TÜBİTAK

Turkish Journal of Medical Sciences

http://journals.tubitak.gov.tr/medical/

Research Article

Turk J Med Sci
(2016) 46: 646-650

doi:10.3906/sag-1503-38

Evaluation of tetanus antibody levels in adults in Yozgat, Turkey

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Received: 05.03.2015 • **Accepted/Published Online:** 21.06.2015 • **Final Version:** 19.04.2016

Background/aim: To assess the immune status against tetanus in relation to self-declared vaccination status among an adult population in Yozgat, Turkey.

Methods: Questionnaires and blood specimens were collected from 267 individuals over 18 years of age in Yozgat, Turkey. Antitetanus antibodies were determined quantitatively by enzyme-linked immunosorbent assay.

Results: Among the 267 subjects (mean age 43.4 ± 15.6; male:female, 133:134) 168 (62.9%) had protective level of tetanus antibody titers. There was a characteristic distribution of the tetanus antibody titers in different age groups as it dropped with increasing age. Protective immunity was obtained in 74.1% of urban and 47.9% of rural participants. The lowest immunity was observed among farmers (53.3%) and housewives (55.6%) when compared to other occupations. Protective antibody titers were detected in 25% of the individuals who had not finished any school, while the rate was 83.7% in those who had graduated from university. While the protection rate in women who had been vaccinated during pregnancy was 78.3%, this rate was 39.2% in nonvaccinated women.

Conclusion: Our findings confirm that only 62.9% of the population is immunized against tetanus, with a lack of coverage in particular for the elderly and in rural areas. It is necessary to follow the recommendations for 10-year boosters for tetanus.

Key words: Tetanus, immunity, Yozgat, Turkey

1. Introduction

Tetanus develops through a neurotoxin called tetanospasmin, which is secreted by *Clostridium tetani* after entering the body through injuries. It is characterized by painful and tonic muscle spasms that progress to become quite heavy, and a high death rate (1). *Clostridium tetani*, an obligate anaerobe, is a gram-positive bacillus with motile and terminal spores. Bacteria, especially in the oxygenic area, halt reproduction and form a resistant spores shape from a vegetative shape. Spores can be found in human, and especially animal, excrement, in sites polluted with feces, in dung-heaps, and in dust and soil. For this reason, it is not possible to completely eradicate tetanus (2). Tetanus can be controlled by vaccination, and is often seen in newborns, children, and young adults in developing countries (1). More rarely, the disease can appear in developed countries and in people over the age of 50 as a result of incomplete vaccination and an irregular descending vaccinated calendar based on reduced antitoxin levels over time (1). With adequate immunization, a serum antitoxin level of 0.1 IU/mL and higher provides protection against the disease. When tetanus toxoid is given 3 times at 1-month intervals for active immunization, at least 5 years of protection is provided (3).

The aim of the present study was to determine the seroprevalence of tetanus antitoxin levels among adults in Yozgat and its relation to vaccination history, age, and residence.

2. Materials and methods

2.1. Selected population, sample size and study design

This cross-sectional epidemiologic study was planned to determine the tetanus antibody levels among adults in Yozgat. With a sample of approximately 328,794 individuals of 18 years of age or older in Yozgat, on the assumption that 65% of the individuals in this age group will have protective levels of antitoxin, the required sample size was calculated as 349 at a confidence interval of 95% and with a deviation

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of 5%. According to this, 267 of those who applied were included in the study, and the distribution of sex and age was taken into account. Data and serum collection was conducted between June 2012 and September 2012. After filling in the informed consent form, the respondents were subjected to face-to-face interviews using a questionnaire. A standard form was filled in, including the subject's age, sex, education level, presence of childhood vaccines, marital status, pregnancy, military service status, current chronic diseases, drug and alcohol use, accident or injury history, and previous tetanus vaccination status. Each participant provided a 10-cc blood sample to determine their tetanus antibody levels, and the serum was separated through centrifugation and kept at −80 °C until the day of study. The study was approved by the Bozok University clinical research ethics committee (Registration number: 03.01.2012/03).

2.2. Enzyme-linked assay

Antitetanus antibodies were determined quantitatively by immunosorbent enzyme-linked assay (ELISA) (Euroimmun, Lübeck-Germany) method in serum samples. A 100-µL serum sample from each patient was put in each well in the test strip. The intention in this regard was to form the immunocomplex by combining the antibodies found in the human serum with the antigens coated on the test strips. After the removal of uncoated immunoglobulin through washing, enzyme conjugates were added. When the substrate solution was added, a blue color appeared as a result of substrate use by the enzyme, after which the color was observed to change from blue to yellow with the addition of a stop solution. Optical density values were obtained by reading the color in a spectrophotometer, and a standard curve was obtained from the optical density values marked on paper. Immunoglobulin G (IgG) levels, corresponding to the absorbance values of the serum samples, were determined in IU/mL. When the test results were interpreted according to the current literature, the respondents with a tetanus IgG level of <0.1 IU/mL were determined to be inadequately immune or sensitive, and those with a tetanus IgG level of ≥0.1 IU/mL were evaluated as definitively immune.

2.3 Statistical analysis

For the statistical analysis, a Student's t test for independent and paired continuous variables and proportional comparisons of categorical variables were performed using chi-square tests, while a Fisher's exact test was used when data were sparse. The results are expressed as mean ± standard deviation (SD), unless indicated otherwise. Spearman's test was used for the correlation analyses. A P value of <0.05 was considered to be statistically significant. Statistics were run using the STATA 11.0 Software Package (College Station, TX, USA).

3. Results

Among the 267 subjects (mean age, 43.4 ± 15.6 years old; male:female, 133:134) 168 (62.9%) had tetanus antibody titers above 0.1 IU/mL. Statistically significant differences in antibody levels were observed between the males and females, with the male participants observing higher protective tetanus antitoxin levels (P = 0.035). There was a characteristic distribution of the tetanus antibody titers among different age groups, which dropped with increasing age. Spearman's correlation analysis showed a significant negative correlation between age and antibody titers (r = −0.5231, P <0.001) (Figure). Seropositivity against tetanus in the different age groups is shown in Table 1. Only 1 subject declared that he had not been vaccinated against tetanus and had no immunity. Protective immunity was obtained in 74.1% of urban and 47.9% of rural participants (P = 0.001). The lowest immunity was observed among farmers (53.3%) and housewives (55.6%) when compared to other occupations. Protective antibody titers were detected in 25% of the individuals who had not finished

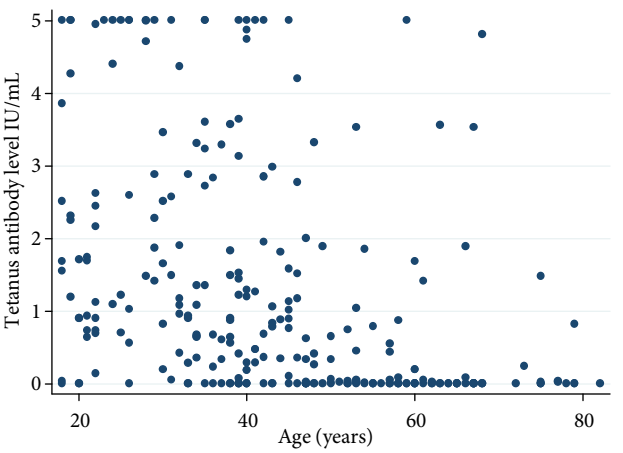

Figure. Tetanus antibody levels and age correlation graph (r = −0.5231, P <0.001).

Table. 1. Seropositivity against tetanus in the different age groups.

Age groups (years)	Seropositivity n (%)
18–20	14 (78%)
21–30	40 (97.6%)
31–40	54 (78.3%)
41–50	39 (69.6%)
51–60	13 (31.7%)
61–70	5 (17.9%)
71–80	3 (21.4%)

any school, while the rate was 83.7% in individuals who had graduated from university (P = 0.001). Spearman's correlation analysis showed a significant positive correlation between education level and antibody titers (r = 0.346, P <0.001). Furthermore, 60 of the 134 women stated that they had been vaccinated for tetanus during pregnancy, and 47 (78.3%) of them were found to be immune. Of the 134 women, 74 said that they had not been vaccinated for tetanus during pregnancy, and 29 (39.2%) of them were found to be immune (P <0.001). In this study, a statistically significant relationship was identified between tetanus immunity and the male sex, education, residence, occupation, comorbidity, vaccination during pregnancy, injury history, and vaccination during military service. In contrast, no significant difference was identified in the rate of immunity between subjects in terms of alcohol use or place of birth. A univariate analysis of variables for tetanus immunity is shown in Table 2.

4. Discussion

Tetanus is a vaccine-preventable disease that has a high mortality rate, especially in people who are unvaccinated or who have an incomplete vaccination program. The age groups affected by tetanus vary according to the development level of the country. Tetanus especially occurs in elderly people in developed countries as a result of antibody titers falling below the protective level, while neonatal tetanus is mostly seen in undeveloped or developing countries (4). In Turkey, tetanus toxoid (TT) vaccinations began in 1935 and, since 1957, it has been used in conjunction with the diphtheria-pertussis-tetanus (DPT) vaccine. TT vaccinations were scheduled across Turkey by the Ministry of Health in 1985, and since then tetanus vaccinations have been given as part of a childhood vaccination program. Vaccinations are given to children in the second, fourth, and sixth months after birth, and at 12 months after the last vaccination.

Table 2. Univariate analysis of variables for tetanus immunity.

Characteristic	Immune cases (n = 168)	Nonimmune cases (n = 99)	P value
Age, years	37.1 ± 12.7	54.1 ± 14.3	<0.001
Sex, male	92 (54.8%)	41 (41.4%)	0.035
Residence			
Rural Urban	34 (20.2%) 134 (79.8%)	37 (37.4%) 62 (62.6%)	0.002
Education			
No school Primary school Secondary school High school University	7 (4.2%) 38 (22.6%) 30 (17.9%) 52 (31%) 41 (24.4%)	21 (21.2%) 43 (43.4%) 13 (13.1%) 14 (14.1%) 8 (8.1%)	<0.001
Occupation			
Farmer Employer Worker Housewife Student Self-employed	20 (11.9%) 30 (17.9%) 30 (17.9%) 48 (28.6%) 17 (10.1%) 23 (13.7%)	16 (16.2%) 9 (9.1%) 13 (13.1%) 42 (42.4%) 1 (1%) 18 (18.2)	0.004
Comorbidity (if any) Diabetes mellitus Chronic renal failure Chronic liver disease Malignancy	35 (20.8%) 18 (10.7%) 3 (1.8%) 11 (6.6%) 3 (1.8%)	39 (39.4%) 15 (15.2%) 6 (6.1%) 7 (7.1%) 11 (11.1%)	0.001 0.105 0.026 0.496 <0.001
Military service (for males only)	79 (85.9%)	40 (97.6%)	0.042
History of injury	71 (42.3%)	27 (27.3%)	0.014
History of vaccination during pregnancy	47 (61.8%)	13 (22.4%)	<0.001

A further dose is given at the age of 6, when the child has reached school age. This application is referred to as primary immunization, and a booster at the age of 10 is recommended, plus tetanus vaccine is administered after 20 weeks of pregnancy in Turkey (http://www.shsm.gov.tr/public/documents/legislation/bhkp/asi/neotetanoz/MaternalveNeonetalTetanozEliminasyonSahaRehberi.pdf). Furthermore, young men of military age are also vaccinated against tetanus through routine vaccinations during military service (5).

This study made an analysis of 267 people above 18 years of age living in Yozgat, among which the protection from tetanus rate was found to be 62.9%. Protective levels of tetanus antibody were found to be 68% to 86.4% in different studies from Turkey (5–7). Different levels of tetanus immunity have also been identified in different countries. The rate of protection in a sample aged 0–95 years of age was found to be 70.7% in a study in Italy (8), along with rates of 82.5% in Canada, 72% in Germany, 76.2% in India, 61.2% in Peru, and 47% in Kenya (9–13).

In terms of the age distribution in our study, tetanus immunity below the age of 40 ranges from 77.8% to 97.6% and it is noted that protection levels decrease gradually with age. Furthermore, the immunity rate against tetanus is 69.9% in the 41–50 age group and 31.7% in the 51–60 age group. After this, the protection rate drops dramatically to 17.9% in the 61–70 age group, and 21.4% in those over 70 years of age. This can be explained by the fact vaccines are administered during pregnancy, school, and military service in those under 50 years of age.

In our study tetanus antitoxin levels significantly decrease after 50 years of age, which is accepted as the limit age. This can be explained by the fact that adult vaccination is not a very common practice in Turkey. Therefore, older people are expected to have a lower tetanus antitoxin level. Tosun et al. in Manisa showed that antitoxin levels significantly decrease with age in both women and men especially after 50 years of age, which is accepted as the limit age at which the antibody is likely to be negative (14). In another study from Turkey a rate of protection of 56% in respondents aged 50 years and above was reported (5). In a further study from Turkey a rate of protection was found of 58.3% between the ages of 40 and 50, 41.7% between 50 and 60, 36.4% between 60 and 70 age, and 20% in those aged above 70 (7). Recent studies in different countries show clearly that the elderly population is not generally immune to tetanus, and tetanus seropositivity was found to be 54% and 29% in people of a mean age of 69 and 82, respectively, in the United States (15). The seropositivity rate in Italy was found to be 43.4%, 26.6%, 27.9% and 17.1% in the 45–65, 65–74, 75–84, and ≥85 age groups, respectively (8). Tetanus seropositivity was found to be 83.6% in Finland, 57.1% in New Zealand, and 63.2% in Israel in people aged over 50 years (16–18).

In our study, a higher level of tetanus IgG positivity was found in males, and similarly high levels were reported in males in studies from Germany and Greece also (10,19). This high rate in the studies may be attributed to fact that men are have more accidents.

We found a conflicting result in this study where immunity was higher in individuals who had not carried out military service than in those who had. Probably, the high immunization rate that was found in individuals who had not done military service is associated with a younger age. The mean age of the individuals who had not completed military service was 26.6 ± 13.7 and of those who had was 46.4 ± 14.4 in this study.

In our study, in contrast to other studies in Turkey (6,7), the immunization rate was significantly lower in people living in rural areas when compared to those living in city centers. We think that this may be attributed to the lack of easy access to health care in rural areas. It is possible that the rate of booster vaccination is lower because, over time, physicians and individuals lose interest in maintaining vaccine coverage in rural areas of Turkey (20). In this study it was observed that immunity to tetanus increased with education level, which was compatible with another study from Turkey (21). According to the information received from the participants, significantly lower tetanus antibody levels were detected in those who had not received routine vaccinations in the childhood period. In our study, tetanus immunity was significantly lower in people with a history of chronic disease. It can be concluded that advancing age and having chronic diseases are associated with reduced immunity to tetanus. In a study that evaluated tetanus immunity in type-2 diabetes mellitus patients, protection from tetanus was significantly decreased in diabetic men (22). Tetanus vaccinations administered during pregnancy directly affect the level of antibodies in the elderly. With the advent of the maternal and neonatal tetanus elimination program in 1995 in Turkey, 2 doses of the tetanus vaccine were administered (1 month apart), followed 6 months later by a third dose, then 2 doses 1 year apart (http://www.shsm.gov.tr/public/documents/legislation/bhkp/asi/neotetanoz/MaternalveNeonetalTetanozEliminasyonSahaRehberi.pdf). Protection was significantly higher in women who were given the tetanus vaccine during pregnancy in this study.

In conclusion, in this study, which was performed with people of different sociocultural levels in Yozgat, we identified a tetanus antitoxin protection level of 62.9%, and it was determined that protection decreases with age. The completion of the primary vaccinations administered

during childhood, the vaccination of women during pregnancy, and vaccinations after an accident or injury protects adults against tetanus up to a certain period. The importance of immunization efforts in both childhood and pregnancy is increasing day by day, and campaigns have been launched to work towards the prevention of neonatal and maternal tetanus nationwide. However, tetanus immunity decreases with age, and the high level of tetanus mortality is an important issue to be kept in mind. This study has shown the necessity of additional doses over the age of 50, when tetanus protection is significantly reduced.

Acknowledgment
This study was supported by the Bozok University Scientific Research Project Units; Project No: 2012TF/A09.

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