

Investigation of Tetanus Antibody Levels in Adults

Erişkinlerde Tetanoz Antikor Düzeylerinin Araştırılması

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ABSTRACT

Aim: Tetanus is an acute and lethal disease caused by exotoxins named tetanospasmin produced by *Clostridium tetani*. Despite being vaccine-preventable, tetanus is still a toxic-infectious disease with high mortality. In this cross-sectional study, it is aimed to determine levels of tetanus anti-toxin IgG and factors affecting it in adults in the region.

Material and Methods: This cross-sectional study was conducted with patients applied to the blood sampling laboratory unit of Düzce University Hospital. Tetanus anti-toxin IgG Enzyme-Linked Immuno Sorbent Assay kits (Catalog number: EI 2060-9601 G, Euroimmun, Germany) were used for detection of tetanus antibodies. Those with tetanus anti-toxin IgG >0.1 IU/ml were considered immunized. Socio-demographic information of participants were collected using a questionnaire during blood collection.

Results: Sufficient tetanus antibody was detected in 140 (39.3%) of 356 patients. Protective antibody ratios were found as 49 (70.0%) in 30-40 age group, 39 (54.9%) in 41-50 age group, 22 (31.0%) in 51-60 age group, 16 (22.2%) in 61-70 age group and 14 (19.4%) in >71 age group. Tetanus immunity ratios were significantly reduced with aging ($p<0.001$). Protective antibodies were found to be higher in the groups who had more education and who were vaccinated in adult ages for any reason than in the other groups (both $p<0.001$).

Conclusion: It was thought that the childhood immunity should be reinforced with the booster doses during adulthood by routine tetanus immunization program. In addition, the high level of tetanus immunity in those with high educational level has shown the importance of education.

Keywords: Adult vaccination; tetanus; tetanus anti-toxin IgG; tetanus vaccine.

ÖZ

Amaç: Tetanoz, *Clostridium tetani* tarafından oluşturulan tetanospazmin isimli ekzotoksinlerin neden olduğu, akut ve ölümcül bir hastalıktır. Aşılama ile önlenebilir olmasına rağmen tetanoz halen mortalitesi yüksek olan toksin-infeksiyöz bir hastalıktır. Bu kesitsel çalışmada, bölgemizdeki erişkinlerde tetanoz anti-toksin IgG seviyelerinin ve tetanoz anti-toksin IgG seviyelerini etkileyen faktörlerin saptanması amaçlanmıştır.

Gereç ve Yöntemler: Bu kesitsel çalışma, Düzce Üniversitesi Sağlık Uygulama ve Araştırma Merkezi Laboratuvarı kan alma ünitesine başvuran hastalar ile yapılmıştır. Tetanoz antikorlarının saptanmasında Tetanus anti-toksin IgG Enzyme-Linked Immuno Sorbent Assay kitleri (Katalog no: EI 2060-9601 G, Euroimmun, Almanya) kullanılmıştır. Tetanoz anti-toksin IgG $\geq 0,1$ IU/ml olanlar bağışık kabul edilmiştir. Çalışmaya katılan kişilere ait sosyodemografik bilgiler kan alma esnasında anket yapılarak toplanmıştır.

Bulgular: Toplam 356 hastanın 140 (%39,3)'ında yeterli tetanoz antikoruna saptanmıştır. Koruyucu düzeyde antikor, 30-40 yaş grubunda 49 (%70,0), 41-50 yaş grubunda 39 (%54,9), 51-60 yaş grubunda 22 (%31,0), 61-70 yaş grubunda 16 (%22,2), >71 yaş grubunda 14 (%19,4) oranlarında saptanmıştır. Tetanoza bağışıklık oranlarının yaşlanma ile birlikte belirgin biçimde azaldığı görülmüştür ($p<0,001$). Eğitim süresi fazla olanlar ve herhangi bir nedenle erişkin yaşlarında aşı yapılan gruplarda koruyucu düzeydeki antikorlar diğer gruplara göre daha yüksek oranda bulunmuştur (her iki $p<0,001$).

Sonuç: Çocukluk çağındaki bağışıklığın, yetişkinlik döneminde rutin tetanoz bağışıklık programı ile rapel dozlarla güçlendirilmesi gerektiği düşünülmüştür. Ayrıca eğitim seviyesi yüksek olanlarda tetanoz bağışıklık oranının da yüksek bulunması eğitimin önemini göstermiştir.

Anahtar kelimeler: Erişkin aşılama; tetanoz; tetanoz anti-toksin IgG; tetanoz aşısı.

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INTRODUCTION

Despite being vaccine-preventable, tetanus is a toxic-infectious disease with high mortality, affecting neonates and young adults in developing countries and elderly in developed countries. Tetanus is an acute disease caused by tetanospasmin named exotoxins produced by *Clostridium tetani*, an anaerobic bacillus; often lethal and characterized by generalized rigidity and spasms in the musculoskeletal (1). It is estimated that the worldwide incidence of tetanus is 18 out of 100000 people, or about a million cases per year in the world (2). In developed countries, the incidence of disease has fallen to less than 2 per million thanks to the implementation of regular vaccination programs. In developing countries, tetanus is an important cause of mortality, especially in newborns, whereas in developed countries it is more common in unvaccinated or under-vaccinated adults (3). Although the incidence of both neonatal and non-neonatal tetanus in Turkey has declined over the years, it remains as an important health problem. The incidence of non-neonatal tetanus was reported to be 0.02 per a hundred thousand in 2006, while it was 0.21 per a hundred thousand in 1990. Of the 923 non-neonatal tetanus cases reported to the Ministry of Health in 1990-2006, 161 died and the ratio of fatality was 17.4%; of the 658 cases of neonatal tetanus, 305 died and the ratio of fatality was 46.4%. Between 2007 and 2015, 119 cases of adult tetanus and 19 cases of neonatal tetanus were observed (4). After tetanus vaccination in childhood, booster doses of tetanus vaccine could not usually be done, and the level of antitoxin could be lost in people over time. Since there is no current adult immunization program in Turkey, only women get vaccinated during pregnancy and men get vaccinated during military service, also, after an accident or injury people get vaccinated. This situation suggests that people, especially those with primer vaccination problems due to reasons such as incomplete vaccination, become susceptible to tetanus in older ages due to decreased levels of antibodies over time (5). In this study, it is aimed to determine the levels of tetanus antitoxin IgG and the factors of affecting tetanus anti-toxin IgG levels in adult patients in the region.

MATERIAL AND METHODS

This study was supported by Duzce University Scientific Research Projects Department with project number 2015.04.01.301. It was done with approval of the Duzce University Ethics Committee, dated 23.12.2014 and numbered 2014/100.

Approximately 120000 patients visit the outpatient clinics of Duzce University Hospital annually. In this study, 356 people with 95% confidence level were included in the study according to the CDC epi info Stat Calc program when it was accepted that 45% of the patients who had come to the hospital for any reason and had had blood test were over 30 years of age. Patients participating in the study were classified as a total of five age groups, 30-40, 41-50, 51-60, 61-70 and >70 years old, with male and female ratios being equal in each age group. In order to obtain sociodemographic information about people who were involved in the study, a questionnaire was conducted during blood drawing. Age, sex, educational status, occupation, involvement in agriculture and livestock breeding, injury history and vaccination against tetanus

were questioned. 7-8 ml of venous blood was collected to tubes without anticoagulant from the subjects who were surveyed. The serum samples obtained from these blood samples after centrifugation for 10 min at 3000 rpm were stored at -20°C until serological analysis. Tetanus antitoxin IG ELISA (Catalog number: EI 2060-9601 G, Euroimmun, Lübeck, Germany) kits were used for detection of tetanus antibodies. Tetanus anti-toxin IgG antibody was accepted as an antibody at a protective level of ≥ 0.1 IU/ml (4).

Statistical Analysis

Descriptive values of categorical variables were given numbers and percentage. Categorical data were analyzed by Pearson Chi-square test and post hoc Bonferroni method. Statistical analyses were done using SPSS for Windows v.16.0 statistical package program and $p < 0.05$ considered as statistical significance level.

RESULTS

A total of 356 patients aged 30 years and over, 178 women and 178 men, who came to the Duzce University Hospital laboratory between November 2014 and December 2015 were included in the study. Sixty two (34.8%) of the women and 78 (43.8%) of the men had protective level of antibodies, while 216 (60.7%) of total 356 patients were found to be susceptible to tetanus. There was no significant difference in terms of protective tetanus antibody proportions according to gender ($p = 0.083$). When the protective tetanus antibody proportions were compared according to the age groups immunity decreased as the age increased ($p < 0.001$). According to the post hoc test results, statistically significant decrease started from 51-60 age group continued in advancing age groups. Protective tetanus anti-toxin IgG level according to age groups was shown in Figure 1. In addition, proportions of protective tetanus anti-toxin IgG in each age groups was shown in Table 1.

When tetanus antibody levels were compared according to the patients' education time, tetanus immunity proportions were increased as the education year increased ($p < 0.001$). Both 6-11 years and ≥ 12 years groups had higher protective proportions than ≤ 5 years group according to the post hoc test results. Protective tetanus anti-toxin IgG level according to education time groups was shown in Figure 2. In addition, proportions of protective tetanus anti-toxin IgG according to the education time groups was shown in Table 2.

When the occupations of patients are categorized as occupations at risk and others in terms of tetanus, 29 (33.0%) of those 88 patients with professions at risk and 111 (41.4%) of those 268 patients with other professions had antibodies at the protective level. There was no relationship between occupational groups and protective tetanus antibody proportions ($p = 0.158$). When the tetanus antibody levels were examined, 31 (36.0%) of 86 farmers had protective level of antibodies, and 22 (44.0%) of the 50 patients who were engaged in livestock breeding had protective level of antibodies. Fifty five (64.0%) of the people engaged in farming and 28 (56.0%) of the people engaged in livestock breeding were found to be susceptible to tetanus disease. There was no difference between farming and livestock breeding in terms of protective tetanus antibody proportions ($p = 0.359$).

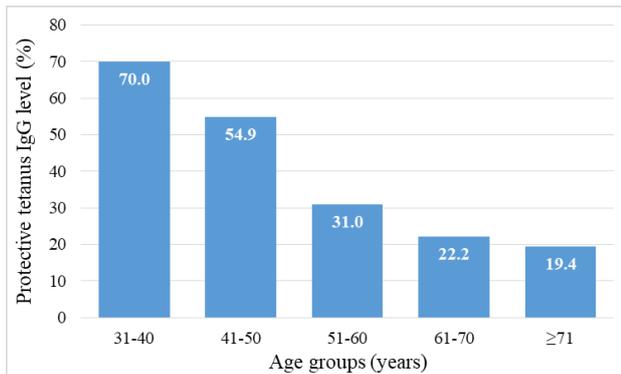


Figure 1. Protective tetanus IgG level according to age groups

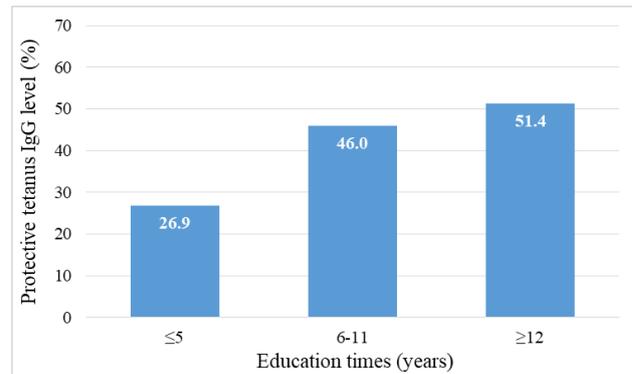


Figure 2. Protective tetanus IgG level according to education

Table 1. Protective tetanus anti-toxin IgG level according to age groups, n (%)

	31-40 (n=70)	41-50 (n=71)	51-60 (n=71)	61-70 (n=72)	≥71 (n=72)	p
<0.1 IU/ml	21 (30.0)	32 (45.1)	49 (69.0)	56 (77.8)	58 (80.6)	<0.001
≥0.1 IU/ml	49 ^a (70.0)	39 ^a (54.9)	22 ^b (31.0)	16 ^b (22.2)	14 ^b (19.4)	

Table 2. Protective tetanus anti-toxin IgG level according to education time, n (%)

	≤5 years (n=145)	6-11 years (n=137)	≥12 years (n=74)	p
<0.1 IU/ml	106 (73.1)	74 (54.0)	36 (48.6)	<0.001
≥0.1 IU/ml	39 ^a (26.9)	63 ^b (46.0)	38 ^b (51.4)	

When the history of injury of the patients was questioned, only 45 (48.9%) of the 92 patients with serious injury story had tetanus antibody at the protective level and it is remarkable that half of them still does not have tetanus immunity. In 264 patients with no history of injury, the immunity rate was 95 (36.0%), and it was found that the immunity rate was lower than the patients with history of injury (p=0.029). Proportions of protective tetanus antibody levels with tetanus vaccination status in adulthood were shown a statistically significant difference (p<0.001). Protective tetanus anti-toxin IgG level according to vaccination status was shown in Figure 3. Significantly more antibodies were detected at protective level in vaccinated people during adulthood. In addition, proportions of protective tetanus anti-toxin IgG according to vaccination status is shown in Table 3.

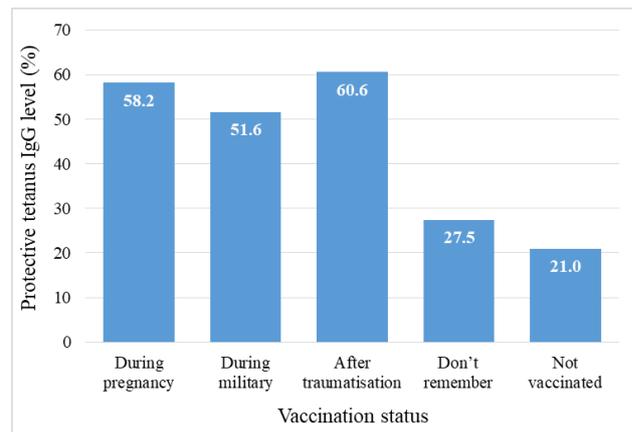


Figure 3. Protective tetanus anti-toxin IgG level according to vaccination status

Table 3. Protective tetanus anti-toxin IgG level according to vaccination status, n (%)

	During pregnancy (n=55)	During military (n=31)	After traumatization (n=66)	Don't remember (n=142)	Not vaccinated (n=62)	p
<0.1 IU/ml	23 (41.8)	15 (48.4)	26 (39.4)	103 (72.5)	49 (79.0)	<0.001
≥0.1 IU/ml	32 ^a (58.2)	16 ^{ab} (51.6)	40 ^a (60.6)	39 ^{bc} (27.5)	13 ^c (21.0)	

DISCUSSION

Most cases of tetanus are not immunized or insufficiently immunized people. The incidence of the disease increases with age (4). According to the current vaccination program in Turkey, primary vaccination is started as diphtheria, acellular pertussis, tetanus, inactive polio and *Haemophilus influenzae* type b vaccine in the second month of life and they administered in three doses with eight week intervals. Booster dose is administered at 18-24 months. Single dose adult tetanus and diphtheria vaccine intensification doses are given in the first and eighth grade of primary school. It is recommended to re-

vaccinate every ten years for the immunity to continue (6). In Turkey, tetanus vaccination is given after injury, during pregnancy and during military service. However, this is not as regular as primer vaccination. Tetanus is one of the vaccine-preventable diseases and vaccinations have been continuing in Turkey since 1968. Three doses are given from birth to one-year-old and booster doses are given at 18 months and 1st grade of primary school. In this way, children in the primary school age are immune to tetanus. However, adult vaccination is not a regular practice in Turkey. In this case, tetanus immunity is gradually decreasing from the age of thirty.

When evaluated in terms of age groups; in this study, when the age groups of patients and tetanus antibody levels were compared, it was observed that the immunity decreased rapidly with age. The protective antibody level was accepted as ≥ 0.1 IU/ml. Protective antibody levels according to age groups were found as follows: 49 (70.0%) in the 31-40 age group, 39 (54.9%) in the 41-50 age group, 22 (31.0%) in the 51-60 age group; 16 (22.2%) in the 61-70 age group and 14 (19.4%) in the ≥ 71 age group. Seroepidemiological field surveys were conducted in Antalya, Diyarbakır and Samsun between February 2000 and October 2001 within the scope of the "Infection Disease Control Project" in Turkey. In this study involving 2094 people over 6 months, the percentage of those in the 40-49 age group who were below the protective level were found as 26.8% in Antalya, 30.8% in Diyarbakır and 20.3% in Samsun. Susceptibility to tetanus in over 50 years of age was found to be 40.6% in Antalya, 51.4% in Diyarbakır and 67.4% in Samsun (7). When other studies in Turkey were reviewed, it was found in a study conducted by Dündar et al. (8) in Kocaeli that the tetanus susceptibility increased in the subjects at the age of 40 and over and that 5% of those under 40 years of age, 23.7% of the 40-60 age group and 34.5% of people over 60 years of age were below the protective value of tetanus antibody levels. Looking at the examples around the world, in a study conducted in Denmark with 30-70 years of age in 1984, 51% of patients were found to have antibody levels below the protective value. Looking at age groups, 20% of the 30-39 age group and 68% of the 50-69 age group were found not to be immune to tetanus (9). In a study conducted in Australia, 2884 individuals of various age groups were investigated for tetanus antibody levels, and the protection levels were reported as 91-97% in 30-39 age group, 67-76% in 50-59 age group and 42-52% over 70 age group (10). Rapisarda et al. (11) reported tetanus seropositivity in Italy as 97% in 18-27 age group, 86% in 28-37 age group, 76% in 38-47 age group, 62% in 48-57 age group, and 49% over 57 age group. Ang et al. (12) reported that the tetanus antibody levels decline with age in the seroprevalence study in Singapore.

When evaluated in terms of sex, women and men were taken on equal numbers in this study. 43.8% of males and 34.8% of females were found to have protective antibodies. Although there was no statistically significant difference for tetanus antibody levels in terms of sex, it was thought that the higher ratio of protective antibodies in men might be due to routine tetanus vaccination in military service. Similar comments were made in some studies where protection was found to be higher. Looking at the studies in Turkey, it was reported in a study conducted with people age range 3-104 years in Ankara that protective tetanus antibody levels were found in 45.2% of females and in 54.8% of males (13). There were no significant differences between males and females in terms of protectiveness in other studies conducted in Turkey (8,13-15). Looking at the examples from the world, it was found that 37% of men and 64% of women were below the protective level of antibody titers in a study conducted in a 30-70 age group in Denmark (9). In the study conducted on individuals over 20 years old in Greece's Northern Halkidiki region, it was found that 82.1% of males and 52.7% of females were immune to

tetanus and this difference was not only in the 21-30 age group but antibody titers were found to be higher in males in all age group over 30 (16).

In this study, when tetanus immunity ratios were examined according to the education time, protective antibody levels were found in 39 (26.9%) of those with less than 5 years of education, in 63 (46.0%) of those with 6-11 years of education and in 38 (51.4%) of those with more than 12 years of education. It was observed that tetanus immunity increased as the years of education increases. Looking at the examples from the world, in studies conducted in the USA by Gergen et al. (17) and McQuillan et al. (18), it was found that as the level of education increased, the ratio of protective antibodies against tetanus also increased. This is thought to be due to the fact that people with higher education levels have more knowledge about the subject and are more sensitive about vaccination. When immunity is evaluated according to occupational status, despite the fact that all individuals in the community are at risk of tetanus, the risk for farmers, construction workers and industrial workers is higher. There was no statistically significant relationship between occupational groups and tetanus antibody levels in this study. In another study conducted in Turkey, protective antibody levels were found 68.0% in soldiers, 53.0% in students, 35.0% in mothers and infants and 35.0% in farmers (19). In a study by Hayney et al. (20), it was found that the percentage of having tetanus antibody titers at the protective level is higher for farmers than non-farmers. In this study, there was no significant relationship between tetanus antibody levels and farmers and livestock breeding. In a study carried out by Papilla et al. (21) in Elazığ, Turkey with 100 construction workers, 100 industrial workers, 100 farmers and 100 control donors, the protectiveness level of the control group was found 49%, 44% in construction workers, 74% in industrial workers and 31% in farmers. They were researched the levels of tetanus antitoxin in construction workers, industry workers and farmers considered as risk groups and 68% of those vaccinated in the last five years, 70% of those who passed 6-10 years after the last vaccination, and 31% of those who passed more than 10 years were detected protective antibody presence. Protectiveness ratios were detected as follows 77% at 10-19 years, 68% at 20-29 years and 29% at 30 years in the same study. Tetanus vaccination and protective level of tetanus antibodies during pregnancy, post-injury and military vaccination were found as follows respectively 32 (58.1%), 32 (48.4%) and 16 (51.6%). The ratio of detection of protective antibodies in those who did not remember about their vaccination and who said that they were not vaccinated are 29 (14.1%) and 13 (20.9%) respectively. It was observed that vaccinated people were significantly more immune. There was a statistically significant correlation between tetanus vaccination status and tetanus antibody levels. It was found that the ones with vaccine history had antibody titers at a 2-fold higher protective level than non-vaccine history. As a result, it is observed that the vaccine has a protective immunity in the 10 years period, whereas it has decreased after 10 years' time. This result shows that vaccination at least every 10 years is necessary. When examples from Turkey reviewed, Ozturk et al. (15) found the percentages of protectiveness against tetanus were 29.2% in those who were vaccinated

once, 47.6% in those who had two or more vaccines, and 16.1% in those without vaccination stories in their study in Kayseri. In Turkey, protectiveness in 100 people aged 1-78 was found as 71.1% in those vaccinated in the last five years and 2.8% of those vaccinated 10 years ago (22). Dundar et al. (8) reported in their studies conducted in adults over 20 years of age that 97.7% of women with a history of vaccination in their pregnancies and 68.5% of those without vaccination stories have protective antibody levels. Aydın et al. (23) studied 21 cases of tetanus who were followed up and treated between 1991-1995 in Karadeniz Technical University, Faculty of Medicine, Department of Infectious Diseases. In the study, when consultation to the doctor was evaluated, it was found that eight cases never consulted to a doctor and the ones who consulted to a doctor were not given immunoglobulin even though they were not immunized and only 4 cases were vaccinated. Looking at the examples around the world, it was found in a study conducted in Germany with people aged 19-90 that the protective effect is especially high in vaccinated young people but it declines rapidly with aging without booster doses (24). Hosseini Shokouh et al. (25) investigated to immunity to diphtheria and tetanus in army personnel and adult civilians in Mashhad, Iran. 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, higher in men than women, and in army personnel than civilians in each age group. In this study, 45 (48.9%) of the 92 patients with serious injury history were found to have tetanus antibody at the protective level when the injury history of the patients was questioned. Since there are high levels of antibodies in the post-injury vaccination, it is important to use the opportunities of any injuries for vaccination for tetanus immunity as there is no routine adult immunization program.

In conclusion, completion of primary vaccinations during childhood, vaccination of women during pregnancy, post-injury vaccination protects adults against tetanus until a certain term, but this protection diminishes with age. With the prolongation of the life span, the elderly immune status will become more important, so that routine tetanus immunization program for adults will be a necessity.

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