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Original Article

Serologic Evidence of Pertussis Infection in Vaccinated Iranian Children

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Abstract

Background: It seems that the incidence of pertussis-like illnesses is considerably increasing despite the wide coverage of immunization with the whole cell pertussis vaccine. We aimed to investigate the occurrence of pertussis in vaccinated children by measuring anti-pertussis antibodies.

Methods: In this cross-sectional study, blood samples were taken from vaccinated children aged 2, 4, 6, 12, 18, and 72 months. Anti-pertussis IgG and IgA were measured by ELISA. $P<0.05$ was considered significant.

Results: 725 children were enrolled in the study. Geometric mean titers for IgG that showed a slight decease after 2 months of age and increased distinctly in children aged 72 months. The frequency of the individuals whose IgG was above the determined cut-off (derived from mean+2SD) was observed in 1% of the 2, 4, and 6-month-old infants, 6% of the 12 and 18-month-olds and 12% of the 6-year -old children. Positive IgA titers were detected in 5, 9, 6, 23, 11, and 8% of children aged 2, 4, 6, 12, 18, and 72 months, respectively.

Conclusion: Since a considerable percentage of children had high levels of anti-pertussis IgG antibodies (≥2 SD), positive anti-pertussis IgA, and most importantly an increased level of anti-pertussis IgG geometric mean titer at 6 years of age, further investigations regarding the protection provided by the presently used pertussis vaccine seems necessary.

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Keywords ● Pertussis ● Whooping cough ● Pertussis vaccine

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Introduction

Reports from the World Health Organization (WHO) reveal that 17.6 million persons contracted whooping cough in 2003 resulting in 279000 deaths; however, some authorities estimate the real figures of cases with pertussis to be around 50 million with 90% living in developing countries.[1] According to the report of WHO Regional Office for the Eastern Mediterranean, 19000 deaths have occurred in under 5-year-old children because of pertussis.

464 patients were reported to have pertussis in Iran, in 2010.[2] Immunization against pertussis is included in the expanded program of immunization developed by the WHO and has been implemented in Iran since 1950. Whole cell pertussis vaccine is used in combination with diphtheria and tetanus toxoids (as DwPT) to vaccinate infants and children against these three deadly diseases.[1] In spite of a coverage of >95% for DwPT, the incidence of pertussis seems to be increasing during recent years. Young

children continue to contract whooping cough and are placed at risk for complications and sometimes mortality associated with this vaccine preventable disease.[1,3,4] Occurrence of pertussis in vaccinated individuals has raised questions about the protection afforded by the whole cell vaccine; numerous researchers have suggested different approaches for assessment of vaccine efficacy including estimating the prevalence of pertussis in vaccinated populations.[1,5-8]

Since pertussis may mimic other respiratory diseases such as adenovirus, influenza and mycoplasma infections, resulting in the so-called "pertussis-like" syndrome, relying solely on the clinical presentation would not be a true measure for documenting the disease, as many cases would be clinically mislabeled as pertussis. Current diagnostic procedures include culture, polymerase chain reaction (PCR), and a rise in antibodies through enzyme-linked immuno-assay (ELISA).[9] Isolation of *Bordetella* pertussis which is the gold standard for diagnosis is a difficult and time consuming procedure, making it impractical for epidemiologic studies.[8] Detecting the organism by PCR is rapid and sensitive but sensitivity decreases with time and with antibiotic treatment.[4,8] Serology, however, appears to be an easily available and reliable technique to document definite infection with *Bordetella* pertussis; a rise in IgG antibodies against pertussis toxin (IgG-PT) is seen in >90% of individuals exposed to *B.* pertussis either through a natural infection or through vaccination.[8-10] Serum IgA, however, does not rise after vaccination and is detectable only in children who acquire natural infection.[9-11]

In vaccinated children, the documentation of natural infection with pertussis would be difficult. Because of the anamnestic response of the immune system after immunization, a rapid increase in anti-pertussis antibodies is seen which prevents a significant difference in antibody concentrations between the acute and recovery sera. Therefore, in vaccinated individuals, detection of anti-pertussis IgA, single values of IgG antibodies above a certain level, and single high values of IgG antibodies 2 to 3 standard deviations exceeding the mean value in vaccinated uninfected individuals have been used to diagnose natural infection.[5,10,12]

We aimed to determine the prevalence of pertussis in vaccinated infants and children at different ages ranging from 2 months to 6 years by measuring the anti-pertussis IgG and IgA antibodies. We aimed to provide an estimate of the protection afforded by the whole cell pertussis vaccine incorporated in the DwPT vaccine currently used in Iran for routine immunization of children.

Subjects and Methods

This cross-sectional study was done in 6 health facility centers affiliated to Tehran and Shahid Beheshti Universities of Medical Sciences, Tehran, Iran. The centers were selected using cluster sampling. The protocol of this study was approved by the Ethics Committee of Shahid Beheshti University of Medical Sciences, Tehran, Iran.

We included disease-free and afebrile infants and children aged 2, 4, 6, 12, 18 and 72 months with a valid vaccination record (card), referring to centers for DwPT vaccination. The children were selected using the convenience sampling method.

Children with incomplete or poorly documented vaccination records, those with a history of blood transfusion, immune-compromised children or those receiving immunosuppressive drugs were excluded from our study.

The sample size was estimated to be 100 samples from each age group (power=80%, confidence interval=95%).

Parental consent was obtained through face to face interview. The children's vaccination cards showed that their vaccination status was up-to-date. After documenting the relevant data, 2 ml venous blood was collected from each child and sent to the laboratory where the sample was centrifuged and the serum stored at -70°C. Samples were then tested by ELISA for the presence of Anti-pertussis IgA (anti-pertussis toxin, anti-filamentous hemaglutinin, and anti-lipopolysaccharides antibodies) and IgG (anti-pertussis toxin, anti-filamentous hemaglutinin, and anti-lipopolysaccharides antibodies) using the kit supplied by the IBL company, Germany (Reference No: RE56131 and RE 56141).

Serum IgG and IgA levels were measured in 2, 4, 6, 12, 18 and 72-month-old children before administering the scheduled DwPT vaccine, imported from the Serum Institute of India and is routinely administered at 2, 4, 6, 18, and 72 months of age. The antibody levels were recorded at different ages and compared with baseline levels at 2 months.

In further analysis, the geometric mean titer (GMT) were classified sequentially for both IgG and IgA at ages 2, 4, 6, 12, and 18 months as the baseline levels and compared with the GMT of the two antibodies at higher ages. The frequency of seropositive subjects was also measured in all age groups.

A natural pertussis infection was determined through any of the following: 1- A positive IgA titer, 2- To have an IgG level above the mean+2SD level. In each age group, after excluding IgA

positive individuals, as naturally infected persons in the remaining children, assumed as being uninfected, the mean+2SD level of IgG was assigned as the upper limit of vaccine induced antibody and also as a cut-off (threshold). Any rise above this level of IgG was assumed as a natural pertussis infection. 3- To have an IgG level ≥100 IU/ml.

Categorical variables were reported as frequency and percentage and for quantitative variables were presented as mean±standard deviation (SD). Antibody GMTs and related standard errors (SE) were calculated by logarithmic transformation of data. The analysis of antibody titers was also done using the logarithmic transformed data.

Linear or logistic regression analyses were done according to the type of dependent variable. To evaluate the level of antibodies against *Burdetella* pertussis between the groups, analysis of variance (ANOVA) and for pair wise comparisons Bonferroni test have been used.

To evaluate the association of categorical data with each other, Chi-square and Fisher's exact tests were used. P<0.05 was considered as statistically significant. In case of performing multiple comparisons to evaluate a single hypothesis, P values were adjusted for the number of comparisons.

Data analysis was done assuming that all data are individually independent from each other.

Results

We included 725 children aged 2, 4, 6, 12, 18, and 72 months. 380 (52%) were boys. Samples were collected from >100 participants in each age group. The most collected samples (n=182) were from the 6-year-old group.

Mean (±SD) IgG levels (GMTs) at 2, 4, 6, 12, 18, and 72 months were 8.43 (±1.07), 6.31 (±1.22), 8.29 (±1.04), 8.58 (±1.08), 7.35 (±1.11), 14.4 (±1.06) U/ml, respectively. The mean (±SD) IgA levels at the same ages were 1.48 (±1.21), 1.43 (±1.23), 1.45 (±1.32), 2.66 (±1.21), 2.24 (±1.19), 2.03 (±1.1) U/ml, respectively (tables 1 and 2).

Table 2: The estimated frequency of natural pertussis infection using different variables

Variable	Cases/population
IgA	64.8/1000
IgG≥100 (6y)	104/1000
IgG>mean+2SD	91/1000

The GMTs for IgG showed a slight fall after 2 months and a definite rise in children aged 72 months (figure 1).

1% of 2, 4, and 6-month-old infants, 6% of the 12 and 18-month-olds and 12% of 6-year-old children had IgG levels above the determined cut-off (derived from mean+2SD). 1% of the 2, 4, and 6–month-old infants, 5% of the 12 and 18–month-olds and 10% of the 6-year-old children had IgG levels ≥100 IU/ml.

GMTs of serum IgA were compared between different age groups, which showed significantly higher GMTs at certain ages (table 2). GMTs for IgA reached the highest levels at 12 and 18 months of age (figure 2).

IgA levels above the assay cut-off were detected in 5, 9, 6, 23, 11, and 8% of the children at the ages of 2, 4, 6, 12, 18 and 72 months, respectively. Considering the equivocal results of IgA as well as the positive ones (IgA≥8 U/ml), the frequency of natural infection were 5, 9, 6, 23, 11, and 8% at the ages of 2, 4, 6, 12, 18 and 72 months, respectively.

Table 1: Comparison of IgA geometric mean±SD titers at different ages with 2, 4, 6, 12, and 18 months considered sequentially as bases for comparison with higher ages

Age (months)	GMT(U/ml)	Age (months)	GMT(U/ml)	P value
2	1.48±1.21	4	1.43±1.23	0.16
2	1.48±1.21	6	1.45±1.32	0.81
2	1.48±1.21	12	2.66±1.21	<0.001*
2	1.48±1.21	18	2.24±1.19	<0.001*
2	1.48±1.21	72	2.03±1.1	0.22
4	1.43±1.23	6	1.45±1.32	0.91
4	1.43±1.23	12	2.66±1.21	<0.001*
4	1.43±1.23	18	2.24±1.19	<0.001*
4	1.43±1.23	72	2.03±1.1	0.21
6	1.45±1.32	12	2.66±1.21	0.008*
6	1.45±1.32	18	2.24±1.19	0.03
6	1.45±1.32	72	2.03±1.1	0.31
12	2.66±1.21	18	2.24±1.19	0.003*
12	2.66±1.21	72	2.03±1.1	0.59
18	2.24±1.19	72	2.03±1.1	0.98

GMT: Geometric mean titer; *Statistically significant

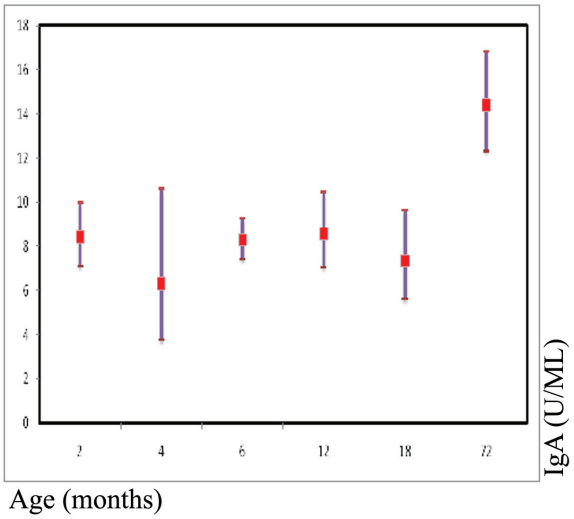

Figure 1: The GMTs for IgG shows a slight fall after 2 months and a definite rise in children aged 72 months. GMT: Geometric mean titer

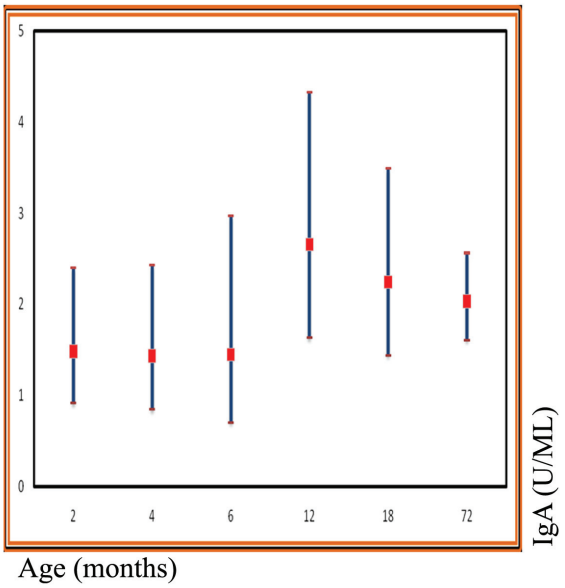

Figure 2: This figure shows the GMT for IgA at different ages of 2, 4, 6, 12, 18 and 72 months. GMTs for IgA reached the highest levels at 12 and 18 months of age. GMT: Geometric mean titer

Discussion

Our findings revealed that IgG titers declined slightly after 2 months, and then remained constant until 18 months of age. However, a sharp rise in the IgG GMT was seen at 72 months, i.e. before receiving the pre-school booster. The plateau in the GMT until 18 months can be explained by the repeated vaccinations including the primary series at 2, 4 and 6 months and the first booster given between 12 and 18 months of age. However, the unexpected sharp rise in IgG before the preschool booster i.e. between 4-5 years after the previous immunization implies recent contact with *Bordetella* Pertussis, which could only be explained through the acquirement of natural infection.

In France, 360 children were tested for pertussis serology 0.5 to 158 months after complete whole cell pertussis vaccination. Antibodies against pertussis antigens decreased rapidly after vaccination but increased secondarily 60 months thereafter. They concluded that unrecognized pertussis is common in France despite massive and sustained immunization in infants and that vaccinated children become susceptible to infection more than 6 years after their last vaccination.[13]

Although a rise in serum IgA is observed only after a natural infection, all infections are not associated with an IgA response. Although, detection of IgA against *Bordetella* pertussis has a low sensitivity with a negative predictive value of 61%, it is highly specific for a definite diagnosis of pertussis with a positive predictive value of almost 100%.[7,10] In our study serum IgA level was elevated in 6-12% of infants <2years of age and in 4% of 6-year-old children. It has been reported that IgA against the PT antigen rises in 20-40% and IgA against the anti-filamentous hemaglutinin is increased in 30-50% of natural infections.[7,10] In this study we measured IgA against three of *Bordetella* pertussis antigens (anti-pertussis toxin, anti-filamentous hemaglutinin and anti-lipopolysaccharides antibodies). Therefore, we presumed that the sensitivity of IgA in our study would be higher than the quoted figures for the measurement of separate antigens. Besides, as the half-life of IgA antibodies is considerably shorter than IgG, the presence of this antibody denotes a recent infection. Based solely on IgA levels, we estimated the prevalence of natural infection in our studied population of vaccinated children at ages of 4, 6, 12, 18, and 72 months to be between 9-11%, with the highest percentage was at 18 months. In infants aged 2 months, yet to receive their DwPT vaccination, 5% revealed evidence of recent exposure to *Bordetella* pertussis. Because of the low sensitivity of IgA, these records are believed to be only a part of the real figures.

Some investigators have used cut-off points for single serum samples derived from the mean+2SDs of anti-pertussis IgG to document natural infection.[13-17] In our study we used a similar strategy to estimate the frequency of the naturally infected vaccinated children through measuring anti-pertussis IgG. IgA positive children were excluded from each age group as naturally infected children. Then, a cut-off point of mean+2SD of the anti-pertussis IgG was assumed in the remaining samples (uninfected group) as the maximum level of vaccine induced antibody. Any rise from this level was considered as a natural pertussis

infection. However, even these figures are an underestimation of naturally infected individuals, because inclusion of IgA negative but IgG positive individuals in the uninfected group would increase the mean IgG levels. Consequently, the cut-off point of mean+2SD would rise, resulting in the underestimation of truly infected children.

Neither natural infection nor vaccination against pertussis provides permanent immunity.[3,4,9] The protective effect of the DwPT vaccine is reported to last for a varying period from 4-12 years. Moreover, only about 52% of children would have a protective level of antibodies 4 years after receiving the vaccine.[3,18] In a recent study from Australia it was noticed that the peak rate of pertussis had shifted from the age of 8-9 years to 12-13 years, after the 5th dose of the DwPT vaccine was introduced as a pre-school booster in 4-5 year-old children. The authors concluded that the protection provided by the DwPT vaccine declines 6-9 years after the last dose.[19]

Immunity against pertussis, either after vaccination or even after natural infection does not bear a direct relationship with the antibody levels, and an individual may be protected against the disease even if the antibodies are undetectable in the blood. On the other hand, antibody levels may rise after an asymptomatic infection in vaccinated individuals.[2]

The WHO reports 98 proven cases of pertussis from Iran in 2004, and 125 cases in 2005.[1] These figures have grossly underestimated the true numbers and result from under-diagnosis and under-reporting, both of which stem from a lack of definite clinical diagnostic criteria and appropriate laboratory methods.

Our figures vary widely from those of Ghanaie and colleagues from Iran who quote an incidence of 318/100000 in 2008 in Tehran among school children between the ages of 6-14 years.[20] This disparity could be explained by the differences in the age of the study population in the 2 studies as well as the use of different criteria used for the diagnosis of pertussis; theirs being clinical manifestation plus a positive PCR.

Conclusion

A considerable percentage of children had high levels of anti-pertussis IgG antibodies, positive anti-pertussis IgA, and most importantly a significant rise of anti-pertussis IgG GMT at 6 years of age, depicting a natural infection in vaccinated children. Further surveys need to be done to study the medium and long-term protection afforded by the current vaccine, in order to prevent the disease in these age group.

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Conflict of Interest: None declared

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