RESEARCH PAPER

Immunogenicity and Safety of Three WHO Prequalified (DTwP -HB-Hib) Pentavalent Combination Vaccines Administered As Per Iranian National Immunization Plan in Iranian Infants: *A Randomized, Phase III Study*

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Background: The pentavalent vaccine Pentavac was officially introduced in the Iranian National Immunization Plan in November, 2014.

Objective: To compare the immunogenicity and safety of Pentavac vaccine (Serum Institute of India Ltd.) with two other pentavalent vaccines available in Iran, i.e., Pentabio (PT Bio Farma (Persero)) and Shan 5 (Shantha Biotechnics Ltd.).

Design: Randomized, phase III study.

Participants: 900 infants attending the study sites to receive the vaccine at 2, 4, and 6 months of age.

Intervention: Infants were randomly assigned to one of the Pentavac, Pentabio, and Shan 5 vaccine groups.

Outcomes: The antibody titers were measured against five antigens, diphtheria, tetanus, pertussis, *Haemophilus influenzae B*, and hepatitis B before receiving the first dose and one month after the last dose. The adverse events following vaccination after each dose were recorded in the adverse events diary.

Results: All vaccines showed similar immunogenicity against four of the five antigens except pertussis. While vaccination with Shan 5 resulted in the highest immunogenicity against pertussis, Pentabio was significantly lower than the other two vaccines (P<0.001). The incidence of local adverse events significantly differed among the three vaccine brands (P<0.001), but the incidence of most of the evaluated systemic adverse events was similar (P>0.05).

Conclusion: Pentavac and Shan 5 had similar immunogenicity, the former having better immunogenicity against pertussis than Pentabio. Pentavac and Pentabio had a comparable safety profile.

Keywords: *Adverse effect, National immunization program, Vaccination.*

Clinical Trial Registration: IRCT2015051222234N1

The use of combination vaccines to immunize children against several diseases simultaneously is a well-known strategy to increase vaccine coverage in the pediatric vaccination program [1]. These vaccines have benefits such as reduced injection number, low cost, and better patient compliance. It is also essential to ensure that adding different components does not change the efficacy, safety, or immunogenicity of each component [2].

Diphtheria (D), tetanus (T), and pertussis (P) antigens are critical components of the World Health Organization's Expanded Program on Immunization (EPI) [2]. In 1992, the World Health Organization (WHO) recommended that the hepatitis B (HB) vaccine be added to the EPI and later recommended the *Haemophilus influenzae B* (Hib) vaccine be included in the pediatric vaccination program in all countries after six weeks of age. The administration of Hib conjugate vaccine led to a decrease of over 90% in the prevalence of severe Hib diseases in countries with universal coverage of the vaccine.

The pentavalent vaccine officially entered the Iranian National Immunization Plan (NIP) in November, 2014 as a DTwP-HB-Hib vaccine administered at the age of 2, 4, and 6 months [3]. The adverse events and immunogenicity of Pentavac (Serum Institute of India Ltd.) were evaluated in two studies in 2017 [3] and 2018 [4], respectively, indicating

that Pentavac was safe, although it did not induce proper immunogenicity against pertussis. Arjamand, et al. [5] indicated acceptable seroprotection against HB by Pentavac six months after three doses of vaccination.

Pentavalent vaccines such as Pentabio [PT Bio Farma (Persero)] and Shan 5 (Shantha Biotechnics Ltd.) have been produced by various countries [6,7]. A study conducted in Indonesia showed that Pentabio is as immunogenic and safe as the Hib monovalent vaccine given simultaneously with DTwP-HB [8]. However, due to lack of research on comparing Pentavac vaccine in Iranian children with other pentavalent vaccines [9-15], this study was designed to evaluate and compare the immunogenicity and safety of Pentavac vaccine with two other pentavalent WHO-prequalified vaccines, Pentabio and Shan 5 [13].

METHODS

In this prospective, randomized, double blind, multicenter, phase III study, we enrolled healthy infants 50 to 70 days of age, born after full-term pregnancy with birth weight ≥2.5 kg, who had not received previous doses of Hib, HB, or DTP vaccines. The protocol and informed consent form were approved by the institutional review board of the study center, and the protocol of the study was registered in the Iranian Registry of Clinical Trials. Written informed consent was taken from one of the parents.

Eligibility of participants was assessed using the infant's health documents archived in the health centers or by interviewing the parents. The exclusion criteria were axillary temperature >37.1°C on the day of inclusion, current or planned involvement in another clinical trial during the clinical trial period, mother with known history of human immunodeficiency virus infection, known immunodeficiency or immunosuppressive conditions, history of blood transfusion or use of blood products or immunoglobulin use since birth, acute symptoms or severe chronic illness that could interfere with conduct or completion of the trial, hypersensitivity to any of the vaccine components, any contraindication to intramuscular injection, and use of any vaccine or research drug other than that of the study during the study period or 30 days before inclusion in the study except for oral polio vaccine, which was allowed at 2, 4 and 6 months of age, along with the study vaccines.

Six health centers in different districts of Tehran city were selected for participant enrolment. The brand of pentavalent vaccine was selected randomly for each health center using block randomization method. The same brand was used for immunization of the study participant in all the three immunization visits. The study was performed between September 2019 and October 2020.The investigated vaccines were Pentavac, Pentabio, and Shan 5.

The participants received intramuscular injection of one of the pentavalent vaccines into the anterolateral aspect of their right thigh at 2, 4, and 6 months of age. All the vaccine vials were covered with the same coating to ensure similar appearance to enable blinding. The parents and laboratory technicians were blinded to the type of vaccine each participant received.

A researcher-made diary was used to record the adverse events of the vaccine [3,7,9]. The parents were taught how to complete the side effect diary. Adverse events were categorized as local adverse events (redness, pain, stiffness, warmth, injection-site lesion/abscesses), and systemic adverse events (fever, drowsiness, skin allergies, lymphadenitis, paralysis, loss of appetite, diarrhea, vomiting, rhinorrhea, cough, asthma, encephalitis, toxic shock syndrome, hospitalization, and death).

Blood samples were collected prior to the first dose of study vaccine and 28 days after the third dose to assess antibody responses. Blood specimens were maintained in a sterile capped test tube and transferred to the main laboratory within 4 hours for serum separation. Serum samples were maintained at -70°C until enzyme-linked immunosorbent assay (ELISA) was done to determine antibody titers.

IgG titers for the DTP and Hib components were determined by ELISA kits (Demeditec). HBV antibodies were measured using ELISA kits (Antisurase, General Biologicals). The cut-off value for seroprotection against diphtheria and tetanus was ³0.1 IU/mL. AntiHBs ³10 mIU/mL was considered protective for Hepatitis B. For HiB, antibody titer ³0.15 g/mL was considered to provide short-term protection, and ³1.0 g/mL was considered to provide long-term protection.

Pertussis specific IgG antibodies (anti-pertussis toxin and anti-filamentous hemagglutinin antibodies) were measured using Bordetella pertussis IgG ELISA- based kit (IBL international kit) according to the manufacturer's instructions, with the lowest detectable level of 1 IU/mL. The cut-off value of >25 U/mL was regarded as a protective value for pertussis [14].

Statistical analysis: Statistical analysis was performed using SPSS (Version 25) (IBM SPSS Statistics for Windows, IBM Corporation). For categorical variables, counts with percentages were presented. Pre- and post-vaccination antibody titers (immune or non-immune; dichotomous variable) were compared using Chi-square test (or Fisher exact test if appropriate) at a significance level of $P<0.05$. Immunogenicity and safety analyses were based on the eligible immunized subjects who completed the study and provided pre- and post-vaccination blood samples.

RESULTS

A total of 900 participants were enrolled, 300 participants in each group. Of these, 292 children (150 males) in the Shan 5 group, 285 (130 males) in the Pentabio group, and 298 (156 males) in the Pentavac group received all the three vaccine doses and were evaluated for immunogenicity and safety of vaccines (**Fig. 1**). The mean (SD) age of infants in the three groups at enrollment was 1.76 (0.36) months in Pentavac group, 1.77 (0.30) months in Pentabio group, and 1.74 (0.32) in Shan 5 group.

The vaccines had the same immunogenicity against all the five antigenic components except for pertussis. In the case of pertussis, the Pentabio vaccine exhibited significantly less immunogenicity than the other two types. **Table II** presents the percentage of immunized subjects in each group in terms of antigen.

After receiving the first to the third doses of each vaccine, the local adverse events (except for the injection-site abscess/ local reactions) were significantly different between the three vaccines. There was no significant difference among various vaccines in most systemic adverse events. Encephalitis, toxic shock syndrome, hospitalization, and death after vaccination were not observed in any of the infants. There was a significant difference in the incidence of anorexia and drowsiness between the vaccines.

DISCUSSION

This study evaluated the immunogenicity and safety of three pentavalent vaccines. Arjmand, et al. [4] showed that only 17.3% of infants receiving the Pentavac vaccine had an acceptable level of antibodies against pertussis six months after the last dose of the injection. A study on the DTP vaccine's immunogenicity against pertussis in Iran showed protective immunity in 76.8% of the participants [14]. In the present study, the most significant change, from 3.3% to 71%, in the pertussis antibody levels was related to the Shan 5 vaccine.

Consistent with the results of Gandhi et al. [1], the results showed that the Shan 5 and Pentavac vaccines were similarly efficient against all the five antigens.

Rusmil, et al. [6] reported protection rates of 99.7%, 100%, 99.3%, and 84.9% against diphtheria, tetanus, hepatitis B and pertussis, respectively. The Pentabio vaccine was reported as a suitable alternative for other certified pentavalent vaccines. [6]. In the present study, pertussis antibodies were measured by the IgG ELISA kit based on sandwich principle, and cut-off values of less than 25 U/mL were considered negative. An important caveat is that antibody testing does not reliably predict protective immunity against pertussis. Although there are no agreements upon the correlates of protection for pertussis, the data of antibody levels have been a basis on which

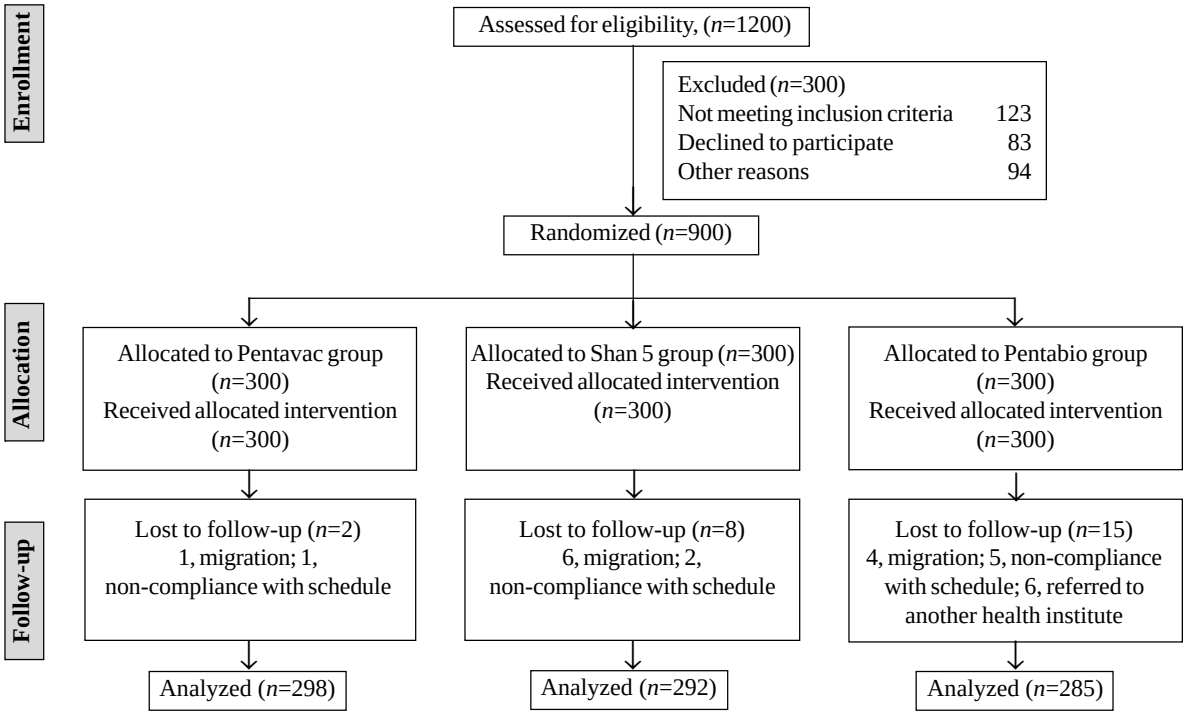

Fig.1 Study flow chart.

Table I Infants Having Post-immunization Antibody Titers Above Minimum Protective Levels After Third Dose of Injection

Immunogenicity	*Before vaccination (at the age of 2 mo)*				*After vaccination (at the age of 7 mo)*			
	A n= 298	*B n=285*	*C n =292*	*P Values*	*A n =298*	*B n=285*	*C n =292*	*P Values*
Tetanus (≥0.1 IU/mL)	245 (82.2)	251 (88)	256 (87.6)	0.15	292 (98)	282 (98.9)	287 (98.2)	0.69
Pertusis (>25 U/mL)	58 (19.5)	45 (15.7)	10 (3.4)	1 (<0.00)	199 (66.8)	131 (43.9)	207 (71)	01 (<0.0)
Diphtheria (≥0.1 IU/mL)	165 (55.4)	149 (52.2)	51 (17.5)	1 (<0.00)	271 (90.9)	255 (89.4)	268 (91.8)	0.79
Hib (≥1.0 g/mL)	262 (87.9)	240 (84.2)	271 (92.8)	0.03	296 (99.3)	280 (98.2)	290 (99.3)	0.39
HB (≥10 mIU/mL)	117 (39.3)	91 (31.9)	110 (37.7)	0.19	272 (91.3)	244 (85.6)	263 (90.0)	0.13

Data presented as no. (%). A, B, C indicate Pentavac, Pentabio, and Shan 5 vaccines, respectively. P values indicate difference differences between the babies in the three groups.

other whole cell pertussis-containing vaccines have been licensed and in routine use [17].

A recent review [18] comparing combined DTP-HepB-Hib vaccine with separately administered DTP-HepB and Hib vaccines showed that minor adverse events such as pain and redness were more common in children given the combined vaccine. Consistent with the results of Sharma, et al. [16], our results showed that stiffness, pain, and redness of the injection site were the most common local adverse events. Dalvi, et al. [19] reported tenderness as the commonest local reaction in infants receiving Pentavac vaccine, followed by swelling, redness and induration. Gandhi, et al. [1] showed a similar rate of redness, pain, and abscess prevalence in infants vaccinated with Pentavac and Shan 5 vaccines. These differences could be due to the differences in how the symptoms were recorded in the the studies.

Rao, et al. [7] showed that one-third of Shan 5 vaccine injections were associated with injection adverse events; the most common local complication was pain and the most common systemic complication was fever. Sharma, et al. [16] reported that Pentavac vaccine recipients had less injection-site pain and limb movement restrictions than those receiving the EasyFive vaccine. In the present study, limb movement restriction was observed only in one Pentavac vaccine recipient and only after the first injection.

A limitation of the present study was that participant's demographics other than age and sex were not recorded. In addition, the vaccine adverse events were not recorded by professionals. Center-to-center reporting bias and failure to assess the geometric mean concentration or titer of antibodies were the other, limitations of this study.

In conclusion, Pentavac vaccine has immunogenicity similar to the Shan 5 vaccine and better immunogenicity against pertussis than the Pentabio vaccine. All the three pentavalent vaccines have a good safety profile.

Ethics clearance: National Institutes for Medical Research Development; No. IR.NIMAD.REC.1395.002 dated: 4 April 2016.

Contributors: All authors approved the final version of manuscript, and are accountable for all aspects related to the study.

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WHAT IS ALREADY KNOWN?

- The comparative evaluation of safety and immunogenicity of licensed pentavalent vaccines in use in Iran is not available.

WHAT THIS STUDY ADDS?

- Pentavac vaccine had immunogenicity similar to that of Shan 5 vaccine and better immunogenicity against pertussis than the Pentabio vaccine.

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