Immunity to Diphtheria and Tetanus in Army Personnel and Adult Civilians in Mashhad, Iran

Seyyed Javad Hosseini Shokouh, Babak Mohammadi, Jalil Rajabi, and Ghasem Mohammadian Roshan*

Infectious Diseases Research Center, AJA University of Medical Sciences, Tehran, Iran

SUMMARY: This study aimed to investigate serologic immunity to diphtheria and tetanus in army personnel and a sample population of adult civilians in Mashhad, Iran. Army personnel (n = 180) and civilians (n = 83) who presented at Mashhad army hospital participated in this study. Diphtheria and tetanus antitoxin levels were determined by enzyme-linked immunosorbent assay. Approximately 77% and 94% of army personnel aged 18–34 years had at least basic protection against diphtheria (antitoxin level ≥0.1 IU/mL) and tetanus (antitoxin level >0.1 IU/mL), respectively. For civilians in this age group, the proportions were 76% for both diseases. Antitoxin levels waned with age. Thus, participants older than 50 years had lower immunity; this decrease in immunity was more pronounced for tetanus than for diphtheria in both army personnel and civilians. For both diseases, geometric mean antitoxin titers and the proportion of participants with at least basic protection were higher in subjects with a history of vaccination in the last 10 years (P < 0.001), higher in men than women, and in army personnel than civilians in each age group. Young army personnel and civilians (18–34 years old) had adequate immunity to diphtheria and tetanus. However, the large number of susceptible older adults (>50 years old) calls for improved booster vaccination protocols.

INTRODUCTION

Almost a century ago, wound tetanus was a major cause of war casualties (1). However, immunization reduced the incidence of this disease. In World War II, only 12 tetanus cases were reported among the 12 million soldiers in the United States Armed Forces (1). However, infectious diseases were responsible for the “sanitary losses” which affected 60% of the Russian army during the invasion of Afghanistan, highlighting the need for vigilance (2). Army personnel live in crowded places, acquire injuries during training and combat, and serve in regions in which they are exposed to new epidemiological threats (3). Thus, they are exposed to certain infectious diseases at a higher rate than civilians, indicating the importance of immunization in military personnel.

Diphtheria is a toxin-mediated bacterial disease that causes respiratory symptoms and damage to the myocardium, kidneys, and nervous system (4). The causative bacterial agent, Clostridium tetani, can grow in wound debris and produce the tetanus neurotoxin that causes muscular spasms (5). In 2013, 190 cases of diphtheria and 13 cases of tetanus were reported in Iran (6). Protocols recommend vaccination against diphtheria and tetanus not only in childhood, but every 10 years in adulthood with booster doses of tetanus-diphtheria (Td) (4,5). Immunity to these diseases wanes over time, making older people more susceptible; this highlights the importance of booster doses for all age groups (7,8). Owing to their occupational risk factors, army personnel in Iran must be vaccinated on entry and are expected to get booster doses every 10 years.

Prior studies have reported on diphtheria and tetanus immunity status in different populations (9–13). Some have studied special age groups (children, adults, or the elderly) (14–16) or special occupation groups (health care workers or military personnel) (17–19). Epidemiological necessity and the lack of studies in the region (to our knowledge) convinced us to conduct the first study of immunity to diphtheria and tetanus in army personnel and a sample population of adult civilians in Mashhad, Iran.

MATERIALS AND METHODS

Study population: This study was designed in Mashhad Army Hospital (Shams al Shamus hospital) in order to access sample populations of both army personnel and civilians. All participants visited the hospital laboratory for elective blood tests between October and December 2014. Participants who provided informed consent were evaluated using a questionnaire about demographic data, Td vaccination history, and exclusion criteria. Participants with a history of tetanus, diphtheria, known immunodeficiency, or blood transfusion in the last year were excluded. Sera from 180 army personnel and 83 civilians were included in the study.

Laboratory tests: Sera were stored at −20°C until analysis. IgG antibodies against diphtheria and tetanus toxoids were measured using commercially available enzyme-linked immunosorbent assay (ELISA) kits (Euroimmun, Luebeck, Germany), and levels were presented as international units (IU)/mL. International

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*Corresponding author: Mailing address: Infectious Diseases Research Center, AJA University of Medical Sciences, No. 101, 4th Fl., Daneshju 19st, 918977361 Mashhad, Iran. Tel: +989155250869, Fax: +985135228589, E-mail: roshan.g2006@yahoo.com
standard serum NIBSC 00/496 (National Institute for Biological Standards and Control, Hertfordshire, England) was used as the reference serum for calibration of the diphtheria assay. World Health Organization 1st International Standard for Human Tetanus Immunoglobulin was used for calibration of the tetanus assay. These reference sera are commonly used for antitoxin measurements in ELISA assays, but they were originally established for use in neutralization assays (20). Optical density was measured at 450/620 nm and antitoxin levels were determined by standard curves.

According to manufacturer-recommended thresholds and the work of previous studies (13,17,21), the results were classified into 3 groups. The diphtheria groups were as follows: susceptibility (antitoxin level <0.1 IU/mL), full protection (antitoxin level >1 IU/mL), and basic protection (antitoxin level >0.1 IU/mL and <1 IU/mL). The groups were defined as follows: susceptibility (antitoxin level ≤0.1 IU/mL), full protection (antitoxin level >0.5 IU/mL), and basic protection (antitoxin levels >0.1 IU/mL and <0.5 IU/mL).

Ethics: The study conformed to the 1995 Declaration of Helsinki and was approved by AJA University of Medical Sciences Ethics Committee.

Statistics: Both army personnel and civilians were classified into the following age groups: 18–34 years, 35–50 years, and >50 years. Immune statuses for the diphtheria groups were compared using Fisher’s exact probability test. For both diseases, geometric mean titers (GMTs) were calculated and compared using Student’s t-test. These reference sera are commonly used for antitoxin measurements in ELISA assays, but they were originally established for use in neutralization assays (20). For diphtheria, a higher tetanus antitoxin GMT was observed in army personnel compared with civilians (P = 0.047; 95% CI, 1%–39%). These differences decreased in the older age groups. The proportion of people with basic or full protection to tetanus decreased according to age in both army personnel and civilians (P = 0.001). Table 2 shows a major drop in immunity in participants older than 50 years.

For diphtheria, a higher tetanus antitoxin GMT was observed in subjects who reported Td vaccination in the last 10 years (P < 0.001), and in army personnel compared with civilians, across all age groups (Table 1 and 2). They differed significantly only in 18–34 years age group (P = 0.047; 95% CI, 1%–39%). These differences decreased in the older age groups. For each age group, the proportion of army personnel who were susceptible to diphtheria did not differ significantly from that of civilians; the greatest difference was observed in participants over 50 years old (Table 2). The rates of basic or full protection were similar in the 35–50 and >50 years groups for both army and civilian participants (Table 2).

The GMT for diphtheria antitoxin decreased as age increased. In army personnel and civilians, the GMT for diphtheria antitoxin was highest in the 18–34 year age group (Table 2). The difference between age groups was only significant among army participants (P < 0.01). For each group, the GMT was higher in army personnel participants than in civilian participants. The GMT differed significantly between participants with a positive vaccination history and those with a negative vaccination history (P < 0.001), but this was only the case for female participants.

Tetanus: The proportions of male and female participants with at least basic protection to tetanus were almost equal (67% and 66%, respectively). These proportions were higher in subjects who reported Td vaccination in the last 10 years (P < 0.001), and in army personnel compared with civilians, across all age groups (Table 1 and 2). They differed significantly only in 18–34 years age group (P = 0.047; 95% CI, 1%–39%). These differences decreased in the older age groups. The proportion of people with basic or full protection to tetanus decreased according to age in both army personnel and civilians (P < 0.001). Table 2 shows a major drop in immunity in participants older than 50 years.

RESULTS

Almost one-third of the total 263 participants comprised civilians. The army personnel were predominantly male; there were more female participants in the civilian group (Table 1). Thirty-six percent (n = 64) of the army personnel reported receiving an injection of the Td vaccine in the last 10 years; this rate was lower in the civilian population (Table 1).

Diphtheria: Table 1 shows that the proportion of male participants with at least basic protection to diphtheria was higher than that of female participants (P = 0.049; 95% CI, 0–27%). This proportion was higher in subjects with positive vaccination histories than in those with negative vaccination histories (P < 0.001). For each age group, the proportion of army personnel who were susceptible to diphtheria did not differ significantly from that of civilians; the greatest difference was observed in participants over 50 years old (Table 2). The rates of basic or full protection were similar in the 35–50 and >50 years groups for both army and civilian participants (Table 2).

Table 1. Immunity to diphtheria and tetanus in all of participants by sex and vaccination history

<table>
<thead>
<tr>
<th></th>
<th>Army</th>
<th>Civilian</th>
<th>Total</th>
<th>Diphtheria&lt;sup&gt;1)&lt;/sup&gt;</th>
<th>Tetanus&lt;sup&gt;2)&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Immune subjects N (%)</td>
<td>GMT (IU/mL)</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>172</td>
<td>27</td>
<td>199</td>
<td>139 (69.8)</td>
<td>0.159</td>
</tr>
<tr>
<td>Female</td>
<td>8</td>
<td>56</td>
<td>64</td>
<td>36 (56.3)</td>
<td>0.101</td>
</tr>
<tr>
<td>Vaccination history</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;10 yr</td>
<td>64</td>
<td>21</td>
<td>85</td>
<td>69 (81.2)</td>
<td>0.300</td>
</tr>
<tr>
<td>&gt;10 yr or unknown</td>
<td>116</td>
<td>62</td>
<td>178</td>
<td>106 (59.6)</td>
<td>0.100</td>
</tr>
</tbody>
</table>

<sup>1</sup): For diphtheria: Immune subjects = Basic or full protection (≥0.1 IU/mL).
<sup>2</sup): For tetanus: Immune subjects = Basic or full protection (>0.1 IU/mL).

GMT, geometric mean titer.
In summary, 51% of participants had basic or full protection to both diseases and 18% had protection to neither of them. The proportions of army and civilian subjects who were susceptible to one of the diseases and had at least basic protection to the other were 30% and 34%, respectively.

**DISCUSSION**

In our study in Mashhad, Iran, the proportion of army personnel participants with at least basic protection to either diphtheria or tetanus was 70%. Surprisingly, similar proportions were observed in the civilian population (59% for either disease) (Table 2). We examined if the participants had protection to both diphtheria and tetanus, but almost a third of the army personnel and the civilian participants demonstrated basic or full protection against one disease and no protection against the other.

Iran is a developing country in the Middle East in which diphtheria is endemic. By 2050, a threefold increase in people older than 60 years is expected (23). Diphtheria and tetanus antitoxins wane with age, making the elderly more susceptible to these diseases (7,8). Therefore, Iran’s health system should be aware of the diphtheria and tetanus immunity status of its population. If we assume a herd immunity threshold of 75% for diphtheria (24), civilians in our study, particularly those older than 35 years, would be prone to a greater epidemic risk. Immunity to tetanus in both the army personnel and the civilian participants aged over 50 years was also insufficient. These results are consistent with those of another study that was performed exclusively on civilians older than 50 years in Kashan, Iran (16).

In Iran, vaccination with a Td booster dose is mandatory for military service enrollment and is available for army personnel throughout their career. This systematic vaccination explains the lower rates of susceptibility to diphtheria and tetanus and the higher antitoxin GMTs that were observed in the army personnel compared with the civilians in each age group. Prior studies have also reported this effect, not only in military participants, but also in civilians who have carried out military service (25). There was a significant difference in the proportion of army personnel who had at least basic protection to tetanus compared with civilians in the 18–34 years age group.

Previous studies have demonstrated that proportions of protected populations and the GMTs of diphtheria and tetanus antitoxins decrease as age increases (11,12). A similar pattern was observed in our study. For tetanus, the decreasing pattern was most prominent in the oldest age group. For diphtheria, proportions of participants with at least basic protection were similar in the 35–50 and >50 years age groups; one potential explanation for this is the wide age range in these groups.

Booster doses during high school, military service, or pregnancy could account for the higher GMTs and the higher proportion of participants with full protection to diphtheria and tetanus in 18–34 year olds. This is consistent with the results of 2 other studies in Iranian populations (26,27), and those of some developed countries (25). Td vaccination history in the last 10 years was a significant contributor to diphtheria and tetanus immunity in our subjects.

One limitation of our study in army personnel is that it is similar to that of other studies (2,17). In Iran, military personnel are predominantly male and military service is mandatory for men. This would result in higher Td vaccination coverage in males. On the other hand, almost 95% of pregnant women (28) or women aged 15–25 years (26) have protection to tetanus in Iran. This high Td vaccination coverage in young females reduces the risk of neonatal tetanus. This may explain why we found no significant difference between male and female participants in terms of tetanus immunity and diphtheria antitoxin levels. However, there was a 14% difference in the proportion of participants susceptible to diphtheria. These results are comparable with those of other studies in Iran and Turkey (which have high vaccination coverage rates for young women) (21,26,27,29). The similar diphtheria and tetanus immune statuses in male and female participants in our cohort may have strengthened the validity of the primary comparison (army personnel versus civilians).
Our sample cohort of laboratory attendees may have resulted in biased data. These participants attended the laboratory for elective blood tests, indicating that they may be more health conscious, with more up-to-date vaccination schedules, than other populations. However, some of the army personnel in this study were chosen and sent for blood tests randomly, in accordance with army surveillance programs, which may have canceled out any potential bias.

Iran has a successful childhood immunization program with a vaccination coverage rate of almost 100% (6). This includes the administration of 5 doses of DTP (diphtheria-tetanus-pertussis) to children up to 6 years old. Immunization is also recommended for high school girls and pregnant women to combat neonatal tetanus. Furthermore, young male adults receive vaccination upon entering military service. However, as people get older, diphtheria and tetanus are not as much of a health priority, as is evident by the immunity status of older adults in this and other studies (11,16). This negligence increases the risk of diphtheria or tetanus in groups like army personnel who are exposed to greater occupational risks (3). Similar studies are required in other regions and with different populations to enforce health measures against diphtheria and tetanus.

This study indicated that a large portion of participants older than 50 years were susceptible to tetanus and diphtheria, not only in the civilian group, but also in army personnel who can be monitored and vaccinated systematically by the army. Inadequate protection against diphtheria in civilians aged 35-50 years increases the level of concern. The acceptable immune status of younger adults suggests that a strict administration of booster schedules for older people should be implemented to prevent diphtheria and tetanus.

**Conflict of interest** None to declare.

**REFERENCES**


