



Evaluating the Immunity to Tetanus in Adults with the Enzyme-Linked Immunosorbent Assay

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Abstract

Background: Tetanus is a worldwide preventable disease with vaccination having an incidence of approximately one million, which causes mortality especially in neonates in developing countries and in individuals over 60 years old in developed countries. The disease particularly affects unvaccinated people or those with an incomplete vaccination schedule. It has been reported in literature that immunity to tetanus diminishes with increased age. The aim of this study was to determine the immunity to tetanus and the need for booster immunization in the adult population in Turkey.

Methods and findings: A total of 339 blood samples were collected from patients aged between 20-81 years. Tetanus antitoxin levels were studied with the Enzyme Linked Immunosorbent Assay (ELISA) method (Genzyme Virotech Germany). Titers <0.1 IU/ml were considered to show insufficient immunity to tetanus.

Of the studied population, 39.5% had insufficient immunity against tetanus. With increasing age, tetanus immunity was seen to decrease. The immunity to tetanus was seen to be higher in patients who had received a booster immunization in the past 5 years compared with the groups with a last booster immunization 5-10 years previously or more than 10 years ago. A higher rate was also determined in the female population with a pregnancy history in the last 5 years compared with the groups with pregnancy 5-10 years previously or more than 10 years ago.

Conclusion: Booster doses for adults are needed. The present vaccination schedule must include boosters every 10 years in order to provide immunity against tetanus for all the population.

Keywords: Tetanus; Immunity; Adults; ELISA

Introduction

COVID-19 Tetanus is an infectious disease caused by *Clostridium tetani*, which is characterized by muscle spasms caused by a neurotoxin named tetanospasmin. The pathogenic bacteria are found in soil, dust and the faeces of animals and humans. It can cause disease by penetrating from a skin wound or through the umbilical cord in the neonatal period. The disease is usually seen in neonates, children and young adults in developing countries, and more rarely in those aged older than 50 years, and in adults with incomplete primary immunization to tetanus in developed countries. The global incidence of the disease is one million per year or 18/100,000. In a tetanus surveillance study conducted between 2009-2017 in the USA, 264 cases were diagnosed of which 64% were between 20-64 years old and one third of the cases were ≥ 65 years old [1-4].

Tetanus is a preventable disease, which can cause mortality in neonates and those aged >65 years. The mortality rate has been reported as 0.1/100,000 and 28/100,000 in North America and developing countries, respectively. Although data in Turkey are limited, Akın et al. reported 164 cases of neonatal tetanus to the Ministry of Health with a mortality rate of 44.2% [5]. According to World Health Organisation (WHO) data, the number of tetanus cases were 25, 8 and 25 in 2010, 2015 and 2017, respectively [6].

The tetanus immunization schedule in Turkey is as follows: primary immunization with 3 doses, followed by booster doses every ten years. It has been stated that immunization to tetanus decreases after the age of 40 years and even further after age 60 [7]. In addition, the co-existence of an immune-compromising disease that affects humoral immunity also makes the population susceptible to the disease. The aim of this study was to evaluate the immunity to tetanus in an adult Turkish population and to determine the need for booster dosing in this age group.

Methodology

This cross-sectional study was conducted in the Faculty of Medicine of Dokuz Eylul University in Izmir, the third largest city in Turkey. The number of cases to be included was decided according to the confidence interval of 95% for tetanus immunity levels. A total of 399 adult were included in the study and this number was determined according to susceptibility rates in prior studies with a standard deviation of 5%. All study participants provided informed consent. A questionnaire was completed by patients attending Dokuz Eylul University Hospital Central Laboratory for blood samples to be taken. The patients were selected at random with the inclusion criteria of age older than 20 years and willingness to participate in the study.

The questionnaire included age, gender, level of education, occupation, place of residence, alcohol consumption, smoking status, income level, the presence of a chronic disease that affects immunity such as renal insufficiency, malignancy, or the use of corticosteroids. Evaluation was also made of pregnancy history, military service status, frequency of admittance to a healthcare service, and vaccination history. Blood samples were collected from the patients presenting at the Central Laboratory. The samples were centrifuged, and then the Enzyme-Linked Immunosorbent Assay (ELISA) (Genzyme GmbH Germany) was performed to detect anti-tetanus IgG levels. The results

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were obtained as IU/ml, in accordance with international standards using the standard curve of Virotech Tetanus ELISA, which was checked with the Diphtheria Antitoxin Human Serum (S1/534) of the Institute for Biological Standards and Control, WHO International Laboratory for Biological Standards in Great Britain. The results were evaluated according to literature as follows: <0.1 IU/ml: Insufficient protection or ≥ 0.1 IU/ml: Full protection. The results were evaluated using the Statistical Package for the Social Sciences (SPSS)-11 software and the χ^2 -test was used in statistical analysis.

Results

The studied population comprised 123 (36.3%) males and 276 (63.7%) females with a mean age of 49.33 ± 15.28 years (range, 20-81 years). Anti-tetanus IgG was determined at an insufficient level in 39.5% of the study sample and at a protective level in 60.5%. There was no statistically significant difference in immunity to tetanus between the genders (χ^2 Yates=0.91, p=0.3412). The levels of immunity according to age are shown in Table 1. Immunity was observed to decrease with age, with a level of immunity 13.03-fold higher in the 20-29 years age group and 2.56-fold higher in the 30-39 years age group compared to the 70-81 years age group (p<0.0001). There was no significant difference in immunity to tetanus between subjects in terms of education level, occupation, income level, living in an urban or rural area, alcohol consumption and smoking. There was no significant difference between subjects in respect of whether or not they remembered their vaccine history. Therefore, the cases who did not remember the vaccine history

were excluded and the Chi-square slope test was performed. The levels of immunity to tetanus of cases who remembered the tetanus vaccine history are shown in Table 2. Immunity to tetanus was 4.30-fold higher in the group with a vaccination in the previous 0-5 years compared to the group vaccinated ≥ 10 years ago (χ^2 linear trend=9.025, p=0.00266).

No significant difference was determined between the subjects in respect of immunity to tetanus and having renal insufficiency, malignant disease or corticosteroid use (χ^2 Yates=0.000, p=0.0996). No statistically significant difference was determined according to the frequency of presenting at healthcare institutions (χ^2 =4.49, p=0.10). Military service is compulsory for men in Turkey at the age of 20 and tetanus vaccination is made at that time. Very few of the male subjects had not completed military service so the relationship between immunity to tetanus and military service was not statistically significant.

The relationships between immunity to tetanus and pregnancy history are shown in Table 3. The level of immunity to tetanus was statistically significantly higher in the group with a history of pregnancy (χ^2 Yates=7.066, p=0.008). The Chi-square test was applied according to pregnancy history (Table 4). The immunity of the group with a pregnancy within the last 5 years was 4.5-fold higher than that of the group with a pregnancy 10 years previously. The group with a pregnancy 5-10 years previously had 2.17-fold higher immunity compared to the group with a pregnancy 10 years previously (χ^2 linear trends=5.903, p=0.0151).

Age	Protection				Total	
	>0.1 IU/ml		<0.1 IU/ml			
	Number	(%)	Number	(%)	Number	(%)
20-29	45	(91,8)	4	(8,2)	49	(100,0)
30-39	31	(68,9)	14	(31,1)	45	(100,0)
40-49	44	(61,1)	28	(38,9)	72	(100,0)
50-59	41	(50,0)	41	(50,0)	82	(100,0)
60-69	25	(50,0)	25	(50,0)	50	(100,0)
70-81	19	(46,3)	22	(53,7)	41	(100,0)
Total	205	(60,5)	134	(39,5)	339	(100,0)

Table 1: The protection rates of cases.

Vaccine	Protection				Total	
	>0.1 IU/ml		<0.1 IU/ml			
	Number	(%)	Number	(%)	Number	(%)
Before 0-5 years	41	(82,0)	9	(18,0)	50	(100,0)
Before 6-10 years	12	(60,0)	8	(40,0)	20	(100,0)
Before 10 years	18	(51,4)	17	(48,6)	35	(100,0)
Total	71	(67,6)	34	(32,4)	105	(100,0)

Table 2: Immunity rate to Tetanus of the cases who remembered vaccination history.

Pregnancy history	Protection				Total	
	>0.1 IU/ml		<0.1 IU/ml			
	Number	(%)	Number	(%)	Number	(%)
Yes	96	(53,9)	82	(46,1)	178	(100,0)
No	30	(78,9)	8	(21,1)	38	(100,0)
Total	126	(58,3)	90	(41,7)	216	(100,0)

Table 3: The relation between pregnancy history of the women participants and tetanus immunity.

	Number	(%)	Number	(%)	Number	(%)
0-5 year before	9	(81,8)	2	(18,2)	11	(100,0)
6-10 year before	13	(68,4)	6	(31,6)	19	(100,0)
10 year before	74	(50,0)	74	(50,0)	148	(100,0)
Total	96	(53,9)	82	(46,1)	178	(100,0)

Table 4: Tetanus immunity rates of women participants having pregnancy period.

Discussion

Tetanus is a rare disease with high mortality rates, which is seen especially in the unvaccinated or partially vaccinated population. Therefore, a vaccination program is important for prevention of the disease. In developed countries, prevention of tetanus is a problem in the older age groups who have diminished immunity. The WHO has stated a tetanus mortality rate of one million per year with almost half of this number comprising neonatal tetanus cases in developing countries. In developed countries the disease is seen in older people with low immunity, and so mortality is higher in the elderly [8,9]. Aydın reported that 95% of tetanus cases were aged ≥ 35 years between 1991-1995 and immunity to tetanus decreased with age [10].

In 1985, the Ministry of Health in Turkey implemented a regular vaccination programme for the paediatric age group. Children are vaccinated at 2, 4, 6 and 18 months old and a further dose is administered in school in grade eight. This is named primary immunization and is followed by additional doses every 10 years [11].

This study was conducted on 399 adults in Izmir. Of them 60.5% were determined to have a sufficient level of immunity to tetanus while 39.5 were susceptible. According to the age groups the immunity declined with the immunity determined in 91.8% of the 20-29 years age group, in 50% of the 50-59 years age group and in 46.3% of those aged >70 years ($p < 0.0001$, Table 1). The immunization program in Turkey was initiated 35 years ago so those aged 50 years or older were not vaccinated at school. Studies in the USA have shown similar results. Alagappan reported 86% immunity in 129 cases aged ≥ 65 years. The authors stated that with age, T cell activity and interleukin 1 and 2 levels decrease, thereby resulting in a decreased response to antigens [12]. Geraldine et al. reported 72.3% immunity to tetanus in a population aged ≥ 6 years and 27.8% in those aged ≥ 70 years, and additional doses every ten years were recommended.

In studies conducted in Turkey the immunity to tetanus has been reported to vary between 8.3%-100%. Cetin et al. found tetanus antitoxin levels to be 75%, 35.7% and 14.3% in age groups of 21-30 years, 31-50 years and >51 years, respectively [13,14]. In a study of 400 cases, Atabey et al. reported immunity in 84.1% of cases aged <5 years and 24.2% in those >30 years [15]. Hacibektasoglu et al. found immunity of 92.9% in cases aged 12-48 years [16]. In another study by Atabey, immunity was found to be 50.7% in cases aged 1-85 years using the indirect hemagglutination method [17]. Ozturk studied 249 cases aged >40 years and reported 25.3% overall immunity and 19.4% immunity in cases >60 years old [18]. Ergonul et al. conducted a study of 100 people and concluded that immunity rates were 93.1% and 20% in groups aged 18-30 and >70 years, respectively [19]. The decrease in antitoxin levels over time is important in deciding the time for additional doses.

There was no significant difference in immunity to tetanus according to gender in the current study. This may be due to the vaccination of males on military service and females during pregnancy. In Finland, there has also been found to be no difference in immunity between the genders as an additional vaccination is given to males at 19 years old [20]. Other papers have also found no difference in immunity to tetanus between the genders [21]. When protective levels were compared according to the level of education, that low immunity was seen in those who did not complete high school, may be due to routine vaccination in high school. There was no significant difference in any other education level group. Studies in the USA have found higher immunity in individuals with a high educational level [7,13]. Similar results in Turkey show that higher educational status is related

with higher immunity to tetanus [18]. When evaluations were made of occupations that constitute a risk for tetanus such as agriculture, industry and construction, no correlations were determined with tetanus immunity. This result may be related to the low number of study participants in high-risk occupations, although other studies have also not shown any relationship [18,19]. Akyol reported 8.3% immunity to tetanus in the 18-68 years age group [22].

No significant difference was determined in immunity to tetanus according to living in an urban or rural area. This can be explained by the easy access to healthcare facilities of the population in this region. Similar results can be seen in Turkey, her studies from Turkey and Germany [18,19,23]. There was no difference in immunity to tetanus according to income level. This result could be attributed to the fact that the income level of the majority of the study participants was in the intermediate group. In a study of 10618 cases in the USA, the immunity rate was determined to be higher in the high income group [7]. There was no significant difference in immunity to tetanus according to smoking status and alcohol consumption.

Of the total study population, 57.3% did not remember their vaccination history. In the analysis of the cases who could recall their vaccination history, immunity was determined in 82% of those immunized within the last 5 years and in 51.4% of those immunized more than 10 years previously (Table 2). These results were in parallel with those of Atabey [17]. These data show that vaccination within the last 10 years provides protection against tetanus, whereas protection is severely diminished after 10 years. Similarly, Yuan et al. found low immunity to tetanus in cases with unclear vaccination history [21]. Ozturk reported 63.6% immunity in those vaccinated within the last 5 years and 28.6% in those vaccinated more than 10 years previously [18]. Ergonul also reported high immunity to tetanus in people vaccinated within the last 10 years [19].

The study participants were questioned in respect of chronic renal insufficiency, malignant disease, and corticosteroid use and none of these were determined to have any effect on tetanus immunity. However, these data may not be of any value in this study as the stage of disease, drug doses and time of therapy were not evaluated. A statistically significant difference was determined in tetanus immunity according to the frequency of presenting at healthcare facilities, which was similar to results published in the USA [13]. In the current study, no significant difference was determined in immunity to tetanus according to military service status because there were few subjects that had not completed military service. In studies in the USA, immunity has been found to be higher in cases that have completed military service [7,13]. In contrast, Yuan reported no relationship between military service and tetanus immunity [21]. In Turkey, immunity to tetanus in soldiers was reported as 68% by Akyol [22] and as 82% by Felek [23].

Female cases were asked about pregnancy history. The rate of tetanus immunity was determined as 81.8% in the group with a pregnancy within the last 5 years and as 50% in the group with a pregnancy more than 10 years previously. Immunity was 4.5-fold higher in those with a pregnancy within the last 5 years and 2.17-fold higher in those with a pregnancy 5-10 years previously compared to the group with a pregnancy more than 10 years previously. The Statcalc program was used in this analysis (χ^2 linear trends=5.903, $p=0.0151$) (Tables 3 and Table 4). Ural et al. reported 73.8% immunity to tetanus in pregnant individuals. When examined according to age groups, the rate was 93.7% in those younger than 21 years, and 41.6% in those aged ≥ 30 years. This was attributed to not applying vaccination boosters to older mothers [24].

Many studies have shown that although the preventive antitoxin titer for tetanus is 0.01 IU/ml, tetanus disease can still be seen [25-27]. According to the WHO, when 2 doses of tetanus vaccine are administered during pregnancy the prevention rate is 80%-90% and if this is followed by a third dose this rate will increase to 95%-98% and be valid for 5 years. It has been said that adding 4th and 5th doses to this scheme will increase the duration of prevention for 10-20 years [28,29]. Similar data have been reported from studies in different countries. Half of the population is susceptible to tetanus in Europe, and one-third in Canada. In the reports of these countries it is said that vaccination schemes have to be reviewed and the elderly population has to be vaccinated immediately. For those who have completed primary immunization, booster doses should be planned. In developing countries neonatal tetanus and tetanus in the elderly is a frequent healthcare problem. In Turkey, attention should be paid to these two vulnerable groups.

Conclusion

Tetanus is a preventable disease with a proper immunization program and mortality can be avoided in the neonatal and geriatric period. The elderly population is clearly more susceptible to tetanus and these results emphasize the need for booster immunization of adults. The present vaccination policy should include re-vaccinations of the adult population every 10 years in order to provide complete protection of the whole population.

Ethics Declarations

Ethics approval and consent to participate

Study approval was given by Ethics Committee of Dokuz Eylül University Medical Faculty and written informed consent was obtained from all participants. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. All the data used in this study was anonymised before its use.

Consent for Publication

Not applicable.

Availability of Data and Materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing Interests

The authors declare that they have no competing interests.

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Author's Contributions

BK, NY and AY contributed to the study design. Data collection was carried out by all the authors, while BK performed the laboratory evaluation. The data analysis and interpretation of results were performed by NY, BK, AY. The initial draft was produced by BK, AY, and NY, and edited by AY. All authors agreed, read and approved the final edited manuscript.

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