

Lower Serum Level of Anti-tetanus Toxin Antibodies in Patients with Type 2 Diabetes Mellitus

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ABSTRAK

Tujuan: untuk menilai kadar antibodi toksin anti-tetanus (anti-TTA) pada pasien dengan diabetes mellitus (DM) tipe 2 dan kelompok kontrol. **Metode:** secara keseluruhan, 100 pasien dengan DM tipe 2 dan 100 orang sehat dengan usia dan jenis kelamin yang sesuai diikutsertakan dalam penelitian. Diagnosis DM tipe 2 dipastikan menurut kriteria klinis dan para-klinis, misalnya kadar glukosa darah puasa di atas 126 mg/dl. Sampel darah perifer diambil dari seluruh subjek penelitian. Sampel serum peserta penelitian diperiksa untuk mengukur kadar anti-TTA dengan metode ELISA. Kadar antitoksin di dalam serum $\geq 0,1$ IU/mL ditentukan sebagai kadar antibodi protektif. **Hasil:** tingkat seroprotektif di kelompok sehat lebih tinggi secara bermakna daripada kelompok diabetes (99% vs. 92%; $p < 0,02$). Titer rata-rata anti-TTA pada kelompok orang sehat ($5,32 \pm 0,26$ IU/ml) secara bermakna lebih tinggi daripada pasien diabetes ($3,46 \pm 0,26$ IU/ml; $p < 0,001$). Pada pria penderita diabetes, nilai rata-rata titer anti-TTA ternyata lebih tinggi secara bermakna dibandingkan dengan wanita penderita diabetes ($3,94 \pm 0,34$ IU/ml vs. $2,59 \pm 0,36$ IU/ml; $p < 0,01$). Pada pasien diabetes, tingkat seroprotektif dan nilai rata-rata titer anti-TTA pada subjek penelitian berusia > 40 tahun juga lebih rendah daripada mereka yang berusia < 40 tahun (89,23% vs. 97,14%; $p < 0,15$ dan $4,57 \pm 0,38$ IU/ml vs. $2,86 \pm 0,32$ IU/ml; $p < 0,002$). Nilai rerata titer anti-TTA lebih tinggi secara bermakna pada pasien yang menderita diabetes < 5 tahun dibandingkan pasien yang menderita penyakit tersebut > 5 tahun ($3,91 \pm 0,35$ IU/ml vs. $2,85 \pm 0,38$ IU/ml; $p < 0,04$). **Kesimpulan:** Hasil penelitian menunjukkan bahwa terdapat kadar anti-TTA yang lebih rendah pada pasien dengan DM tipe 2, pada wanita penderita diabetes, pada pasien berusia > 40 tahun, dan pada pasien yang telah menderita diabetes > 5 tahun.

Kata kunci: imunitas, tetanus, antibody, diabetes tipe 2

ABSTRACT

Aim: to evaluate the serum levels of anti-tetanus toxin antibodies (anti-TTA) in patients with type 2 diabetes mellitus (DM) and in a control group. **Methods:** totally, 100 patients with type 2 DM and 100 age- and sex-matched healthy individuals were enrolled to study. The presence of type 2 DM confirmed according to the clinical and para-clinical criteria such as fasting plasma glucose above 126 mg/dl. A peripheral blood sample was collected from all subjects. The serum samples of participants tested for the levels of anti-TTA by

ELISA method. The serum antitoxin concentration ≥ 0.1 IU/mL was considered as a protective level of antibody. Results: the seroprotective rate in healthy group was significantly higher than diabetic group (99% vs. 92%; $p < 0.02$). The mean titer of anti-TTA in healthy group (5.32 ± 0.26 IU/ml) was also significantly higher than diabetic patients (3.46 ± 0.26 IU/ml; $p < 0.001$). In diabetic men the mean titer of anti-TTA was significantly higher in comparison to diabetic women (3.94 ± 0.34 IU/ml vs 2.59 ± 0.36 IU/ml; $p < 0.01$). In diabetic patients the seroprotective rate and the mean titer of anti-TTA in subjects with age > 40 years was also lower in comparison to those with age < 40 years (89.23% vs 97.14%; $p < 0.15$ and 4.57 ± 0.38 IU/ml vs 2.86 ± 0.32 IU/ml; $P < 0.002$, respectively). The mean titer of anti-TTA was significantly higher in patients with diabetes duration < 5 years in comparison to patients with disease duration > 5 years (3.91 ± 0.35 IU/ml vs 2.85 ± 0.38 IU/ml; $p < 0.04$). Conclusion: these results showed lower levels of anti-TTA in patients with type 2 DM, in diabetic women, in patients aged > 40 years and in diabetic patients with disease duration > 5 years .

Key words: immunity, tetanus, antibody, type 2 diabetes.

INTRODUCTION

Tetanus is an infectious disease caused by neurotoxin secreted from the gram-positive anaerobic bacillus *Clostridium (C) tetani*. Despite being preventable with an effective vaccine, tetanus remains a significant cause of morbidity and mortality worldwide.¹

Contamination of wounds with spores of *Clostridium tetani* usually causes tetanus, due to release of neurotoxin. Tetanus is rare in people with history of complete vaccination course. However, inadequate tetanus toxoid vaccination and wound prophylaxis are associated with tetanus. Risk for fatal disease is also reported to be higher in patients with 65 years of age and older.² The current Advisory Committee on Immunization Practices and Centers for Disease Control and Prevention recommendation propose diphtheria, tetanus, and acellular pertussis (DTaP) at ages 2, 4, 6, 15-18 months with a booster at 4-6 years.¹ In Iran, immunization against diphtheria, tetanus and pertussis has been applied since 1950 with using a local vaccine manufactured by Razi Institute (Razi-DTWP).³ In most countries, booster immunization of diphtheria and tetanus is recommended to be performed every 10 years. Booster Td vaccine is recommended to start at the age of 11-12 years old.⁴

The prevalence of type 2 DM in Iran has been reported to be 24% in individuals with age > 40 years and 5.5% in those aged 15-65 years.^{5,6} Similar results have been reported in other studies.⁷ It has been reported that the ulceration and some infectious diseases are common in

diabetic patients.⁸ This susceptibility to infections has been associated to impaired immune system in diabetic patients.⁹ Foot ulcers are also sizable in diabetic patients.⁸ The vulnerability of diabetic patients to slow-healing foot ulcers may provide a route for *C. tetani* spores and subsequent tetanus infection.^{8,10} Contamination of a these ulcers with spores of *C. tetani* may result to tetanus. Accordingly, it is expectable that the incidence of tetanus among diabetics patients is greater than non-diabetics subjects. The results of the some studies have demonstrated the diabetic patients were more susceptible to tetanus.^{11,12} Moreover, it has been reported that the incidence of tetanus was higher in diabetic patients as compared to non-diabetics.¹³ The results of a study from United States have demonstrated the highest average annual incidence rate of tetanus among persons aged ≥ 60 years (0.35 cases/million population) and also among old adults (aged ≥ 60 years) with diabetes (0.70 cases/million population).¹³ These studies indicate a higher risk of tetanus in diabetics and therefore the implication of the vaccination against tetanus has been recommended for diabetic patients, especially in those over 50 years of age.^{11,13,14} This study is conducted to evaluate the serum levels of anti-TTA in patients with type 2 DM and in a healthy control group.

METHODS

From August 2012 to December 2012, a cross-sectional seroprevalence study was carried out in Rafsanjan (a city that located in South-East

of Iran in Kerman province). 100 type 2 DM patients (mean age: 42.09 ± 6.51 years; range: 24 to 54 years; 64 men and 36 women) and 100 healthy subjects (mean age: 41.12 ± 7.65 years; range: 26 to 57 years; 61 men and 39 women) were included in the study.

As mentioned, the universal vaccination of infants and children against diphtheria, tetanus and pertussis has been incorporated in the national immunization scheme in Iran since 1950.¹⁵ Accordingly all subjects born after this timepoint (including subjects enrolled to this study) received DTwP vaccine. Primary vaccination has been done by using 3 doses of vaccine at 2, 4, and 6 months of age with 3 booster doses at age 18–24 months, 5–6 years and 18–24 years. There was no any other vaccination records regarding tetanus for participants.

The type 2 DM patients selected from subjects referring to the Pathobiological Laboratory of Rafsanjan University of Medical Sciences. The type 2 DM patients had disease, according to the fasting plasma glucose above 126 mg/dl (as a major criteria defined by the American Diabetes Association) and physician's examination. The healthy control group was recruited among blood donors of Rafsanjan Blood Transfusion Center. The healthy controls were basically health, with normal fasting plasma glucose and no acute or chronic illnesses. Subjects with disease such as history of recurrent infections, asthma, allergy and atopic diseases, any suspected immunological disorders, cigarette smoking and use of any drugs were all excluded from the study. Other exclusion criteria were malignancy, surgery and major trauma within 6 months prior to blood collection. The participants did not even received any immunomodulating treatment during 6 month prior to sampling. All participants had normal CBC, normal liver and normal renal function tests. Moreover, the blood lipids profile (including cholesterol and triglyceride) of all subjects were within normal range.

This study was evaluated and approved by the Ethical Committee of Rafsanjan University of Medical Sciences. All of participants gave written informed consent to take part in the study. Peripheral blood (2–4 milliliter) were collected from the subjects of 2 groups and the serum

separated and stored at -20°C .

Determination of Anti-tetanus Toxin Specific Antibodies in Serum

Serum levels of anti-TTA was measured by an enzyme-linked immunosorbent assay (ELISA) method by using commercial kits (IBL-Hamburg GmbH, Hamburg, Germany). Principally, the wells of ELISA plate are coated with tetanus toxin as antigen. Specific antibodies of the sample binding to the antigen coated wells are detected by a secondary enzyme conjugated antibody specific for human IgG. After the addition of substrate the intensity of the color is proportional to the amount of IgG-specific antibodies. Results of samples can be determined directly using the standard curve.

In the test procedure, after pipetting 100 μL of each standard and diluted serum (1/100) into the respective wells, plates were incubated for 60 min at 25°C . At the end of the incubation period, plate strips were washed four times with 300 μL washing buffer per well. Then, 100 μL conjugate was added into each well. After the incubation period (for 30 min at 25°C), plate strips were washed as previously described. Thereafter, 100 μL of Tetramethylbenzidine (TMB) substrate solution was added into each well and incubated for 20 min at 25°C in dark. The substrate reaction was stopped by addition 50 μL of sulfuric acid into each well. Optical density of each well measured with a photometer at 450 nm within 60 min after pipetting of the stop solution.

Anti-TTA was measured by using standard samples with known concentrations of anti-TTA expressed as IU/mL, provided by the manufacturer. According to manufacturer guideline, an antitoxin concentration ≥ 0.1 IU/mL was considered as protective level of antibody. Tetanus antitoxin levels less than 0.1 IU/ml were considered to denote susceptibility. The sensitivity of ELISA kit was 0.004 IU/ml.

Statistical Analysis

Differences in variables were analyzed using t-test and chi-square test as appropriate and P-values of less than 0.05 were considered significant. All data were analyzed by SPSS software (version 15, Chicago, IL).

Table 1. Serum concentrations of anti-tetanus toxin antibody in healthy and diabetic groups according to gender

Group	Gender	Number	Protection rate N (%)	anti-tetanus toxin antibody (IU/mL)
Healthy group	Men	61	60 (98.36%)	5.51±0.33†
	Women	39	39 (100%)	5.02±0.45
	Total	100	99 (99%)	5.32±0.26
Diabetic patients	Men	64	59 (92.18)	3.94±0.34
	Women	36	33 (91.66)	2.59±0.36
	Total	100	92 (92%)	3.46±0.26

† The serum levels of anti-tetanus toxin antibody expressed as mean±SEM

Table 2. Serum concentrations of anti-tetanus toxin antibody in healthy and diabetic groups according to age group

Group	Age (year)	Number	Protection rate N (%)	anti-tetanus toxin antibody (IU/mL)
Healthy group	<40	38	37 (97.36%)	5.85±0.40†
	>40	62	62 (100%)	4.99±0.35
	Total	100	99 (99%)	5.32±0.26
Diabetic patients	<40	35	34 (97.14%)	4.57±0.38
	>40	65	58 (89.23%)	2.86±0.32
	Total	100	92 (92%)	3.46±0.26

† The serum levels of anti-tetanus toxin antibody expressed as mean±SEM

RESULTS

The seroprotective rate of anti-TTA in healthy control group (99%) was significantly higher than that observed in diabetic group (92%, $P<0.02$). The mean titer of anti-TTA in healthy control group (5.32 ± 0.26 IU/ml) was also significantly higher than that in diabetic patients (3.46 ± 0.26 IU/ml; $P<0.001$).

In diabetic men the mean titer of anti-TTA was significantly higher in comparison to diabetic women (3.94 ± 0.34 IU/ml vs 2.59 ± 0.36 IU/ml; $P<0.01$). In healthy men the mean titer of anti-TTA was higher than that observed in healthy women but the difference was not statistically significant (**Table 1**). In both diabetes and control groups no significant difference was observed between men and women with respect to the seroprotection rate (**Table 1**).

In diabetic patients the mean titer of anti-TTA in subjects with age >40 years was significantly lower in comparison to those with age <40 years (4.57 ± 0.38 IU/ml vs 2.86 ± 0.32 IU/ml; $p<0.002$). In healthy control group the mean titer of anti-TAA in subjects with age >40 years was also

lower than that observed in those aged <40 years but the difference was not statistically significant (5.85 ± 0.40 IU/ml vs 4.99 ± 0.35 IU/ml; $p=0.12$). (**Table 2**). In diabetes group the seroprotection rate was lower in patients with age >40 years as compared with patients with age <40 years but the difference was not statistically significant (89.23% vs 97.14%; $p=0.15$). Moreover, in healthy control groups no significant difference was observed between subjects with age >40 years and subjects with age <40 years with respect to seroprotection rate (**Table 2**).

According to the duration of the diabetes the patients divided into two subgroups, 57 patients were diabetic for <5 years and 43 patients were diabetic for >5 years. The mean titer of anti-TTA was significantly higher in patients with diabetes duration <5 years in comparison to patients with disease duration >5 years (3.91 ± 0.35 IU/ml vs 2.85 ± 0.38 IU/ml; $p<0.04$). No significant difference was observed between patients with disease duration <5 years and patients with diabetes duration <5 years with respect to seroprotection rate, although this parameter

Table 3. Distribution of subjects according to anti-tetanus toxin antibody in healthy and diabetic group

Groups	<0.1 IU/mL	0.1-1 IU/mL	1-2 IU/mL	2-3 IU/mL	3-4 IU/mL	4-5 IU/mL	>5 IU/mL
Healthy group	1%	8%	7%	8%	6%	11%	59%
Diabetic Patients	8%	11%	20%	19%	7%	5%	30%

was found to be higher in patients with disease duration <5 years (94.7% vs 88.4).

The distribution of subjects according to their serum levels of anti-TTA has been demonstrated in **Table 3**. The rate of subjects with high serum levels of anti-TTA (>4 IU/mL) in healthy control group was significantly higher in comparison to diabetic group (70% vs 35%; $p < 0.001$). On the other hand the rate of subjects with low serum levels of anti-TTA (<1 IU/mL) in diabetic group was significantly higher in comparison to healthy control group (19% vs 9%; $p < 0.05$).

DISCUSSION

The results of the present study showed that both seroprotective rate and the mean titer of anti-TTA were lower in diabetic patients as compared with healthy control subjects. These findings represent that diabetic patients have a greater susceptibility to tetanus infection. There is a few studies regarding the tetanus immunity among type 2 DM patients. Two studies from Turkey have observed that the mean titer of anti-TTA was significantly lower in patients with DM as compared with healthy subjects.^{14,16}

The lower levels of anti-TTA in diabetic subjects can be attributed to some immune dysfunction in these patients. The lower antibody responses to other vaccines such as hepatitis B vaccine and influenza A vaccine have been also reported in patients with type 2 DM.^{17,18} Moreover, it has been reported that diabetic patients are susceptible to infection and sepsis.¹⁹ Furthermore, some immunologic disorders have been shown in patients with type 2 DM. Dendritic cells (DC) play a key role in initiating innate and adapted immune responses. It has been demonstrated that absolute frequency of DC significantly were diminished in patients with type 2 DM.²⁰ Some defects in humoral immunity and complement system also demonstrated in diabetic patients.¹⁹ Moreover, it has been demonstrated that the

phagocytic activity of monocytes is attenuated in experimentally-induced type 2 diabetes in rat.²¹ Furthermore, enhanced functions of regulatory T-cells demonstrated in experimentally-induced diabetes in mice.²² Impaired functions of polymorphonuclear leukocyte have been also reported in patients with type-2 DM.²³

It should be noted that the vaccination is one of the most significant tools for induction the prophylaxis against infection diseases. This prophylaxis mediated via inducing of immunologic memory.²⁴ In addition to several immune dysfunctions in diabetic patients that mentioned above, the low levels of anti-TTA in diabetic patients may be also attributed in part to impairment of immunological memory in this patients.

The results of the present study also showed that mean titer of anti-TTA was higher in men as compared to women. Similar findings have been reported in other studies.^{25,26} This may be due to higher accident rates among men that increase the higher exposure to *Clostridium tetani* which results in higher protective immunity. The results of a study from United State have demonstrated the differences in the tetanus incidence rates between men and women that varied by age group. For subjects aged <20 years, the incidence of tetanus among men reported as 2.7 times more than the incidence among women and for persons aged 20–59 years, the incidence of tetanus among men reported as 2.9 times more than the incidence among women.¹³ The results of a ten-year retrospective study from Nigeria showed that the male: female ratio in tetanus patients was 3:1.²⁷ It has been also reported that the men who had a history of dirty wounds or frequent soil exposure have more levels of anti-TAA.²⁶ Moreover, a proportion of men may served in the army as young adults where they vaccinated against tetanus.^{25,28} Further investigation is needed to clarify the reasons for greater tetanus immunity among men than woman. However,

there was no significant difference between men and women of healthy control group with respect to the seroprotection rate, probably due to the tetanus booster vaccination in women performed during pregnancy.

The results of the present study also showed that the mean titer of anti-TTA in subjects with age >40 years was lower in comparison to those with age <40 years. These finding represent that the anti-TAA levels declined with age so that there is the greater the necessity for the older persons to receive a tetanus booster because of waning immunity in the older age group.

In diabetes group, the seroprotection rate was observed in 89.23% of patients with age >40 years and 97.14% of patients with age <40 years. Moreover, in healthy control groups the seroprotection rate was observed in 100% of subjects with age >40 years and 97.36% of subjects with age <40 years. It has been reported that the inadequate tetanus toxoid vaccination and wound prophylaxis are associated with tetanus. Risk for fatal disease is also reported to be higher in patients 60 years of age and older.^{2,29} It should be noted that seroprotective rate against tetanus varies between countries. For example, seroprotective rate was noted in 33.9% of people aged >50 years from Turkey³⁰, in 53% of people aged 20-50 years from India³¹, in 84.7% of people aged 20-64 years from Brazil³², in 70% of people aged >80 years from Germany³³, in 53% of people aged >60 years from England and Wales³⁴, and in <75% of people aged >50 years from Australia.³⁵ This discrepancy may be attributed largely to differences in the age, job and race of participants, the primary vaccination schedule, and time intervals between vaccine administration and collection of blood samples.

The results of the present study also showed that the mean titer of anti-TTA were significantly higher in patients with diabetic duration <5 years as compared to patients with disease duration >5 years. These results represent a negative association between the duration of diabetes and the mean titer of anti-TTA. The results of a investigation from Korea demonstrated that that the type 2 DM patients with longer duration of diabetes had lower seroconversion rate after vaccination with a type of influenza A vaccine.¹⁷

According to serum levels of anti-TTA we have arbitrary classified the subjects to several subgroups. In healthy control group 59% of subjects have the anti-TTA >5 IU/mL whereas 30% of diabetic subjects have the anti-TTA >5 IU/mL. On other hand the proportion of subjects with low anti-TTA was higher in diabetes group in comparison to healthy control group. This classification can be use for designing of the re-vaccination program. According to manufacturer guideline, for individuals with antitoxin level <0.1 IU/ml (no protection), vaccine should be administered. Individuals with antitoxin level 0.1-1.0 IU/ml should be control after 1-2 years. Individuals with antitoxin level 1.1-5.0 IU/ml should be control after 2-4 years. Individuals with antitoxin level >5.0 IU/ml should be control after 8 years. Similar guideline has been presented for revaccination by Centers for Disease Control and Prevention.³⁶

CONCLUSION

The results of the present study showed lower levels of anti-TTA in patients with type 2 DM, in diabetic women and in patients aged >40 years. Moreover, a reverse association was also observed between the duration of diabetes and the mean titer of anti-TTA.

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