Jpn. J. Infect. Dis., 62, 275-278, 2009

Original Article

Community-Based Seroepidemiology of Diphtheria and Tetanus in Edirne, Turkey

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(Received November 17, 2008. Accepted May 18, 2009)

SUMMARY: The aim of this study was to evaluate the seroprevalence and correlates of diphtheria and tetanus in Edirne, Turkey. Tetanus and diphtheria antitoxin levels were determined by enzyme-linked immunosorbent assay. Among 99 participants, a diphtheria antitoxin level of ≥0.1 IU/mL was found in 97 (98%), while 2 (2%) had antitoxin levels of 0.011 - 0.099 IU/mL. The geometric mean titers (GMTs) in men were statistically higher. Among 295 participants, a tetanus antitoxin level of ≥0.1 IU/mL was found in 291 (98.6%), while 4 (1.4%) had antitoxin levels of 0.011 - 0.099 IU/mL. Participants who had completed secondary school or higher education showed higher GMT values. Additionally, participants vaccinated within the previous 5 years had higher GMT values and the percentage of participants who had completed secondary school or higher education was higher among them. GMTs decrease with increasing age and increase as the poverty index increases. The average socioeconomic status index of the participants was high for both diphtheria and tetanus seroepidemiology. In this community-based study, antitoxin levels of diphtheria and tetanus were high. However, revaccination of adults with tetanus-diphtheria toxoids at every opportunity (military service, pregnancy, post-injury prophylaxis, etc.) together with a single booster every 10 years should be considered as an immunization policy.

INTRODUCTION

Tetanus and diphtheria are serious diseases with high mortality rates, but they are easily prevented by a single vaccination program applied as one intramuscular injection.

From the 1960s to 1990, the incidence of diphtheria decreased significantly in Europe and less dramatically worldwide, however, the disease remained endemic in many developing countries. In 1990, a diphtheria epidemic broke out in Russia and particularly involved children under 15; more recent outbreaks have also affected unimmunized and poorly immunized adults (1,2).

The global incidence of tetanus is estimated at 1 million cases annually, or about 18 people in every 100,000 (3,4). Tetanus is sometimes seen in neonates but more often occurs in older people who have not received adequate booster doses of vaccine.

People who have not been adequately vaccinated against tetanus and diphtheria are most likely to get the disease. The importance of continuous vaccination programs for preventing the mortality and morbidity associated with tetanus and diphtheria is therefore clear.

In Turkey, the administration of tetanus toxoid has been a recommended part of the childhood immunization program since 1962, and diphtheria since 1968. Nation-wide systematic vaccination programs were initiated after 1985. The recommended national vaccination schedule requires the administration of 3 doses of diphtheria-tetanus-pertussis (DTP) vaccine at 2, 3, and 4 months as primary immunization. Booster doses include one DTP at 18 months of age, one DT in the 7th year (the 1st year of primary school) and one tetanus-adult diphtheria (Td) in the 14th year (the 8th year of primary school) (5). Although health authorities recommend a booster dose of Td every 10 years, there is no comprehensive Td vaccination program for adults in Turkey (3,5). Despite the lack of a formal program, many women receive 2 doses of tetanus toxoid during pregnancy and all men are vaccinated with Td during their military service.

In order to achieve adequate levels of immunity and prevent the mortality and morbidity caused by diphtheria and tetanus, it is necessary to monitor the serum antibody levels of the general population, and identify and vaccinate insufficiently protected groups (6). The aim of the present community-based study was to evaluate immunity to diphtheria and tetanus in Edirne, Turkey, and to assess factors influencing this immunity.

MATERIALS AND METHODS

Study population: Turkey is geographically located between Europe and Asia. Edirne is a city on the northwest border of Turkey, and is a neighbor of Greece and Bulgaria. The population of Edirne was 110,542 according to national population data obtained in 2005. The present study was cross-sectional. We used the Epistat package program to form a sample representative of Edirne, and a multistage sampling technique was used in the selection of the study group. Edirne was divided into 11 different subregions so that the socioeconomic differences present in the city would be reflected in the sample. The regions were determined according to the Primary Health Care Units (PHCUs) of the Turkish health system. The minimum sample size was calculated for diphtheria at 92 according to a prevalence of 60%, deviation 10% and confidence level of 95%, and the final sample size was set at 99. The minimum sample size was calculated

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for tetanus at 288 on the assumption that 25% of people will have protective levels of antitoxin, at a confidence interval of 95% and with a deviation of 5%. The final sample size was set at 295. The age range of the participants was 15 - 80. The participants were selected from patients applying for various purposes to one of the 11 PHCUs in the different regions of the city.

The study was performed with the permission of the Local Ethics Committee of the Medical Faculty of Trakya University and local administration. For each participant, a questionnaire was completed to provide information on sociodemographic characteristics such as age, residency (urban, slum), education (illiterate, literate, primary school, secondary school, high school, university), occupation, number of house-hold members, poverty index, diphtheria and tetanus vaccination status, and diphtheria and tetanus history. The poverty index was calculated by taking 586 New Turkish Liras (Yeni Türk Lirasi, YTL; about $400, according to the value announced by the Turkish government in September 2006 based on the monthly income of a three-person family as the upper limit of poverty).

To evaluate the socioeconomic level of the family, a Socio-Economic Status (SES) index adapted from Toukan et al. was used (7). SES score was counted by adding the scores of the features of the house where the participant lived and the goods inside the house. SES scores ranged from 0 to 42 and socioeconomic levels were grouped as follows: 0 - 20, low; 21 - 25, low middle; 26 - 30, high middle; and 31 - 42, high.

Laboratory methods: Blood samples were taken and the serum was stored at −20°C until tested. The serum levels of tetanus and diphtheria antitoxin were determined by enzyme-linked immunosorbent assay (ELISA; Virotech System GmbH, Rüsselsheim, Germany) following the manufacturer's instructions.

Three levels of immunity to diphtheria and tetanus were defined as follows: "no immune protection or seronegativity or susceptibility" for a diphtheria and tetanus antibody level of ≤0.01 IU/mL; "basic immunity or low seropositivity or basic protection" for 0.011 - 0.099 IU/mL; and "full protection or seropositivity" for ≥0.1 IU/mL (6,8-10).

Statistical methods: Geometric mean titers (GMTs) were calculated using the log transformation of serological titers and taking the antilog mean of these transformed values. The relationships between GMTs and other parameters (education, vaccination history, etc.) were assessed using an independent samples *t* test, correlation analysis, and the chi-square test. All analyses were performed using MINITAB Release 13.1 (license no. wcp 1331.00197). Differences were considered statistically significant when the obtained *P* value was less than 0.05.

RESULTS

The sample for diphtheria consisted of 99 participants (49 males and 50 females) aged 15 - 80 years (mean age, 41.0 ± 18.0). A diphtheria antitoxin level of ≥0.1 IU/mL (full protection) was found in 97 subjects (98%), while 2 (2%) had antitoxin levels of 0.011 - 0.099 IU/mL (basic protection); no subject had an antitoxin level of ≤0.01 IU/mL (no immune protection). No participants reported a past history of diphtheria or booster diphtheria vaccination after the age of 7. The mean SES index of the 99 participants in the diphtheria seroprevalence study was high at 33.6 ± 8. GMTs were found to be 0.404 IU/mL. The GMTs in men (0.468 IU/mL) were significantly higher than those in women (0.350 IU/mL; $P < 0.05$; Table 1). No significant relationships were identified between diphtheria antitoxin levels and any other parameter such as age, education or poverty index ($P > 0.05$).

The sample for tetanus consisted of 295 participants (133 males and 162 females) aged 15 - 80 years (mean age, 42.6 ± 17.9). A tetanus antitoxin level of ≥0.1 IU/mL (full protection) was found in 291 (98.6%), while 4 (1.4%) had antitoxin levels of 0.011 - 0.099 IU/mL (basic protection) and nobody showed antitoxin levels of ≤0.01 IU/mL (no immune protection). The mean SES index of the participants was high at 33.6 ± 7.4, and 52% of the participants had completed secondary school or higher education. Slightly more than half (51.5%) of the participants were unvaccinated for tetanus or could not remember vaccination status. The mean GMTs of the 295 subjects was 0.69 IU/mL, with GMTs for men at 0.73 IU/mL, and for women at 0.66 IU/mL. There was no statistically significant difference between genders ($P > 0.05$; Table 1). The GMTs of the participants who were illiterate or had completed their education only through primary school were significantly lower than those of participants who had completed secondary school or higher education ($P < 0.001$). The

Table 1. Diphtheria and tetanus immunity status of the participants with respect to different parameters

Characteristic		Tetanus	Diphtheria
Mean age		42.6 ± 17.9	41 ± 18
Gender	male	133 (45.1%)	49 (49.5%)
	female	162 (54.9%)	50 (50.5%)
GMTs	male	0.73	0.468 ($P = 0.02$)
	female	0.66	0.35
	mean	0.69	0.404
Mean of SES		33.6 ± 7.4	33.6 ± 8
Education		no. (%)	no. (%)
Illiterate		25 (8.5)	5 (5.1)
Literate		2 (0.7)	1 (1.0)
Primary school		113 (38.3)	35 (35.3)
Secondary school		35 (11.9)	13 (13.1)
High school		70 (23.7)	28 (28.3)
University		50 (16.9)	17 (17.2)

GMTs, geometric mean titers; SES, socioeconomic status.

Table 2. Comparison of geometric mean titers (GMTs) with education and vaccination status of the participants

Parameter	Tetanus		Diphtheria	
	GMTs	*P*	GMTs	*P*
Education				
Illiterate	0.47		0.37	
Literate	0.21	<0.001[1)]	0.43	>0.05
Primary school	0.49		0.41	
Secondary school	0.82		0.41	
High school	1.13		0.39	
University	0.82		0.41	
Vaccination history				
No vaccination	0.62			
5 years ago	1.10	<0.001[2)]		
10 years ago	0.74			
Unknown	0.45			

No participants had a booster diphtheria vaccination history.
[1)]: The GMTs of the secondary school and upper graduates were higher.
[2)]: The GMTs of the vaccinated within 5 years were higher.

GMTs of the participants who were not vaccinated or who could not remember being vaccinated and those of participants vaccinated at least 10 years earlier were found to be significantly lower than those of participants who had been vaccinated within the previous 5 years ($P < 0.001$; Table 2). GMTs increased significantly with increasing poverty index ($r = 0.56$, $P = 0.014$) and decreased as the mean age increased ($r = -0.47$, $P = 0.031$). We investigated the ratio of vaccination within 5 years according to education level: illiterate (24%), literate (0%), primary school (22%), secondary school (57%), high school (60%), university (34%). The ratio of vaccination within 5 years was significantly higher in participants who had completed secondary school or higher education ($P < 0.001$).

DISCUSSION

In some countries, the proportion of individuals susceptible to diphtheria with antitoxin concentrations of <0.01 IU/mL varies widely (e.g., 37.6% in the UK, 27.1% in Austria, 26% in Denmark, 20.3% in France, 12% in Netherlands, 9.9% in Italy, 4% in Germany, 34.1% in Egypt, 5.3% in Israel) (2,9-16). In the present study, none of our participants had an antitoxin level of ≤0.01 IU/mL (no immune protection). These variations can be explained by different vaccination programs and booster schedules, awareness among populations (the specific division of age groups), and by different study designs and methods. A 98% positive immunity rate against diphtheria (≥0.1 IU/mL) was found in the present study; rates vary in other cities in different regions of the country: 62% in Ankara and 79.1% in Izmir, for example (17,18).

In Edirne, 13 diphtheria cases were reported between 1970 and 1985 and there have been no other cases after the first nationwide vaccination campaign in 1985. To achieve the elimination of diphtheria from Europe, a minimum immunization coverage rate of 90% in children and 75% in adults is required (19). According to the Ministry of Health of Turkey, the immunization coverage rates achieved with 3 doses of DPT vaccination in childhood in the Marmara region, where Edirne is located, were 94, 94, 95, 93 and 92% for 1997, 1998, 1999, 2000 and 2001, respectively. The high positive immunity against diphtheria in the present sample population can be explained by the high socioeconomic status of the participants in Edirne and the high rate of childhood vaccination.

Women had lower levels of full protection and GMTs than men. In addition to receiving booster injections of diphtheria toxoid in their childhood, many men had served in the military, where they received booster injections, and they were more likely to suffer frequent injuries and thus receive more routine Td vaccinations. Women generally received booster injections of diphtheria toxoid only in childhood. Lower antitoxin levels for women have also been reported in other studies conducted in different cities in Turkey (5,18). The authors of one study hypothesize that either vaccination is less efficient in women, or that diphtheria immunity following vaccination might be less long-lasting among women (18). However, some researchers from other countries have found similar immunity levels for men and women, while others have reported lower levels for men (2,11,14,15).

The prevalence of protective titers for tetanus obtained in this study (98.6%) is higher than those reported in other studies. Immunity status (≥0.1 IU/mL) differs among countries: with respect to immunity to tetanus, 97.2% of the population of the Czech Republic is reported to be immune, 90.4% in Germany, 76% in Poland, 68.3% in Spain, 64.4% in Greece, 68.3% in Egypt, and 69.7% in the US (20-26). These rates change depending on the developmental level of the country, the success of immunization programs, and studies conducted with different methods and designs.

Other studies conducted in different cities in Turkey show the ratio of seropositivity for tetanus as 75% in Samsun, 73.5% in Antalya, 59.9% in Diyarbakir and 25.3% in Kayseri (8,27). In Edirne, 17 adult and neonatal tetanus cases were reported between 1970 and 1985 and at the time of writing, no cases have been reported for the last 3 years. The high positive immunity against tetanus can be explained by the high SES of the participants in Edirne and the high rate of DPT vaccination in the Marmara (northwest) region of Turkey, which includes Edirne.

The SES level of the participants in the tetanus study was high and the GMT value increased significantly with increasing SES level. We can conclude that the present participants were highly likely to be informed about tetanus and could make contact with health institutions conveniently because of living in Edirne.

The GMTs for tetanus were similar in both genders. Men are typically immunized during military service, and are more subject to trauma outside and during work, leading to more frequent vaccination. However, this situation is balanced by tetanus vaccinations given to women, particularly during pregnancy. Our results are consistent with the findings reported by other investigators in Turkey (27-29).

The present results show that the percentage of protective GMTs for tetanus decreases with increasing age in Edirne. The expanded immunization program was established in Turkey in 1985. The low prevalence of antibody levels against tetanus in the elderly might be related to the fact that adults born before 1960 did not receive any scheduled tetanus vaccinations during childhood, while younger people would have received vaccinations at school, and/or during military service or pregnancy. This phenomenon of decreasing rates of immunity to tetanus among adults is consistent with reports from Turkey and other countries, despite variations in the methods used for testing antibody concentrations and despite the use of different vaccination and booster schedules (8,24,25,29). Antitoxin levels also decrease in a manner inversely proportional to age in other countries that have not yet fully established immunization programs for adults.

The GMT values for tetanus antibodies of participants whose had completed secondary school or higher education were significantly higher than those of participants who were illiterate or had graduated only from primary school. Additionally, the ratio of vaccination within the previous 5 years was statistically higher among participants who had completed secondary school or higher education. Individuals who attend schools are routinely vaccinated against tetanus during their education (in Turkey in the 1st and 8th years of primary school). They also attend health institutions more often and receive more boosters during pregnancy and military service and when injured than people who are illiterate. The illiterate population is generally older and their antitoxin levels are lower. Antitoxin levels have been found to be higher with more education in several studies (26,27,29). The GMTs for tetanus among the participants who were not vaccinated or do not remember having been vaccinated and in participants vaccinated at least 10 years ago were low, indicating the importance of a booster schedule. Although in this community-

based study we found high antitoxin levels for diphtheria and tetanus in Edirne, we must strongly encourage booster injections every 10 years for both diphtheria and tetanus. The diphtheria and tetanus immune status of the population should also be investigated in other regions of Turkey. We must effectively apply an appropriate schedule of immunization and booster injection in all regions of Turkey in order to achieve high childhood and adult immunization ratios.

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