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Original Article (Pages: 7023-7031)

Study of Serologic Response Rate to Pertussis after Administration of the Third Dose of Pentavalent Vaccine in Children 12 Months Old in Karaj City, Iran

Reza Arjmand[1], Mehri Gholami[2], *Fariba Shirvani[3], Omid Safari[1], Mohammad Javad Gharavi[4], Homa Sadri[5], Mostafa Qorbani[6]

[1]Department of Pediatric, Emam Ali Hospital, Alborz University of Medical Sciences, Karaj, Iran. [2]Alborz University of Medical Sciences, Karaj, Iran. [3]Pediatric Infections Research Center, Research Institute for Children Health, Shahid Beheshti University of Medical Sciences, Tehran, Iran. [4]Department of Parasitology, Faculty of Allied Medicine, Iran University of Medical Sciences, Tehran, Iran.[5]Allergy and Clinical Immunology Department, Emam Ali Hospital, Alborz University of Medical Sciences, Karaj, Iran. [6]Non-Communicable Diseases Research Center, Alborz University of Medical Sciences, Karaj, Iran.

Abstract

Background: After substitution of Pentavalent vaccine with diphtheria, tetanus, pertussis (DTP) in the Iranian National Vaccination program with 3 Pentavalent (three times vaccination with Pentavalent vaccine at months 2, 4, and 6) in 2014 and the lack of published research in the field of immunogenicity of pertussis component of this vaccine, the efficacy of pertussis vaccine was studied 6 months after the last dose of Pentavalent vaccine in Iranian infants.

Materials and Methods: Five hundred blood samples were collected from healthy one-year-old children who attended 18 health care centers of Karaj, Iran for routine vaccination selected by cluster sampling (2016). Sampling checklists contained demographic information and risk factors. The blood samples were sent to the laboratory for determination of Immunoglobulin G (IgG) and IgA anti-pertussis antibody titer by ELISA method. Data were analyzed by STATA software (version 14.0).

Results: 82.7% (n=413) of children (95% confidence interval [CI]: 79.49-86.11) had IgG titer less than 16 IU/ml against pertussis (no immune response), and 17.3% (n=87) had equal or greater than 16 IU/ml IgG titer against pertussis (95% CI: 13.89-20.51). IgA titer against pertussis was less than 8U/ml in all cases. Anti-pertussis IgG geometric mean titer (GMT) was 15.80 U/ml (95% CI: 15.26-16.36), and IgA GMT was 6.26 U/ml (95% CI: 6.22-6.30). There was not a significant correlation between titer of pertussis antibody and demographic factors.

Conclusion: Based on low IgG titer in vaccinated children, immunogenicity of pentavalent vaccine in Iranian children needs more investigation. In this study, 100 % of children had negative serologic response (IgA <8 U/ml); therefore, natural infection did not occur in the study cases.

Key Words: Antibodies, Bordetella Pertussis, Child, Pentavalent Vaccine, Pertussis.

*Please cite this article as: Arjmand R, Gholami M, Shirvani F, Safari O, Gharavi MJ, Sadri H, et al. Study of Serologic Response Rate to Pertussis after Administration of the Third Dose of Pentavalent Vaccine in Children 12 Months Old in Karaj City, Iran. Int J Pediatr 2018; 6(2): 7023-31. DOI: **10.22038/ijp.2017.26000.2217**

Corresponding Author:

Fariba Shirvani, Subspecialist in Pediatric Infectious Diseases, Associate Professor, Pediatric Infectious Diseases Research Center. Research Institute for Children Health. Mofid Children's Hospital, Shahid Beheshti University of Medical Sciences, Tehran, Iran.

Email: shirvanifariba@rocketmail.com

Received date: Oct.23, 2017; Accepted date: Nov.22, 2017

1- INTRODUCTION

Pertussis or whooping cough is the disease of respiratory tract caused by a virulent bacteria living in throat and nose of children and adults with prolonged cough, but the complication is more prevalent in infants. There were 142,512 reported cases with Pertussis over the world and 89,000 cases in countries of eastern Mediterranean region, and 18,903 deaths were attributed to be caused by pertussis in 2008. However, a recent publication modeling of pertussis number of cases and mortality has estimated 24.1 million pertussis cases and 160,700 deaths worldwide in children younger than 5 years in 2014, contributing to 2% mortality of children under 5 years of age (1, 2).

Since 86 % of at risk persons are being vaccinated globally, it seems the disease has not been well controlled till now (3). World Health Organization (WHO) reported localized outbreak of Pertussis in Iran as a regional place in Eastern Mediterranean Region (EMRO) in 2010 with 464 acute cases (2, 4). The number of acute cases increased to 856 cases in 2011 (5); 3,629 suspected cases were reported during 2011-13 period and 239 (6.6 %) of these cases were laboratory confirmed (6); and 115 cases were reported in 2014 (7). WHO report of pertussis in USA in 2014 and 2015 were 18,166 cases (8).

This variable report of pertussis incidence may be due to cyclic pattern of the disease and underreporting of pertussis because of the belief of pertussis eradication by high vaccination coverage among health workers and variation in clinical presentation of the disease in different ages and modification of the disease by vaccination (9). The exact mortality rate due to pertussis in some regions as Iran is not determined accurately, and conflicting interpretations of results are reported (10). Expanded program of immunization with Diphtheria, Tetanus, and Whole Cell Pertussis (DTwP) was developed by WHO since 1950 in Iran (11). Indian Pentavalent vaccine was introduced to Iran vaccination program from 18th Nov. 2014, and it was replaced with DTwP vaccine in 2, 4, and 6 months routine vaccination (12). In a study by Zarei et al. (2007), geometric mean titers (GMT) of anti-pertussis antibody 2 to 4 weeks after the third dose of DTP (Diphtheria and Tetanus Toxoids and Pertussis Vaccine Adsorbed USP, DTaP) vaccination showed lower results, and by changing the vaccine to Indian type in 2009, this vaccine still had not sufficient efficacy against pertussis (9, 13).

In order to determine the serologic response to whole cell Pertussis component of the new pentavalent vaccine from Serum institute in India, the anti-pertussis antibody in 500 one-year-old healthy children after six months of pentavalent vaccination was investigated. This study provides valuable information about pentavalent vaccine efficacy in Iranian children.

2- MATERIALS AND METHODS

A cross-sectional study was conducted on healthy children attending health care centers in Karaj city, Alborz province, Iran for routine vaccination. Hosting a population of around 1.96 million, as recorded in the 2011 census, Karaj is the fourth-largest city in Iran, after Tehran, Mashhad, and Isfahan. Based on the estimated prevalence (P) of 80 % immunogenicity rate and estimation of the prevalence that is clinically worthwhile to detect (*d*) as 4% and with estimation of design effect of 1.3, the sample size was indicated 500 cases using ($P (1-P) Z2 (1-\alpha/2)/d^2$) formula. The number of cases by calculation was 384, and by multiplying it to design effect, the number of cases was found 500. The vaccine used from 2014 in Iran is Pentavac (DTP/HB/Hib) in which Sii HibPRO (Haemophilus Type b Conjugate Vaccine I.P.) is reconstructed with Diphtheria, Tetanus, Pertussis and

Hepatitis B Vaccine Adsorbed (SII Q-VAC) (DTP-HB Vaccine), supplied by Serum Institute of India Ltd., and is a pentavalent vaccine consisting of diphtheria toxoid, tetanus toxoid, killed Bordetella pertussis bacilli, and Hepatitis B surface antigen adsorbed on aluminum gel and suspended in isotonic sodium chloride solution. Each dose of vaccine (0.5 ml) contains Diphtheria Toxoid 20 Lf (Floculation units of Toxoid) to 30 Lf, Tetanus Toxoid 5 to 25 Lf, B pertussis (whole cell), 4 IU; HBsAg (rDNA) 10 mcg/ml (Hepatitis B Surface Antigen [Ridosomal DNA]), purified polyribosyl ribitol phosphate capsular polysaccharide (PRP) of Hib 10 mcg, and Tetanus Toxoid (carrier protein) 19 to 33 mcg adsorbed on aluminium phosphate, Al 1.25 mg Preservative: Thiomersal 0.005% (Serum Institute of India vaccination brochure).

Sampling was done using multi-stage cluster sampling method; firstly, 12 restricts of Karaj were listed, and six restricts were selected, and from each restrict, three health centers were chosen randomly. The number of cases selected from each center by random sampling method was 28. A check list was filled for each case, and the birth weight, weight, height, exclusively breast feeding status, duration of breast feeding, the number of siblings, mother and father education, place of child maintenance, history of parental cigarette smoking, and history of respiratory tract infections were recorded by an educated co-worker to be used for final statistical analysis. Each case had one blood sampling. After obtaining the informed consent from parents, blood samples were obtained from wrist and forearm veins by educated heath workers; considering hand hygiene with antiseptic other than chlorhexidine, the child was warmed and immobilized by the parents or other educated health workers, so positioning was done. Using sterile steel butterfly 23 to 25 gauge needles and an appropriate tourniquet, the skin was punctured 3 to 5 mm distal of the vein, and after the needle entered alongside the vein, 2 milliliters of blood was drawn slowly and steadily to prevent hemolysis. Blood specimens maintained in a sterile cupped test tube and transferred to the main laboratory in 4 hours for serum separation. Serum samples were maintained at -70 °C until ELISA test was done. This study had ethical certification from Karaj University Research Council and Ethics in Research Committee, and permission from heath officials to enter health centers of Karaj (Ethic code= Abzums.rec.1394.69). After description of the study objectives to the parents, everybody who volunteered to enter the study signed the informed consent, and sampling was performed for their children.

Inclusion criteria were: one-year-old children (between 0 days and 11 months and 29 days and 11 months of age), history of complete vaccination confirmed by vaccination card, and normal growth checked on growth chart. Exclusion criteria were: parents' dissatisfaction, non-cooperative children, history of congenital or acquired immunodeficiency, history of blood and blood products transfusion, malignancy and consumption of immunomodulators and Intravenous immunoglobulin (IVIg) reception 3 months before sampling. Quantitative variables were shown by mean and standard deviation (SD). The significance between study variables and dependent qualitative and quantitative variables were analyzed using logistic and linear regression analysis. Vaccine efficacy was measured by 95 % Confidence Interval (95%CI); logistic regression was used for demographic data, and p-value less than 5% were considered significant. Bordetella pertussis Anti Pertussis Toxin (PT), Immunoglobulin G (IgG), and Immunoglobulin A (IgA) measurements were done using ELISA method and IBL

international kit Germany (Catalogs Reference No: RE56141 and 56131). Specific antibodies of the patients' samples were banded to the antigen coated wells and were detected by a secondary enzyme conjugated antibody (E-Ab) (enzyme antibody) specific for human IgG. The color developed after substrate reaction was detected. The results of optic density of samples were determined directly using the standard curve. The results were reported as IU/ml; the lowest detectable level was 1 IU/ml.

3- RESULTS

The present study set to estimate anti pertussis toxin (PT) IgG and IgA antibodies in 500 one-year-old children and their relation to gender, exclusively breast feeding status, the number of siblings, history of respiratory infections, mother and father educational status, parental cigarette smoking, and the living place of child. Results showed that 82.7% (n=413) cases (confidence interval [CI]: 79.4-86.1) had anti pertussis IgG titer lower than 16 IU/ml, and 17.3% (CI: 13.9-20.5) (n=87) had higher titer lower than 16 IU/ml antibody titer. Geometric Mean Titer for IgG was 15.8 (CI: 15.3-16.3). IgA (Immunoglobulin A) anti pertussis antibody measurement showed that 100% (n= 500) of children had lower than 8 IU/ml equal to low response.

Geometric Mean Titer for IgA was 6.26 (CI: 6.22-6.3); 51% of cases (n=255) were female; 84.4% (n=422) had exclusive breast feeding in their first 6 months of life; 27.3% (n=137) had a history of more than once recurrent respiratory tract infection; 23.8% (n=119) had one or more smoker parents, and 1.4 % (n=7) attended kindergarten. There was no significant relationship between antibody titer and demographic characteristics such as weight and height at the time of sampling, birth weight, type of milk used at the first year of life, duration of breast feeding, parents' smoking behavior, presence of one sibling older than one year, the number of family members, parental education, recurrent respiratory infections, and history of kindergarten attendance (P>0.05).

Table 1: Anti-Pertussis antibody levels and demographic data in 500 one-year-old children in Karaj, Iran.

Variables		No response <16	Positive response ≥ 16	Total	P-value
Weight (gr)*	-	9.604 (1124)	9.676 (1018)	-	0.58
Height (cm)*	-	75.99 (3.60)	76.30 (3.05)	-	0.45
Breastfeeding duration (month)*	-	10.27 (3.65)	10.44 (3.79)	-	0.70
Gender**	Male	206 (84.1)	39 (15.9)	245	0.45
	Female	208 (81.6)	47 (18.4)	255	
Siblings number**	<2	357 (82.6)	75 (17.4)	432	0.81
	≥2	57 (83.8)	11 (16.2)	68	
Mother education**	Non academic	319 (82.4)	68 (17.6)	387	0.68
	Academic	95 (84.1)	18 (15.9)	113	
Father education**	Non academic	323 (83.9)	62 (16.1)	385	0.23
	Academic	91 (79.1)	24 (20.9)	115	
Place of care child**	House	407 (82.6)	86 (17.4)	493	0.22
	Kindergarten	7 (100)	0 (0)	7	

Parent smoking**	Yes	97 (81.5)	22 (18.5)	119	0.67
	No	317 (83.2)	64 (16.8)	381	
Exclusive breastfeeding**	Yes	348 (82.5)	74 (17.5)	422	0.64
	No	66 (84.6)	12 (15.4)	78	
Brest feeding**	Yes	382 (82.7)	80 (17.3)	462	0.81
	No	32 (84.2)	6 (15.8)	38	
Frequent URI**	Yes	118 (86.8)	18 (13.2)	136	0.15
	No	296 (81.3)	68 (18.7)	364	
Birth weight (gr)**	≤ 2.500	34 (91.9)	3 (8.1)	37	0.3
	2.500 - 4.000	372 (82.1)	81 (17.9)	453	
	≥ 4.000	8 (80.0)	2 (20.0)	10	

*are presented as mean (SD); ** are presented as mean (%); URI: Upper Respiratory Tract Infections.

4- DISCUSSION

This study investigated anti-pertussis IgG and IgM in Iranian children (Karaj city) after primary series of vaccination and before 18 months DTwP first booster. It was expected that IgG antibody against pertussis increase to a high level 6 months after primary routine vaccination against pertussis. Only, 17.3% of cases had acceptable protective antibody against pertussis. Because of short time lapsed (three years) after introduction of Indian vaccine in Iran, study on antibody response on pertussis component of Indian vaccine is limited in Iran, but in the recent years Iranian studies have revealed similar results. In a study by Safar et al. (2005) on 69 infants, 6 to 8 weeks after DTP vaccination with 25 IU/ml cutoff, seropositivity rate was 76.8% (14). In the study of Zarei et al. (2007) on 350, 4-6 years old children, 2 to 4 weeks after Razi DTP vaccination with 16 IU/ml cutoff, 70.3% positive cases were recorded, and the mean GMT was 24 IU/ml (22.18±44) (9). In Zarei et al.'s study (2009) on 672 4-6 years old children, GMT was 8.7 IU/ml 2-4 weeks after Razi DTP vaccination (15). Two years after using Indian DTwP vaccine instead of Razi Institute vaccine for routine vaccination of children in Iran, a study by Sannei et al. on 725 12-month-old children showed low mean antibody titer as 8.58 IU/ml; this measurement was 6.31 IU/ml at 4 months of age before the second primary vaccine injection. IgG and IgA antibody titer in 72-month-old children showed upward increment because of natural infection (13). Seroepidemiologic study on Iranian teenagers (2007) showed a positive result in 47.6% of cases with mean age of 19.48 years. This finding may indicate the need of supplemental vaccination for 16-year-old individuals with Acellular Pertussis in Iran which is not available now (16). In another study on 1,101 subjects in different age groups in Tehran, seropositivity was 50% in the 8-month, and decreased to 40% in the 6- to 10-year and increased to 70% in the 16- to 20-year group without any vaccination history (17). Reports from other countries show high postvaccination DTwP or DTaP antibody level. Post vaccination IgG GMT after the 3rd dose of pentavalent vaccine (6 months) with acellular pertussis component was 146U/ml in New York (1994) (18); this measurement in Chorea 2010, 4 weeks after three-dose vaccination, was 247U/ml (19). Pentaxim™/Pentavac™ (DTaP-IPV//PRPT) was approved to be used in >85 countries including India in 2007; Indians reported a high antibody response

1 month after the third vaccine dose of Pentaxim™. Following a primary vaccination Expanded Programme on Immunization (EPI) (6, 10, and 14 weeks), the response rate to pertussis antigen (PT) increased fourfold in 93.7% of Indian children (20). In many studies Quantitative antibody shown higher titers immediately after vaccination. Tregnghi et al., Gatchalin et al., and Clemens et al. studies reported antibody level after the third dose as 133.7, 101, 4, and 117.5 IU/ml, respectively with high response after vaccination and marked antibody increase after the booster dose (21-23). In Castarica (2002), 9 months after DTPw-HB/Hib, 50% of children had protective antibody against Tetanus and pertussis, and they showed a strong response after booster injection (24). In a study in Pio Preto Sao Paulo in Brazil, high level of anti-pertussis antibody continued at least 2 years after DTWP vaccination (25).

In a local pediatric practice center in Madison after initial series and booster vaccination, a fourfold increase in antibody titer regarding all four antigens occurred. Post booster the GMT of anti-PT was 58.0 (46.3–72.3) (26). After change of DTwP to DTaP in the 1980s, because of high adverse reaction to DTwP, the incidence of pertussis began to increase despite mass vaccination (27, 28). In a local epidemic of pertussis in Florida 2013, 39 cases showed the confirmed national case definition of pertussis, but only two persons did not complete the series of vaccination with pertussis. The attack rate was 50% (29). Recent studies on anti-pertussis antibody level show its decrease a long time after DTaP vaccination (30). Pertussis disease after one to 20 years past vaccination was reported from Greece (2008), Denmark (2010), and Turkey (2009-2010) (31). In a pertussis outbreak in 10 to 17-year-old teenagers, a case control study on individuals born from 1994-1999 in California compared three groups of DTwP, Mixed DTwP/DTaP, DTaP received cases; the DTwP group was found to have lower risk for Pertussis acquisition (32). A systematic review on acellular DTaP vaccines shows that lower number of components in DTwP vaccine has less immunologic power (33). Based on these results, it seems that DTwP may have higher immunogenicity. T- Cell response increase occurs in whole cell vaccine primed and naturally infected children after boosting, but this response may not occur in acellular vaccine primed children (34**)**. Despite higher immunogenicity, the protective effect of whole cell pertussis is at the level of 71% and does not reach 100% even one month after vaccination (35). As it is known, vaccine used in Iran is whole cell pertussis as a component of Pentavalent (DTwP-hepB-Hib), but the response of antibody to the pertussis component is not optimal. This study showed a low level of antibody against pertussis in one year old Iranian children 6 months after the last dose of pertussis vaccination as a component of pentavalent vaccine. Based on Iranian post vaccination serologic studies and higher antibody level after vaccination in studies from other countries, it is the time for another revision on the immunogenicity and cost effectiveness of Iranian vaccine used for our routine vaccination.

4-1. Limitations of the study

The study suffered from the lack of information about antibody response after the first pertussis booster in Iranian children. Strong booster response can be a good indicator of acceptable immunogenicity of pentavalent vaccine.

5- CONCLUSION

This study showed low level antibody 6 months after the last routine pentavalent vaccination. Low IgA level in all cases indicates that, despite low IgG level, natural infection did not occur. The results

may show that other components of immunologic system such as T cell immunity can play an important role in vaccine induced protection.

6- CONFLICT OF INTEREST: None.

7- ACKNOWLEDGMENT

Our special thanks go to Shahid Bahonar Hospital Clinical Research Development Unit (Alborz University of Medical Sciences) and Fardis Medical Laboratory (Karaj) for their comprehensive cooperation.

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