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Primary Immunization with a Triple Diphtheria-Tetanus-Whole Cell Pertussis Vaccine in Iranian Infants: An Analysis of Antibody Response

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ABSTRACT

Universal vaccination of neonates and children against diphtheria, tetanus and pertussis has had a tremendous impact on the control of these infectious diseases worldwide. Immunization by the triple diphtheria, tetanus and whole cell pertussis vaccine (DTwP) has been applied in Iran for almost 50 years. Periodic assessment of immunogenicity of this vaccine is an important aspect of successful mass vaccination programs. The present study was performed to assess the antibody response against tetanus, diphtheria and pertussis in a group of Iranian infants vaccinated with a local DTwP vaccine.

In this prospective study, 330 infants received primary vaccination at 2, 4 and 6 months of age with DTwP vaccine manufactured by Razi Institute of Iran. Blood samples were taken 2-4 weeks after the third dose to assess seroprotection and geometric mean titers (GMT) of specific antibodies.

Among the 283 infants who completed the vaccination course, 98.2% and 100% developed antibodies against diphtheria and tetanus, respectively. The GMT of antibodies to tetanus, diphtheria and pertussis, were 2.09 IU/ml, 2.08 IU/ml and 8.73 EU/ml, respectively.

Comparison of the results obtained from this study with those from previous studies performed in other countries revealed a similar GMT and protection rates for diphtheria and tetanus components. In the absence of well-established serological criteria, judgment about protection rate against pertussis has not been possible. A prospective vaccination study using the local DTwP vaccine in parallel to a WHO approved standard vaccine, could enable assessment of immunogenicity of the pertussis component.

Key words: Enzyme-linked immunosorbent assay; Diphtheria-tetanus-pertussis vaccine; Immunization; Infant; Vaccination

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INTRODUCTION

Today, immunization of infants against diphtheria, tetanus and pertussis is a common practice in most regions of the world. As early as 1925, the vaccine was shown to be effective for control of these diseases. Whole-cell pertussis vaccine was developed in the 1940s and has been used in many countries as a part of the WHO Expanded Program on Immunization (EPI) since its launch in 1974.[1] *Bordetella pertussis* infection continues to be endemic worldwide, with an estimated 50 million cases occurring annually, 90% of which are in developing countries with a high vaccination coverage.[1] An increase in the number of pertussis cases, particularly in infants and adults, has been described since 1980 in many countries.[2-6] Tetanus is readily preventable through immunization and tetanus toxoid-containing vaccines are included in childhood immunization programs all over the world. In 2004, an estimated 40 million pregnant women were still in need of immunization against birth-associated tetanus, and about 27 million children did not complete their primary tetanus immunization series.[7] Diphtheria is a potentially acute disease caused by exotoxin-producing *Corynebacterium diphtheriae*. Devastating diphtheria epidemics affecting mainly children have been described in many countries throughout history. In spite of great discrepancies in the rate of incidence, the dramatic reduction in the number of reported cases of diphtheria, from 98,000 in 1980 to 9,000 in 2000, is probably a consequence of the impressive EPI achievements [8]. It was the predecessor of the current diphtheria, tetanus and whole cell pertussis (DTwP) vaccine, that was in general use for nearly 50 years in many countries, which resulted in a drop in the incidence of the related diseases to very low levels.[7-9] In 2002, WHO estimated that fewer than 4000, 198000 and 294000 children less than 5 years would die from diphtheria, tetanus and pertussis, respectively.[10] Concerns about safety of DTwP vaccine have led to the development of acellular pertussis vaccines in the 1970s. Acellular vaccines (DTaP), consisting of up to five specific *B. pertussis* antigens, have been reported to induce more immunogenicity and lower incidence of both local and systemic complications.[11-15]

In Iran, the number of approved reported cases of diphtheria, tetanus and pertussis was totally 15, 8 and 125 cases in 2005 and 26, 11 and 89 in 2006, respectively, as reported by WHO.[16] Vaccination against diphtheria, tetanus and pertussis began in Iran since 1950s by using a local vaccine manufactured by Razi Institute (Razi-DTwP). The DTwP vaccination consists of a three dose primary series given at 2, 4 and 6 months of age, with a fourth dose given at 18 months and a fifth dose, administered between 4 and 6 years of age. Although the efficacy of the vaccine was confirmed by previous studies,[17-21] however, periodic assessment of the vaccine is essential for universal vaccination programs. The present study was undertaken to investigate the immunogenicity of Razi-DTwP vaccine in a group of Iranian infants vaccinated routinely in a number of health centers of Tehran.

PATIENTS AND METHODS

Population

The study population comprised of 330 healthy infants aged 2 months at the time of entry into the field trial and not previously vaccinated against diphtheria, tetanus and pertussis. Infants were excluded if they had any serious adverse events related to a previous vaccination, i.e., hypersensitivity, encephalopathy, or hypotonia-hyporesponsiveness; fever > 40^{o}C, history of seizures or other neurological disorders, a birth weight of <2500 g, known or suspected immunocompromised individuals receiving treatment with immunosuppressive drugs, intravenous immunoglobulins (IVIG) and/or any blood products.

Study Design

This prospective study was conducted in four health centers affiliated to Shahid Beheshti University of Medical Science in Tehran city from April 2006 to June 2007. After receiving written informed consent from the parents, health center nurses administered Razi-DTwP vaccine to the infants. The vaccine was given at 2, 4 and 6 months of age. Subjects were monitored for any immediate reactions for 30 min following vaccination. Serum samples were obtained 2-4 weeks after the third vaccination and stored at −20^{o}C until analysis. The level of specific antibodies against diphtheria, tetanus and pertussis was measured by enzyme-linked immunosorbent assay (ELISA).

Vaccine

Based on the information provided in the instruction sheet, the Razi-DTwP vaccine consists of 15 Lf (limes flocculation) diphtheria toxoid, 10 Lf tetanus toxoid, 16

IU (international units) inactivated *B. pertussis* bacterial cells, which are combined together with 0.3 to 0.6 mg aluminum phosphate as adjuvant, (DTP, Razi Vaccine & Serum Research Institute, Tehran, Iran). Each dose of vaccine was administered as deep intramuscular injection in the antero-lateral side of the thigh using a 23 gauge, 25mm length needle by AD syringes (Soloshot IX, Becton Dickinson, Fraga, Spain).

Serologic Evaluations

Antibody concentration was determined in serum by commercial ELISA kits (IBL-Hamburg GmbH, Hamburg, Germany). The cut-off values for protective levels of diphtheria and tetanus antibodies were set at 0.1 IU/ml, based on the EPI recommendation.[7,8] Since there is no defined serological correlate of protection for pertussis, no criteria for the pertussis vaccine threshold were defined. The sensitivity of ELISA kits for diphtheria and tetanus was 0.004 IU/ml and the sensitivity of pertussis ELISA kit was less than 1 EU/ml.

Ethics

The study protocol was approved by Avicenna Research Institute Ethics Committee.

The Food and Drug Administration and Health Administration of Ministry of Health, Treatment and Medical Education of Iran approved the study protocol, and the trial was conducted in accordance with the Declaration of Helsinki and Good Clinical Practice guidelines. Written informed consent was obtained from the parents of all infants before enrollment into the study.

Statistical Analysis

Two-tailed statistical analyses were performed using the SPSS software (SPSS Inc., Chicago, Illinois). The GMT, 95% confidence interval for geometric mean, the proportion of infants with antibodies above the defined seroprotection threshold and classification of antibody titers were computed and comparisons of antibody levels for each gender were done using the Mann-Whitney *U*-test.

The effect of sex and birth weight on the antibody titers was assessed using linear regression with backward method. P-values less than 0.05 were considered significant.

RESULTS

Study Population

A total of 330 infants were enrolled in to the study of whom 283 (85.7%) completed the study. Twenty subjects in the second dose and 27 subjects in the third dose vaccination withdrew from the study but none due to an adverse event.

The mean age at enrolment was 9 weeks with a range of 7.91–10.9 weeks and a male to female ratio of 1:1.08.

Mean birth weights, weights in the first dose, the second dose and the third dose of vaccination were 3.24±0.40, 5.12±0.72, 6.57±0.90 and 7.76±0.90 kg, respectively.

Measurement of Serum Antibodies Specific for Diphtheria, Tetanus and Pertussis

Classification and GMT for diphtheria, tetanus and pertussis antibodies are shown (Table 1). The antibody responses to diphtheria and tetanus vaccines were classified into four groups of "No Response" (≤0. 1 IU/ml),[7,8] "Low Response" (>0. 1 and ≤1.0 IU/ml), "Intermediate Response" (>1.0 and ≤5.0 IU/ml) and "High Response" (>5.0 IU/ml). Most of cases in primary vaccination samples were in the Intermediate Response group (Table 1).

The classification for antibody response to pertussis also included the "No Response" (≤5 EU/ml),[22,23] Low Response" (>5 and ≤24 EU/ml), "Intermediate Response" (>24 and ≤100 EU/ml) and "High Response" (>100EU/ml). The majority (71.7 %) of the samples checked for anti-pertussis antibody titer were in the "Low Response" group (Table 1). Classification into low, intermediate and high responses for pertussis as well as tetanus and diphtheria was performed arbitrarily. Box plot presentation of antibody titers obtained after primary vaccination for all the immunizing components of the vaccine are shown (Figure 1).

There was no significant difference in immunogenicity of any components of Razi-DTwP vaccine between the two groups of gender (Table 1). With multiple linear regression analyses (see Materials and Methods), we did not find any correlation between sex and birth weight with primary vaccination antibody titers of diphtheria, tetanus or pertussis (data not shown).

Table 1. Classification and geometric mean titers for diphtheria, tetanus and pertussis following primary vaccination with DTwP vaccine in Iranian infants

Antibody	Sex	No. of subjects	Classification (n=283)				GMT	95% confidence interval for GMT		P- value[e]
			No Response[a] (%)	Low Response[b] (%)	Intermediate Response[c] (%)	High Response[d] (%)		Lower limit	Upper limit	
Diphtheria	Male	138	3(2.2)	22(15.9)	83(60.1)	30(21.7)	2.08	1.71	2.54	
	Female	145	2(1.4)	27(18.6)	91(62.8)	25(17.2)	2.10	1.79	2.47	0.58
	Total	283	5(1.8)	49(17.3)	174(61.5)	55(19.4)	2.09	1.85	2.38	
Tetanus	Male	138	0(0)	37(26.8)	63(45.7)	38(27.5)	2.11	1.73	2.58	
	Female	145	1(0.7)	39(26.9)	77(53.1)	28(19.3)	2.05	1.71	2.46	0.72
	Total	283	1(0.4)	76(26.9)	140(49.5)	66(23.3)	2.08	1.82	2.38	
Pertussis	Male	138	31(22.5)	91(65.9)	14(10.1)	2(1.4)	8.75	7.61	10.06	
	Female	145	22(15.2)	112(77.2)	10(6.9)	1(0.7)	8.71	7.65	9.91	0.95
	Total	283	53(18.7)	203(71.7)	24(8.5)	3(1.1)	8.73	7.94	9.60	

[a] *No Response is defined as < 0.1 IU/ml in diphtheria and tetanus and <5 EU/ml in pertussis*

[b] *Low Response is defined as > 0.1 and <1 IU/ml in diphtheria and tetanus and >5 and <24 EU/ml in pertussis*

[c] *Intermediate Response is defined as >1 and <5 IU/ml in diphtheria and tetanus and >24 and <100 EU/ml in pertussis*

[d] *High Response is defined as >5 IU/ml in diphtheria and tetanus and >100 EU/ml in pertussis*

[e] *The statistical significance of differences between male and female was assessed using the Mann-Whitney U test*

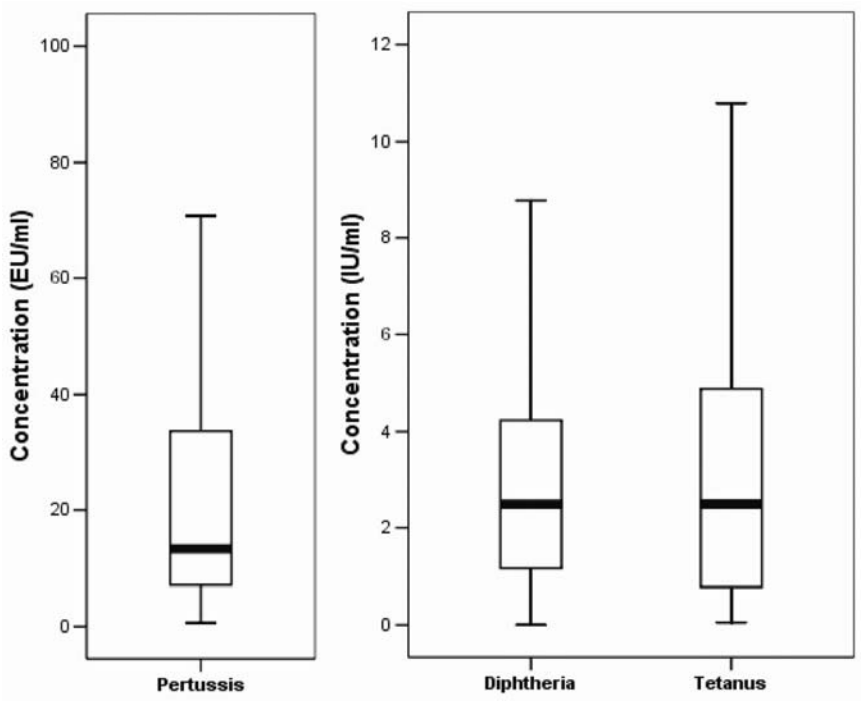

Figure 1. Box plot presentation of antibody titers against diphtheria, tetanus and pertussis in primary vaccination serum samples from Iranian infants

The box length is the interquartile range. Bars show the range from 10th to 90th percentiles. (—), median

Table 2. Geometric mean titers and seroprotection rates of anti-diphtheria and anti-tetanus antibodies retrieved from previous studies compared with the present study

References	Study (year)	Vaccine (manufacturer)	GMT		Seroprotection rate	
			Diphtheria (IU/ml)[h]	Tetanus (IU/ml)[h]	Diphtheria	tetanus
35	Nolan (1998)	DTwP-Hib-Hep [a] (CSL)	7.2	0.83	NI [i]	NI [i]
36	Mills (1998)	DTwP-IPV [b] (Pasteur Merieux)	0.29	0.63	80%	100%
37	Gyhrs (1999)	DTaP-Hep [c] (GlaxoSmithKline)	1.6	5.33	NI [i]	NI [i]
38	Richie (1999)	DTwP-Hib [d] (Swiss Serum)	4.45	9.34	99%	100%
39	Araujo (2000)	DTwP [e] (Pasteur Merieux)	0.54	10.91	100%	100%
40	Santos (2002)	DTwP-Hep [f] (GlaxoSmithKline)	1.9	1.6	98%	98%
41	Clemens (2003)	DTwP-Hib [d] (GlaxoSmithKline)	1.47	6.68	98%	100%
42	Botet Asensi (2003)	DTwP [e] (Chiron Behring)	0.19	2.24	100%	100%
43	Buttery (2005)	DTwP-Men C [g] (Sanofi Pasteur)	1.47	5.86	100%	100%
44	Hla (2006)	DTwP-Hep [f] (GlaxoSmithKline)	1.17	2.91	97.6%	100%
28	Tregnaghi (2007)	DTwP-Hep [f] (GlaxoSmithKline)	2.52	2.86	98.9%	100%
34	Kerdpanich (2007)	DTwP [e] (GlaxoSmithKline)	0.93	3.70	96%	100%
45	Gatchalian (2008)	DTwP-Hep [f] (GlaxoSmithKline)	0.81	2.57	94.9%	100%
Present study	Zarei	DTwP [e] (Razi)	2.09	2.08	98.2%	100%

[a] *diphtheria, tetanus, whole cell pertussis, haemophilus influenzae type b, hepatitis B vaccine*
[b] *diphtheria, tetanus, whole-cell pertussis, inactivated polio vaccine*
[c] *diphtheria, tetanus, acellular pertussis, hepatitis B vaccine*
[d] *diphtheria, tetanus, whole cell pertussis, haemophilus influenzae type b vaccine*
[e] *diphtheria, tetanus, whole cell pertussis vaccine*
[f] *diphtheria, tetanus, whole cell pertussis, hepatitis B vaccine*
[g] *diphtheria, tetanus, whole cell pertussis, neisseria meningitidis group c vaccine*
[h] *International unit*
[i] *not identified*

Table 3. Comparison of geometric mean titers retrieved from previous studies with those of the present study

References	Study (year)	Vaccine (manufacturer)	GMT of pertussis (EU/ml) [d]
40	Santos (2002)	DTwP-Hep a (GlaxoSmithKline)	170.1
41	Clemens (2003)	DTwP-Hib b (GlaxoSmithKline)	124
44	Hla (2006)	DTwP-Hep a (GlaxoSmithKline)	82.6
28	Tregnaghi (2007)	DTwP-Hep a (GlaxoSmithKline)	133.7
34	Kerdpanich (2007)	DTwP c (GlaxoSmithKline)	72.8
45	Gatchalian (2008)	DTwP-Hep a (GlaxoSmithKline)	110.2
Present study	Zarei	DTwP c (Razi)	8.70

[a] *diphtheria, tetanus, whole cell pertussis, hepatitis B vaccine*
[b] *diphtheria, tetanus, whole cell pertussis, haemophilus influenzae type b vaccine*
[c] *diphtheria, tetanus, whole cell pertussis vaccine*
[d] *ELISA unit*

DISCUSSION

Immunization has an essential impact on public health, worldwide.[24] Numerous studies have shown the efficacy of different vaccines to protect infants leading to either eradication or significant reduction of the related diseases in many countries thanks to universal immunization.

It is well-known that the immune system is partially immature at birth, resulting in a deficiency of cellular and humoral immunity: T-cell function is diminished, including T cell-mediated cytotoxicity and T cell help for B cell differentiation.[25,26] Therefore, neonatal immunization does not generally lead to rapid antibody responses, but it may result in an efficient immunologic priming which can act as a basis for future responses.[27] Accordingly, immunogenicity of DTwP vaccine was reported at a higher frequency following administration of the booster dose compared to the primary course.[28-32] We have previously observed a similar finding after booster vaccination with DTwP in preschool children,[33] where comparison of GMTs between pre-booster and post-booster vaccinations, showed a significant rise in antibody titers after booster injections. In that study, the GMTs of the antibodies produced against diphtheria, tetanus and pertussis by DTwP vaccine were 7.76, 9.37 IU/ml and 30.20 EU/ml after booster vaccine dose, respectively.

The current study was conducted to evaluate the immunogenicity of DTwP vaccine in healthy Iranian infants following primary vaccination. There has been no published report on the immunogenicity of this vaccine in Iranian infants following a mass primary vaccination for almost 30 years.

Kerdpanich et al. (2007) compared the immunogenicity of a locally manufactured DTwP vaccine with a WHO-approved vaccine (*Tritanrix*™) manufactured by GlaxoSmithKline (GSK), in a group of Thai infants.[34] They showed that by administration of *Tritanrix*™, the GMT of diphtheria was 0.93 IU/ml (current study 2.09) and that of tetanus was 3.70 IU/ml (current study 2.08). The seroprotection of *Tritanrix*™ was 96% (current study 98.2%) and 100% (current study 100%) against diphtheria and tetanus, respectively. Other studies have evaluated DTwP and DTaP vaccines in different groups of infants with different ethnic backgrounds, but these vaccines were largely used in combination with other vaccines. Table 2 summarizes these studies with respect to GMT and seroprotection rates for diphtheria and tetanus.[28,34-45] The variable response may depend on genetic factors, cytokine immunoregulatory network, highly polymorphic human leukocyte antigen system, maternal antibodies and application of combined vaccines,[46] though combined vaccines are generally well tolerated and induce seroprotection and seroconversion rates comparable to those of single vaccines.[47]

It seems that the immunogenicity observed in the present study against diphtheria and tetanus was similar or even better than those of other vaccines (Table 2).

Anti-pertussis antibody titer was also determined in vaccinated infants by ELISA technique.

The results, however, could not be compared because of the lack of appropriate standard preparation and cut-off value which can identify protectively immunized subjects. In the absence of a protective correlate for pertussis immunization, the results obtained by ELISA are reported as ELISA units per ml (EU/ml) with different arbitrary assignments for seroconversion. Taking this into consideration, the results from some recent studies[28,34,40,41,44] have been shown in Table 3 and compared with our results which imply a lower immunogenicity for pertussis component of the Razi-DTwP vaccine.

Data analysis performed in the present study revealed no effect of gender and birthday weight of the infants on the immunization outcome (Table 1), a finding already reported by other investigators.[48]

In summary, comparison of our results with those reported by other investigators implies a similar immunogenicity of the diphtheria and tetanus components of the Razi- DTwP vaccine compared to the standard WHO-approved commercial vaccines. In the absence of a well-established serological correlate for protection against pertussis, we could not compare our results whit other studies. To resolve these differences, we are performing a prospective case-control vaccination study using Razi-DTwP vaccine in parallel to a WHO-approved standard vaccine in a large cohort of Iranian infants.

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