Seroprevalence of *Bordetella pertussis* immunoglobulin G antibodies among children in Samsun, Turkey

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SUMMARY: Özkal A, Şensoy G, Acuner Ç, Belet N, Güney AK. Seroprevalence of *Bordetella pertussis* immunoglobulin G antibodies among children in Samsun, Turkey. Turk J Pediatr 2012; 54: 15-19.

In this study, we aimed to investigate anti-pertussis immunoglobulin (Ig) G antibodies in the serum of children in our region vaccinated against pertussis with four doses. Between August 2008-2009, antibody levels to *Bordetella pertussis (B. pertussis)* antigens were studied in 385 serum samples from healthy children aged 1.5-18 years (y) vaccinated against pertussis in Samsun, Turkey. The study population was divided into six groups according to ages: 1.5-3 y; 4-5 y; 6-8 y; 10-12 y; 13-15 y; and 16-18 y. IgG antibodies to *B. pertussis* antigens were measured with a commercial ELISA kit. Mean age of the children was 9.6 ± 5.3 y. Anti-pertussis IgG titers were positive in 48.3% of the cases. The lowest positivity rate was determined in the 4-5 y age group (28.1%) and the highest rate in the 16-18 y age group (64.2%). Geometric mean titer of anti-pertussis antibodies was 39.2 IU/ml, and again the lowest value was obtained in the 4-5 y age group (23.3 IU/ml) and the highest in the 16-18 y age group (51.4 IU/ml). The antibody levels to *B. pertussis* antigens significantly decrease 4-6 years after vaccination and again increase in school children, possibly due to natural infection.

Key words: vaccination, booster, pertussis.

Pertussis, caused by *Bordetella pertussis (B. pertussis)*, is an acute and highly contagious respiratory infection. It is estimated that 20-40 million cases of pertussis occur every year worldwide, accounting for about 200,000-400,000 deaths[1]. It primarily affects infants and young children. However, pertussis cases among adolescents and adults have been increasing as a result of waning immunity after natural infection or immunization[2-8]. Although they have mild disease, they are the main source of infection in infants and young children, who have the highest rates of pertussis hospitalizations, complications and mortality[4,9-12]. Recently, some developed countries such as the United States (US), Australia, Canada, France, and Germany have incorporated an adolescent booster dose into their current immunization schedules nationally[2,4,10]. In Turkey, pertussis vaccine has been administered as three primary doses and one booster dose combined with diphtheria and tetanus vaccines (DTP), since 1968. After participation in the Expanded Program on Immunization of the World Health Organization (WHO), immunization accelerated in 1985 and the DTP vaccination coverage increased to 83% in 2001 and 90% in 2005[13]. In 2008, DTP vaccine switched to diphtheria, tetanus and acellular pertussis vaccine including 25 mcg *B. pertussis* toxoid and 25 mcg filamentous hemagglutinin as pertussis antigens (DTaP), and began to be given with inactive polio and *Haemophilus influenzae type b* vaccines at the ages of 2, 4, 6 and 18-24 months. Recently, the Ministry of Health in Turkey added the fifth dose of acellular pertussis vaccine to the National Immunization Program for 1st grade children.

In this study, we aimed to investigate anti-*B. pertussis* immunoglobulin (Ig)G antibodies in the serum of children fully vaccinated against pertussis and to determine whether or not a second booster dose and adolescent vaccination are necessary in Turkish children.

Material and Methods

Between August 2008-2009, antibody levels to *B. pertussis* antigens were studied in 385

serum samples from healthy children (194 boys, 191 girls) aged 1.5-18. None of the children had a complaint of coughing for more than two weeks in the last three months. The study population was divided into six groups according to ages as: 1.5-3 y; 4-5 y; 6-8 y; 10-12 y; 13-15 y; and 16-18 y. Serum samples of children aged 1.5-3 y and 4-5 y were obtained from children who applied to Ondokuz Mayıs University Children's Hospital for reasons such as trauma, intoxication or medical certificate. Serum samples of the other age groups were obtained from children in primary school (grades 1, 5, 8) and high school (grade 4) in three schools in Samsun. The children and parents were informed about the study and written consent forms were obtained. All of the study population had four doses of pertussis vaccine according to the vaccination schedule of the Health Ministry. The Ethical Committee of the university approved the study.

Serum samples were stored at -20°C until the study day, and IgG antibodies to *B. pertussis* antigens (pertussis toxin, filamentous hemagglutinin and lipopolysaccharides) were measured with a commercial enzyme-linked immunosorbent assay (ELISA) kit (Demeditec Diagnostics GmbH, Kiel, Germany) with the manufacturer's stated sensitivity of 100%. Results were evaluated according to the manufacturer's classifications, and values of <18 IU/ml, 18-22 IU/ml and >22 IU/ml were considered as negative, borderline and positive, respectively.

Statistical Analysis

Data were analyzed using the Statistical Package for the Social Sciences (SPSS) 16.0 for Windows. Kruskal-Wallis variance analysis and Mann-Whitney U tests were used for the comparisons between more than two groups and two groups, respectively. A value of $p<0.05$ was considered to be significant.

Results

The mean age of the children was 9.6 ± 5.28 y (min: 1.5 y, max: 18 y), and 191 (49.6%) were female and 194 (50.4%) male. There was no significant difference between genders. Anti-pertussis IgG titers were positive in 48.3% of the cases, borderline in 3.1% and negative in 48.6%, and the lowest positivity rate was determined in the 4-5 y age group (28.1%) and highest in the 16-18 y age group (64.2%). Geometric mean titer (GMT) of anti-pertussis antibodies was 39.2 IU/ml, and again the lowest values were in the 4-5 y age group and the highest in the 16-18 y age group (Table I). GMTs of the antibodies of age groups 4-5 y and 6-8 y were significantly lower than in the other age groups ($p<0.05$). Figure 1 shows GMT of antibodies according to the age groups. Antibody titer >125 IU/ml was detected in 9.1% of the children, most frequently in the 16-18 y age group (14.7%). Figure 2 shows titers of antibody to anti-pertussis antigens according to the age groups.

Discussion

Pertussis cases among adolescents and adults have been increasing recently as a result of waning immunity after natural infection or immunization. It was reported that pertussis cases in the US, in the age groups of 10-19 years and over 20 years in 2004-2005 were 15.6- and 16.7-fold higher than in the

Table I. Distribution of the Seropositivity Rates and GMTs of the Antibodies According to the Age Groups

Age groups (y)	N	Seropositivity rates of the antibodies (%)	GMTs of anti-pertussis antibodies (IU/ml)
1.5-3	55	52.7	45.2
4-5	64	28.1	23.3
6-8	63	30.2	24.5
10-12	75	57.3	48.9
13-15	61	55.7	40
16-18	67	64.2	51.4
Total	385	48.3	39.2

N: Number. GMT: Geometric mean titer.

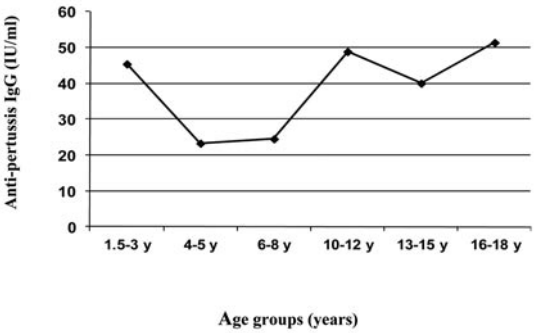

Figure 1. Geometric mean titers of antibodies according to the age groups.

period 1990-1993, respectively[2]. Konda et al.[6] reported that anti-pertussis antibodies tended to decrease until 6-8 years of age in Japan and again increased in adolescents and young adults. Similarly, a study in Belgium confirmed waning of vaccine-induced antibody levels and suggested pertussis resurgence during adolescence and young adulthood[14].

While infants and children have typical symptoms of pertussis such as paroxysmal cough, posttussive vomiting, inspiratory whoop, and prolonged cough, adolescents and adults usually have less typical symptoms with only prolonged cough. Thus, pertussis may be undiagnosed among adolescents and adults, but they are a major source of infection in neonates and infants, especially for those not yet fully immunized, and most of the infant pertussis deaths occur in babies younger than two months[3,9]. Recently, Tdap vaccine for adolescents has been incorporated into the immunization schedules in some developed countries[2,4]. According to the data of the Health Ministry, adolescent and adult pertussis cases are increasing in Turkey as well[13]. The frequency of pertussis in Turkish children with prolonged cough was reported as approximately 16% recently[15,16].

Protective immunity after natural pertussis infection wanes after 7-20 years. Estimates of the duration of immunity provided by whole-cell vaccine range from 4-12 years[17,18]. Although there is a study that suggests a longer duration of protective immunity acquired by whole-cell pertussis vaccination than by acellular pertussis vaccination, other studies did not find a difference between these two vaccines, and estimate of protection acquired by acellular vaccine is around six years[17,19-22]. In this study, we investigated the seroprevalence of anti-pertussis IgG antibodies in vaccinated children, and we determined that the seroprevalence was very low at the age of 4-6 years and in primary school grade 1 children, in accordance with the literature. The positivity rate of antibodies increased in the primary school children in grades 5 and 8, and the highest positivity was detected in grade 4 high school students (age 16-18 y). Furthermore, GMT of antibodies was highest in the older children. These findings suggest natural immunization occurs after the age of 6-8 years. Surprisingly, the seropositivity rate in 1.5-3-year-old children was also lower than we expected, but a literature search revealed that other studies also support the rapid decrease in the anti-pertussis titers[23,24]. Grimprel et al.[23] investigated the long-term serum antibody responses after complete whole-cell pertussis vaccination in France. They reported that detection rates of anti-pertussis toxin antibodies were 50%, 31%, and 11.5% at 3.7, 28, and 57 months postvaccination, respectively. De Greeff et al.[24] studied pertussis disease burden in the household of young infants with pertussis in the Netherlands, and they detected that 46% of children vaccinated with whole-cell vaccine and 29% of children vaccinated with acellular vaccine had pertussis 1-3 years after completion of the primary series. Genetic and immunologic factors may be influential on the rapid decrease of antibodies or vaccine failure.

In Turkey, two other studies in children were also done to detect the seroprevalence of anti-pertussis antibodies[25,26]. Cevik et al.[25] investigated children aged 4-24 years in Ankara and determined the lowest GMT in children aged 4-6 years. The frequency of antibodies was

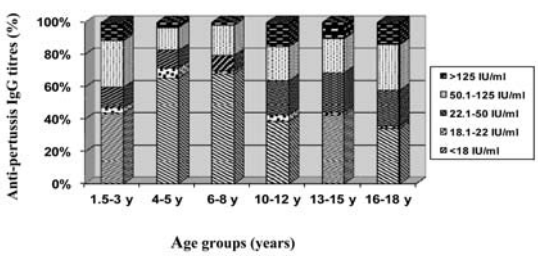

Figure 2. Anti-pertussis antibody titers according to the age groups.

only 38% at 4-6 years, similar to our study, and increased to 84% in children aged 13-18 years. In another study in Ankara, Ozkan et al.[26] reported a higher (64.1%) seropositivity at the age of 6 years, and a sharp decline to 38.1% at the age of 11 years, and again a sharp increase to 97% in 12-year-olds. There may be some local differences in the seropositivity rates according to ages, but these three studies support permanent circulation of *B. pertussis* in the community, and there is a need for a booster dose in school children.

In conclusion, we suggest that a second booster dose should be given to Turkish children as well. This study was performed between August 2008-2009 before the new pertussis vaccination schedule of the Health Ministry of Turkey in 2011, which added a fifth dose of vaccine. Our findings support this vaccination strategy. However, it might be necessary to vaccinate children earlier than primary school grade 1 due to the rapidly waning immunity after vaccination. Further studies about the rate of waning of vaccine-acquired immunity will help determine the optimal age and frequency of booster doses. In addition, adolescent vaccination will help to prevent the continuation of pertussis in the population and protect the young infants.

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