

Full Length Research paper

Response to tetanus vaccination in patients with type 2 diabetes mellitus

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Diabetes mellitus is an endocrine disease that profoundly affects the immune system. Inadequate regulation of blood sugar leads to immune defects and results in more frequent infections. Tetanus is an infectious disease that occurs more frequently in developing countries. Antitoxin levels against tetanus are reduced to inadequate levels 10 years after the last vaccination. The aim of the present study was to investigate the response to tetanus vaccination at 0, 1 and 12 months in 45 patients with type 2 diabetes mellitus and in 37 healthy volunteers who were not immune against tetanus. Immune response to tetanus vaccination was compared between the two groups at the fourth week after each vaccination. The p values were 0.207, 0.036 and 0.437 when antibody levels between groups were compared after the first, second and third vaccinations, respectively. A statistically significant difference was found between patients with disease duration of ≤ 5 years versus ≥ 11 years only after the second vaccination ($p=0.02$). The response of diabetic patients was similar to that of the healthy population probably because of the strong antigenic structure of the tetanus toxoid vaccine.

Key words: Tetanus, immunity, diabetes mellitus, immune response, vaccination.

INTRODUCTION

Although tetanus is an infectious disease that is preventable by vaccination, deaths due to tetanus are still reported throughout the world, notably in developing countries. The high fatality rate and unfavorable course of the disease clearly reveal the importance of vaccination against tetanus (McQuillan et al., 2002, Cook et al., 2001, Ergönül et al., 2001).

Diabetes mellitus is an endocrine disease that profoundly influences the immune system. Hyperglycemia largely influences neutrophil and macrophage functions. Impairment in functions such as chemotaxis, phagocytosis, and adherence and killing of microorganisms within the cell occurs during this endocrine disease. In diabetic patients in whom their blood sugar level is well

controlled, continuous disturbance is not generally observed in host resistance against infections; however, in those in whom blood sugar levels are not controlled, impairment in chemotaxis, phagocytosis and microbicidal functions has been demonstrated (Molenaar et al., 1976). Excessively high blood sugar levels, acidosis, ketosis and hyperlipidemia are presumed to be the responsible factors for disturbances in lymphocyte functions, which are suppressed in patients with an irregular course of illness (Jirkovska et al., 2002, Eibl et al., 2002). In type 1 diabetes mellitus, alterations have been found in some lymphocyte subgroups: decrease in the total number of T lymphocytes and specific CD4 phenotype that results in a fall in the ratio of CD4/CD8.

Specific antibody response after immunization was found within normal values in patients with diabetes mellitus (Beam et al., 1980). However, patients with long-duration type 1 diabetes mellitus were reported to respond poorly to hepatitis B vaccination. This deficient res-

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Table 1. Clinical characteristics of diabetic and control groups

Diabetic Group	Number
Mean age	57
Sex (Female/Male)	29 / 16
Total	45
Treatment of diabetes mellitus	
Oral antidiabetics	32
Insulin	13
Mean duration of disease (year)	10.4 ± 4.7
Control Group	
Mean age	54
Sex (Female/Male)	21 / 16
Total	37

Table 2. Age distribution of the groups

Age groups	Diabetics (%)	Controls (%)
40-49 years	7 (15.6)	8 (21.6)
50-59 years	22 (48.9)	22 (59.5)
60-69 years	14 (31.1)	6 (16.2)
70-79 years	2 (4.4)	1 (2.7)
Total	45 (100)	37 (100)

ponse was thought to be related to inadequate recognition of the antigens by the macrophages (Eibl et al., 2002). Functional failure in other cells that serve in specific immune response, like B lymphocytes, has also been reported to occur. Decrease in blood IgM levels was reported in type 1 and type 2 diabetes mellitus patients (Alexiewicz et al., 1997). All of the above factors, acting separately or together, can result in a more severe course of infections and poorer response to vaccination in patients with diabetes mellitus than in healthy individuals. The aim of this study was to investigate the response of diabetic patients to tetanus vaccination in comparison with that observed in healthy people non-immune against the disease.

MATERIALS AND METHODS

Thirty-seven healthy adults and 45 patients with type 2 diabetes mellitus referring to the Infectious Diseases and Clinical Microbiology and Internal Medicine Departments of Kirikkale University were included in the study. The tetanus antitoxin levels of all participants were below 0.1 IU/ml.

Type 2 diabetes mellitus (formerly called NIDDM, type 2 or adult-onset) has feature of insulin resistance in peripheral tissue and an insulin secretory defect of the beta cell. The diagnosis of diabetes mellitus in this study are described following criteria;

1. plasma glucose concentration that above 200 mg/d (11.1 mmol/l) at any time of day without regard to time since last meal. The classic symptoms of diabetes include polyuria, polydipsia, and unexplained weight loss.

2. or fasting plasma glucose (no caloric intake or at least 8h) that above 126 mg/dl (7.0 mmol/l).

3. 2-h post load blood glucose 200 mg/dl (11.1 mmol/l) during an Oral Glucose Tolerance Test (OGTT) is another analysing method for diagnosis. The proprieties is by using a glucose load containing the equivalent of 75g anhydrous glucose dissolved in water (American Diabetes Association, 2005).

Patients with chronic or autoimmune diseases were excluded from the study. Each patient completed a standard questionnaire including information about age, sex, telephone contact information, duration of disease, and treatment modality. Participants were vaccinated at 0, 1, and 12 months and venous blood samples were taken at the fourth week after each vaccination. Serums were kept at -70 °C until analysis. Tetanus antitoxin levels were detected using a commercial ELISA kit (Genzyme Virotech GmbH, Germany). Interpretation of results: Antitoxin level <0.1 IU/ml: no protection, primary vaccine should be administered. Antitoxin level 0.1-0.5 IU/ml: partial immunity is present; re-vaccination should be administered. Antitoxin level 0.6-1.0 IU/ml: adequate immunity is present; re-vaccination should be administered within 2 years. Antitoxin level 1.1-5.0 IU/ml: long-term immunity is present; re-vaccination should be administered within 5-10 years. Antitoxin level >5.0 IU/ml: re-vaccination should be administered after 10 years (CDC, 1998). Patients were stratified into three groups according to the duration of diabetes mellitus as ≤ 5 years, 6-10 years and ≥11 years. The vaccine material used in study was Sii Tetanus Toxoid (Adsorbed) as (Serum Institute Of India Ltd). This vaccine fulfils the I.P. requirements for Tetanus Toxoid (Adsorbed).

Statistical analysis was carried out using SPSS 10.0 program. Mann-Whitney U, X², Pearson correlation analysis, paired sample t test and Wilcoxon signed rank tests were used in the analysis of the data. A p value of <0.05 was considered to indicate statistical significance.

RESULTS

In total, 82 patients were included in the study (50 female, 32 male). The 45 patients diagnosed with type 2 diabetes mellitus (29 females, 16 males) ranged in age between 40-70 years (median 57 years). The duration of disease varied between 5-24 years (mean 10.4 years). Oral anti-diabetics were used in 32 patients (71.1%) and insulin was applied in 13 patients (28.9%) (Table 1). The control group (21 female, 16 male) ranged in age between 40-76 years (median 54 years) (Table 2). No statistically significant difference was found between the two groups in terms of age distribution (p=0.101).

Vaccination against tetanus was performed with three doses at 0, 1 and 12 months in both groups. Tetanus antitoxin levels were investigated in serum samples drawn at the fourth week after each vaccination. No statistically significant difference was found between antitoxin levels of the two groups after the first and third vaccinations (p values 0.218 and 0.542, respectively). However, there was a statistically significant difference between the groups after the second vaccination (p=0.006). The difference in antitoxin levels between sexes was investigated in control and diabetic groups and a significant difference was found in titrations after the second vaccination only in the control group (Table 3). After the third vaccination, no statistically significant difference was found between genders.

In the patient group, serum antitoxin levels were comp-

Table 3. Antitoxin levels (IU/ml) after vaccination between groups after each vaccination

Control group	Median	Min-max	p
First dose	0,31	0,06-5,50	NS*
Second dose	1,23	0,12-6,12	0,006 [§]
Last dose	2,36	0,38-6,95	NS [#]
Diabetic group			
First dose	0,17	0,06-4,84	
Second dose	0,42	0,07-5,46	
Last dose	2,28	0,15-7,36	

Mann Whitney- U test was used for pair wise comparisons.

Comparisons: * First dose of control group vs first dose of diabetic group.[§] Second dose of control group vs second dose of diabetic group.

[#]Last dose of control group vs last dose of diabetic group

ared between individuals according to disease duration of ≤ 5 years or ≥ 11 years, and a statistically significant difference was found only after the second dose; no correlation between disease duration and titration values was determined after the first and third doses.

DISCUSSION

Although tetanus is an infectious disease that is preventable with immunization, approximately one million deaths from the disease are reported throughout the world annually. In elderly individuals with inadequate motor functions, adults with disease influencing humoral immunity and people who work with soil or in non-sanitary environments, heightened susceptibility to tetanus was demonstrated (Hsu and Groleau, 2001, Omoigberale and Abiodun, 2005, Bethell et al., 2004, Kanchanapongkul, 2001, Coplu et al., 2004).

Diabetic patients are at higher risk for infections, which can be attributed to functional impairment in B lymphocytes and remaining leukocytes and other disturbances in the immune system. In addition, soft tissue complications that predispose to infections, like neuropathy, vascular complications, trauma and injection, are reported frequently during the disease course. Tetanus secondary to diabetic foot infection was described in a patient without tetanus prophylaxis (Geerlings and Hoepelman, 1999, Panning and Bayat, 1999). Clostridium tetani has also been reported as the isolated organism from diabetic wounds (Hsu and Groleau, 2001). Therefore, immunization against tetanus is of particular importance in diabetic patients. Furthermore, type 2 diabetes mellitus appears after middle age and adult vaccination programs in advanced ages are neglected in developing countries (Ozturk et al., 2003, Stark et al., 1999). Disease prognosis is also worse in cases developing after the age of 40 years (Geerlings and Hoepelman, 1999, Greco et al., 2001). In patients with chronic, long-duration skin ulcers, the frequency of progression

to tetanus increases, again highlighting the increased importance of immunization against tetanus in this patient group (Greco et al., 2001). It has also been established that advanced age leads to weakness in T cell activity and decrease in interleukin 1 and 2 levels. These disturbances affect response to antigens, result in impairment in the ability to generate antibody response and decrease antibody concentration after antigenic stimulation (Alagappan et al., 1997).

In the present study, no significant difference was found between the healthy control and patient groups with respect to the immune response against tetanus vaccination. Brydak and others reported good response against influenza vaccine in 70% of patients with type 1 diabetes mellitus. Investigators determined a statistically insignificant difference in response between the healthy group and type 2 diabetes mellitus patients in the research (Brydak and Machala, 2000). For benefit provings about human, vaccination has positive effect on health. This process is especially fundamental praxis in certain patients grups like diabetes mellitus (Spier, 2004).

On the other hand, inflammatory responses to microbial invasion may be diminished in these persons. Specific defects in innate and adaptive immune function have been identified in diabetic patients (Peleg et al., 2007). Chemokines, inflammatory system and immune cells diabetic patients are examined at different times, especially in diabetic foot infections (Schiel et al., 2005). Hyperglycemia induced oxidative stress and negatory impact at cellular functions like bacterial balance, sustained proteases, cytokine release, leukocyte function are also explained (Gary and Woo, 2008). Infection-related complications are affected by the age, peripheral vascular disease, low transcutaneous oxygen, smoking, and poor glycemic control. Vaccine efficacy seems adequate in most diabetic patients, but those with type 1 diabetes and high glycosylated haemoglobin levels are most likely to exhibit hypo-responsiveness to vaccines and infections (Peleg et al., 2007).

In our previous study and other research performed by Tamer and co-workers, it was found that tetanus antibody titers were lower in diabetic patients than in age-matched healthy controls. Tamer and co-workers demonstrated that tetanus antibody levels started to decrease at earlier ages in diabetic patients (Tamer et al., 2005). It is thought that this difference may be a result of the impairment of B lymphocytes or of another component of the immune system, separately or acting together. It has also been emphasized that this detriment is more pronounced in advanced ages (Dodet, 2000, Murr et al., 2003). In literature, it enunciated that duration of disease probably culminate regression of the immunological system (Gardner and Frantz, 2008, Alba-Loureiro et al., 2007). Nevertheless, further inquiries is required for determining of duration the diabetes at antibody and other immunological response.

In the study of Eibl and co-workers, diabetic patients

(20 type 1 and 20 type 2 diabetes mellitus) were vaccinated with hepatitis A, diphtheria and pneumococcus vaccines. In the type 1 diabetic group, response to hepatitis A and diphtheria was found to be decreased, whereas in the type 2 diabetic group, responses were normal after all vaccines. The response of both groups to pneumococcus vaccination was reported to be within normal ranges as in healthy individuals (Eibl et al., 2002).

El-Madhun and co-workers investigated the response of type 1 diabetic patients to influenza vaccine and compared the antibody level with that in normal individuals. They found no difference between healthy and diabetic groups in terms of humoral immune response (El-Madhun et al., 1998). Arslanoğlu and co-workers established that the anti-HBs titer of children with type 1 diabetes mellitus was the same as the response of healthy children after vaccination with hepatitis B vaccine (Arslanoğlu et al., 2002). Nevertheless, there are also publications reporting that the response to vaccine is weaker in diabetic patients. In the study of Muszkat others, the response of elderly individuals, resident in the community and in nursing homes, was investigated and a weaker response was determined in persons with type 2 diabetes mellitus (Muszkat et al., 2003).

While in one study no difference was found in the response to phytohemagglutinin (PHA) and *Candida* antigens by peripheral blood lymphocytes between diabetic patients whose blood sugar level was well regulated and healthy people (Ragab et al., 1972), in another study, impaired response to PHA in patients in whom blood sugar level was not under control was demonstrated (Plouffe et al., 1978). Casey and co-workers reported that in non-insulin dependent diabetic patients, peripheral blood lymphocytes responded inadequately to tetanus toxoid. They suggested the impairment may be caused by failure of the antigen preparation through macrophages in addition to the defect in cell-mediated immunity (39).

Tetanus toxoid is a vigorous and effective antigen and high level response by the mediation of antitoxin titer can be obtained after two doses of vaccination (Casey and Sturm, 1982). The weakening in immunity is related to the antibody titer and the number of doses of the vaccination. For prolonged and adequate immunity, three vaccination doses should be applied. In our study, investigation of groups revealed satisfactory response to the vaccination, and protective serum antibody levels were achieved in both groups.

Conclusion

In conclusion, although there are equivocal reports in the literature regarding the response to vaccine in diabetic patients, especially in patients with type 2 diabetes mellitus, in this study, a comparable response to the tetanus vaccine was observed between the diabetics and the

healthy population. This response may be due to the strong antigenic structure of the tetanus toxoid vaccine. However, further studies are required in order to evaluate the immunologic alterations and immune response to various antigens in type 2 diabetic patients.

REFERENCES

- Alagappan K, Rennie W, Narang V, Auerbach C (1997). Immunologic response to tetanus toxoid in geriatric patients. *Ann. Emerg. Med.* 30:459-462.
- Alba-Loureiro TC, Munhoz CD, Martins JO, Cerchiaro GA, Scavone C, Curi R, Sannomiya P (2007). Neutrophil function and metabolism in individuals with diabetes mellitus. *Braz. J. Med. Biol. Res.* 40: 1037-1044.
- Alexiewicz JM, Kumar D, Smorgorzewski M, Massry SG (1997). Elevated cytosolic calcium and impaired proliferation of B lymphocytes in type II diabetes mellitus. *Am. J. Kid. Dis.* 30:98-104.
- American Diabetes Association (2005). Diagnosis and classification of diabetes mellitus. *Diabetes Care.* 28:S37-42.
- Arslanoğlu I, Çetin B, İggüven P, Karavus M (2002). Anti-HBs response to standard hepatitis B vaccination in children and adolescents with diabetes mellitus. *J. Pediatr. Endocrinol. Metab.* 15:389-395.
- Beam TR, Crigler ED, Goldman JK, Schiffman G (1980). Antibody response to polyvalent pneumococcal polysaccharide vaccine in diabetics. *JAMA* 244: 2621-2624.
- Bethell D, Parry CM, White NJ, Day NP, Farrar JJ (2004). Impact of improved vaccination programme and intensive care facilities on incidence and outcome of tetanus in southern Vietnam, 1993-2002. *Trans. R. Soc. Trop. Med. Hyg.* 98: 671-677.
- Brydak LB, Machala M (2000). Humoral immune response to influenza vaccination in patients from high risk groups. *Drugs* 60:35-53.
- Casey J, Sturm C (1982). Impaired response of lymphocytes from non-insulindependent diabetics to staphage lysate and tetanus antigen. *J. Clin. Microbiol.* 15:109-114.
- Centers for Disease Control and Prevention (1998). Neonatal testing-Montana, 1998. *MMWR* 47: 928-930.
- Cook TM, Protheroe RT, Handel JM (2001). Tetanus: a review of the literature. *Br. J. Anaesth.* 87: 477-487.
- Coplu N, Esen B, Gozalan A, Miyamura K, Yoshida I, Kurtoglu D, Dogan NO, Afacan G, Unal A, Ishida S, Takahashi M (2004). Tetanus antibody assay combining in-house ELISA and particle agglutination test and its serosurvey application in a province in Turkey. *Jpn. J. Infect. Dis.* 57: 97-102.
- Dodet B (2000). Immunity in the elderly. *Vaccine* 18: 1565.
- Eibl N, Spatz M, Fischer GF, Mayr WR, Samstag A, Wolf HM, Scherthaner G, Eibl MM (2002). Impaired primary immune response in type 1 diabetes: results from a controlled vaccination study. *Clin. Immunol.* 103: 249-259.
- El-Madhun AS, Cox RJ, Seime A, Sovik O, Haaheim LR (1998). Systemic and local immune responses after parenteral influenza vaccination in juvenile diabetic patients and healthy controls: results from a pilot study. *Vaccine* 16:156-160.
- Ergönül Ö, Sözen T, Tekeli E (2001). Immunity to tetanus among adults in Turkey. *Scand. J. Infect. Dis.* 33:728-730.
- Gardner SE, Frantz RA (2008). Wound bioburden and infection-related complications in diabetic foot ulcers. *Biol. Res. Nurs.* 10: 44-53.
- Gary Sibbald R, Woo KY (2008). The biology of chronic foot ulcers in persons with diabetes. *Diabetes. Metab. Res. Rev.* 24: S25-30.
- Geerlings SE, Hoepelman AI (1999). Immune dysfunction in patients with diabetes mellitus (DM). *FEMS Immunol. Med. Microbiol.* 26: 259-265.
- Greco JB, Sacramento E, Tavares-Neto J (2001). Chronic ulcers and myiasis as ports of entry for *Clostridium tetani*. *Braz. J. Infect. Dis.* 5: 319-323.
- Hsu SS, Groleau G (2001). Tetanus in the emergency department: a current review. *J. Emerg. Med.* 20: 357-365.
- Jirkovska A, Fejfarova V, Hosova J, Striz I, Kalanin J, Skibova J (2002).

- Analysis of the inflammation reaction and selected indicators of immunity in patients with an infected diabetic ulcer. *Cas. Lek. Cesk.* 141: 483-486.
- Kanchanapongkul J (2001). Tetanus in adults: a review of 85 cases at Chon Buri Hospital. *J. Med. Assoc. Thai.* 84: 494-499.
- McQuillan GM, Kruszon-Moran D, Deforest A, Chu SY, Wharton M (2002). Serologic immunity to diphtheria and tetanus in the United States. *Ann. Intern. Med.* 136: 660-666.
- Molenaar DM, Palumbo PJ, Wilson WR, Ritts RE (1976). Leukocyte chemotaxis in diabetic patients and their nondiabetic first-degree relatives. *Diabetes* 25: 880-883.
- Murr C, Hainz U, Asch E, Berger P, Jenewein B, Saurwein-Teissl M, Grubeck-Loebenstein B, Fuchs D (2003). Association of increased neopterin production with decreased humoral immunity in the elderly. *Exp. Gerontol.* 38: 583-587.
- Muszkat M, Friedman G, Dannenberg HD, Greenbaum E, Lipo M, Heymann Y, Zakay-Rones Z, Ben-Yehuda A (2003). Response to influenza vaccination in community and in nursing home residing elderly: relation to clinical factors. *Exp. Gerontol.* 38: 1199-1203.
- Omoigberale AI, Abiodun PO (2005). Upsurge in neonatal tetanus in Benin City, Nigeria. *East. Afr. Med. J.* 82: 98-102.
- Ozturk A, Gokahmetoglu S, Erdem F, Mysguroglu Alkan S (2003). Tetanus antitoxin levels among adults over 40 years of age in Central Anatolia, Turkey. *Clin. Microbiol. Infect.* 9: 33-38.
- Panning CA, Bayat M (1999). Generalized tetanus in a patient with a diabetic foot infection. *Pharmacotherapy* 19: 885-890.
- Peleg AY, Weeraratna T, McCarthy JS, Davis TM (2007). Common infections in diabetes: pathogenesis, management and relationship to glycaemic control. *Diabetes. Metab. Res. Rev.* 23: 3-13.
- Plouffe JF, Silva J Jr, Fekety R, Allen JL (1978). Cell-mediated immunity in diabetes mellitus. *Infect. Immun.* 21: 425-429.
- Ragab AH, Hazlett B, Cowan DH (1972). Response of peripheral blood lymphocytes from patients with diabetes mellitus to phytohemagglutinin and *Candida albicans* antigen. *Diabetes* 21: 906-907.
- Schiel R, Heinrich S, Steiner T, Ott U, Stein G (2005). Post-transplant diabetes mellitus: risk factors, frequency of transplant rejections, and long-term prognosis. *Clin. Exp. Nephrol.* 9: 164-9.
- Spier RE (2004). Ethical aspects of the methods used to evaluate the safety of vaccines *Vaccine* 22: 2085-90.
- Stark K, Schonfeld C, Barg J, Molz B, Vornwald A, Bienzle U (1999). Seroprevalence and determinants of diphtheria, tetanus and poliomyelitis antibodies among adults in Berlin, Germany. *Vaccine* 17: 844-850.
- Tamer A, Karabay O, Ekerbicer H, Tahtaci M, Selam B, Celebi H (2005). Impaired immunity against tetanus in type 2 diabetes. *Med. Sci. Monit.* 11: CR580-584.