Clinical Research

Determination of *Bordetella pertussis* Seroprevalence and Colonization in Adolescents

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ABSTRACT

Objective: Pertussis is a contagious respiratory disease caused by *Bordetella pertussis (B. pertussis)* which can affect all age groups. We aimed to identify *B. pertussis* infection and determine age-specific antibody levels and provide epidemiologic data for national immunization strategies.
Material and Method: Children aged 10-18 years with cough were examined for *B. pertussis* PCR (polymerase chain reaction), nasopharyngeal specimen culture, serum *B.pertussis* IgG by ELISA (enzyme-linked immunosorbent assay).
Results: Of 429 subjects, mean age was 13,04 ± 2,16 years; 229 (53,4%) were male, 200 (46,6%) were female. Cough duration' median was 4 days (minimum 1 day and maximum 90 days). Of 405 (94%) fully vaccinated children, we accepted 27% of subjects were applied five, 67% were applied four doses of *B.pertussis*. Since fifth dose of *B.pertussis* is applied in school age, we accepted all subjects in appropriate age had five doses with hesitation. Subjects were negative for the culture. Three subjects (0,69%) were positive with *B. pertussis* PCR. Forty three subjects (10%) were positive for *B. pertussis* IgG. There was a linear correlation between age and *B.pertussis* IgG absorbance value (r =0,112; p =0,020). Cough duration was significantly longer in *B. pertussis* IgG positive group compared to those negative (p =0,012). The highest seropositivity was in 13-15 ages.
Conclusion: To decrease the frequency of pertussis infection, to prevent pertussis infection transmisson to infants via adolescents; we recommend sustaining infanthood vaccinations and applying a booster dose at 10-13 years of age in addition to the fifth dose.

Keywords: *Adolescent, Booster, Pertussis, Seroprevalence, Vaccine.*

ÖZET

Adolesanlarda *Bordetella pertussis* Seroprevalansının ve Kolonizasyonunun Belirlenmesi
Amaç: Boğmaca, *Bordetella pertussis (B. pertussis)* infeksiyonu olduğu her yaştan kişileri etkileyebilen bulaşıcı bir solunum sistemi hastalığıdır. Çalışmamızda *B. pertussis* infeksiyonu olan adolesanları tanımlamayı, yaşa özgü antikor düzeylerini saptamayı ve ulusal aşı stratejileri için epidemiyolojik veri sağlamayı hedefledik.
Gereç ve Yöntem: Öksürük yakınması olan 10-18 yaş arası çocukların nazofarengeal sürüntü örneklerinde *B. pertussis* PCR (polimeraz zincir reaksiyonu) ve kültürü, serumlarında *B. pertussis* IgG çalışıldı.
Bulgular: Çalışmadaki 429 adolesanın 229'u (%53,4) erkek, 200'ü (%46,6) kızdı, yaş ortalaması 13,04 ± 2,16 yıldı. Öksürük süresi median 4 gün (minimum 1 gün ve maksimum 90 gün) olarak saptandı. Katılımcıların 405'i (%94) boğmaca için tam aşılı olarak saptandı. Boğmaca aşısının 2010 yılında başlatılan beşinci dozu okul çağında uygulandığı için uygun yaştakilerin tümüne beş doz aşı uygulandığının kabulü hususunda çekincemiz olmakla beraber boğmaca aşılı hastaların %27'si 5; %67,4'ü 4 doz *B.pertussis* aşısı uygulanmış olarak kabul edildi. Kültürde üreme olmadı, 3 adolesanda *B. pertussis* PCR pozitif, 43 adolesanda *B. pertussis* IgG pozitif saptandı. *B. pertussis* IgG absorbans değeri ile yaş arasında doğrusal korelasyon saptandı (r =0,112; p =0,020). *B. pertussis* IgG pozitif olan grupta, öksürük süresi antikor negatif olan gruba göre daha yüksek bulundu (p =0,012). Seropozitiflik 13-15 yaş grubunda en yüksekti.
Sonuç: Ülkemizde boğmaca enfeksiyon sıklığını azaltmak, adölesanlar aracılığıyla boğmaca enfeksiyonunun bebeklere geçişini önlemek amacıyla yaşamın ilk iki yılındaki aşılara devam edilmesi, ilkokul birinci sınıfta yapılan beşinci doza ek olarak 10-13 yaş grubuna boğmaca aşısının pekiştirme dozunun yapılmasının uygun olacağı kanısındayız.
Anahtar Sözcükler: *Adolesan, Pekiştirme, Boğmaca, Seroprevalans, Aşı.*

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Pertussis, caused by *Bordetella pertussis (B. Pertussis)*, is a contagious respiratory infection affecting all ages but it can be severe in newborns and infants. Pertussis of babies and infants is typically characterized by paroxysmal cough; it can cause cough lasting for weeks in older children (1). Pertussis can be prevented by vaccination. In 2008 World Health Organisation (WHO) estimated that global vaccination against per-

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tussis has prevented approximately 687000 deaths. The main aim of pertussis vaccination is to reduce the risk of severe pertussis in infancy. By widespread vaccination programmes and increasing vaccination rates, a significant reduction in pertussis incidence has been achieved but epidemiological studies in recent years show that *B.pertussis* has high circulation in adolescents and adult age groups (2). As the protection of pertussis vaccine decreases significantly 5-10 years after the last vaccination, pertussis susceptibility is increasing among adolescents and adults (3). In addition adolescents and adults are leading to the spread of the disesase to all age groups during endemics and epidemics and they are the source of infection for incompletely vaccinated babies (4). Increasing prevalence of the disease despite high vaccination rates, has changed pertussis management strategies. In developed countries acellular booster doses have been added to vaccination schedule for children aged 10 -18 years. By new diagnostic methods, attempt for identifying asymptomatic and atypical adolescent and adult cases has increased (5).

Pertussis is still an infectious disease which affect all age groups in our country. It has cyclic epidemics and it causes outbreaks every 2-5 years. In Turkey pertussis vaccination was first administered in 1968 as three doses of Diphteria-whole cell Pertussis-Tetanus (DPT) in first year of life and the booster dose was applied in 16-24 months. Because of adverse reactions with whole cell pertussis vaccine; acellular pertussis vaccine (aP) has been administered since 2008. By October 2010 the fifth dose of pertussis vaccine (DaPT-IPA) has been added to national vaccination schedule to be applied to students in first class of primary school. In last ten years studies show an increase in pertussis incidence in our country as in many other countries (6). Epidemiologic studies have gained importance for suffering with pertussis which is a public health problem at these ages. Age-specific seroepidemiology of pertussis should be known to determine the appropriate time for booster doses of pertussis.

This study was achieved with children aged 10-18 years with cough in Mersin, by identifying pertussis with clinical, serological and bacteriological methods; in order to determine differences in antibody levels for pertussis due to age, establish necessity of additional booster dose of pertussis and determine the appropriate age for booster dose.

MATERIAL AND METHOD

In our study 429 children aged 10-18 years applied to hospital with cough between January-July 2015 were included. Exclusion criteria were having tuberculosis, cystic fibrosis, ciliary dyskinesia, bronchiectasis and other pulmonary diseases causing chronic cough; having immunodeficiency and receiving chemotherapy. Patients' vaccination status was assessed. Because the fifth dose of pertussis was added to the vaccination schedule by October 2010; fully vaccinated cases aged 10-11 years were accepted as having five doses while cases aged 12-18 as four doses. The study was achieved with approval of local ethical committee (MEU 2015/28).

One nasopharyngeal swab (NS) and 5 ml venous blood was taken to EDTA (etilen diamin tetra asetikasit) tubes by aseptic methods. Nasopharyngeal sample was taken from each patient by inserting one Dacron swab through nostrils and rotating the swabs that have flexible to the posterior wall of nasopharynx for 3-5 seconds. Cotton swabs were not used. Samples were transported within a few hours by Amies transport medium with charcoal to the microbiology laboratory. Nasopharyngeal samples were used for culture and PCR (polymerase chain reaction). For isolation of *B. Pertussis*, nasopharyngeal specimens were added to Bordet Gengou (BG) agar (Lot: 2226449, Becton Dickinson, Germany) containing 15% sheep blood supplemented with cephalexin.

Patients coughed at a coughing plate containing BG agar with cephalexin. Nasopharyngeal samples' plates and the other plates which patients coughed into were incubated at 36 ° C in humidified, aerobic conditions for ten days. Positive control strain was used for medium. PCR kits were obtained from Infectious Diseases Laboratory of Turkey Public Health Agency. Deoxyribonucleic acid (DNA) was purified from nasopharyngeal samples by using modified classic phenol-chloroform and chloroform method. For detecting *B. pertussis*, DNA BP1/BP2 primer sequences (BP-1: 5'-GAT TCA ATA GGT TGT ATG CAT GGT- 3 'and BP-2: 5'-TGG ACC ATT TCG AGT CGA CG-3') were used. Specimen obtained from patients was kept at -30 ° C until ELISA (enzyme-linked immunosorbent assay) tests done. Serum samples were tested for *B.pertussis* IgG antibodies by using commercially available *Bordetella pertussis* IgG ELISA (NovaLisa™, Novatec Immunodiagnostica, Germany). Results were assessed by calculating the NovaTec Units (NTU: [Patient absorbance value/cut off] x 10). The absorbance is determined spectrophotometrically at 450/620 nm. The NTU of <9 was considered as negative, 9-11 was considered as equivocal or grey zone and >11 was considered as positive.

Minimum sample size was determined as 416 patients by considering the number of patients aged 10-18 year applied with cough to pediatric policlinics the year before this study was planned. Descriptive statistics were used for statistical analysis of the data. Frequency and percentage values were given for the categorical variables. Chi-square test was used for checking the relation between two categorical variables. Independent –Samples T test was used to compare two independent groups of normally distributed continuous variables. Nonparametric Mann-Whitney U test was used for non-normal distributed variables. ANOVA was performed to compare more than two independent groups of normally distributed continuous variables. Pearson's correlation coefficient was used to examine relation

between two continuous variables. ROC analysis was used for the continuous variable whose cut off point is needed. Statistical significance level was accepted as p <0,05.

RESULTS

Of 429 cases 53,4 % were (n =229) male and mean age was 13.04 ± 2.16 years. Four hundred and five patients were fully vaccinated for pertussis, 27% (n = 116) were applied five doses while 67% (n =289) were applied four doses. All patients were negative for the culture. Three cases (0,7%) were positive for *B. pertussis* PCR (Table 1).

Table 1. *Demographic characteristics of the study group.*

	n	%
Female	200	46.6
10-12 years	89	20.7
13-15 years	89	20.7
16-18 years	22	5.1
Male	229	53.4
10-12 years	93	21.6
13-15 years	89	20.7
16-18 years	47	10.9
Pertussis vaccine dose		
4 doses	289	67.4
5 doses	116	27.0
unknown	22	5.1
not immunized	2	0.5
Time after the last vaccine dose		
<6 years	127	29.6
≥6 years	302	70.4
Culture		
positive	0	0,0
negative	429	100.0
PCR		
positive	3	0.7
negative	426	99.3
***B.pertussis* IgG**		
high positive	36	8.4
low positive	7	1.6
negative	386	90.0

Two of these patients were 13, and one was 16 years old. Of PCR positive patients, two were negative for *B.pertussis* IgG while one was low positive. Median cough duration was 4 days (minimum 1day and maximum 90 days). Median of *B.pertussis* IgG absorbance value was 1,4 NTU (minimum 0,1 and maximum 34). There was a linear correlation between age and *B.pertussis* IgG absorbance value (r =0,112; p =0,020) (Figure 1).

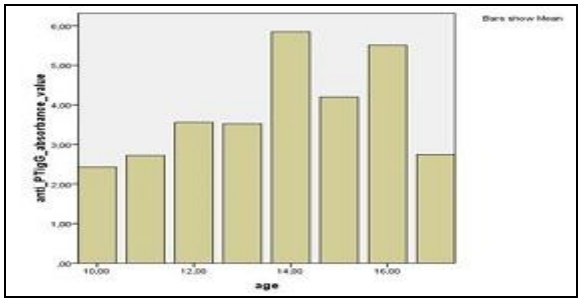

Figure 1. *B.pertussis IgG absorbance levels by age.*

There was statistically significant difference in the mean value of *B.pertussis* IgG absorbances between age groups when they were divided into two groups by age 14 (p <0,001). *B .pertussis* IgG value was significantly higher in group received four vaccine doses compared to those received five doses (p =0,016). The cases were divided into two groups by the time passed over the last pertussis dose; the difference between *B. pertussis* IgG absorbance levels between groups was statistically significant (p =0,019) (Table 2).

Table 2. *B. pertussis IgG absorbance values according to vaccination status.*

	***B.pertussis* IgG absorbance value (mean)**	***p***
Pertussis vaccine dose		
4 doses	4,07±6,88	0,016
5 doses	2,65±4,58	
Time elapsed after the last vaccine dose		
≥6 years	4,12±6,94	0,019
<6 years	2,77±4,63	

The cases were divided into two groups considering their *B.pertussis* IgG seropositivity status, mean age differed statistically with 12,95 ± 2,17 years in seronegative group and 13,8 ± 1,83 in seropositive group (p =0,011) (Table 3).

Table 3. *B. pertussis IgG absorbance values due to age.*

	***B. pertussis* IgG absorbance value (median)**	***p***
Age		
<14 years	1,1	<0,001
≥14 years	1,7	

A significant correlation was observed between seropositivity and cough duration. Long cough duration (>14 days) was more common in *B. pertussis* IgG positive group compared to those negative (p =0,012). Seronegativity was higher in group receiving five doses of pertussis compared to those receiving four doses (p =0,044). No correlation was found between time elapsed after the last pertussis vaccination and seropositivity (p =0,096). The cases divided into two groups by the age; antibody positivity was increasing statistically in both genders by age 14 (p =0,001) (Table 4).

Table 4. *Characteristics of B. pertussis specific antibody positive and negative groups.*

***B. pertussis*IgG level**	**Positive (n =43,%)**	**Negative (n =386,%)**	***p***
Age (year)	13,83±1,83	12,95±2,17	0,011
Age group			
<14 years	15 (34,8)	233 (60,3)	0,001
≥14 years	28 (65,1)	153 (39,6)	
Age range			
10-12 years	11 (25,5)	171 (44,3)	0,055
13-15 years	22 (51,1)	156 (40,4)	
16-18 years	10 (23,2)	59 (15,2)	
Pertussis dose			
4 doses	34 (79,0)	255 (66,0)	0,044
5 doses	6 (13,9)	110 (28,4)	
Time elapsed after the last vaccine dose			
≥6 years	35 (81,3)	267 (69,1)	0,096
<6 years	8 (18,6)	119 (30,8)	
Duration of cough			
≥14 days	10 (23,2)	40 (10,3)	0,012
<14 days	33 (76,7)	346 (89,6)	

Seropositivity was the highest at age of 14 (27,9%) followed by age of 16 (20,9%) and 15 (14,0%).

The cases were grouped according to *B .pertussis* IgG values as high positive, low positive and negative; mean age among these groups was statistically significant (p =0,033). Mean age was 13,75 ± 1,81 years in high positive group while it was 12,95 ± 2,17 in negative group.

Notably 61,1% of patients with high positive *B. pertussis* IgG were 14 years or older and 38,9% were under 14 years (Table 5).

Table 5. *Characteristics of high positive, low positive and negative groups for B. pertussis IgG.*

***B. pertussis IgG* level**	**High positive (n =36, %)**	**Low positive (n =7, %)**	**Negative (n =386,%)**	***p***
Gender				
male	17 (47,2)	6 (85,7)	206 (53,3)	0,175
female	19 (52,7)	1 (14,2)	180 (46,6)	
Age(years)	13,75±1,81	14,28±2,05	12,95±2,17	0,033
Age group				
<14 years	14 (38,8)	1 (14,2)	233 (60,3)	0,003
≥14 years	22 (61,1)	6 (85,7)	153 (39,6)	
Age range				
10-12 years	10 (27,7)	1 (14,2)	171 (44,3)	0,154
13-15 years	18 (50)	4 (57,1)	156 (40,4)	
16-18years	8 (22,2)	2 (28,5)	59 (15,2)	
Pertussis dose				
4 doses	28 (77,7)	6 (85,7)	255 (66)	0,104
5 doses	5 (13,8)	1 (14,2)	110 (28,4)	
Unknown/none	3 (8,3)	0 (0,0)	21 (5,4)	
Time elapsed after last vaccine dose				
≥6 years	30 (83,3)	5 (71,4)	267 (69,1)	0,173
<6 years	6 (16,6)	2 (28,5)	119 (30,8)	

The patients were grouped by age as 10-12, 13-15, 16-18 years; both high and low positivity were common in 13-15 years group (51,2%). High positive *B. pertussis* IgG values were frequent at 14 and 16 years for male; 14 and 15 years for female. High positivity for both genders was seen at age of 14, with ratio of 31,6% for male and 23,5% for female.

DISCUSSION

Despite immunization, worldwide pertussis incidence is increasing in children younger than 1 year old since 1990s (7). Studies of pertussis with adolescents and adults over last 20 years have been a guide for pertussis epidemiology. According to recent studies; cyclic pattern of epidemic pertussis in prevaccine era is similar to postvaccine era. In this respect pertussis differs from the other diseases controlled by vaccination (8).

Neither vaccination for pertussis nor natural disease can provide lifelong or complete immunity against disease and reinfection. Three - five years after vaccination, protection for typical disease starts to decrease and after twelve years antibodies can't be measured. In the United States of America (USA) despite effective vaccination and having disease in natural way, pertussis outbreaks have been reported in elderly people, nursinghomes, in the places where pertussis exposure is uncommon, in suburbans with high rates of vaccination. Pertussis outbreaks have been reported with adolescents and adults who were vaccinated long time ago. Adolescents and adults with cough who are not generally considered as having pertussis are major reservoirs for *B.pertussis*; these are also index cases for pertussis of babies and children (9). According to studies carried abroad, it is crucial to improve immunization policies for adolescents, adults and health workers to control pertussis infection and related mortality (10).

In our country despite high immunization rates achieved over the years; pertussis infections still remain common. By the fifth dose of pertussis was administered; pertussis infection began to occur in elder ages. Age-specific seroepidemiology of pertussis must be known to determine when the pertussis vaccine' protection ends. According to CDC (centers for disease control and prevention) and WHO; a patient should meet these criteria; coughing for at least two weeks accompanied by paroxysmal coughing, inspiratuar stridor and vomitting after coughing to be considered as pertussis clinical case. In our study cough duration was longer than two weeks in 11,7% (n =50) and vomitting after coughing was 14,2% (n =61) of all patients. Mean cough duration of all patients was 6,8 days; whereas it was 10,8 days for *B.pertussis* IgG positive cases. Cough duration in our study is longer than reported in some studies conducted with seropositive infants in our country; while it is shorter compared to 18 days reported in a study conducted with adults in abroad (11).

Confirmed case is defined by CDC as the case whose laboratory tests are positive or the case with pertussis clinic which has a connection with a case who has one positive laboratory test. We applied all three laboratory tests to participants; including culture, PCR and serology, considering case definitions of Ministry of Health, CDC and WHO. In adults' studies reported from abroad, culture positivity was observed in a ratio of 0%-30% among those with 90% -100% seropositivity of Ig G or IgA with ELISA (12). In our study no patient was positive for culture. This result may be due to *B.pertussis* being a fastidious bacterium, possible mistakes in transporting nasopharingeal swaps, taking specimens after the early weeks of the infection.

In literature there is PCR positivity for adults ranging between 0%-30%; and anti-PT or anti-FHA (filamentous hemagglutinin) positivity is 57-100% ELISA (12,13). There are data showing that culture or PCR positivity is 10% less compared to approved serological results in adolescents and adults (14). In our study PCR positivity is 0,7% (n =3) and seropositivity is about 10%. According to CDC; PCR gives definite results up to four weeks after the onset of cough. After the fourth week of the infection; decreasing DNA amounts can lead to false negative results. Furthermore PCR test is less sensitive to previously immunized individuals (15). Therefore low PCR positivity rate is an expected result.

Significant increase of serum antibody titers between acute and convalescent phases must be shown for making pertussis diagnosis. High levels of *B. pertussis* IgG and IgA in single serum sample also points infec-

tion in adolescents and adults (16). According to CDC, the best time for taking serum sample is two-eight weeks after cough onset when the highest antibody levels are detected. In our study *B. pertussis* IgG levels of patients were assessed qualitatively with single serum sample by ELISA. Increasing *B.pertussis* seropositivity by the age was remarkable. These results are similar to previous studies in Turkey (17-20). High positive antibody levels were interpreted as a recent or ongoing pertussis infection or colonization especially in patients who received last pertussis vaccine dose more than six years ago. High positive antibody levels which were assessed as acute infection peaked at age of 14 with a ratio of 27,8% . For both genders increase in rate of high positivity was noticeable by the age 14 (AUC:0,625; p :0,0085) (figure 2).

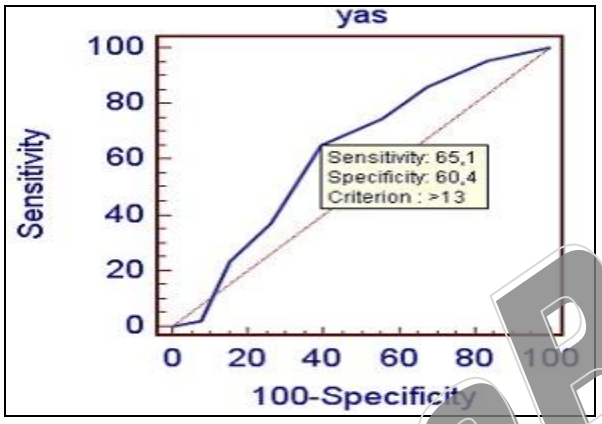

Figure 2. *Age-ROC curve; AUC: 0,625 p: 0,0085.*

Considering decreasing protection of vaccine over age of 14 years; high positive antibody levels were evaluated as recent infection. Seronegativity of cases aged 14 years and older with a ratio of 84% was interpreted as decreasing antibody levels to an unmeasurable level and waning of vaccine-induced immunity.

In our study 83,3% of high seropositive cases were found to had the last pertussis dose more than six years ago and it was interpreted as this group has become more susceptible to infection due to decreasing vaccine protection. This group of adolescents which is susceptible to pertussis infection isn't affected seriously from the disease but since this group transmits the infection to younger populations; immunization and protection of this group against pertussis is important.

By the 2000's, pertussis incidence has increased especially among persons aged 10-19 years both in our country and worldwide (17). Seroprevelance studies show *B. pertussis IgG* seropositivity has increased with age (21, 22). Antibody titers were demonstrated to have folded three times in cases aged 7-12 years and peaked at age of 13-17 years. According to our study's results *B.pertussis* seropositivity tends to increase after age of 13 and makes the peak at age of 14. Both positive and high positive levels of *B. pertussis'* antibodies are clustering in group of 14-16 years. Increasing positivity of *B. pertussis* IgG by age 14, noted as acute pertussis infection due to decreasing immunity of vaccine in this group because they received their last pertussis dose more than six years ago. These results are consistent with previous literature suggesting that vaccine protective effect is vanishing within 5-12 years after the last vaccine dose (23).

In 2004, a Japanese study with 320 patients aged 0-80 years showed that the highest anti-PT antibody titers were in 11-15 age group (24). In the study conducted by Ozkan et al. (20) with 317 students aged 6-14 years; pertussis seropositivity was reported as 70,3% (68,5% for female; 71,9% for male). Low seropositivity was observed at 6-10 years whereas the highest seropositivity (86,7% - 97%) was observed at 12-14 years in the same study. Low seropositivity in 6-10 years group indicates decreasing vaccine protection because this group received last vaccine dose at age of two years.

In our country some studies point at 10-14 years while some point at 13-16 years for pertussis seropositivity becoming significant. Because the cases included in these studies haven't received pertussis vaccine in primary school, high antibody levels were assessed as having natural infection (17,18). In Kafes' study (18) with 460 cases aged 13-30; anti-PT IgG seropositivity was reported as 81,7% in 13-18 age group and it was observed that seropositivity was rising correlated with age till age 19. Our study also shows *B. pertussis* seropositivity is increasing with age and seropositivity is higher in 13-15 age group without statistical significance. The highest seropositivity was detected at age of 16 in Kafes' study (18) while it was 16 for male and 14 for female in our study. Regardless of gender, the highest *B. pertussis* IgG seropositivity was at the age of 14 in our study. These results indicate antibody titers are decreasing after last pertussis vaccine dose; because these children are getting susceptible to pertussis; *B. pertussis* IgG levels are rising depending on recent pertussis infection.

Due to addition of the fifth dose of pertussis to national immunization schedule by October 2010; some of the cases enrolled in our study had received four, while some had five doses of pertussis. This difference was noted and the results were evaluated considering the time passed after the last dose of pertussis.

The fifth dose of pertussis vaccine was introduced into childhood schedule for children aged 4-6 years in addition to infant vaccination in USA by 1996. Pertussis incidence has raised by the years and a booster dose for children aged 11-12 years has been established since 2006 (25). In our country due to applying pertussis fifth dose at age of 6-7 years; pertussis infection tends to occur in older ages. Adolescents are generally composed of school-aged children and social activities are preliminary in adolescents so adolescents have important role in spreading pertussis infection (26). Adolescent- targeted vaccine studies will be effective in development and protection of public health.

Vaccination for healthcare servers to protect against pertussis is recommended by CDC; but this recommendation hasn't yet been implemented. A Japanese

study has shown that many infants get pertussis infection from their caregivers. Hospital outbreaks may be another source of infant pertussis (27). Although there are a few goals in immunization for protecting infant health, the most sensible one seems to be immunizing adolescents.

Immunity acquired against pertussis is not long lasting. Studies have shown that immunity is decreasing even running out 7-20 years after natural infection; 4-12 years (average 5 years) after vaccination (28). Importance of vaccination is being understood since pertussis incidence still remains high in developing countries while it is rising again in countries where vaccine coverage is low and ineffective vaccines are used.

In conclusion to decrease the frequency of pertussis infection in our country, to prevent pertussis transmission to babies via adolescents; we recommend keeping on vaccination schedule of the first two years of life and administering a booster pertussis dose to children aged 10-13 years who are accessible in schools in addition to the fifth dose being applied in the first year of primary school and introducing the booster dose into national vaccination schedule.

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