

Is there any difference in tetanus IgG levels of diabetic patients with respect to the presence of foot ulcers?

Fatih TEKİN^{1*}, Mehmet SÜRMEİ², Zeynep ÇİZMECİ³, Özlem TEKİN⁴,
Ergin IŞIK⁵, Ömer Faruk TANER¹, Derun Taner ERTUĞRUL⁶

¹Department of Plastic and Reconstructive Surgery, Keçiören Training and Research Hospital, Ankara, Turkey

²Department of Plastic and Reconstructive Surgery, Bağcılar Training and Research Hospital, İstanbul, Turkey

³Department of Microbiology and Clinical Microbiology, Bakırköy Dr. Sadi Konuk Training and Research Hospital, İstanbul, Turkey

⁴Department of Dermatology, Keçiören Training and Research Hospital, Ankara, Turkey

⁵Department of Plastic and Reconstructive Surgery, Muş State Hospital, Muş, Turkey

⁶Department of Internal Medicine, Keçiören Training and Research Hospital, Ankara, Turkey

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Background/aim: The aim of this study was to reveal the tetanus immunization status of diabetic patients and to determine whether diabetic patients with foot ulcers have different TIG levels.

Materials and methods: A cross-sectional study was designed that included diabetic patients with foot ulcers (n = 30) and diabetic patients without ulcers (n = 30). The groups were compared for serum TIG levels along with total serum protein, albumin, C-reactive protein (CRP), and total immunoglobulin G (Ig G).

Results: For diabetic patients without foot ulcers, 17 of 30 (56.6%) patients were found to have nonprotective TIG levels whereas for diabetic patients with foot ulcers, 28 of 30 (93.3%) patients were found to have nonprotective TIG levels. The mean value of TIG for diabetic patients without foot ulcers was 0.345 ± 0.281 IU/mL and for diabetic patients with foot ulcers the mean TIG value was 0.055 ± 0.033 IU/mL. Statistically significant differences were observed in TIG (P = 0.008), total protein (P < 0.001), albumin (P < 0.001), and CRP levels (P < 0.001) between the two groups.

Conclusion: The majority of the diabetic patients had low TIG levels and they were significantly lower in diabetic patients with ulcers. A booster dose of tetanus vaccine should be considered for diabetic patients with and without diabetic foot ulcers.

Key words: Diabetes mellitus, diabetic foot ulcer, tetanus immunoglobulin

1. Introduction

Clostridium tetani is a drumstick-shaped gram-positive anaerobic bacterium that is abundantly found as spores in soil, human skin, and the gastrointestinal tract. It is known to synthesize two potent biological toxins: tetanolysin and tetanospasmin (1). Tetanolysin is responsible for tissue destruction observed during the course of infection. Tetanospasmin is a neurotoxin responsible for tetanus, which is characterized by painful muscular spasms, leading to respiratory failure and associated with a very high mortality rate. Tetanospasmin is known to act by interfering with the release of inhibitory neurotransmitters (glycine and gamma-aminobutyric acid), thereby increasing resting motor neuron activity, leading to muscular rigidity and spasms (2,3).

C. tetani cannot survive in the presence of oxygen during its growth, which makes necrotic and vascularly compromised tissue a suitable host for proliferation of this bacterium.

Diabetes mellitus (DM) is a chronic systemic disease characterized by impaired wound healing and increased tendency for the patients to develop infections as a result of immune dysfunctions.

Although there has been an overall decrease in the total number of cases of tetanus and its morbidity as a result of effective vaccination programs, it is still a matter of major concern with an annual incidence of 0.1 per one million population overall and 0.23 per million among people aged ≥ 65 years in United States with almost one million mortalities worldwide annually (4,5).

* Correspondence: drfatihTekin@hotmail.com

Tetanus is particularly more common in patients with chronic wounds. There are increasing numbers of studies that show a decrease in tetanus immunoglobulin (TIG) levels with age and with the presence of chronic ulcers (6,7).

Contaminated wounds and chronic leg ulcers are generally the main portals of entry of *C. tetani* spores and therefore regarded as the main etiologic factors in the pathogenesis of tetanus. Diabetic patients are prone to develop chronic leg/foot wounds that can easily be complicated with tetanus. Therefore, vaccination and immunization status are important parameters in assessing the risk of tetanus and preventing tetanus infections. Although there are studies about the tetanus immunization status of patients with DM or chronic leg ulcers of any cause, there is no comparison of TIG levels between diabetic patients with and without leg ulcers. The findings of our study may, in turn, affect the tetanus vaccination protocols for these patients.

In the present study we measured and compared TIG levels among patients with diabetes mellitus type 2 who have leg ulcers and who do not, and thereby to determine the vaccination needs of the two different groups.

2. Materials and methods

The study was approved by Keçiören Training and Research Hospital Regional Ethics Committee (Decision Number: 22.02.2012 /B.10.4.İSM.4.06.68.49/09). A cross-sectional study was designed and total protein, albumin, C-reactive protein (CRP), hemoglobin A1c (HbA1c), total immunoglobulin G (IgG), and TIG levels of 60 diabetic patients were assessed. Only patients without a vaccination history in the previous 5 years were included in this study. Two groups were formed, each of which contained 30 patients. Group 1 was defined as patients with DM but without diabetic foot ulcers. Group 2 was defined as patients with DM accompanied by diabetic foot ulcers with Wagner class 2 and 3. For group 2, patients with a chronic wound history of more than 3 months and more

than 2 cm diameter without acute purulent discharge were selected for the study. The diabetic patients with ischemic or venous lower extremity wounds, smoking history, and systemic complications (chronic renal failure, congestive heart failure, etc.) were excluded from the study. The inclusion criteria for the study are summarized in Table 1.

Serum levels of total protein, albumin, CRP, HbA1c, total IgG, and TIG levels were compared between the two groups. The parametric variables were compared using the independent sample t-test and nonparametric variables were compared using the Mann–Whitney U test. The comparison of frequencies among the two groups was analyzed using the chi-squared test. Statistical significance was determined as $P < 0.05$ with a confidence interval of 95%.

3. Results

In group 1, there were 13 male and 17 female patients and in group 2 there were 24 male and 6 female patients (Table 2). The mean age in group 1 was 60.5 ± 13.9 years and in group 2 it was 56.2 ± 9.5 years. The mean duration of DM was 7.7 ± 4.3 years in group 1 and 8.5 ± 6.1 years in group 2. There was no statistically significant difference in terms of age or duration of diabetes between the groups (Table 2). The mean wound diameter was 3.9 ± 0.9 cm and mean wound duration was 7.3 ± 3.7 (range: 3–15) months.

The normal laboratory values for the parameters used in the study were based on our laboratory's reference values and are listed in Table 3.

Diabetic patients with and without foot ulcers were compared in terms of their serum TIG, total protein, albumin, CRP, total IgG, and HbA1c levels.

Mean serum total protein, albumin, CRP and HbA1c, total IgG, TIG levels and standard deviations are summarized in Table 3 and graphically presented in the Figure.

TIG levels were significantly lower in diabetic patients with foot ulcers (0.055 ± 0.033 IU/mL) when compared to patients without foot ulcers (0.345 ± 0.281 IU/mL) (P

Table 1. Inclusion criteria for the study.

No finding of ischemia or venous insufficiency of the extremity
No finding of acute cellulitis, no acute purulent discharge
At least 3-month duration of lower extremity wound
Wound diameter > 2 cm
No tetanus vaccination history in the previous 5 years
No other systemic disease complicating diabetes (chronic renal failure, congestive heart failure, vasculitis, immune disorder)
No smoking history

Table 2. Age, sex, mean duration of diabetes, and mean wound diameter data collected in the study.

Age (years)		Group 1 (mean ± SD*)	Group 2 (mean ± SD*)
			60.5 ± 13.9
Sex	Male	13	24
	Female	17	6
Mean duration of DM (years)		7.7 ± 4.3	8.5 ± 6.1
Mean duration of wound (months)		No wound	7.3 ± 3.7
Mean wound diameter (centimeters)		No wound	3.9 ± 0.9

*SD: Standard deviation

Group 1 = DM patients without ulcers; Group 2 = DM patients with ulcers

Table 3. Mean and standard deviation values of the laboratory tests in our study.

	Group 1 (mean ± SD)	Group 2 (mean ± SD)	Reference values ^c
Total protein	7.50 ± 0.30 g/dL	6.48 ± 0.41 g/dL	6–8 g/dL
Albumin	4.23 ± 0.18 g/dL	2.82 ± 0.55 g/dL	3.5–5.5 g/dL
CRP	0.37 ± 0.19 mg/dL	9.17 ± 8.1 mg/dL	<0.5 mg/dL
HbA1c	70.5 ± 4.9 mmol/mol IFCC ^a (8.6 ± 2.6% DCCT ^b)	83.6 ± 6.0 mmol/mol IFCC ^a (9.8 ± 2.7% DCCT ^b)	<48 mmol/mol IFCC ^a (<6.5% DCCT ^b)
Total IgG	1330 ± 189 mg/dL	1428 ± 369 mg/dL	640–1430 mg/dL
Tetanus IgG	0.345 ± 0.281 IU/mL	0.055 ± 0.033 IU/mL	>0.15 IU/mL*

^aThe International Federation of Clinical Chemistry and Laboratory Medicine^bThe Diabetes Control and Complications Trial (DCCT)^cNormal laboratory values for the tests used in the study according to our laboratory's reference values

* >0.15 IU/mL is regarded as protective

= 0.008). When the laboratory reference value of TIG is accepted as the cut-off point to classify patients as protected and nonprotected, it is clearly demonstrated that 17 of the 30 patients (56.6%) among the diabetic patients without foot ulcers had nonprotective TIG levels, whereas 28 of the 30 patients (93.3%) among the diabetic patients with ulcers had nonprotective TIG levels (Table 4).

Moreover, 45 of the 60 patients (75%) overall and 13 of the 15 (86.6%) patients over the age of 70 had TIG values below protective levels.

Total protein and albumin were significantly lower and CRP levels were significantly higher in the diabetic patients with foot ulcers ($P < 0.001$).

4. Discussion

The incidence of tetanus is 0.1 per million of the population. Twelve percent of all tetanus patients have diabetes. Diabetes is clearly associated with an increased risk of tetanus, especially in the elderly, with tetanus incidence reaching up to 0.70 cases per million for diabetic patients over the age of 60. According to Centers for Disease Control and Prevention (CDC) data, diabetic foot ulcers and gangrene are a major route for tetanus infections. Chronic wounds, which are more commonly observed in diabetic patients, were shown to be the etiologic factor in about 26% of tetanus cases (4,8,9).

Diabetic patients are more prone to *C. tetani* infections because of several risk factors associated with diabetes. The microvascular angiopathy in diabetic patients together with

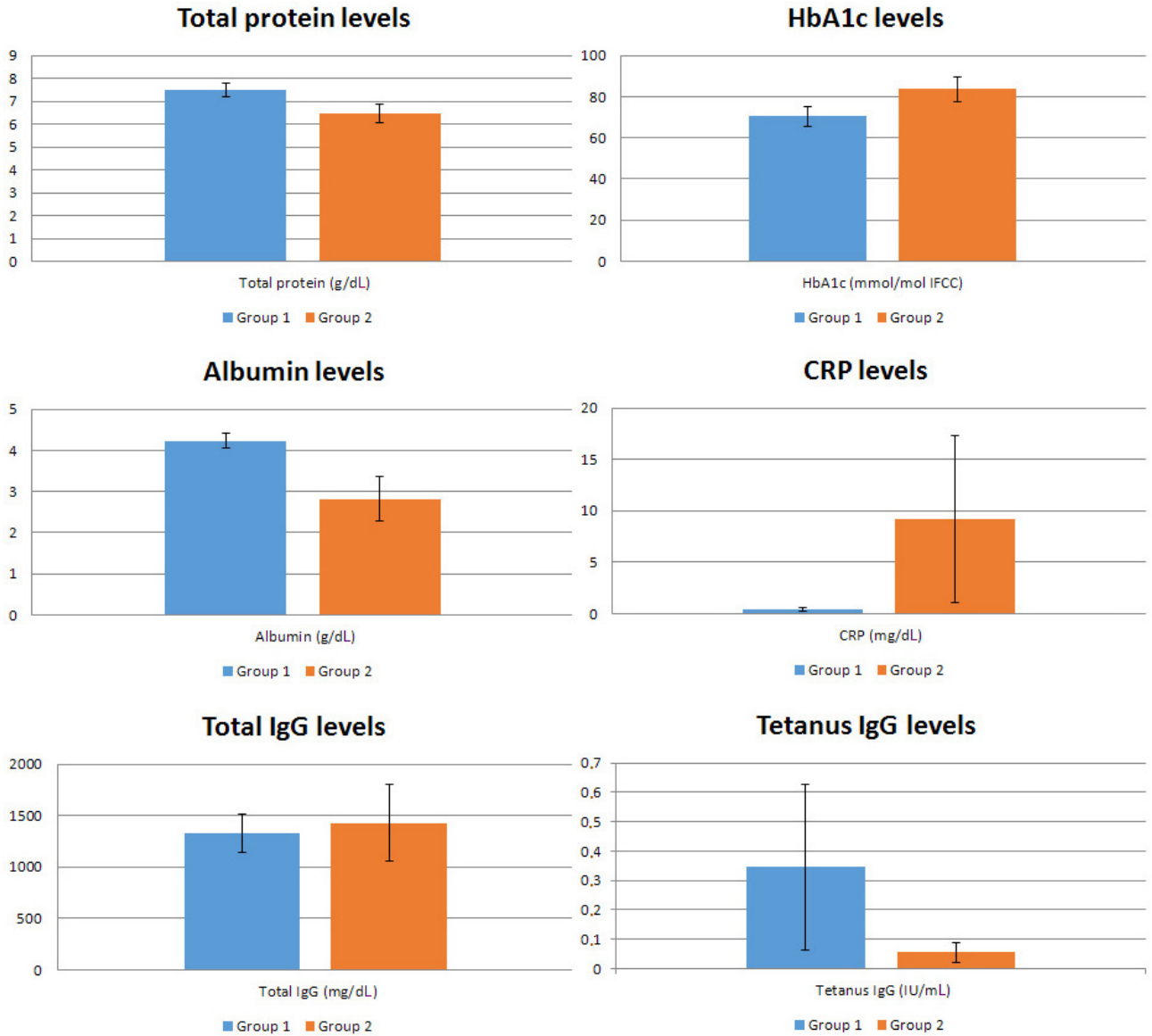


Figure. Comparison of laboratory values between the groups with chart graphs and standard deviation bars.

Table 4. Tetanus IgG levels among the groups.

		Tetanus IgG levels		
		Nonprotective	Protective	Total
Group	Diabetics without ulcers	17	13	30
	Diabetics with ulcers	28	2	30
Total		45	15	60

increased atherosclerosis affecting macrovasculature leads to decreased tissue perfusion and oxygen partial pressure. This vascular insufficiency together with infection causes tissue necrosis, which is called gangrene. This creates a

culture medium for the growth of *C. tetani* (10). Tetanus spores need to penetrate the skin and proliferate only in anaerobic conditions. Almost 15% of diabetic patients have been shown to develop diabetic foot ulcers during

their lifespan (11). The decreased perfusion also limits the total number of immune cells delivered to the infection site (12).

Diabetes has been shown to impair cellular and humoral immunity, which is characterized by abnormalities in antibody titers associated with possible B-cell dysfunction and impairments in margination, chemotaxis, phagocytosis, and bactericidal functions, which are vital in immune defense (13–18).

In various studies about TIG levels comparing diabetic and nondiabetic patients, it has been reported that type 2 DM is associated with decreased TIG levels in patients who have previously been immunized (6,7,19,20).

In our study, when the two groups (DM patients with and without diabetic foot ulcers) were compared in terms of TIG values, diabetic patients with ulcers were found to have significantly lower TIG levels when compared to patients without ulcers. Although the underlying mechanisms have not yet been fully enlightened, diabetic immunopathy, chronic inflammation, continuous exposure to the relevant bacteria, and antibody consumption are the suggested pathophysiological mechanisms that might cause decreased TIG levels.

One of the limitations in our study was the lack of a healthy control group for comparison of TIG levels. The healthy control group was excluded because of the significantly lower mean age of this group, which made the statistical comparison unmeaningful.

Poorly controlled blood glucose levels are strongly associated with diabetic peripheral neuropathy, diabetic immune dysfunction, and poor wound healing, all of which eventually cause foot ulcers in diabetic patients. In our study there was no significant difference in HbA1c levels between the groups and there was no correlation between HbA1c and TIG levels.

In the current literature, increased age seems to correlate with decreased serum IgG levels (21,22). Cook et al. reported that 49%–66% of people over the age of 60 have TIG levels below the protective range (23). Nemati et al. reported that TIG levels were significantly lower in patients over 40 years old in their younger patient series

(7). Tamer et al. reported that TIG levels were decreased in diabetic patients older than 50 years of age, whereas this decrease was seen at the age of 65 in healthy individuals (20).

In our study there was no correlation between increased age and TIG levels, possibly because of the relatively narrow age range of patients, making it difficult to demonstrate a clear correlation between increased age and decreased TIG levels. The patients included in our study were already at older ages and yet 45 of the 60 patients (75%) had TIG levels below the protective range.

Although humoral immunity is known to be affected by uncontrolled DM, there are studies with different findings regarding immunization response to vaccines. Type 2 diabetic patients have been reported to have decreased vaccine responses to hepatitis B virus (24) and influenza A virus (25); however, they have been reported to have almost normal vaccination responses to tetanus toxoid vaccine (26).

Tetanus vaccination with a booster dose is already in use for diabetic patients with acute foot infections with purulent discharge. In our study it is clear that the majority of the diabetic foot patients attending our clinics have not received proper tetanus prophylaxis probably because of the chronic and insidious course of these ulcers. Those patients with no sign of acute infection are often neglected for tetanus prophylaxis. Therefore, a tetanus booster should be administered to all patients with diabetic foot ulcers even though they do not manifest signs of acute infection, osteomyelitis, or partial foot necrosis.

In conclusion, the majority of patients with chronic wounds are known to be vulnerable to tetanus infections. Yet the major protective mechanism against *C. tetani* infections is tetanus IgG. Diabetic patients with foot ulcers have lower TIG values. The majority of diabetic foot patients do not receive proper tetanus prophylaxis since these patients are usually neglected because of the lack of signs of acute infection. Hence, a tetanus booster should be administered to all diabetics regardless of presence or status (acute vs. chronic) of foot ulcers.

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