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Original Article

Investigation of tetanus seropositivity levels in adult patients with rabies risk exposure admitted to a hospital in Ankara

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Abstract

Introduction: This study aimed to assess tetanus seropositivity levels among adult patients admitted to a tertiary care hospital following rabies risk exposure, and to explore potential factors influencing their immunological status.

Methodology: This cross-sectional descriptive epidemiological study included 182 adult individuals (68 females and 114 males) who presented to the hospital following rabies risk exposure. The demographic data was collected during a face-to-face interview, and the tetanus antibody concentrations were assessed using a micro-enzyme-linked immunosorbent assay (ELISA) kit. Serum antibody levels of ≥ 0.1 IU/mL were defined as "seropositive", while values below this threshold were considered "seronegative".

Results: Seropositivity was identified in 81.9% of the patients. There was a significant decline in antibody levels with age ($p < 0.001$). The Spearman correlation analysis showed a moderately significant negative correlation between age and antibody titers ($r = -0.404$, $p < 0.001$). In addition, there were significantly higher tetanus antibody levels in patients from urban areas, those vaccinated during pregnancy, and those vaccinated within the past 10 years ($p = 0.025$, 0.036, and 0.013, respectively).

Conclusions: Overall, the results highlight a reduction in tetanus antibody levels with age, emphasizing the importance of receiving a booster dose every 10 years. In addition, rabies risk exposure, particularly in older adults, presents a valuable opportunity to administer tetanus vaccination.

Key words: tetanus; seroprevalence; vaccine; immunity; rabies; antibody.

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Introduction

Tetanus is a life-threatening condition caused by the neurotoxin-producing bacterium *Clostridium tetani* [1]. Global immunization efforts have led to a notable reduction in incidence; however, tetanus remains a pressing public health concern, particularly in regions with limited access to vaccination. It continues to be a major cause of neonatal mortality in developing countries. In contrast, cases predominantly occur among unvaccinated adults in high-income nations. Although the incidence of tetanus has declined markedly in Türkiye since the 1980s, isolated cases still emerge, suggesting that the disease persists as a residual but preventable health threat [2].

Vaccination remains the most efficient strategy for preventing tetanus [3]. The national tetanus immunization program was launched in Türkiye in 1935 [4]; and since then, tetanus vaccination has been included in the routine childhood immunization schedule. Four doses of the combined diphtheria-tetanus-acellular pertussis-inactivated polio–*Haemophilus influenzae* type b (DaBT-IPA-Hib) vaccine have been administered at 2, 4, 6, and 18 months of age, since 2008; following the guidelines of the American Committee on Immunization Practices (ACIP). The current practice is to administer booster doses as DaBT-IPA at age 4 years, followed by a single dose of adult-type diphtheria and tetanus vaccine (Td) at age 13 years.

The primary immunization schedule comprises three doses for adults who have never been vaccinated: two Td doses administered 4 weeks apart, followed by a third dose 6 months later. Thereafter, booster doses are recommended every 10 years, with at least one of them being the tetanus, adult diphtheria, and acellular pertussis (Tdap) formulation. Among the vaccinations

that target adults, tetanus immunization is offered to women of reproductive age (15–49 years), pregnant women, and military personnel [5].

Despite the established childhood immunization infrastructure, adult tetanus vaccination coverage in Türkiye remains suboptimal [6]. For instance, the booster vaccination rate among individuals aged 65 years and older was reported to be merely 15.4% in Ankara [7]. Another study found that only 35.7% of individuals older than 50 years were seropositive for tetanus antibodies [8]. Although this rate has gradually improved, a 2019 study reported a seropositivity rate of just 57% in the same age group [9].

Injuries such as puncture wounds, lacerations, and crush traumas resulting from animal bites can lead to tissue damage, localized ischemia, and contamination with environmental agents such as soil, animal waste, or saliva. The abundance of *Clostridium* species in the oral microbiota of dogs and in soil underscores the risk of tetanus transmission through such injuries. Therefore, animal bites with a potential risk of rabies transmission are classified as contaminated wounds. It is recommended that all patients presenting with rabies risk exposure should be assessed for tetanus prophylaxis [1,3,10]. However, clinical practice has inconsistencies and errors. For instance, tetanus vaccines are often administered automatically to children and adolescents during rabies post-exposure prophylaxis, without verifying their vaccination history. Conversely, although it is well established that tetanus antibody levels decline with age, vulnerable older adults are frequently overlooked in tetanus immunization strategies [6]. Therefore, this study aimed to determine tetanus seropositivity levels among adult patients admitted to a tertiary hospital in Ankara due to rabies risk exposure, and to explore the potential factors influencing their immune status.

Methodology

Ethical approval

The Ethics Committee of the Health Sciences University Ankara Numune Training and Research Hospital granted ethical approval for this study (Decision Number: 2569).

Case selection and sample collection

This study employed a cross-sectional descriptive epidemiological design. About 4,000 patients, aged 18 years and older, present annually to Ankara Training and Research Hospital due to rabies risk exposure. Based on an anticipated seropositivity rate of 78.9% in this age group, the minimum required sample size was calculated to be 241, with a 95% confidence interval and a 5% margin of error [11]. However, ultimately data from 182 participants could be collected. Accordingly, this study was conducted with 182 patients (68 females and 114 males) who were older than 18 years of age, presented to the hospital with suspected rabies exposure, were neither immunocompromised nor under immunosuppressive therapy, and voluntarily consented to participate in the study. Data and blood samples were collected between April and December 2019. Patients' sociodemographic characteristics—including age, gender, educational background, and prior vaccination history—were collected during face-to-face interviews using a questionnaire, at the time of admission. Each patient was assessed for both rabies and tetanus prophylaxis and received appropriate treatment. The hospital staff gathered venous blood samples for serological analysis prior to prophylaxis.

Serum analysis

The tetanus-specific immunoglobulin G (IgG) levels were analyzed using a commercially available quantitative sandwich enzyme-linked immunosorbent assay (ELISA): the NovaLisa *Clostridium tetani* toxin IgG kit (NovaTec Immundiagnostica, Dietzenbach, Germany). IgG concentrations were expressed in international units per milliliter (IU/mL), corresponding to the absorbance values of the serum samples. Serum antibody levels ≥ 0.1 IU/mL were classified as "seropositive," whereas levels < 0.1 IU/mL were considered "seronegative"; in accordance with the manufacturer's guidelines.

Statistical analysis

The Statistical Package for the Social Sciences (SPSS) 23.0 (IBM Corp., Released 2015, Armonk, NY, USA) was used for statistical analyses. The data did not follow a normal distribution; therefore, nonparametric methods were employed throughout the analyses. Categorical variables were analyzed using the Pearson Chi-square test or Fisher's exact test. When significant differences were detected, Bonferroni correction was applied as a post hoc procedure. Moreover, the Spearman's rank-order correlation analysis was to assess the association between antibody titers and age. The coefficients were interpreted as follows: 0.00–0.25 as weak, 0.26–0.50 as moderate, 0.51–0.75 as strong, and 0.76–1.00 as very strong. All findings were reported within a 95% confidence interval (CI) and a p value < 0.05 was accepted as statistically significant.

Results

A total of 182 patients were included in the study, comprising of 68 females (37.4%) and 114 males (62.6%), with a median age of 42 years (range: 18–83). Among them, 149 (81.9%) were seropositive for tetanus antibodies. The findings showed no significant difference in seropositivity by gender (p = 0.986). Seropositivity rates across age groups were as follows: 94.4% in patients aged 18–30 years, 90.0% in those aged 31–40 years, 86.5% in those aged 41–50 years, 76.3% in those aged 51–60 years, 50.0% in those aged 61–70 years, and 45.5% in those who were > 70 years old. When dichotomized by age, seropositivity was 90.0% in patients < 50 years, whereas it dropped to 65.5% in those aged ≥ 50 years. Overall, there was a significant decline in antibody levels with age (p < 0.001). The post-hoc test indicated that the decline became evident in the beginning in the 51–60 years age group, and continued progressively in older cohorts. Spearman's rank-order correlation analysis also demonstrated a moderately significant negative correlation between age and antibody titers (r = – 0.404, p < 0.001).

The analysis did not reveal a significant association between education and seropositivity (p = 0.220). However, patients with no formal education or only primary school education had a lower seropositivity rate (75.7%) compared to those with higher levels of education. A significant difference was noted in antibody levels by residential location—68.8% of patients from rural areas were seropositive while 85.3% of those living in urban areas were seropositive (p = 0.025). In addition, 87.5% of the 24 pregnant vaccinated patients were seropositive (p = 0.036). Military service history in male patients did not have a significant effect on their seropositivity (p = 1.000); 82.2% of male patients who completed military service were seropositive.

No significant difference in seropositivity was identified between patients vaccinated due to an accident or injury, and those who were not (p = 0.141). The majority of those vaccinated for such reasons (88.3%) were seropositive.

Seropositivity was further evaluated by the number of tetanus boosters received in adulthood. The self-reported data of patients' vaccination histories was used for this analysis, due to the absence of a centralized electronic vaccination registry. The seropositivity between those who were never vaccinated were compared those who had received a single dose, and those who had received two or more doses. Although there was no significant difference between the groups (p = 0.577), the seropositivity was lower in the unvaccinated group (77.6%), than in the vaccinated group. In addition, 38 patients were unable to recall the timing of their last tetanus booster. Seropositivity was significantly higher in individuals who had received a booster within the past 10 years (92.2%) compared to those vaccinated more than 10 years ago (77.6%; p = 0.013). Finally, no significant difference was observed in antibody levels in those who had previously presented to the hospital with rabies risk exposure, and those who had not (p = 0.675). Tetanus seropositivity by sociodemographic characteristics is shown in Table 1, and vaccination-related characteristics are summarized in Table 2.

Discussion

Tetanus remains a serious and potentially life-threatening disease, despite being preventable through

Table 1. Tetanus seropositivity by patients' sociodemographics.

Characteristics	Seropositive		Seronegative		Total		*p* value
	n	%	n	%	n	%	
Gender							
Female	56	82.4	12	17.6	68	100	0.986[1]
Male	94	82.5	20	17.5	114	100	
Age groups							
18–30 years	51	94.4	3	5.5	54	100	**< 0.001[1]**
31–40 years	27	90.0	3	10.0	30	100	
41–50 years	31	86.5	6	13.5	37	100	
51–60 years	29	76.3	9	23.7	38	100	
61–70 years	6	50.0	6	50.0	12	100	
70 years older	5	45.5	6	54.5	11	100	
Education status							
Primary school and lower	28	75.7	9	24.3	37	100	0.220[1]
Secondary school	24	82.8	5	17.2	29	100	
High school	38	82.6	8	17.4	46	100	
Higher educational instutions	60	85.7	10	14.3	70	100	
Place of residence							
Urban	128	85.3	22	14.7	150	100	**0.025[1]**
Rural	22	68.8	10	31.3	32	100	

[1]Chi-square; Statistically significant values are indicated in **bold**.

Table 2. Tetanus seropositivity by patients' vaccination characteristics.

Characteristics	Seropositive		Seronegative		Total		*p* value
	n	%	n	%	n	%	
Tetanus vaccination at pregnancy							
Yes	21	87.5	3	12.5	24	100	**0.036**[2]
No	6	54.5	5	45.5	11	100	
Tetanus vaccination at military							
Yes	74	82.2	16	17..8	90	100	1[2]
No	20	83.3	4	16.7	24	100	
Admission to health facilities after injury							
Yes	53	88.3	7	11.7	60	100	0.141[1]
No	97	79.5	25	20.5	122	100	
Tetanus vaccination status*							
In the last 10 years	71	92.2	6	7.8	77	100	**0.013**[1]
More than 10 years	52	77.6	15	22.4	67	100	
Booster in adulthood							
0	38	77.6	11	22.4	49	100	
1	65	84.4	12	15.6	77	100	0.577[1]
≥ 2	47	83.9	9	16.1	56	100	
History of admission with previous rabies risk contact							
Yes	15	78.9	4	21.1	19	100	0.675[2]
No	135	82.8	28	17.2	163	100	

*38 people with unknown tetanus vaccination status were excluded from the analysis; [1]Chi-square; [2]Fisher's exact test. Statistically significant values are indicated in **bold**.

vaccination. Although national immunization programs in Türkiye have expanded in recent decades, new tetanus cases continue to be reported. It is well-established that antibody levels induced by vaccination wane over time, prompting global health authorities to recommend booster doses at 10-year intervals to maintain immunity. In adults, tetanus vaccines are typically administered during specific life events (e.g., pregnancy and military service). However, unlike the structured and comprehensive childhood immunization schedule, adult vaccinations tend to be irregular and opportunistic. Injuries and potential rabies exposures also serve as common triggers for tetanus prophylaxis. Notably, there is currently no centralized surveillance system in Türkiye to systematically track adult tetanus vaccination status, which poses a significant challenge for public health monitoring and policy development [6,12].

The overall tetanus seropositivity rate was 81.9% in this study. This finding aligns with the results of a 2019 Ankara-based study that reported a seropositivity rate of 78.9% among individuals older than 20 years [11]. Similarly, a 2017 study identified a seropositivity rate of 75.3% among 267 adult trauma patients admitted to emergency departments across Türkiye [13]. In contrast, a Düzce-based study focusing on adults over 30 years of age reported a notably lower seropositivity rate of 60.7% [14], while research conducted in Yozgat found the rate to be 62.9% among adults over 18 years of age [15]. Conversely, a remarkably high seropositivity level of 98.6% was reported among individuals aged 15 to 80 years in Edirne [16]. Earlier (2001) data from Ankara indicated a seropositivity rate of 68% in a sample of 100 adults [8].

These regional discrepancies highlight that antibody prevalence varies considerably not only across countries and income levels but also within different regions of the same country. The relatively higher seropositivity observed in this study may be attributed to the fact that the study was conducted in the capital city, where socioeconomic indicators are stronger, healthcare access is more consistent, and routine antenatal care practices (e.g., tetanus vaccination during pregnancy) are more likely to be followed. While a prior study in the same city reported even lower rates, the findings of this study are consistent with other recent research conducted in Ankara reinforcing the reliability of the results [11].

There was no significant difference in tetanus antibody levels by gender, which is consistent with previous findings reported from Ankara and Düzce [11,14]. However, data from Yozgat indicated higher antibody levels among the male patients [15]. It is well established that tetanus antibody titers wane over time in the absence of booster vaccinations, increasing the risk of infection, particularly among older adults [11]. Similarly, a previous multicenter study revealed that 60.5% of tetanus-related fatalities occurred in individuals over the age of 60 years [9].

The results of this study further confirm the age-related decline in immunity: while seropositivity was 90% among individuals younger than 50 years, it dropped to 76.3% in those aged 51–60 years, to 50% in those aged 61–70 years, and to 45.5% in those older than 70 years. Seroepidemiological research, as part of the "Infectious Diseases Control Project" between 2000

and 2001, demonstrated similar trends. Among the participants of that study, those aged 40–49 years had seropositivity rates 73.2% in Antalya, 69.2% in Diyarbakır, and 69.7% in Samsun [17]. Likewise, a study conducted in a nursing home in Bolu reported a seropositivity rate of only 15.7% among residents over 60 years of age [18]. The antibody levels were over 90% among children and adolescents (< 20 years) in Ankara, but declined sharply to 16.3% in individuals over 60 years of age [19]. In another study, 65.5% of participants older than 60 years in Kocaeli were reported to be seropositive [20].

These findings are not unique to Türkiye. Similar age-related declines have been observed in countries where childhood tetanus vaccination is routine, but adult boosters are not systematically implemented. For example, Mizuno *et al*. reported that while most individuals under 40 years of age in Japan were seropositive, this rate dropped to 30.8% in those over 50 years [21]. A Korean study from 2012 found that 92% of participants aged 11–20 years were seropositive, compared to only 19.3% in individuals over 61 years [22]. Additional seroepidemiological data from Singapore, Italy, and the United Kingdom consistently demonstrated that tetanus antibody levels decrease with age, emphasizing the global relevance of adult booster vaccination [23–25].

Existing literature suggests that tetanus seropositivity tends to increase with higher educational attainment [9,11,26]. Although this study did not find a significant association between education and seropositivity, patients with no formal education or primary school education exhibited lower antibody levels (75.7%), compared to patients with higher education. Unlike the findings from previous studies conducted in Yozgat, Kayseri, and Italy—which reported a clear positive correlation between education level and antibody levels [15,24,27]—the results of this study may reflect the more standardized vaccination coverage and follow-up protocols implemented in urban areas. Besides, the results revealed lower seropositivity rates among individuals residing in rural areas compared to their urban counterparts. This aligns with the findings of a previous study from Ankara in 2001 [8]. The lower rates observed among rural populations could be attributed to limited access to healthcare services, logistical barriers in reaching immunization programs, and generally lower levels of vaccine literacy.

Tetanus vaccination is routinely administered as part of antenatal care in Türkiye, and is included in the national expanded immunization program. Neonatal tetanus was officially eliminated in 2009 as a result of these efforts [3]. As anticipated, the pregnant women in this study who had received a tetanus vaccine during their pregnancy exhibited higher seropositivity rates compared to those who had not. Similarly, a previous study in Ankara reported that 69% of 493 women who had recently delivered and who had been vaccinated during pregnancy were seropositive, while this rate was only 46.4% among those who had never received a pregnancy-related tetanus vaccine [28].

The results also showed that military service did not significantly affect antibody levels in male patients. This finding aligns with earlier studies by Dündar *et al*. and Tosun *et al*. [20,26]. The lack of a difference may be explained by the relatively young age of male patients who had not completed military service, potentially reducing the time-dependent decline in antibody levels. Furthermore, there was no significant difference in antibody levels between patients who were vaccinated following an injury and those who were not. Although a previous study in Düzce indicated higher seropositivity among individuals who presented to healthcare facilities after injury, the findings of this study are consistent with those reported in Kocaeli that concluded no such difference [14,20]. The lack of a significant association in this study may be attributed to the relatively small number of patients falling into this category.

The tetanus antibody levels were noted to be lower among individuals who were never vaccinated in adulthood, independent of the number of vaccine doses they had previously received. However, this difference was not statistically significant. The vaccination history of participants reporting not receiving any adult vaccinations could not be verified due to the absence of a centralized electronic vaccination registry. It is possible that they may not have recalled having received a booster dose during adulthood. Seropositivity in adults is influenced not only by the number of doses administered, but also by the time elapsed since the last vaccination and age [1]. In this study, the seropositivity rate was 92.2% among those who had been vaccinated within the past 10 years, whereas this rate declined to 77.6% among those whose last vaccination was more than a decade ago. Approximately 17% of the patients were unable to recall when they last received a tetanus booster. Similarly, research conducted in Kayseri, Edirne, and Bolu reported higher seropositivity when the time since the previous vaccination was shorter [16,18,27]. Research from Taiwan, Austria, and Japan also corroborate this trend [21,29,30].

Since tetanus vaccination is not mandatory for adults, rabies risk exposure in cases other than military service, pregnancy, accidents, or injuries, represents another important opportunity for tetanus vaccination. The seropositivity rates in patients who sought vaccination due to potential rabies exposure were similar to those who did not. To the best of our knowledge, no prior research has investigated tetanus antibody levels in individuals exposed to rabies risk in Türkiye. Yet, the relatively limited significant findings in this study may be attributed to the low number of patients seeking vaccination after rabies risk exposure and the voluntary nature of the study's participation.

One potential limitation of the findings may be attributed to the fact that this study was conducted with a relatively small number of patients with rabies risk exposure, all of whom were admitted to a training and research hospital in a large city over a 9-month period. Future studies may consider focusing on the subject in a larger and more diverse patient population, to improve generalizability of the results. Moreover, the limited sample size prevented optimization of the homogeneous distribution of subgroups. Multivariate analysis could not be performed due to insufficient number of patients across the groups. Despite these limitations, the use of a quantitative kit to study antitoxin levels strengthens the reliability and impact of the findings.

Conclusions

This study identified a decrease in tetanus antibody levels with age. In addition, the seropositivity rates were higher in individuals who lived in urban areas, were vaccinated during pregnancy, and within the last 10 years. The results emphasize the need for a booster dose every 10 years in order to maintain adequate immunity.

Tetanus antibody levels are generally higher in the younger population, as childhood vaccinations are completed and boosters are administered for several reasons (e.g., school, pregnancy, and military service). However, antibody levels decrease with age. Therefore, rabies risk exposure, particularly in old age, should be considered a key opportunity for tetanus vaccination. Moreover, a common electronic vaccination information system is recommended to track the vaccination status of patients, and this could greatly assist in determining their vaccination history. In addition, it is recommended to raise public awareness about the importance of tetanus booster vaccines through educational initiatives, and administer these boosters systematically.

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Conflict of interests

No conflict of interests is declared.

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