Immunogenicity of a Triple Diphtheria-Tetanus-Whole Cell Pertussis Vaccine in Iranian Preschool Children

Saeed Zarei[1], Mahmood Jeddi-Tehrani[1,2], Mohammad Mehdi Akhondi[3], Hojjat Zeraati[4], Tahere Kheirkhah[5], Morteza Ghazanfari[6], Fazel Shokri*[1,7]

[1]Monoclonal Antibody Research Center, [3]Reproductive Biotechnology Research Center and [6]Nanobiotechnology Research Center, Avesina Research Institute, Iranian Academic Center for Education, Culture & Research, Tehran, Iran, [2]Immune and Gene Therapy Lab, Karolinska Cancer Center, Karolinska University Hospital, Stockholm, Sweden, [5]Deputy of Health, Shahid Beheshti University of Medical Sciences, Tehran, Iran, [4]Department of Epidemiology and Biostatistics and [7]Department of Immunology, School of Public Health, Medical Sciences/University of Tehran, Tehran, Iran

ABSTRACT

Background: Immunization against diphtheria, tetanus and pertussis has been applied in Iran since 1950. WHO suggests periodical evaluation of effectiveness of the triple diphtheria-tetanus-whole cell pertussis (DTwP) vaccine, worldwide. **Objectives:** To determine the immunogenicity of locally manufactured DTwP vaccine administered to preschool children in a number of health centers of Tehran in 2006. **Methods:** In this prospective study, 350 children aged 4-6 years were injected with DTwP vaccine manufactured by Razi Institute of Iran. Blood samples were collected before and 2-4 weeks after the vaccination. The immunogenicity of the vaccine was assayed by measurement of specific antibodies using enzyme-linked immunosorbent assay (ELISA) technique. **Results:** Of the 337 children who were vaccinated, 99.4% and 100% had protective anti-diphtheria and anti-tetanus antibody titers, respectively. The vaccine response and seroconversion for pertussis was achieved in 70.3% of the subjects. The geometric mean titers (GMT) 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. **Conclusions:** Comparison of the results obtained from this study with those of previous studies performed in other countries reveals that immunogenicity of diphtheria and tetanus components is similar to other vaccines, but the immunogenicity of pertussis vaccine was less efficient. The lower immunogenicity of DTwP against pertussis may be related to the bacterial strain used or the formulation protocol adopted for the vaccine preparation.

Keywords: Diphtheria-Tetanus-Pertussis Vaccine, Immunization, ELISA

*Corresponding author: Dr. Fazel Shokri, Monoclonal Antibody Research Center, Avesina Research Institute, Tehran, Iran, P.O. Box: 19835-177, Tehran, Iran. Tel: (+) 98 21 2243 2020, Fax: (+) 98 21 2243 2021, e-mail: fshokri@tums.ac.ir

INTRODUCTION

The incidence of infectious diseases such as diphtheria, tetanus and pertussis has declined significantly over the years as a result of universal infant immunization programs. Pertussis is an important cause of infant death and continues to be a global public health concern even in countries with high vaccination coverage. Recent estimates from WHO suggest that, in 2003, about 17.6 million cases of pertussis occurred worldwide, 90% of which were in developing countries, and that about 279 000 patients died from this disease. It is estimated that, in 2003, global vaccination against pertussis averted about 38.3 million cases and 607 000 deaths (1). Since the end of the 1980s, about 80% of all infants worldwide have received pertussis vaccine (1). In 2002, the total number of deaths caused by tetanus was estimated to be 213 000, of which neonatal tetanus was estimated to represent about 180 000 and maternal tetanus possibly as many as 15 000–30 000 deaths (2). In 2004, about 27 million children, world wide, did not complete their primary tetanus immunization series (2). Throughout history, diphtheria has been one of the most feared childhood diseases, characterized by devastating outbreaks(3). Although the figures are quite uncertain, the dramatic reduction in the number of reported cases of diphtheria, from 98 000 cases in 1980 to 9000 in 2000, is probably a consequence of the impressive EPI (Expanded Program on Immunization) achievements (3). Whole cell pertussis vaccines have been effective, as demonstrated by controlled studies of efficacy done in countries such as the United Kingdom in the 1940s, Kenya in the 1970s, and Indonesia, Sweden, and the United Kingdom in the 1980s. Extensive reviews of whole cell pertussis vaccine efficacy studies (e.g. retrospective analyses or outbreak investigations and clinical trials) have also been published (4- 8). Whole-cell pertussis vaccines were developed in the 1940s and have been used worldwide for many years, and are a part of the WHO program since its launch in 1974 (9). In 2002, among diseases for which vaccines are universally recommended, WHO estimated that fewer than 4,000 children aged less than 5 years died from diphtheria; 198,000 from tetanus and 294,000 from pertussis (10). In Iran, immunization against diphtheria, tetanus and pertussis has been applied since 1950 using a local vaccine manufactured by Razi Institute (Razi-DTwP) and the efficacy of the vaccine was confirmed by previous studies (11- 15). In 2004 WHO and UNICEF reported that the incidence of diphtheria, tetanus and pertussis in Iran were 6, 11 and 98 cases, respectively while in 2005, the respective incidences were 15, 8 and 125 (16, 17). Therefore, WHO suggested to evaluate the vaccines once every few years (1). The present article reports on the immunogenicity of Razi-DTwP in a group of 4-6 year old Iranian children.

MATERIALS AND METHODS

Population. Three hundred and fifty healthy 4–6 years old children, who had received four doses of DTwP vaccine (D.T.P., Razi Vaccine & Serum Research Institute, Tehran, Iran) at 2, 4, 6 and 18 months of age according to the national vaccination schedule, were included in this study. The subjects were excluded if they had a history of diphtheria, tetanus or pertussis at any time. Additional exclusion criteria were a history of allergic diseases or reactions likely to be exacerbated by any component of the vaccine or were previously recorded following DTWP vaccination, or a history of any serious adverse

reactions following previous DTWP vaccination. Children were excluded if they had a history of administration of immunosuppressive agents, immunoglobulins or blood products and were also excluded if they had any underlying conditions such as major congenital defects, neurological disorders including seizure disorders or acute febrile illness at the time of enrollment.

Study Design. The prospective study was conducted at 3 health centers affiliated to Shahid Beheshti University of Medical Sciences in Tehran from April to October 2006. To check for immediate adverse reactions, the children were under observation for 30 minutes at the health centers. Blood samples were collected before (pre-booster) and 2-4 weeks after the vaccination (post-booster). The immunogenicity of the vaccines was assessed by enzyme-linked immunosorbent assay (ELISA), using commercial kits.

Vaccine. Each dose of a 0.5 ml of Razi-DTwP vaccine contained 15 Lf diphtheria toxoid, 10 Lf tetanus toxoid, 16 IU inactivated Bordetella pertussis bacterial cells, 0.3 to 0.6 mg aluminum phosphate (metal ion) and 0.01% merthiolate according to the instruction sheet provided by the manufacturer. Each dose of vaccine was administered by deep intramuscular injection in the deltoid region of the non-dominant side by AD syringes (Soloshot IX, 23 G, 0.6 × 25 mm, Becton Dickinson, Fraga, Spain). All children enrolled in this study also received MMR vaccination at 4-6 years of age according to the national vaccination schedule of Iran.

Serologic Evaluations. Antibody measurements were performed on serum samples taken immediately before and 2-4 weeks post-booster. Sera were separated and stored at -20 ^{o}C until analysis. All antibody levels were measured by commercial ELISA kits (IBL-Hamburg GmbH, Hamburg, Germany). Optical density was measured at 450 nm using ELISA reader (Anthos Labtec Instruments, Austria). Based on the EPI Program of WHO (2, 3) the assay cut-offs for protective levels of diphtheria and tetanus antibodies were set at 0.1 international units per ml (IU/ml). Pertussis antibody titer was used with an assay cut-off of 16 ELISA units per ml (EU/ml) as defined by the manufacturer. For diphtheria and tetanus, concentrations above the assay cut-offs were considered to be seroprotective. For pertussis, sera with titers above the assay cut-offs were considered seroconverted. As there is no defined serological correlate of protection for pertussis, criteria for a vaccine response were defined (18). A vaccine response (VR) was defined as two-fold increase of antibody titer in post-booster compared to pre-booster samples.

Ethics. Written informed consent was obtained from parents of all children. The study protocol was approved by Avesina Institute Ethics Committee, and was conducted according to the Declaration of Helsinki and Good Clinical Practice. The study was approved by Food and Drug Administration and Health Administration of Ministry of Health, Treatment and Medical Education of Iran.

Statistical Analysis. Two-tailed statistical analyses were performed using the SPSS software (SPSS Inc., Chicago, Illinois). For the booster injection given at 4–6 years of age, the geometric mean of antibody titers (GMT), the proportions of children with antibodies above the defined seroconversion/seroprotection thresholds and classification of antibody titers were computed before and 2-4 weeks after the booster dose. The frequencies of children with a two-fold rise in antibody titers were computed. Comparisons between pre- and post-booster titers were analyzed with the Wilcoxon signed rank test for paired data. The effects of age, sex, weight, birth weight and pre-booster antibody titers on post-booster antibody titers were assessed using linear regression with backward method.

RESULTS

Study Population. As planned, a total of 350 subjects entered the study of whom 337 (96.3%) subjects completed the study. The study population included 144 males (42.7%) and 193 females (57.3%). Mean age at inclusion was 6.08±0.64 years. Mean weight and birth weight were 20.57 ± 3.42 and 3.17 ± 0.46 kg, respectively.

Measurement of Serum Antibodies Specific for Diphtheria, Tetanus and Pertussis. Table 1 shows pre-booster and post-booster seroprotection rates and mean titers of diphtheria, tetanus and pertussis antibodies. The antibody responses to diphtheria and tetanus vaccines were classified into four groups of "No Response" (<0.01 IU/ml), "Low Response" (>0.01 and <1.0 IU/ml), "Intermediate Response" (>1.0 and <5.0 IU/ml) and "High Response" (>5.0 IU/ml). Accordingly, the pre-booster antibody response against diphtheria and tetanus was predominantly categorized into "No Response" and "Low Response" profiles. However, the majority of cases in post-booster samples were in the "High Response" group (Table 1). Comparison of GMT between pre-booster (0.25 and 0.28 IU/ml for diphtheria and tetanus, respectively) and post-booster (7.76 and 9.37 IU/ml), showed highly significant rise of antibody titers after vaccination (p<0.0001).

Table 1. Classification and geometric mean titers (GMT) for diphtheria, tetanus and pertussis following booster vaccination with Razi-DTwP vaccine in 4-6 year old Iranian children

Antibody		Classification(n=337)				Mean±SD[e]	GMT
		High Response[d] (%)	Intermediate Response[c] (%)	Low Response[b] (%)	No Response[a] (%)		
Diphtheria	Pre-booster	60(17.8)	256(76)	21(6.2)	0	0.41±0.34	0.25
	Post-booster	2(0.6)	12(3.6)	50(14.8)	273(81)	9.88±5.59	7.76
Tetanus	Pre-booster	103(30.)	168(49.9)	56(19.3)	1(0.3)	0.64±0.86	0.28
	Post-booster	0	3(0.9)	79(23.4)	255(75.7)	12.32±8.14	9.37
Pertussis	Pre-booster	252(74.)	31(9.2)	30(8.9)	24(7.1)	22.18±44.24	8.41
	Post-booster	100(29.)	53(15.7)	128(38)	56(16.6)	50.42±54.16	30.20

[a] No Response is defined as < 0.01 IU/ml in diphtheria and tetanus and <16 EU/ml in pertussis
[b] Low Response is defined as > 0.01 and <1 IU/ml in diphtheria and tetanus and >16 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] SD refers to standard deviation

Pre-booster seroconversion rate of pertussis was 25.2% and changed to 70.3% upon vaccination (Table 1). The classification for antibody response to pertussis also included the "No Response" (<16 EU/ml), Low Response" (>16 and <24 EU/ml), "Intermediate Response" (>24 and <100 EU/ml) and "High Response" (>100EU/ml) groups. The majority (74.8%) of pre-booster samples for anti-pertussis antibody titer were in the "No Response" group. Interestingly, 100 cases of these remained in the "No Response" category even after booster vaccination (Table 1). However, the overall response to pertussis vaccination showed that the GMT of the samples increased from 9.37 EU/ml in pre-booster to 30.20 in post-booster samples (p<0.0001). The vaccine response to pertussis was measured to happen in 70.3% of the children. Figure 1 shows the box plot presentation of pre- and post-booster antibody titers for all the immunizing components of the vaccine. Statistical comparison of antibody titers between pre- and post-booster samples of diphtheria, tetanus and pertussis showed a significant increase in post-booster samples for all three bacterial components (p<0.0001). The regression model (see Materials

and Methods) showed that there was no statistically significant association between age, sex, weight and birth weight with post-booster antibody titers of diphtheria, tetanus and pertussis. However, the regression model strongly confirmed the above mentioned significant differences between antibody titers in pre- and post-booster samples for the three immunizing components ($p<0.0001$).

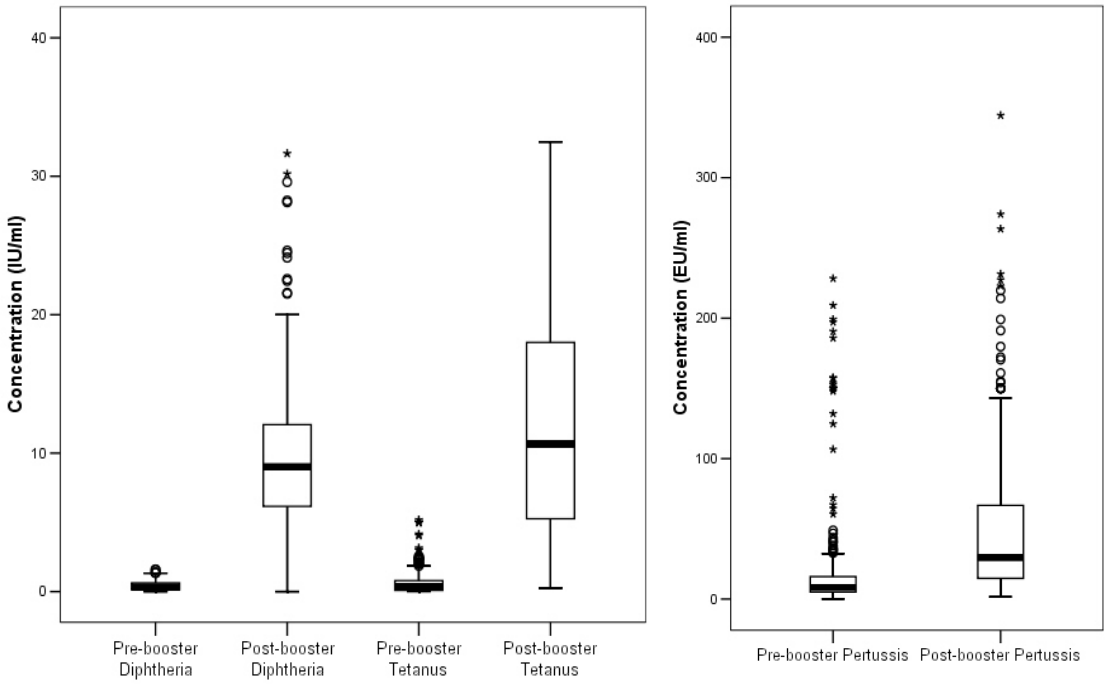

Figure 1. Box plot presentation of antibody titers against diphtheria, tetanus and pertussis in pre-booster and post-booster serum samples from 4-6 year old vaccinated Iranian children.
The box length is the interquartile range. Bars show the range from 10th to 90th percentiles. (**—**), median; (○), cases with values between 1.5 and 3 box lengths from the upper or lower edge of the box; (*), cases with values more than 3 box lengths from the upper or lower edge of the box.

DISCUSSION

Worldwide mass vaccination of neonates with combined DTP vaccine has significantly reduced the incidence of these diseases to a level that natural exposure in childhood is currently uncommon in many countries and immunity is acquired almost exclusively through vaccination. Immunity, however, wanes over time showing an age-related decrease in serum antibody concentrations to all three components of DTP vaccine (19, 20). Waning of pertussis immunity after primary immunization suggests the need for booster immunization of 4–6 year-old children to ensure continuing immunity and prevent the development of a reservoir of infection which could affect non-immune infants (21). Therefore, pre-school fifth dose booster immunization at 4–6 years of age has been established in many countries around the world, including Iran where DTwP vaccine

has been given to children aged 2, 4, 6, and 18 months and 4–6 years. Supplementary vaccination of adults with an extra booster dose of pertussis vaccine has also been proposed and is being adopted by some countries to control transmission of disease to neonates and children (22).

The current study was conducted to evaluate the immunogenicity of DTwP vaccine in healthy Iranian pre-school children. A previous investigation in Iranian children using the same vaccine, indicated high immunogenicity of pertussis and low immunity against tetanus and diphtheria (23). In that study it was claimed that pertussis showed a better efficacy than other standard WHO approved DTwP vaccines ($p<0.0001$), with similar GMT (23). Conversely, it was also reported that the diphtheria and tetanus vaccines of DTwP-Razi had an inferior performance ($p<0.0001$) with GMT less than half of the standard vaccines. These findings are unexpectedly different from ours, despite the use of the same vaccine. Although the reasons underlying such discrepancy are not clearly understood, a number of variables and technical pitfalls may account for this discrepancy. The most important parameter is the method of antibody measurement. In the previous study custom-designed micro hemagglutination tests using antigen-coated sheep erythrocytes were employed. These assays have long been known to have low sensitivity, specificity and reproducibility compared to ELISA which was employed in the present study. Furthermore coupling of sheep erythrocytes with different preparations of bacterial particles at different time intervals may lead to substantial inter-assay variations. The samples collected in the above mentioned study were taken as dried blood spots on filter papers which were dissolved in a given volume of diluent prior to performance of the assay. The other important parameter is the age of the vaccines; the subjects enrolled in that study were neonates receiving triple doses of primary vaccination, as opposed to our 4-6 years old children who received that fifth vaccine dose as a booster vaccination.

Kosuwon et al. compared the immunogenicity of diphtheria, tetanus, acellular pertussis (DTaP) vaccine with that of diphtheria, tetanus, whole cell pertussis (DTwP) vaccine (both approved by WHO and produced by GlaxoSmithKline (GSK) Biologicals, Belgium), used as booster administrations (18). They showed that the GMT of pre-booster diphtheria was 0.40 IU/ml (current study 0.25), post-booster diphtheria 4.98 IU/ml (current study 7.76), pre-booster tetanus 0.97 IU/ml (current study 0.28) and post-booster tetanus 9.42 (current study 9.37). The post-booster seroprotection of GSK vaccine was 99.4% (current study 99.4%) and 100% (current study 100%) against diphtheria and tetanus, respectively. Other studies have used DTwP and DTaP vaccines but they mainly used in combination with other vaccines (18, 24-28). Table 2 summarizes these studies with respect to GMT and seroprotection rates for diphtheria and tetanus and compares them with the present study. It seems that the immunogenicity against diphtheria and tetanus was similar or even better than that of other vaccines.

Kosuwon et al. evaluated anti-pertussis antibody titers by ELISA technique with a cut-off level of 15 EU/ml (current study 16 EU/ml). The GMT of pre-booster pertussis antibodies was 16.4 EU/ml (current study 8.4) and that of post-booster was 109 EU/ml (current study 30.2). For pertussis, the vaccine response was 94.4% (current study 70.3%). Considering that recent studies on immunization against pertussis have been mainly performed using DTaP vaccines, the results may not basically be comparable to our results. Data from two recent studies (18, 27) with emphasis on GMT, cut-off levels and seroconversion rates have been compared with our results in Table 3 implying a less comparable immunogenicity of Razi pertussis vaccine.

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

References	Study (year)	Vaccine (manufacturer)	GMT				Seroprotection rate	
			Pre-booster diphtheria (IU/ml)[g]	Post-booster diphtheria (IU/ml)[g]	Pre-booster tetanus (IU/ml)[g]	Post-booster tetanus (IU/ml)[g]	Diphtheria	Tetanus
18	Kosuwan (2003)	dTaP[a] andDTwP[b] (GSK)	0.401	4.981	0.972	9.421	99.4%	99.4%
20	Halperin (2003)	DTwP-IPV[c] and DTaP-IPV[d] (Aventis Pasteur)	0.14	23.9	0.31	9.47	NI[h]	NI[h]
21	Langue (2004)	DTaP-IPV[d] (Aventis Pasteur)	0.08	8.62	0.21	21.50	100%	100%
22	Huang (2005)	dTaP[a] (GSK)		4.7		20.3	100%	100%
23	Tregnaghi (2006)	DTwP-HB[e] (GSK)	0.171	7.013	0.305	9.821	100%	100%
24	Meriste (2006)	DTaP-HB-IPV[f] (GSK)	0.07	2.78	0.41	18.67	100%	100%
Present study	Zarei (2007)	DTWP(Razi)	0.25	7.76	0.28	9.37	99.4%	100%

[a] reduced antigen content diphtheria, tetanus, acellular pertussis vaccine
[b] diphtheria, tetanus, whole cell pertussis vaccine
[c] diphtheria, tetanus, whole-cell pertussis, inactivated polio vaccine
[d] diphtheria, tetanus, acellular pertussis, inactivated polio vaccine
[e] diphtheria, tetanus, whole cell pertussis, hepatitis B vaccine
[f] diphtheria, tetanus, acellular pertussis, hepatitis B, inactivated poliovirus
[g] International unit
[h] not identified

Table 3. Geometric mean titers (GMT), cut-off levels and seroconversion rates of anti-pertussis antibodies retrieved from previous studies and compared with the results of the present study

References	Study (year)	Vaccine (manufacturer)	GMT		Cut-off (EU/ml)[d]	Vaccine response or Seroconversion rate
			Pre-booster pertussis (EU/ml)[d]	Post-booster pertussis (EU/ml)[d]		
18	Kosuwan (2003)	dTaP[a] and DTwP[b] (GSK)	16.4	109	15	94.4%
23	Tregnaghi (2006)	DTwP-HB[c] (GSK)	13.8	284.9	15	100%
Present study	Zarei (2007)	DTWP(Razi)	8.4156	30.2030	16	74.8%

[a] reduced antigen content diphtheria, tetanus, acellular pertussis vaccine
[b] diphtheria, tetanus, whole cell pertussis vaccine
[c] diphtheria, tetanus, whole cell pertussis, hepatitis B vaccine
[d] ELISA unit
[h] not identified

The regression model of data in the present study revealed no effect of age, sex, weight and birth weight of the children on the immunization outcome, a finding already reported by many other investigators (29).

Comparison of our results with those reported by other investigators (see Table 2), indicates similar immunogenicity of the diphtheria and tetanus components of the DTwP vaccine manufactured by Razi Institute compared to the standard WHO approved com-

mercial vaccines. In addition, comparison of the immunogenicity against pertussis with the results of a previous study in Iran (23) and other studies that used commercial DTwP vaccines showed that the immunogenicity of the pertussis component of Razi-DTwP was inferior to other commercial DTwP vaccines. This may be related to the bacterial strain, bacterial cell preparation and/or the process of vaccine formulation. To resolve these differences, we are planning to perform a prospective vaccination study using Razi-DTwP vaccine in parallel with a WHO-approved standard whole cell DTP vaccine in a group of Iranian children.

ACKNOWLEDGEMENTS

The authors are grateful to the parents who allowed their children to enter this study. We are also indebted to the personnel of Mohammadian, Dawazdah Bahman and Salavati Health Centers of Shahid Beheshti University of Medical Sciences for their assistance in vaccination and sample collection. The authors wish to thank Dr. Ali Ramezankhani from the deputy of Health of Shahid Beheshti University of Medical Science for cooperation and Dr. Mohammad Ali Akhavizadegan and Mohammad Ali Mansori for consultation. This work was supported by a grant from Food and Drug Administration of the Ministry of Health, Treatment and Medical Education of Iran.

REFERENCES

1 WHO. Pertussis vaccines--WHO position paper. Wkly Epidemiol Rec. 2005;80:31-9. Available from:URL: http://www.who.int/immunization/topics/wer8004pertussis_Jan_2005.pdf
2 WHO. Tetanus vaccine-WHO position paper. Wkly Epidemiol Rec. 2006;81:198-208.Available from:URL: http://www.who.int/immunization/wer8120tetanus_May06_position_paper.pdf
3 WHO. Diphtheria vaccine-WHO position paper. Wkly Epidemiol Rec. 2006; 81:21-32. Available from:URL: http://www.who.int/wer/2006/wer8103.pdf
4 Plotkin SA, Cadoz M. The acellular pertussis vaccine trials: an interpretation. Pediatr Infect Dis J. 1997;16:508-17.
5 Ramsay ME, Farrington CP, Miller E. Age-specific efficacy of pertussis vaccine during epidemic and non-epidemic periods. Epidemiol Infect.1993;111:41-8.
6 Muller AS, Leeuwenburg J, Pratt DS. Pertussis: epidemiology and control. Bull World Health Organ 1986; 64:321-31.
7 Fine PE, Clarkson JA. Reflections on the efficacy of pertussis vaccines. Rev Infect Dis. 1987;9:866-83.
8 Wintermeyer SM, Nahata MC, Kyllonen KS. Whole-cell and acellular pertussis vaccines. The Ann pharmacother. 1994; 28:925-39.
9 Crowcroft NS, Pebody RG. Recent developments in pertussis. Lancet 2006;367:1926-36.
10 Vaccine preventable deaths and the Global Immunization Vision and Strategy, 2006-2015.Morb Mortal Wkly Rep 2006; 55:511-515. Available from: URL:http://www.who.int/vaccines-documents/DocsPDF05/GIVS_Final_EN.pdf
11 Mirchamcy H. Study on diphtheria, tetanus combined immunization in children in some elementary school of Tehran. Arch Inst Razi. 1960; 12:9-18.
12 Mirchamcy H. The use of dried whole blood absorbed on filter paper for the evaluation of diphtheria and tetanus antitoxine in mass survey. Arch Inst Razi. 1969;21:7-15.
13 Mirchamcy H. Resultats De immunisation collective des infants En Iran avecles immunogenes De production locale. Arch Inst Razi. 1982; 33:73-78.
14 Nazari F MH. Mass immunity against diphtheria and tetanus in some urban and rural areas in Iran. Arch Inst Razi. 1973;25: 49-55.
15 Nazari F MH, Aleagha S,Mahinpour. A model for developing countries of mass serological survey of children vaccinated against diphtheria and tetanus. Arch Inst Razi. 1977;29:3-10.
16 Immunization summary 2006. UNICEF.2006. Availabe from:URL: http://www.unicef.org/publications/files/Immunization_Summary_2006.pdf
17 Immunization summary2006. WHO. 2006. Available from:URL: http://www.who.int/vaccinesdocuments/GlobalSummary/GlobalSummary.pdf
18 Kosuwon P, Warachit B, Hutagalung Y, Borkird T, Kosalaraksa P, Bock HL et al. Reactogenicity and immunogenicity of reduced antigen content diphtheria-tetanus-acellular pertussis vaccine (dTpa) administered as a booster to 4-6 year-old children primed with four doses of whole-cell pertussis vaccine. Vaccine. 2003; 21:4194-200.
19 Kjeldsen K, Simonsen O, Heron I. Immunity against diphtheria and tetanus in the age group 30-70 years. Scand J Infect Dis. 1988;20: 177-85.
20 Konda T, Kamachi K, Iwaki M, Matsunaga Y. Distribution of pertussis antibodies among different age groups in Japan. Vaccine. 2002; 20:1711-7.

21 Forsyth K, Nagai M, Lepetic A, Trindade E. Pertussis immunization in the global pertussis initiative international region: recommended strategies and implementation considerations. Pediatr Infect Dis J. 2005; 24:S93-7.
22 Van Damme P, Burgess M. Immunogenicity of a combined diphtheria-tetanus-acellular pertussis vaccine in adults. Vaccine. 2004; 22:305-8.
23 Akhavizadegan M. Evaluation and comparison of immunogenicity and reactogenicity of DTP vaccines produced in Iran and other countries in Iranian infants. PhD thesis of Medical Epidemiology, School of Public Health, Tehran University of Medical Sciences, Tehran, 1996.
24 Halperin SA, Scheifele D, Mills E, Guasparini R, Humphreys G, Barreto L et al. Nature, evolution, and appraisal of adverse events and antibody response associated with the fifth consecutive dose of a five-component acellular pertussis-based-combination vaccine. Vaccine. 2003; 21:2298-306.
25 Langue J, Matisse N, Pacoret P, Undreiner F, Boisnard F, Soubeyrand B. Persistence of antibodies at 5-6 years of age for children who had received a primary series vaccination with a pentavalent whole-cell pertussis vaccine and a first booster with a pentavalent acellular pertussis vaccine: immunogenicity and tolerance of second booster with a tetravalent acellular vaccine at 5-6 years of age. Vaccine. 2004; 22:1406-14.
26 Huang LM, Chang LY, Tang H, Bock HL, Lu CY, Huang FY et al. Immunogenicity and reactogenicity of a reduced-antigen-content diphtheria-tetanus-acellular pertussis vaccine in healthy Taiwanese children and adolescents. J Adolesc Health. 2005; 37: 517e1-517e5.
27 Tregnaghi M, Lopez P, Rocha C, Rivera L, David MP, Ruttimann R et al. A new DTPw-HB/Hib combination vaccine for primary and booster vaccination of infants in Latin America. Rev Panam Salud Publica. 2006; 19:179-88.
28 Meriste S, Lutsar I, Tamm E, Willems P. Safety and immunogenicity of a primary course and booster dose of a combined diphtheria, tetanus, acellular pertussis, hepatitis B and inactivated poliovirus vaccine. Scand J Infect Dis. 2006; 38:350-6.
29 Christy C, Pichichero ME, Reed GF, Decker MD, Anderson EL, Rennels MB et al. Effect of gender, race, and parental education on immunogenicity and reported reactogenicity of acellular and whole-cell pertussis vaccines. Pediatrics. 1995; 96:584-7.