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Diabetic Foot Wounds: Which Patients are More Prone to Tetanus?

Diyabetik Ayak Yarası: Hangi Hastalar Tetanoza Daha Yatkın?

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Abstract

Introduction: Patients who have tetanus-prone wounds, diabetes mellitus, or chronic wounds are more likely to contract tetanus. In this study, we aimed to evaluate the tetanus immunization status of patients with diabetic foot wounds (DFW) and to determine factors that may predict insufficient immunity to tetanus.

Materials and Methods: Patients who presented to our outpatient clinics with DFW were included. Tetanus antibody levels below 0.1 IU/ml were defined as "no reliable protection" and levels of 0.1 IU/ml and above were defined as "reliable protection".

Results: Ninety-one patients were enrolled in the study, 66 (72.5%) were male and the mean age was 62±11 years. Sixty-five (71.4%) of the patients had no reliable protection, while 26 (28.6%) had reliable protection. Age, duration of diabetes mellitus, education level, and residency status were significantly different between the immune and nonimmune groups in univariate analysis. In the multivariate logistic regression analysis, age ≥65 years [Odds ratio (OR): 8.33; 95% confidence interval (CI): 2.03-34.16], diabetes mellitus duration ≥11 years (OR: 6.52; 95% CI: 1.97-21.59), and lowest educational level (OR: 10.51; 95% CI: 1.61-68.52) were identified as independent predictors for insufficient tetanus immunity.

Conclusion: We found that elderly patients and patients with lower education have a very low rate of immunity against tetanus. Therefore, tetanus vaccination should be conducted in elderly patients with DFW.

Keywords: Vaccine, educational level, epidemiology, ELISA, *Clostridium tetani*

Öz

Giriş: Tetanoza meyilli yarası, diyabeti veya kronik yaraları olan hastaların tetanoza yakalanma ihtimali daha yüksektir. Bu çalışmada, diyabetik ayak yaraları olan hastalarda tetanoza karşı bağışıklık durumunun araştırılması ve yetersiz immüniteyi öngördürebilecek faktörlerin araştırılması amaçlandı.

Gereç ve Yöntem: Diyabetik ayak yarası ile polikliniklerimize başvuran hastalar çalışmaya dahil edilmiştir. Tetanoz antikör seviyelerinin 0,1 IU/ml'nin altında olması durumu yeterli bağışıklığın olmadığı; 0,1 IU/ml'nin üstünde tespit edilmesi durumunda ise bağışıklık yeterli olarak kabul edildi.

Bulgular: Doksan bir hasta çalışmaya dahil edildi ve 66'sı (%72,5) erkekti. Yaş ortalaması 62±11 yıldır. Altmış beş hastada (%71,4) yeterli düzeyde koruyucu antikör yokken 26 (%28,6) hastada vardı. Yaş, diyabet yaşı, eğitim seviyesi, yaşadığı bölge açısından tetanoza karşı bağışık olan ve olmayan iki grubun univariate analizinde, gruplar arasında istatistiksel olarak anlamlı farklılık mevcuttu. Lojistik regresyon analizinde ise yaş ≥65 yıl olması [Odds oranı (OR): 8,33; %95 güven aralığı (GA): 2,03-34,16], diyabet yaşının ≥11 yıl olması (OR: 6,52; %95 GA: 1,97-21,59) ve düşük eğitim seviyesi (OR: 10,51; %95 GA: 1,61-68,52) yetersiz bağışıklığın öngördürücüsü faktörler olarak tespit edildi.

Sonuç: Çalışmamızda yaşlı ve düşük eğitim seviyesi olan hastaların tetanoza karşı çok düşük oranda bağışıklığa sahip olduklarını tespit ettik. Bu nedenle, diyabetik ayak yaraları olan yaşlı hastalarda serolojik kontrol bakılmaksızın tetanoz aşılmasını yapmalarını öneriyoruz.

Anahtar Kelimeler: Aşı, eğitim seviyesi, epidemiyoloji, ELISA, *Clostridium tetani*

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Introduction

Diabetic foot wound (DFW) is one of the important complications of diabetes mellitus (DM) and may significantly reduce quality of life^[1]. Diabetics, patients with chronic wounds, and intravenous drug users are more prone to tetanus than other populations. The prevalence of DM among patients diagnosed with tetanus was reported as 15%, nearly three times the average expected prevalence of DM in the United States^[2]. There are several factors that lead to tetanus in a patient suffering from DFW. One of them is macrovascular or microvascular disease, which affects the delivery of immune cells to the wound and which can also create an anaerobic environment due to lower oxygen tensions in DFW. Gangrenous tissue is another factor that is frequently seen in DFWs and predisposes patients to tetanus^[3,4]. Over 70% of all diagnosed tetanus infections occur in people over 50 years of age, and 11% of all tetanus cases were found in patients suffering from chronic wounds^[5].

In this study, we aimed to evaluate the tetanus immune status of patients with DFWs and identify factors which may predict insufficient immunity against tetanus.

Methods

Study Settings and Patients

The study was conducted between 01 January 2016 and 31 December 2016. Our hospital is a regional referral hospital with 1,200-bed capacity. Patients who were presented to the diabetic foot council of our hospital and were willing to take part in the study were included. During the study period, a total of 127 patients were evaluated by the council and 91 participated in the study. Diabetic foot wounds were classified according to the Wagner Classification^[6].

All patients provided written informed consent and the study was approved by the Local Ethics Committee of Çukurova University Medical Faculty (09.01.2016/49). All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and the 2008 revision of the Declaration of Helsinki^[7]. Demographic characteristics of the patients were recorded and a standard questionnaire was applied to all patients to collect a detailed diabetes and tetanus vaccination history.

Serological Analysis

Venous blood was drawn from all patients and centrifuged for 5 minutes at 3000 rpm; the resulting serum samples were stored at -40 °C until testing. Tetanus antibody levels were measured using a commercial *Clostridium tetani* 5S immunoglobulin G (IgG) enzyme-linked immunosorbent assay (ELISA) kit (Novatec Immun Diagnostica GmbH, Germany) in the ELISA laboratory

of our hospital. The results were evaluated according to levels previously defined by Schröder and Kuhlmann^[8]. Antibody levels below 0.1 IU/ml were defined as "no reliable protection" and antibody levels of 0.1 IU/ml and above were defined as "reliable protection but may require booster dose." Antibody levels of 0.5 IU/ml and above were considered "reliable protection, no booster dose required" according to manufacturer suggestions. All patients were referred to their family physician to be evaluated for tetanus vaccination after clinical assessment and vaccinated if required.

Statistical Analysis

Statistical analysis was performed using MedCalc Statistical Software version 17.4 (MedCalc Software bvba, Ostend, Belgium) and SPSS version 20.0 (SPSS Inc., Chicago, IL, USA). Descriptive analyses were presented using percentages, median, minimum-maximum, means, and standard deviations. The variables were investigated using Kolmogorov-Smirnov test to determine whether they were normally distributed. Patients were divided into two groups depending on whether they had reliable protection against tetanus. The two groups were compared according to age, gender, diabetes duration, marital status, residency status (rural/urban), education level, previous history of DFW, whether they had received tetanus vaccination every 10 years, and their self-reported tetanus immunization status (fully covered, not covered, unknown). Receiver operating characteristic (ROC) curve analysis was used to detect a cut-off value for diabetes duration. Student's T-test for continuous variables and chi-square or Fischer's Exact test for discrete variables were used between groups for univariate analysis. For multivariate analysis, the possible predictive factors ($p < 0.20$) identified in univariate analysis were further entered into logistic regression analysis to determine the independent predictors of insufficient tetanus immunity. Hosmer-Lemeshow goodness-of-fit statistics were used to assess model fit. Spearman's test was used for correlation analyses between age and tetanus antibody titers. A p value of less than 0.05 was considered statistically significant.

Results

Demographics

Ninety-one patients were enrolled to the study, 66 (72.5%) were male and 25 (27.5%) were female. Mean age was 62 ± 11 years. Seventy-two (79.1%) of the patients lived in urban areas and 19 (20.9%) in rural areas. Regarding marital status, 81 (89%) of the participants were married and 10 (11%) were single.

A total of 75 patients (82.4%) were using insulin, 15 (16.5%) were using an oral antidiabetic agent, and one patient (1.1%) was not using any antidiabetic medicine. Mean diabetes duration was 15.3 ± 8.2 years. Sixty-eight patients (74.7%) had one or more

comorbidities concomitantly with diabetes and 23 patients (25.3%) had no comorbidity other than diabetes. Comorbidities, Wagner classification, and wound locations of the patients are presented in Table 1. Forty-nine patients (53.8%) had a history of previous DFW.

When asked about their tetanus immunization status, 79.1% of the patients (n=72) were unsure of their vaccination status, 14.3% (n=13) believed they were not covered, and 6.6% (n=6) believed they were covered. However, 97.8% of the patients (n=89) stated that they did not get vaccinated every 10 years. Only 20 patients (22%) remembered being vaccinated with at least one dose of tetanus vaccine.

Serological Results

Using a cut-off value of 0.1 IU/ml for reliable protection against tetanus, 65 patients (71.4%) had no reliable protection and 26 (28.6%) had reliable protection. Only 10 patients (11%) had tetanus antibody levels above 0.5 IU/ml and did not require booster vaccination. However, at least one dose of booster vaccination was required for the rest (89%) of the patients, even though some of them had reliable protection (>0.1 IU/

ml). When assessing the association between booster dose requirement and patients' self-reported tetanus immunity, booster dose vaccination requirement was significantly higher in patients who stated that they were not covered or did not know their tetanus vaccine coverage than among those who stated that they were fully covered against tetanus (p<0.002) (Table 2).

Spearman's correlation analysis showed a significant negative correlation between age and tetanus antibody titers (r=-0.393, p<0.0001) (Figure 1). The patients' protection rates that were determined according to age (in decade units) were as follows: <39 years: 100%; 40-49 years: 62.5%; 50-59 years: 35.3%; 60-69 years: 28.2%; and ≥70 years: 0% (Figure 2).

Patients were divided into two groups based on whether or not they had reliable protection against tetanus. Factors including age, diabetes mellitus duration, education level, and residency status were significantly different between the two groups (Table 3). In ROC analysis, 11 years was determined as the cut-off value for diabetes duration (AUC: 0.641, p=0.033).

In the multivariate logistic regression analysis, age ≥65 years [Odds ratio (OR): 8.33; 95% confidence interval (CI): 2.03-34.16], diabetes duration ≥11 years (OR: 6.52; 95% CI: 1.97-21.59) and lowest education level (OR: 10.51; 95% CI: 1.61-68.52) were identified as independent predictors for insufficient tetanus immunity (Table 4).

Table 1. Patients' comorbidities, Wagner classification, and wound locations

| | n | % |
|-----------------------|----|------|
| Comorbidities | | |
| CVD | 46 | 50.5 |
| CRF | 24 | 26.3 |
| Hypertension | 21 | 23.0 |
| COPD | 4 | 4.3 |
| Wagner classification | | |
| 1 | 16 | 17.6 |
| 2 | 21 | 23.1 |
| 3 | 27 | 29.7 |
| 4 | 20 | 22 |
| 5 | 7 | 7.7 |
| Wound location | | |
| Toe | 63 | 69.2 |
| Calcaneal | 12 | 13.2 |
| Plantar | 8 | 8.8 |
| Dorsal | 8 | 8.8 |

CVD: Cardiovascular disease, CRF: Chronic renal failure, COPD: Chronic obstructive pulmonary disease

Discussion

Diabetes is considered as one of the greatest emerging threats to human health in the 21st century. Besides the classical complications of the disease, diabetes has been associated with reduced T cell response, neutrophil dysfunction, and disorders of humoral immunity^[9,10]. A study regarding tetanus epidemiology between 1972 and 2001 indicated a higher risk of tetanus in diabetic patients. Overall, diabetic patients are 3.2 times more likely to contract tetanus than nondiabetic patients. In addition, case mortality is higher in diabetic patients than nondiabetics (44% vs. 28%). Rather than the more typical puncture or cut, these patients had chronic wounds, such as an ulcer or gangrene^[11]. In various studies carried out in North America, DFW, and gangrene represented a main risk factor, accounting for up to 25% of all tetanus infections^[3]. In another case series of 25 tetanus patients from Uganda, the most

Table 2. Patients' self-reported tetanus immunization status and their booster vaccination requirements

| Self-reported tetanus immunization status | Booster vaccination, required (<0.5 IU/ml) | | Booster vaccination, not required (≥0.5 IU/ml) | | p |
|---|--|----------------------------|--|---------|-------|
| | Fully covered, n (%) | Not covered/unknown, n (%) | Total, n (%) | | |
| | 3 (50) | 78 (91.8) | 81 (89) | 3 (50) | 0.002 |
| | | 7 (8.2) | | 10 (11) | |
| | | | | | |

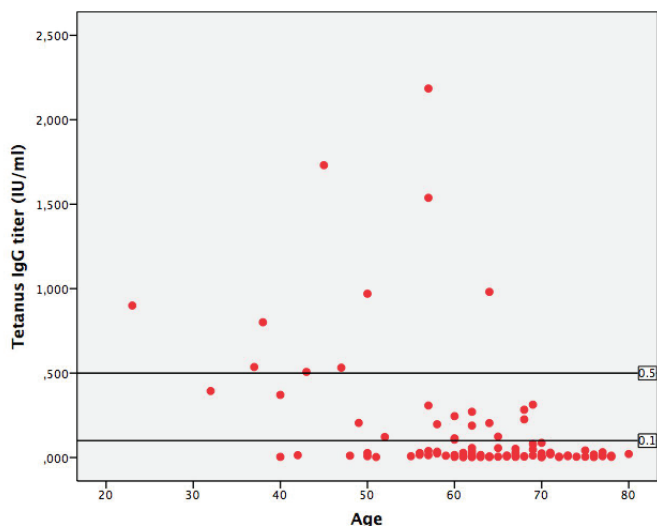


Figure 1. Age and tetanus immunoglobulin G level correlation graph ($r=-0.393$, $p<0.0001$)

IgG: Immunoglobulin G

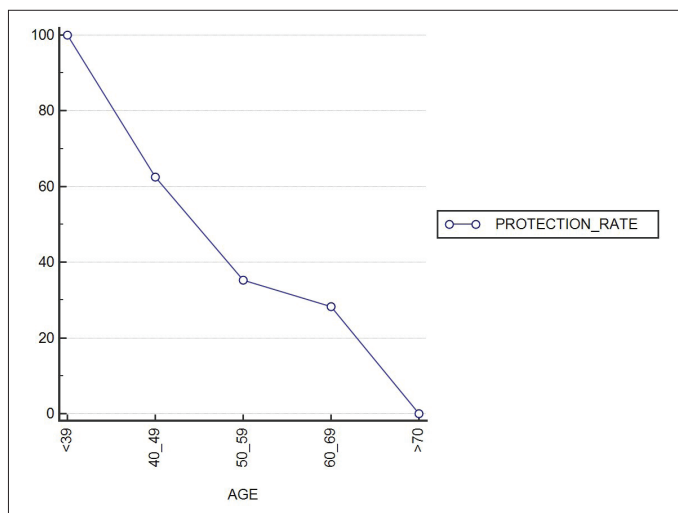


Figure 2. Tetanus protection rates of the patients according to age (by decade)

common tetanus entry wounds were due to traffic accidents, followed by DFWs^[12]. In light of these epidemiological data, it is expected that older patients with diabetes are more prone to tetanus. Unsurprisingly, if the patients have chronic wounds like DFWs, the risk will increase. Due to the higher mortality rate, we wanted to investigate tetanus immunization status of our patients suffering from DFW and we found a very low rate (28.6%) of reliable protection against tetanus in these patients.

The site of entry is known in more than 90% of patients presenting with tetanus and lower limb injuries, and wounds are one of the main causes of tetanus^[13,14]. Because of the necrotic and dirty nature of DFWs, it may provide a suitable environment for *C. tetani* spores. Therefore, it is essential to know the tetanus immunity status of DFW patients.

Table 3. Univariate analysis of variables for tetanus immunity

| Characteristics | Immune patients (≥ 0.1 IU/ml) (n=26) | Nonimmune patients (< 0.1 IU/ml) (n=65) | p |
|--|--|--|--------|
| Age | | | |
| <65 years, n (%) | 22 (43.1) | 29 (56.9) | 0.0001 |
| ≥ 65 years, n (%) | 4 (10.0) | 36 (90.0) | |
| Diabetes duration | | | |
| <11 years, n (%) | 16 (47.1) | 18 (52.9) | 0.003 |
| ≥ 11 years, n (%) | 10 (17.5) | 47 (82.5) | |
| Gender | | | |
| Male, n (%) | 21 (31.8) | 45 (68.2) | 0.265 |
| Female, n (%) | 5 (20.0) | 20 (80.0) | |
| Marital status | | | |
| Married, n (%) | 25 (30.9) | 56 (69.1) | 0.271 |
| Single, n (%) | 1 (10.0) | 9 (90.0) | |
| Residency status | | | |
| Urban, n (%) | 24 (34.3) | 46 (65.7) | 0.028 |
| Rural, n (%) | 2 (9.5) | 19 (90.5) | |
| Education level | | | |
| Primary/secondary school, n (%) | 20 (24.7) | 61 (75.3) | 0.029 |
| High school/university, n (%) | 6 (60) | 4 (40) | |
| Vaccination every 10 years | | | |
| Yes, n (%) | 1 (50) | 1 (50) | 0.492 |
| No, n (%) | 25 (28.1) | 64 (71.9) | |
| Previous diabetic foot wounds | | | |
| Yes, n (%) | 15 (30.6) | 34 (69.4) | 0.642 |
| No, n (%) | 11 (26.2) | 31 (73.8) | |
| Self-reported tetanus immunization status | | | |
| Fully covered, n (%) | 3 (50) | 3 (50) | 0.461 |
| Not covered, n (%) | 4 (30.8) | 9 (69.2) | |
| Unknown, n (%) | 19 (26.4) | 53 (73.6) | |

Table 4. Predictive factors for insufficient immunity against tetanus

| | Odds ratio | 95% CI | p value |
|-----------------------------------|------------|------------|---------|
| Age ≥ 65 years | 8.33 | 2.03-34.16 | 0.003 |
| Diabetes duration ≥ 11 years | 6.52 | 1.97-21.59 | 0.002 |
| Rural residency | 5.67 | 1.00-32.16 | 0.050 |
| Lower education level* | 10.51 | 1.61-68.52 | 0.014 |

CI: Confidence interval, *Primary and secondary school

It is known that the majority of tetanus cases involve people over 60 years of age in developed countries^[15]. A study conducted by Srivastava et al.^[11] revealed that 52% of all tetanus cases were

in patients over age 60, and 74% of all deaths occurred in this age group. In another study including 117 tetanus patients from Turkey, the mean age was 58 ± 16 and 62 patients (53%) were over 60 years old^[16]. Because of the high incidence of tetanus among elderly patients, seroepidemiological studies should be performed periodically to investigate tetanus immunity, and vaccination campaigns should be initiated. The rate of tetanus immunity differs from region to region due to countries' varying vaccination policies. In the United States, the proportion of adults reporting having received any tetanus toxoid-containing vaccination during the past 10 years was 61.6% overall for adults aged ≥ 19 years, and 56.9% for adults aged ≥ 65 years^[17]. In a study from Iran, the immunity rate was reported as 35% in patients over 50 years of age^[18]. In various studies from Turkey, immunity rates were reported to range between 15.7% and 33.8% in elderly patients^[19-22]. In the present study, we found an immunity rate of 28.6% overall and 10% for patients who were ≥ 65 years old, consistent with previous studies in Turkey. In addition to older age, we also found that low education level might predict insufficient tetanus immunity, which was also compatible with other studies^[22,23].

In Turkey, the National Vaccination Campaign accelerated tetanus immunization among children in 1985. The rate for three doses of primary vaccination increased from 20-30% to over 80% with the campaign and thereafter the rate was maintained between nearly 70% and over 80% as a nationwide average^[24]. Turkish people who were born before 1985 are more likely to have incomplete primary vaccination series against tetanus. Therefore, all physicians should know when childhood national vaccination campaigns against tetanus were launched in their country. This information may help physicians decide whether to administer only one tetanus booster dose or the three-dose primary vaccination series in adult patients.

There are numerous studies in the literature concerning immunity rates against tetanus in various patient populations, but studies including patients suffering from DFW or chronic leg ulcers are very limited. Korber et al.^[5] reported that 47% of all patients suffering from chronic leg ulcers showed an insufficient tetanus antibody concentration, and their subanalysis revealed that 70% of patients aged over 80-year-old had insufficient protection against tetanus. In the present study, we found that sufficient protection rates in DFW patients decreased with increasing age, as in the aforementioned study. Another study conducted by Farnworth et al.^[25] included 100 patients with chronic leg ulcer, nearly half of whom had DM. When the patients were asked about their tetanus immunization status, 48% were unsure of their status, 30% believed that they were not covered, and 22% believed that they were covered. After confirming with vaccination records, they found that 43% of the patients were not covered, while 33% of them were covered. In contrast, we determined sufficient immunity in only 50%

of the patients who thought they were covered, 30.8% of the patients who thought they were not covered, and 26.4% of the patients who were unsure of their status. In addition, only 22% of the patients remembered being vaccinated at least once and 97.8% of the patients reported not receiving a routine tetanus booster every 10 years. All of these results suggest high level of unawareness regarding tetanus vaccination in patients and low routine adult vaccination rates.

The medical records of adult vaccination were generally poor, and although patients claimed to be immune, half of them did not have sufficient immunity against tetanus. Our data suggest that we should not rely on verbal claims if the records are not complete. A booster dose was required for the 89% of the patients who were not vaccinated or did not know about their vaccinations status. Therefore, we propose that all patients presenting with DFWs should be vaccinated with at least one dose of tetanus vaccine without testing for IgG status and a three-dose primary vaccination schedule should be considered for those who do not know their vaccination history and whose medical records are incomplete or inaccessible.

We did not find any difference in tetanus immunity between patients who had a history of previous DFW and those who did not. This was thought to be because tetanus vaccination did not come to the physicians' minds when treating the earlier DFWs, so the patients were not vaccinated. We believe that this is evidence of the low awareness level of both patients and treating doctors in terms of tetanus vaccination.

One of the limitations of this study was the small number of patients and the fact that it was conducted in only one institute. However, it was encouraging to find the results were similar to larger and more inclusive epidemiologic studies about adult immunization rates against tetanus.

Conclusion

As the immunity rate against tetanus decreases with advanced age, DM duration, and lower education in DFW patients, attending physicians should immunize these patients for tetanus at the first possible opportunity in addition to the other required vaccines for DM (pneumococcal, influenza, hepatitis B, and zoster vaccine).

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Ethics

Ethics Committee Approval: This study was approved by the Local Ethics Committee of Çukurova University Medical Faculty (09.01.2016/49).

Informed Consent: Informed consent was filled out by all participants.

Peer-review: Externally and internally peer-reviewed.

Medical Practices: F.K., S.Ç., **Concept:** F.K., **Design:** F.K., B.K., **Data Collection or Processing:** F.K., M.E., A.U., S.K., **Analysis or Interpretation:** F.K., A.S.İ., **Literature Search:** F.K., Y.T., **Writing:** F.K., B.K., M.E., A.S.İ.

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