queried throughout the whole 3-mo study period.

Conclusions: Since both immunogenicity and reactogenicity of the two vaccines were almost identical, the Indian vaccine poses a good alternative to the costlier competitor vaccines.

Introduction

Despite the wide availability of effective vaccines, hepatitis B (HB) is still of a major concern globally. By 2004, almost one third of the world's population was infected and 350 million suffered from chronic infection.[1] The annual mortality due to HB complications (chronic hepatitis, cirrhosis and hepatocellular carcinoma) range from 0.5 to 1.2 million, making it the 10th leading cause of death worldwide. In contrast to the western countries, chronic HB in Asia and most of Africa is common and usually acquired perinatally or in childhood.[2]

In India, perinatal transmission contributes significantly to HB cases.[3-5] Globally also, perinatal transmission may account for 15% of HBV-related deaths, even in low-endemic areas. Thus, there is a strong need for HB vaccination in the developing countries. Since the World Health Organization (WHO) recommends routine vaccination for all infants, HB immunization should become an integral part of the national schedules.[6] Because of the high mother-to-infant transmissibility, neonatal HB vaccination is necessary soon after birth, but the further doses can be combined with other vaccines.[6]

In most developing countries, diphtheria-tetanus-whole cell pertussis (DTwP) vaccines, given at age 6, 10 and 14 weeks, compose a part of the Expanded Programme of Immunization (EPI). The options for HB vaccination are 3 doses, each administered at 0, 6 and 14 weeks, at 6, 10 and 14 weeks, or four doses at 0, 6, 10 and 14 weeks. The four-dose schedule may be accomplished by using a monovalent vaccine, or combined with, for example, DTPw and/or *Hemophilus influenzae* type b (Hib).[6]

Because the whole cell pertussis vaccine is considerably cheaper than acellular, we setup a study in which a low-cost Indian DTwP-HB vaccine was put face-to-face against an European competitor vaccine. Especially, we were interested in the immunogenicity and reactogenicity of these products. All infants had already received the first dose of HB vaccine at birth.

Results

Out of the total of 287 enrolled subjects, 1 child interrupted the study after the first dose, 3 children were lost from follow-up, serum samples were lost by leakage or breakage of the tube in 6, the sample was inadequate in 14, while 13 children proved

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Table 1. Proportion of seroprotection/seropositivity

Seroprotection levels	Indian vaccine (Q-Vac™) (n = 123)		European vaccine (Tritanrix-HB™) (n = 127)	
	Pre (%)	Post (%)	Pre (%)	Post (%)
Diphtheria (≥0.1 IU/ml)	35 (29)	122 (99)	39 (31)	127 (100)
Pertussis (>30 FDA-U/ml)	15 (12)	109 (89)	24 (19)	119 (94)
Tetanus (≥0.1 IU/ml)	123 (100)	121 (98)	126 (99)	121 (95)
Hepatitis B (≥10 mIU/ml)	34 (28)	123 (100)	26 (21)	127 (100)

Table 2. Geometric mean titres

Component	Item	Indian vaccine (n = 123)		European vaccine (n = 127)	
		Pre	Post	Pre	Post
Diphtheria	GMT (IU/ml)	0.12	3.83	0.11	3.62
	95% CI	0.10–0.13	3.43–4.27	0.10–0.14	3.26–3.99
Pertussis	GMT (FDAU/ml)	23.63	184.71	27.61	233.69
	95% CI	17.37–31.62	151.35–218.77	21.87–33.88	190.54–281.83
Tetanus	GMT (IU/ml)	3.95	18.45	3.63	14.36
	95% CI	3.38–4.57	15.84–20.89	3.09–4.16	12.30–16.59
Anti-HBs	GMT (mIU/ml)	5.69	1022.76	6.43	1019.77
	95% CI	3.01–10.47	912.01–1122.01	3.23–12.30	933.25–1096.47

HBsAg positive. Thus, 250 vaccinees remained to be analyzed; 123 had received the Indian and 127 the European vaccine. Of them, 48% and 57% were males, and the mean age was 46 d (±3.6 d) and 46 d (±3.4 d), respectively.

The baseline antibody levels were rather similar in both vaccine groups. After 3 doses, the seroprotection rate against diphtheria was 99% in the Indian and 100% in the European vaccine group. For pertussis, the rates were 89% and 94%, for tetanus, 98% and 95%, and for hepatitis B 100% and 100%, respectively. All these differences between the two groups were insignificant. The details are given in **Table 1**.

The GMTs are given in **Table 2**. Initially, GMTs for all 4 antibodies were comparable. The 3 vaccine doses elicited a significant response for all antigens in both groups, between which the only difference was in tetanus antibodies: 18.45 IU/ml vs. 14.36 ($p < 0.05$) in recipients of the Indian vs. European vaccine, respectively.

Of the 287 subjects who had received ≥1 vaccine dose, local adverse reactions like pain, redness and swelling were commonly reported, in both, Indian and European vaccine recipients. The cumulative incidence rate for all three doses was as follows; pain was reported in 62% vs. 64%, redness in 32% vs. 31%, swelling in 34% vs. 34%, respectively. Four nodules were reported. As far as solicited systemic adverse reactions are concerned, fever was found in 18% vs. 16%, irritability in 44% vs. 41%, unusual crying in 42% vs. 43%, drowsiness in 9% vs. 12%, anorexia in 7% vs. 5%, vomiting in 3% vs. 3% and diarrhea in 2% vs. 2%, respectively.

Some unsolicited events, including solicited reactions occurring beyond 7 d follow up period, were reported in both the groups. These were cough and cold (2% vs. 0.69%), common cold (0.23% vs. 0.46%), constipation (0.23% vs. none), seborrheic dermatitis on face (0.23% vs. none), fever (0.23% vs. 0.92%), unusual crying (none vs. 0.23%), vomiting (7% vs. 7%), diarrhea (4% vs. 3%), anorexia (4% vs. 5%) and drowsiness (13% vs. 9%), respectively, in the Indian and European vaccine arms. No unsolicited event that one would put in a causal association with either vaccine was reported and all resolved without any sequelae.

One girl developed high fever 7 d after the 2nd dose of the European vaccine, was hospitalized and recovered within 3 d without special treatment. The reason remained open. The event was not deemed causally related to the vaccine.

Discussion

A number of logistic, economic and other reasons justify the use of the combination vaccines,[7] specially that—with a few exceptions—immunogenicity of the various vaccine antigens is not impaired and reactogenicity is not much increased.[6,8-10] With this background we were happy to find that the Indian-made tetravalent DTwP-HB combination competed well in these respects with the European vaccine. In fact, GMT of anti-tetanus antibodies was higher with the Indian vaccine.

Our vaccinees had already rather high anti-tetanus antibody levels prevaccination—the India's Universal Immunization program (UIP) recommend 2 doses of tetanus vaccine to all pregnant women—but still GMT increased from baseline 3.95 and 3.63 IU/ml to 18.45 and 14.36 IU/ml in recipients of the Indian or European vaccine, respectively.

Neonatal HB vaccination, obviously, did not interfere with later antibody production since the seroresponse was good with both vaccines. Also, reactogenicity was very low. The immunological results are in line with earlier studies on DTPw-HB.[8-10] In Czech Republic, a new DTPw-HB vaccine

(GSK Biologicals Gödöllö, Hungary) was administered at age 3, 4 and 5 mo, and the seroprotection rates were 100%, 99%, 95% for diphtheria, tetanus and hepatitis B, respectively, seropositivity to *B. pertussis* being 99%.[8] Virtually a 100% response was observed also in Lithuania, when the same European vaccine we used here was tested; the schedule in Lithuania was 0–1.5–3 mo, beginning at 3–4 mo of age.[9] In Spain, all subjects responded well, when a tetravalent vaccine (SmithKline Beecham Biologicals, Belgium) was given at 3, 5 and 7 mo of age.[10] However, opposite to our study, neonatal HB vaccination was not accomplished in these studies.

More than 80 countries give HB vaccine at birth. However, the schedule is completed with 2 more doses (at varying intervals) to 3 more doses (17 countries). These are given as separate shots, or combined with components such as DTP/Hib/IPV (following the birth dose).[11] We are convinced that using either one of the tetravalent vaccines we tested here is a good continuation to neonatal monovalent HB vaccination.

All the subjects in our study received a birth dose of HB vaccines, the names of which are not available nor was it the aim of the study to categorize responses by vaccines.

Importance of perinatal transmission of HB cannot be overemphasized. In a study on agewise HBsAg prevalence in children in India, the prevalence rate was found 4.35% in less than 1 y age group.[12] In a study in Delhi, overall HBsAg positivity in children below five years was 2.25% which indicated that a large proportion of HBV infection in children of this age is acquired via vertical transmission.[3] It is estimated that about one-third of the adult asymptomatic HB carriers in India evolve directly from perinatal infection.[4] On the other hand, in a recent study on 4,000 pregnant women, 0.9% tested positive for HBsAg, of which 56.8% women were positive for HBeAg. Of the 25 babies delivered vaginally, 15 (60%) developed vertical transmission.[5] In any case, considering India's birth cohort, the actual numbers can be very high, which makes a strong case for protection at birth followed by routine three dose schedule, which seems to work as shown by the present study.

Although immunogenicity and reactogenicity of these vaccines are almost identical, the low cost (≈US $ 1.7 vs. US $ 4.5 are the maximum retail prices when enquired to an Indian stockist) of the Indian alternative makes it an especially attractive choice also in countries outside the developing world. As the vaccine is pre-qualified by the World Health Organisation for sale to the United nation's agencies for public health sector markets, the prices are still are more attractive for large volumes of vaccine, which can ensure a wider coverage.

Materials and Methods

This was a Phase IV, open label, randomized, 1:1 comparative clinical study among 287 normal healthy infants aged 6 to 8 weeks of either gender in India. For each subject, the study lasted for 3 mo.

Enrollment. The subjects were recruited through an oral campaign among parents who attended the well-baby clinic of King Edward Memorial (KEM) Hospital Research Centre, Pune, India. After obtaining an informed consent from the legal guardian, the children were screened. If found healthy, the infant was enrolled if he/she was of age 6 to 8 weeks, and had received HB vaccination at birth. The following criteria led to an exclusion from the study: HB surface antigen (HBsAg) seropositivity, participation in another clinical trial, use of any other investigational or non-registered drug or vaccine, history known hypersensitivity, family history of congenital or hereditary immunodeficiency, receipt of an immune-modifying agent, major congenital defect or chronic illness, an ongoing acute disease, known pulmonary, cardiovascular, hepatic or renal abnormality, thrombocytopenia or bleeding disorder and receipt of immunoglobulins or any other blood product.

Vaccination. The randomization number assigned, and the first blood sample taken, the child received the first vaccine dose intramuscularly in the anterolateral aspect of the thigh. The doses were administered at age 6, 10 and 14 weeks. As per the hospital policy, paracetamol syrup was given prophylactically after each dose.

Vaccines. The first DTwP-HB vaccine used was Q-VAC™ (Batch No. EU-60501-A and EU-60504-A), manufactured locally in India by Serum Institute of India (SIIL), Pune. Each dose of 0.5 ml contained diphtheria toxoid (≤25 Lf), tetanus toxoid (≥5 Lf), *Bordetella pertussis* (≥4 IU), purified HBsAg (≥10 μg), this being produced in *Hansenula Polymorpha* (yeast), adsorbed on aluminum hydroxide (0.53 mg. Al^{+++}) and having thiomersal ≤ 0.01 as preservative. Each vaccine vial contained 10 doses.

The other vaccine used was from Europe, Tritanrix-HB™ (Batch no. AT15B066AC and AT 15B350AA), manufactured by GlaxoSmithKline (GSK). Each 0.5 ml contained diphtheria toxoid (≥30 IU), tetanus toxoid (≥60 IU), *B. pertussis* (≥4 IU), and purified HBsAg (≥10 μg). The vaccine arrived in 1-dose vials.

Both vaccines are liquid and were stored in the refrigerator at +2°C to +8°C before use. The temperature was checked daily.

Serology. The second blood sample was collected one month after the third dose. The samples were coded and measured blindly by the Department of Microbiology, KEM Hospital Research Centre, Pune. Enzyme-linked immunosorbent assay (ELISA) was used to measure the total levels of anti-diphtheria, anti-tetanus, anti-pertussis and anti-HBs antibodies. Commercial ELISA kits of Virion/Serion GmbH were used for the anti-diphtheria, -pertussis and -tetanus antibodies, while the DiaSorin kits were used for measuring the anti hepatitis-B antibodies. The seroprotection level for diphtheria and tetanus was any titer ≥ 0.1 IU/ml, this for HB being anti-HBs titer of ≥10 mIU/ml. For pertussis, a titer >30 FDA-U/ml was taken as seropositive, while the titers <20 FDA-U/ml were deemed seronegative.

Follow-up. After vaccination, the children were observed for 30 min for potential immediate reactions. The study personnel advised the parents/legal guardians how to document at home adverse events in diary, should they develop. The recording lasted for 7 d after each of the 3 doses. On the 3 visits to the vaccination site (hospital), the diaries were checked, and the children were examined physically. The information obtained was transcribed in the case record forms. In all, the follow-up was 3 mo.

All solicited events were graded on the basis of predefined severity criteria.

Ethical aspects. The study was approved by the institutional ethics committee of the KEM Hospital Research Center, Pune, and was conducted according to the Declaration of Helsinki, International conference on harmonization (ICH) Good clinical practices guidelines, and the Indian regulatory guidelines. An information sheet in vernacular languages was given to parents/legal guardians. Only children whose parents/legal guardian consented in writing were enrolled.

Statistical analysis. The study was designed as a one-sided non-inferiority trial. The sample size was based on the formula given by Jones et al.[13] ($n = [2p\{100 - p\} \{z[1 - (\alpha)] + z(1 - \beta)\}2]/\Delta^2$). The primary endpoint was the percentage of the seroprotection/seropositivity, and the reactogenicity rates for each of the test vaccines after 3 doses. For a significance level of 5% (i.e., $\alpha = 0.05$), 80% power ($1 - \beta = 0.80$), and 7% delta (Δ) for all components except pertussis ($\Delta = 8\%$), we arrived at a total of 250 evaluable subjects. Expecting 15% of the vaccinees to drop out, we recruited 287 children in the study.

The postvaccination seroprotection/seropositivity rates were compared by chi-square test. When calculating geometric mean titers (GMT), using unpaired t-test, all negative values were taken as zero and were excluded from analysis. A p value ≤ 0.05 was considered significant. All the solicited reactions occurring with 7 d of vaccination were calculated and an average was presented by percentage with respect to the total number of doses administered. Reactogenicity was analyzed on the intention-to-treat and immunogenicity on the per-protocol basis, these latter including only subjects who had received all 3 doses, completed the study protocol, and gave both blood samples.

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Author Contributions

Kulkarni P.S. designed and supervised most of research and wrote the paper, Amita Sapru, Ashish Bavdekar and Anand Pandit performed most of research, S.S. Naik performed the antibody assay, Moreshwar Patwardhan monitored the study and Prajakt Barde analyzed data.

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