Immunity against diphtheria among children and adults in Izmir, Turkey

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The aim of this study was to evaluate diphtheria immunity in a sample of the Turkish population having high childhood immunization coverage, including a booster dose of diphtheria toxoid at 12–15 years of age. A total of 599 persons aged 1–70 years were selected with cluster sampling. The information on socio-demographic characteristics, vaccination status and diphtheria history was gathered for each participant. Diphtheria antitoxin levels were measured qualitatively by using micro-enzyme immune assay. Of studied population, 72.3% had fully protective antitoxin levels (≥0.1 IU/ml). The rate of protection was 92.5% in the children aged 0–2 years, 93.2% in the primary school children aged 7–9 years, and 86.0% in the adolescents aged 15–19 years. After 20 years of age, diphtheria protection rates showed a significant age-related decrease, reaching minimum in the 30–39 age group, in which 47.3% of these subjects had fully protective antitoxin levels. The diphtheria antitoxin geometric mean titer (GMT) was highest in the 0–2 year age group (1.18 IU/ml). In the adolescents aged 15–19 years, diphtheria antitoxin GMT was 0.71 IU/ml. Then, geometric mean titer decreased with increasing age, and reached the minimum level in the 40–59 years age group (0.18 IU/ml). The protection rate among females was significantly lower than males (67.1% vs. 80.9%). The difference was apparent in the 20–29 and the 30–39 years age group: 80% of the males and 46.2% of the females in the 20–29 years age group, and 60% of males and 44.1% of females in the 30–39 years age group were fully protected against diphtheria (p < 0.0001). These results suggest that in Izmir, Turkey, full serological protection against diphtheria is only detectable in <50% of the young adult population, even though childhood immunization coverage is relatively high. Potentially, there is still risk of diphtheria outbreaks among the adults in our country. Therefore, a revaccination of adults with reduced doses of diphtheria toxoid should be considered to sustain diphtheria immunity.

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1. Introduction

Diphtheria is an acute bacterial disease caused by exotoxin-producing Corynebacterium diphtheriae, which can result in obstruction of the airway and, eventually, death. It is estimated that before the World Health Organization (WHO) Expanded Programme on Immunization (EPI) which began in 1977, about 1 million cases of diphtheria and 50,000–60,000 deaths due to the disease and its complications occurred globally each year. The decline in diphtheria cases in the 1980s is consistent with the increasing DTP3 (diphtheria, tetanus and pertussis) coverage. The sudden increase in incidence during the 1990s is due to an epidemic in the countries of the former Soviet Union. In this outbreak, over 150,000 cases and 5000 deaths were reported between 1990 and 1997 and more cases occurred in young adults than in children [1]. Since 1994, with the initiation of aggressive immunization efforts, the number of reported cases has decreased. However, outbreaks were also reported from Algeria, Iraq, the Lao People’s Republic, Mongolia, India, Papua New Guinea, the Sudan, and Ecuador [2–4].

A characteristic feature of recent diphtheria outbreaks concerns the age group; most cases have occurred in adolescents and adults, rather than in children. When diphtheria was endemic, it primarily affected children younger than 15 years; recently, the epidemiology has shifted to adults who lack natural exposure to toxigenic C. diphtheriae in the vaccine era and those who have low rates of receiving booster injections. In the 1990–1997 epidemics in countries of the former Soviet Union, adults were accounted for 38–82% of all cases [5,6]. At least up to 1986, most of these countries had high childhood immunization coverage, including a booster dose of diphtheria toxoid at 14–16 years of age [7]. These findings have demonstrated the importance of maintaining high vaccination coverage against diphtheria among both children and adults. Therefore, most industrialized countries have added booster doses of diphtheria toxoid to the primary immunization series, and subsequently every 10 years throughout life. Revaccination of adults with tetanus toxoid and a low dose of diphtheria vaccine (Td) every 10 years are maintained systematically in some industrialized countries such as US, Austria, Finland, and Germany [2].
Diphtheria toxoid has been used in Turkey since 1937. An immunization program against diphtheria has been implemented in Turkey since 1968, using combined diphtheria, whole-cell pertussis and tetanus (DTwP) vaccine. Since 2008, combined diphtheria, acellular pertussis, tetanus, haemophilus influenzae type b and poliomyelitis (DTaP-IPV-Hib) is administered. In Turkey, primary immunization includes doses at 2, 4, 6 and 18 months of age, using DTaP-IPV-Hib vaccine containing ≥30 IU of adsorbed diphtheria toxoid. A booster dose, diphtheria-tetanus (DT) vaccine containing ≥30 IU of adsorbed diphtheria toxoid, is administered at 7 years of age (at the first class of primary school). In 1997, because of diphtheria outbreaks in the neighboring countries, a second booster dose of tetanus-diphtheria (dT) vaccine containing ≥2 IU of adsorbed diphtheria toxoid was introduced at 12–15 years of age. However, today no further routine revaccination for adults is considered [8].

Data from Europe are particularly noteworthy because the childhood immunization rate exceeds 95% in some countries (e.g., Sweden), but approximately 20% of persons younger than 20 years and as many as 75% of persons older than 60 years lack the protective antibody. Other broad serosurveys have identified large subgroups of underimmunized individuals in the United States and other countries in which immunization is believed to be universal; these individuals would be at risk if the organism were introduced [2]. Therefore, European Advisory Group of World Health Organization Expanded Programme on Immunization (EAG/EPI) proposed that the diphtheria immune status of adult population should be assessed in all countries [9]. There are few epidemiological studies in the literature on the diphtheria immune status of population in Turkey [8,10]. The aim of the present study was to evaluate the current seroepidemiology of diphtheria in 1–70 years age group in Izmir, Turkey, and to assess factors influencing immunity against diphtheria.

2. Patients and methods

The study was conducted in Izmir, the third largest city of Turkey. Izmir is located in the Aegean part of Anatolia. According to 2006 census, total population of the city was about 3.8 million. About three quarter of the population live in urban area.

A cluster sample design developed by EPI of the World Health Organization for the surveys of immunization was carried out for the selection of the study population [11]. State Institute of Statistics of Turkey had a list of all the communities (villages for rural sector and city blocks for urban sector), and selected a sample of 30 clusters for both urban and rural sectors. Selection procedure was carried out by creating a cumulative list of community populations and selecting a systematic sample from a random start.

The study population consisted of 599 healthy subjects, and the study was carried out in July–November 2008. Subjects were classified into ten age groups. A starting household was selected in each community by locating the ward’s center, randomly selecting a house from a list of all houses falling along the line drawn from the ward center to the periphery in the chosen direction. The house was then examined to determine if the subject of eligible age and sex was living there. Subsequently, the nearest household to the right was visited and the steps repeated until the desired number of persons was obtained.

For each participant, a questionnaire was completed to provide information on socio-demographic characteristics, diphtheria vaccination status, and past diphtheria history. The information was obtained from the parents of the children and from themselves of the adults. Immunization status of the study subjects was verified with their vaccination records if available.

After the signed informed consent was obtained, blood samples were taken from each participant (3 ml from children <10 years and 5 ml from subjects ≥10 years) and the serum stored at −80 °C until tested. The tissue culture toxin neutralization assay is the more accurate method for measuring low antitoxin levels. However, the diphtheria antitoxin levels can also be measured by the enzyme immunoassay. This method has been used to detect full serological protection against diphtheria in some studies [12,13]. In this study, micro-enzyme immunoassay (micro-EIA) test (Virotech, Rußelsheim, Germany) was also used to determine the diphtheria antitoxin levels. For quantification, six standard sera were used. Diphtheria antitoxin levels were expressed in international units (IU/ml), as compared to the International Standard for diphtheria antitoxin (S1/534, WHO International Laboratory for Biological Standards in Great Britain).

According to the international criteria, the antitoxin levels below 0.01 IU/ml are regarded as susceptibility, from 0.01 to <0.1 IU/ml as basic protection and levels ≥0.1 as full protection [14]. In this study, persons with diphtheria antitoxin levels equal or more than 0.1 IU/ml were considered as ‘fully protected’, as generally accepted [14–16].

Statistical analysis was performed using SPSS for Windows 18.0. Testing for statistical significance between seropositivity and sociodemographic characteristics was performed by using χ² and Fisher’s Exact test. Multivariable analysis was performed using a logistic regression model containing the following independent variables: age, sex, residential area, socio-economic status (low, moderate, high), and number of household members (<4, >4). Full protection against diphtheria was used as the dependent variable. A p value of <0.05 was taken to be statistically significant.

3. Results

A total of 599 subjects (aged 1–70 years; 225 males and 374 females) were enrolled in the study. No subject had a past diphtheria history. All of the children below 12 years of age were immunized against diphtheria. However, sufficient information about vaccination status of persons over 12 years of age could not be taken because vaccination records were not available in most of them.

Table 1 shows diphtheria immune status of the participants with respect to different characteristics. Of the 599 subjects tested, 433 (72.3%) had fully protective antitoxin levels against diphtheria (≥0.1 IU/ml). The rate of protection was different by age-groups: 92.5% of the children aged 0–2 years, 93.2% of the primary school children aged 7–9 years, and 88.0% of the adolescents aged 15–19 years had full serological protection against diphtheria (≥0.1 IU/ml). However, the protection rate showed a significant age-related decrease (p < 0.01) after 20 years of age, up to the 30–39 years age group, in which 47.3% of subjects were fully protected against diphtheria. As can be seen in Fig. 1, the lower protection rate corresponds to the 30–39 years age group. The protection rate began to slightly increase after 40 years of age and reached about 66% in the 60–69 years age group.

The overall geometric mean antitoxin level (GMT) level was 0.58 IU/ml. The GMT was highest in the 0–2 years age group (1.18 IU/ml). Geometric mean antitoxin level was 0.71 IU/ml in the adolescents aged 15–19 years. The GMT began to decrease after 20 years of age and reached the minimum level in the 40–59 years age group (0.18 IU/ml). The diphtheria antitoxin GMTs for males and females were 0.69 and 0.52 IU/ml, respectively.

The protection rate among females was significantly lower than males (67.1% vs. 80.9%) (p < 0.001). The difference was apparent in the 20–29 and the 30–39 years age group. In the 20–29 years age group, 80% of the males and 46.2% of the females in the
30–39 years age group, 60% of males and 44.1% of females were fully protected against diphtheria (Fig. 2).

Logistic regression analysis showed that among the several independent variables, only age and sex \((p < 0.001)\) were significantly associated with diphtheria protective antitoxin levels. No significant influence of the other factors (socio-economic status, residential area, the number of household members) on protection rates was observed.

### 4. Discussion

The present study indicates that in adults in the study population, diphtheria immunity is not satisfactory, both for prevalence of immune subjects and for the antibody levels. Only 53.4% of the all adult population and 47.3% of young adults in the 30–39 years age group have full serological protection against diphtheria \((\geq 0.1 \text{IU/ml})\). The results lie within the same range as international studies reported from European countries assessing diphtheria protection among adults \([2,17–19]\). In Germany, low full protection rate of 42.2% was found in adults \([20]\). On the contrary, much higher full protection rates of 74.7% and 87% have been observed in East European countries such as Poland and Romania, respectively \([21,22]\). The differences may be explained by different vaccination schedules. A less circulation of toxigenic \(C. diphtheriae\) organisms in the community may also contribute low protection rates in industrialized countries.

The results of the studies both in developed and developing countries suggest that protection against diphtheria decrease with increasing age \([6,8,17,22–25]\). Before the widespread immunization with diphtheria toxoid, most of cases were in children younger than 15 years of age. Whereas, a characteristic feature of recent outbreaks in Africa, Asia, Europe and South America has been the high percentage of adult cases. In recent diphtheria outbreaks in the newly independent states of the former Soviet Union, adults were accounted for nearly 70% of all cases \([5,6]\). Because primary immunization coverage against diphtheria is approximately 95% in infants and preschool children in our country, we detected high protection rates in these age groups. Similarly, the majority (88.0%) of the adolescents aged 15–19 years had full serological protection against diphtheria. Whereas, in our previous study, which was performed by using the same epidemiological method on the same population in 1998, full protection rates against diphtheria were found to be 77.2% among adolescents \([8]\). Over the last 10 years, the increase of the protection rates in the adolescents may explain with implementation of a booster dose of diphtheria toxoid at 12–15 years of age in Turkey since 1997.

However, after 20 years of age, the protection rate was gradually decreased by age, reaching the minimum in the 30–39 year age group, in which only 47.3% of the subjects had antibody titer above the full protective level. A recent study from Turkey also reported...
that only 43.7% of adults aged between 20 and 81 had immunity against diphtheria [26]. These findings suggest that, despite high (>90%) childhood immunization coverage including a booster dose of diphtheria toxoid at 12–15 years of age, the immunity against diphtheria is insufficient among Turkish adult population. Therefore, the potential risk of diphtheria outbreaks among adults still exists in our country. In fact, even though no diphtheria cases had been reported in Turkey since 2003, a case of 33 y/o female died because of the disease in January 30, 2011. This may be a warning for reconsideration of booster vaccination in adult population for every ten years in the risky countries.

The lower antitoxin levels and protection rates among females were reported in many studies [16,19,20,25]. Authors conjectured that either vaccination is less efficient in women or diphtheria immunity following vaccination might be less long-lasting among women. However, no clear difference was reported both in a Poland [27] and a Taiwan study [28]. We observed that the protection rates among females were significantly lower. Especially, in the 20–29 and 30–39 years age groups, this difference was very obvious. In our country, men take booster vaccination against diphtheria during their military duty, which may be also the cause of this higher protection rates.

Protection rates against diphtheria could change according to regions within the same country depending on the disease among the regions, being a carrier and the difference of immunization rates. Since the frequency of diphtheria cases and immunization rates between both rural and urban areas and among regions are different, protection against diphtheria might be expected to be different. In this study, any difference of protection rates between rural and urban areas is not observed. In an Italian study, protection rates in the northern region which have high socio-economical level were found to be higher than that in southern region and it emphasizes that difference could originate from high vaccination coverage [18]. In Germany, in a study performed, adult population diphtheria protection rates are found higher in East Berlin than west part (34.4%; 20.3%) [29].

The results of this study suggest that in Izmir, Turkey, a large proportion of the adult population is gradually rendered susceptible to diphtheria as a result of waning immunity. Potentially, there is still risk of diphtheria outbreaks among the adults in our country. To demonstrate the regional differences, similar seroepidemiological studies should be also performed in the other regions of Turkey. High immunity levels of children should be continued by high immunization coverage against diphtheria. Revaccination of adults against diphtheria every 10 years should be considered to sustain diphtheria immunity. Routine use of tetanus-diphtheria (Td) vaccine, rather than monovalent tetanus toxoid, also helps maintaining immunity in adults.

Conflicts of interest statement

All the authors declare that there are no conflicts of interest.