Prevalence of High Antibody Titers of Pertussis in Turkey: Reflection of Circulating Microorganism and a Threat to Infants

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Acute pertussis infection among adults can cause its transmission to the larger population, especially to infants and young children, who can develop severe disease. In order to determine an age-dependent pertussis immune response, anti-pertussis toxin (PT) antibody was detected by the indirect enzyme-linked immunosorbent assay (ELISA) method in serum samples from 2,085 healthy subjects ranging in age from 6 months to ≥ 60 years. Also included in the evaluation were responses to a questionnaire including sociodemographic characteristics, vaccination, and infection history. Titers of 50–99 ELISA units (EU)/mL and of ≥ 100 EU/mL were accepted as indicative for recent exposure or infection. In addition, 30 EU/mL was estimated to be a sufficient titer in women of childbearing age to protect their newborns until administration of their first dose of pertussis vaccine. After the age of 4–5 years, presence of high-titered antibodies that

increase with age might be a reflection of circulating infection and indicate the magnitude of the threat to infants. According to the questionnaires, in the groups younger than 15 years old, three to four doses of diphtheria toxoid-whole cell pertussis-tetanus toxoid (DwPT) were administered in 47.2 to 77.4%, 91.2 to 100.0%, and 83.5 to 100.0% of participants in Diyarbakir, Samsun, and Antalya, respectively. In addition, up to half of the expectant mothers we studied lacked a sufficient level of estimated antibody titers. To protect infants from life-threatening pertussis infection, improving vaccination coverage to ensure herd immunity and uniformly establishing coverage throughout the country are essential. Furthermore, revaccination with acellular vaccine for schoolchildren as well as for the households of pregnant women is recommended. *J. Clin. Lab. Anal.* 21:154–161, 2007. © 2007 Wiley-Liss, Inc.

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INTRODUCTION

Pertussis is a vaccine-preventable disease. In Turkey, pertussis vaccine coverage (three doses) reached a level of about 80% among children younger than 6 years of age following the National Vaccination Campaign of 1985, and it was maintained over the following years with improvement in vaccination procedures by the Ministry of Health. Nevertheless, coverage is not homogeneous throughout the country, with lower coverage rates in eastern regions (1). The incidence of disease dropped from 21/100,000 in 1970 to 0.27/100,000 in 2001 as a result of this vaccination campaign, but there may be missing cases, especially among older children and adults, because of insufficient identification

and reporting (2,3). Although the routine immunization of infants has been effective in reducing the occurrence of clinical disease in children, it has not eliminated the circulation of the microorganism. There are several reports from various countries indicating infection among schoolchildren, adolescents, and adults (4–9). In Turkey, pertussis vaccine is administered at the 2nd, 3rd, and 4th months of life and a booster is given to

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18–24-month-old children as diphtheria toxoid-whole cell pertussis-tetanus toxoid (DwPT) (1). Consequently, schoolchildren, adolescents, and adults are expected to be vulnerable to the infection due to waning of the vaccine-acquired immunity, and this plays a role in the transmission of the disease to infants prior to the completion of their routine immunization schedule, unless passively acquired antibodies are present in sufficient titers (10). Although the disease is mild and even asymptomatic among adults, adult immunization with acellular vaccine is being considered in an effort to protect newborns and younger children, since pertussis is severe and life-threatening in infants (8,10,11).

Recent advances in serological techniques have made it possible to diagnose *B. pertussis*, including asymptomatic infections and atypical presentations of the disease (6,11,12). Indirect enzyme-linked immunosorbent assay (ELISA) is one of these serological techniques and is used widely to diagnose disease and immune responses to vaccines and to evaluate immunity in populations. The quantitative results of ELISA can distinguish low, intermediate, and high antibody levels; the latter might be a reflection of rates of circulating infection in a seroepidemiological study. In addition, ELISA provides the opportunity to estimate the pertussis antibody titer required for mothers to protect their newborns until administration of the first dose of vaccine following birth (4–6,9,11,12).

“The Infectious Disease Control Project in Turkey” was implemented by the Ministry of Health of Turkey, the General Directorate of Primary Health Care, and Refik Saydam National Hygiene Center (RSNHC), with the cooperation of the Japan International Cooperation Agency, to determine the age-specific seroprevalence of six vaccine-preventable diseases (diphtheria, tetanus, pertussis, measles, hepatitis B, and polio). Surveys in three different regions were performed in 2000 and 2001 on sera collected from individuals whose ages ranged from 6 months to over 60 years, and questionnaires were administered (13). An in-house ELISA method was performed in RSNHC to assess the prevalence of *B. pertussis* pertussis toxin (PT) antibody because anti-PT antibody is protective against typical pertussis infection and high titers are indicative of acute/recent infection in symptomatic patients (7,9,12,14).

The aim of this study was to determine the percentage of individuals with high antibody titers, which might reflect the magnitude of circulation of *B. pertussis* and indicate the risk to infants, as well as the vaccination status among young children, who are an at-risk population. We also aimed to measure the percentage of mothers with antibody levels believed to provide sufficient protection from pertussis infection for their

infants. This evaluation was expected to provide data relevant to the discussion on vaccination procedures.

MATERIALS AND METHODS

Study Population

Eight health centers from Antalya (four rural and four urban), eight from Diyarbakir (four rural and four urban), and 10 from Samsun (five rural and five urban) (26 health centers in total) were selected by the Provincial Health Directorates of each city following practical considerations, such as which health centers were willing and capable of participating at the required level. A total of 16 individuals and three additional individuals were selected randomly from each age group among individuals older than 6 months (6–11 months, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10–14, 15–19, 20–24, 25–29, 30–34, 35–39, 40–44, 45–49, 50–54, 55–59, 60+ years), using the list in which all household members are systematically registered by the health center. Midwives of a related region informed the selected individuals via door-to-door visits and requested their consent. In every city, two surveys were performed, in 2000 and 2001, and 672 subjects were aimed to be selected for each province. However, due to the participation of some additional individuals, the number of subjects was increased. Individuals were able to participate in the survey as an eligible subject if they were free of infectious disease on the survey day (13,15).

The physicians of the health centers examined the subjects, and face-to face interviews were conducted to complete questionnaires composed of 20 queries about sociodemographic characteristics, vaccination history, and infectious disease history. Unfortunately, dates of vaccination doses or infection were not requested. Some of the data collected by questionnaire regarding sociodemographic characteristics has been published elsewhere (13,15). Blood specimens were collected in health care centers. The collection of the sera included 2,085 samples: 703, 714, and 668 from Diyarbakir, Antalya, and Samsun, respectively. There were no statistically significant differences in numbers of the subjects based on age group ($\chi^2 = 14.048$; $P = 0.998$), gender ($\chi^2 = 1.601$; $P = 0.449$), and residence ($\chi^2 = 0.044$; $P = 0.978$). The analyses were performed in 11 age groups: 0–1, 2–3, 4–5, 6–7, 8–9, 10–19, 20–29, 30–39, 40–49, 50–59, and ≥ 60 years old. The number of subjects in each age group were 194, 192, 205, 183, 177, 194, 230, 212, 202, 179, and 117, respectively. The number of male/female subjects for each group were 94/100, 94/98, 104/101, 84/99, 89/88, 87/107, 94/136, 102/110, 100/102, 87/92, and 54/63, respectively.

A total of 406 subjects were women of reproductive age (15–49 years old). The distribution of females

according to age groups is mentioned above. Among these subjects, 84 (20.7%) were single, whereas 322 (79.3%) were “ever married”; 124 (30.5%) had no children, and 282 (69.5%) had one or more children. The distribution of these subjects in Diyarbakır, Antalya, and Samsun were 124 (30.5%), 155 (38.2%), and 127 (31.2%), respectively; distribution according to urban/rural localizations was 207/199 (49.0%/51.0%).

Measurement of Immunoglobulin G (IgG) Anti-PT Antibody by Indirect In-House ELISA Method

The immunoglobulin G (IgG) anti-PT antibody was measured by in-house indirect ELISA, as previously described (9,16). In short, ELISA was applied using purified PT (JN1H-5, Biken, Japan), Fc-specific alkaline phosphatase-conjugated goat anti-human IgG (Seikagaku Kogyo, Tokyo, Japan), and p-nitrophenyl phosphate (Wako, Tokyo, Japan). Anti-PT IgG antibody concentrations were calculated by the parallel line assay and expressed in ELISA unit (EU)/mL according to the reference anti-sera for PT (JN1H-10; 250 EU/ampule for anti-PT) (17). In addition, a sera with a known antibody titer was included in each test for quality control. The limit of detection for anti-PT antibody was 1.0 EU/mL.

Evaluation of Immune Status

Titers of 50–99 EU/mL and of ≥ 100 EU/mL were accepted as indicative for recent exposure or infection, and 30 EU/mL was estimated to be a sufficient titer in women of childbearing age to protect their newborns

until administration of their first dose of pertussis vaccine.

Data Analysis

Statistical analyses were performed with SPSS 10.0 for Windows, and P values < 0.05 were considered statistically significant. A chi-squared test was used to examine the differences between age groups, gender, and urban/rural regions. Correlations between two variables such as age and anti-PT titers were calculated using Pearson correlation.

RESULTS

The anti-PT antibody distribution by age groups in the total study group is shown in Fig. 1 ($N = 2,085$). We found a weakly positive correlation between age and anti-PT titers ($r = 0.075$; $P = 0.001$). The frequencies of antibody presence at a level of 50–99 EU/mL and of ≥ 100 EU/mL were found to be lowest in 0–1 year olds, at 5.7% and 5.2%, respectively. These percentages increased with age; the maximum frequency for 50–99 EU/mL was 25.0% in ≥ 60 -year-olds and for ≥ 100 EU/mL it was 18.5% in 50–59-year-olds. Totally, 15.3% of the entire study population had 50–99 EU/ml and 12.5% had ≥ 100 EU/ml anti-PT antibodies.

According to the questionnaires, the vaccination history was based on cards in the groups younger than 15 years old, and three to four doses of DwPT were administered in 47.2 to 77.4%, 91.2 to 100.0%, and 83.5 to 100.0% of participants in Diyarbakır, Samsun, and Antalya, respectively. The history of vaccination was

Fig. 1. The distribution of anti-PT antibodies in < 50 , 50–99, ≥ 100 EU/mL levels among age groups from 6–11 months old (*) to ≥ 60 years old ($N = 2,085$).

based on recall in the older age groups and was not reliable, with 44.6 to 62.5%, 43.3 to 80.0%, and 60.0 to 92.5% having unknown history of vaccine in the aforementioned provinces, respectively. Pertussis infection history was present in 35, 16, and seven subjects in the same provinces, respectively, who were clinical cases diagnosed by physicians; laboratory confirmations were missing.

The distribution of anti-PT antibodies considering the 6-month to 6-year-olds is shown in Fig. 2a, b, and c for Diyarbakir, Samsun, and Antalya, respectively, and the distribution of DwPT doses in the same age groups is shown in Fig. 3a, b, and c, respectively. In Fig. 2a, b, and c, although the trends were observed to be similar for anti-PT antibody, there was a statistically significant difference in antibody levels ($\chi^2 = 28.086$; $P = 0.000$) among the three provinces; when data for Antalya was excluded, however, there was no significant difference ($\chi^2 = 1.743$; $P = 0.418$). In Fig. 2, the levels of 50–99/ ≥ 100 EU/mL for Diyarbakir, Samsun, and Antalya were 9.0%/9.0%, 10.2%/12.4%, and 3.1%/3.1%, respectively, with the lowest percentages shown in Antalya. On the contrary, 3–4 doses of DwPT were received in 64.8%, 98.7%, and 95.0% of participants in the same provinces, respectively.

Pertussis infection history was positive in 12 children among subjects 6-years-old or younger; 11 were from Diyarbakir and one was from Samsun.

The percentage of 15–49-year-old women with anti-PT titers ≥ 30 EU/mL was 57.1%.

DISCUSSION

High titer of anti-PT antibody at levels of 50–99 EU/mL and ≥ 100 EU/mL were the assigned cutoffs for recent exposure or infection in this study, based on other studies in which a titer of ≥ 100 EU/mL was considered indicative for acute or recent infection, with a mean persistence of 4.5 months (range: 4 weeks to 1 year after onset of the disease), and a level of 50–99 EU/mL was reached in the 2nd week after the onset of the disease and persisted at this level for 6–11 months (6,11,12). However, symptomatic patients were evaluated in these studies, whereas the subjects of this study were a healthy population. On the other hand, exposure to the microorganism may cause an increase in antibody titer without any apparent symptoms, which is not a potential risk because the microorganism is not spread to others; nonetheless, it indicates the amount of circulating microorganism and can be accepted as an indirect threat. Furthermore, infection might occur as well, and when the disease is symptomatic in adults and adolescents, physicians rarely diagnose it correctly since patients may not present with the characteristic

whooping cough, and their infection as a result can lead to transmission of the microorganism to susceptible individuals (8). The latter might have happened in this study group because only the present infections were examined and history of the vaccine-preventable diseases included in this project was queried, but possibly the history had been limited by typical whooping cough. High titers of anti-PT antibody were observed throughout the study groups, with the lowest percentage in the youngest age groups, increasing by age, and the highest percentages reached in the oldest age groups. This suggests high infection rates in the past with repeated exposure and booster effects. The presence of high levels of anti-PT antibody is thought to be indicative of the circulating microorganism and is threatening to infants, who are susceptible. In addition, the vaccination histories showed that coverage of vaccination in Diyarbakir is considerably lower than in the other two provinces; and national coverage is around 80%. Thus, there must be a large number of children who are susceptible to pertussis infection, and therefore improvement in vaccination procedures is essential.

Vaccination is supposed to be another cause of high titers. The vaccine administered in Turkey contains whole-cell pertussis vaccine and in the case of any delay, it cannot be administered to children who are older than 6 years of age. Based on this event and the questionnaires, the antibody titers in young age groups up to 6 years old, which should be affected by the vaccine, were evaluated in detail, together with DwPT doses (Figs. 2 and 3). It was observed that the lowest percentage of high titers was present in the youngest age group, who are the target of the primary vaccination. Some investigators have mentioned that high titers were likely to decline shortly after vaccination, so measurement of such titers might be minimally influenced by previous exposure to vaccine (11,12,18). Thus, the response to the vaccine might show lower titers of antibodies than the indicated levels in this study. Furthermore, one of the highest vaccination percentages was in Antalya, where the high titers were the lowest in percentage, which can be explained by low circulation of *B. pertussis* in this province, but not by the vaccination rate. Delay in vaccination might be an explanation of the higher titers in the older age groups in Fig. 2, but unfortunately, the date of vaccination was not queried, and the duration since the last dose was unknown. Likewise, there were only 12 clinically defined cases and the date of infection was unknown. Therefore, it was likely that the high titers were due to the booster effect of the exposure to circulating microorganism in the subjects with existing anti-PT antibody, in addition to any delayed vaccine doses or infection.

Fig. 2. The distribution of anti-PT antibodies < 50 , 50–99, ≥ 100 EU/mL levels among from 6–11-month-olds (*) to 6-year-olds by 1-year interval. The results of subjects from Diyarbakır (a), Samsun (b), and Antalya (c).

(a)

(b)

(c)

Fig. 3. The distribution of DwPT doses 0, 1, 2, 3, 4, and unknown among 6–11-month-olds (*) to 6-year-olds by 1-year interval. The results of subjects from Diyarbakir (a), Samsun (b), and Antalya (c).

The age of the subjects in this study was older than 6 months, and the younger children, i.e., those not included in this study population, are likely to present with severe and even fatal illness prior to the administration of primary immunization (10,19). During this period, passively acquired antibody is expected to protect newborn babies. Accordingly, the titer of the antibodies present in mothers' sera must be sufficient to offer protection, at least until the first dose of the vaccine is administered. It has been shown that passively acquired anti-PT antibody has a half-life of 36 days, and the geometric mean titers (GMTs) for the antibody in cord sera were shown to be more than 150% of maternal delivery values by means of active placental transfer (7,10,19). In addition, based on the lowest measured antibody titer among children recovering from pertussis, 10 EU/mL had been tentatively considered as the threshold of protection by some authors (5,12). An estimated titer of 30 EU/mL for anti-PT antibody in mothers' sera would therefore correspond with a level of approximately 45 EU/mL in the infant at birth, falling to around 22 EU/mL in the 36th day and to 11 EU/mL in the 54th day, and would thus be expected to be protective until the administration of the first dose of vaccine in the 2nd month of life (9). The presence of estimated titers of protective antibody for newborns was absent in 42.9% of the women of childbearing age in this study. The proportion lacking enough titers was found to be one-third in another study concerning adolescent girls in Thrace, Turkey (9). This high rate of nonprotection among possible mothers-to-be is alarming for newborns. To protect this age group, very early vaccine administration is not advantageous and may even result in "immunological paralysis." Therefore, vaccination beginning after 4 weeks of age is suggested to be ideal for satisfactory antibody response and protection against disease (7,19). There are some studies suggesting vaccination during pregnancy to provide IgG antibodies to the newborn (10,19), but data to support the safety and efficacy of maternal vaccination are lacking (20). For diseases spread from person to person, if a high enough proportion of the population is immunized (i.e., "herd" immunity), transmission may be interrupted in the community, and it serves to provide protection to those who have not been immunized. The herd immunity threshold for pertussis in developed countries is approximately 92 to 94% (21). Therefore, there is need to improve the vaccination coverage percentages to over 90% and to apply coverage uniformly throughout the country in order to protect the vulnerable infants and young children against pertussis. Selective vaccination of household contacts of pregnant women, including siblings, to reduce the transmission of the microorganism to the newborn, and revaccination with

acellular vaccine for schoolchildren have been recommended (8), and might be reasonable in our country.

In conclusion, according to the data gathered in this study, nearly half of the newborns seem to be vulnerable to pertussis infection, vaccination percentages are not sufficient, and the populations in older age groups can be an important source of infection. Therefore, an increase in the coverage rate of vaccination to over 90% in order to ensure herd immunity and improvement in primary vaccination procedures, especially in southeast Anatolia, should be instituted so that the coverage will be uniform nationwide. Immunization of both schoolchildren and households of pregnant women might be considered as well.

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