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Diphtheria Vaccine Immunity in Patients with Type 1 Diabetes Mellitus

Tip 1 Diyabetes Mellituslu Hastalarda Difteri Aşısının İmmunitesi

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Abstract / Özet

Objective: Patients with Type 1 diabetes mellitus (DM) may have abnormalities in immune function and presumed increased morbidity and mortality from infections. The aim of this study was to compare the diphtheria antitoxin levels in immunized children with Type 1 DM and healthy children.

Methods: Diphtheria antitoxin levels were measured in serum samples of 40 patients and 40 age and sex matched healthy subjects.

Results: Overall, 23% (9/40) of the patients and 25% (11/40) of healthy controls had insufficient immunity against diphtheria. There was no statistical significant difference for diphtheria antibody levels between patients and controls (X^2=0.267, p=0.797).

Conclusion: Our results indicate that the impairment of the immune response in Type 1 DM patients could be antigen specific and not a general event, and require further investigation in a larger study.

Key Words: Type 1 diabetes mellitus, diphtheria antitoxin, vaccine

Amaç: Tip 1 diyabetes mellituslu (DM) hastaların immun sistemlerinde bozukluklar olabilir ve enfeksiyonlardan kaynaklanan morbidite ve mortalite artmıştır. Bu çalışmanın amacı difteriye karşı aşılandığı bilinen tip 1 DM'lu ve sağlıklı çocuklarda difteri antitoksin düzeylerini ölçerek antikor oluşturma yeteneklerini karşılaştırmaktır.

Yöntemler: Difteri antitoksin düzeyleri 40 hasta ile yaş ve cins bakımında eş olan 40 sağlıklı çocuğun serum örneklerinde ölçüldü.

Bulgular: Hastaların %23'ü (9/40) ile sağlıklı çocukların %25'inde (11/40) difteriye karşı yetersiz immunite saptandı. Hasta ile kontrol grupları arasında difteri antikorları düzeyleri açısından istatistiksel olarak anlamlı bir fark saptanmadı (X^2=0,267, p=0,797).

Sonuç: Bizim sonuçlarımız tip 1 DM'daki bozulmuş immun cevabın antijene spesifik olabileceğini, yaygın bir olay olmadığını ve gelecekte daha geniş çalışmalar gerektiğini düşündürdü.

Anahtar Kelimeler: Tip 1 diyabetes mellitus, difteri antitoksini, aşı

Introduction

Type 1 DM is a syndrome of disturbed energy homeostasis caused by a deficiency of insulin or its action and resulting in abnormal metabolism of carbohydrate, protein, and fat. This disease is thought to result from chronic cell-mediated, autoimmune islet cell damage (1). Patients with diabetes may have abnormalities in immune function and they have an increased risk of infections. Eibl et al. (2) described a reduced proliferative response of CD4·T-cells to primary antigens in patients with Type 1 DM. This reduced proliferative response was suggested to be the reason underlying animpairment in the production of T-cell-dependent antibodies after vaccination in this group of patients.

Diphtheria is an acute toxic infection caused by Corynebacterium diphtheriae. It was the first infectious disease to be conquered on the basis of principles of microbiology and public health. Reduced from a major cause of childhood death in the west in the early 20th century to a medical rarity, modern reminders of the fragility of such success underscore the need to assiduously apply those same principles in an era of vaccine dependency and single global community. Since the introduction of the diphtheria vaccine in the 1940s and improvements in social conditions, the disease has become very rare in the world in which mass immunization campaigns have been carried out (3, 4).

People with diabetes generally have appropriate humoral immune responses to vaccination (5). There are insufficient clinical trials of diphtheria vaccine efficacy in patients with diabetes. The aim of this study was to demonstrate the immune status of immunized patients with Type 1 DM and healthy controls against diphtheria by measured serum diphtheria antibody (DAb) levels.

Methods

Patients: A total of 40 patients with Type 1 DM were vaccinated against diphtheria. These patients were treated with insulin given in 2 injections per 24 hours. This group of patients consisted of 22 boys and 18 girls, ranging in age from 7 to 18 years (average age12.7±3.2), and the duration of diabetes ranged from 0 to 14 years (average 3.4±3.3 years). Controls were healthy individuals

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(n=40, 19 girls, 21 boys) age-matched with the Type 1 diabetes patients (average age: 12.9±2.5 years) (Table 1).

Primary immunization schedule of children in Turkey for diphtheria-tetanus and pertussis (DTP) begins at the 2nd month of life and consists of 3 doses at intervals of 4 weeks. Booster doses include one DTP in the 18th month and one DT in the 7th year of life. Another booster dose of tetanus-diphtheria (Td) vaccine was introduced at 12 years of age.

All patients and healthy controls gave written consent to participate in the study, and when the child's age was less than 16 years, informed consent of a parent or guardian was obtained. Exclusion criteria were as follows: (a) primary or secondary immune deficiency at entry, (b) any active infection at entry.

Fasting venous samples were collected between 7.00-8.00 a.m. for measurement of diphtheria toxoid IgG antibodies from patients and healthy children. All samples were centrifuged at 3000xg for 10 minute at 4°C, aspirated and stored at -80°C until analysis. Quantitation of diphtheria specific antibodies was done by a commercially available IgG-specific ELISA kit (ELISA Diphtheria/Diphtheria IgG, Virotech, Genzyme Virotech GmbH Rüsselsheim, Germany) according to manufacturer's instructions. Patients' sera were diluted 1:100 in PBS dilution buffer and 100 ml samples were pipetted into microtiter wells previously coated with diphtheria antigen. Standards (0.001 IU/mL, 0.002 IU/mL, 0.005 IU/mL, 0.01 IU/mL, 0.02 IU/mL and 0.05 IU/mL IgG diphtheria antitoxin antibodies) were run at the same time as positive controls and dilution buffer alone was run as a negative control. Standards included with each kit were calibrated against "Diphtheria Antitoxin Human Serum, S1/534", of the Institute for Biological Standards and Control (WHO International Laboratory for Biological Standards, Great Britain). Serial dilutions of these were used to create a standard curve.

Statistical analysis

All analyses were performed using the SPSS Win 10.0 statistical package. Testing for statistical significance of immunity rates in Type 1 DM patients and healthy children were performed by using the chi-square test. A p-value of < 0.05 is considered statistically significant.

Results

None of the patients and healthy subjects had a past history of diphtheria. All of the children below 12 years of age were immunized against diphtheria 4 times and the others were immunized 5 times. Classifying sero-immunity against diphtheria toxin of the examined subjects, the internationally accepted criteria (7, 8) were applied to our result: antitoxin <0.01 IU/mL (no immune protection); 0.01-0.099 IU/mL (basic immune protection); >0.1 IU/mL (full protection).

Distribution of immunity rates in patients and controls are shown in Table 2. In 9 (23%) patients and 10 (25%) controls, DAb levels were below 0.01 IU/mL which signifies insufficient immunity against diphtheria. Partial protective levels of antibody titer against diphtheria were found in 12 (30%) of the patients and 10 (25%) of the controls respectively. In addition, fully protective levels of antibody titer against diphtheria were found in 19 (47%) of the patients and 20 (50%) of the controls respectively. Protection rates did not differ significantly between patients and healthy controls (X^2=0.654, p=0.721).

In contrast, 77% (31/40) of patients and 75% (29/40) of normal subjects had partial or full protection antibody levels. Partial or full protection was not significantly different between patients and healthy subjects (X^2=0.267, 95%CI=0.277-2.114, p=0.797).

There was a positive correlation between DAb levels and leukocyte count (p=0.029, r=0.345) in all patients. In contrast, there was no correlation between DAb levels and age, weight, height, duration of diabetes, HA1c, fructosamine, daily dose of insulin and c-peptide in all patients (p>0.05). There was a negative correlation between DAb and age (p<0.001, r=0.598), weight (p<0.001, r=-0.654) and height (p<0.001, r=-0.592), but no correlation between DAb levels and leukocyte count in control subjects (p>0.05).

Discussion

Diabetes is a common metabolic disorder with significant morbidity and mortality. Most clinicians are convinced that diabetics are more susceptible to bacterial, viral and fungal infections. This may be connected with immune system disorders and the resultant defective production of antibodies, as well as immunity disorders, complement and granulocyte malfunctions, etc. The results demonstrate a non significant impairment of the primary humoral immune response to T-cell-dependent antigens in Type 1 DM (6, 7).

Previous clinical studies on the antibody response to various vaccinations in diabetic patients were inconclusive, with studies describing impaired responses (8, 9) and others showing normal responses (10). Pozzilli et al. (11) have reported that Type 1 DM patients had similar increases in the percentage of activated B lymphocytes after influenza vaccination compared to control subjects. There was no clinical study about diphtheria vaccination in Type 1 DM patients. Thus we studied DAb levels in these patients. We showed no sig-

Table 1. Characteristics of the patients and healthy controls

	All Patients (n=40)	Healthy controls (n=40)
Age (year) (mean±SD)	12.7±3.2	12.9±2.5
Weight (kg) (mean±SD)	42.9±12.2	46.0±15.5
Height (cm) (mean±SD)	150.7±17.2	151.5±15.5
Sex (Girl / Boy)	18/22	19/21

SD: standard deviation; n: number of patients

Table 2. İmmune protection status against diphtheria vaccine in patients and healthy controls

Group	No immune protection (<0.01IU/mL)		Basic immune protection (0.01-0.099 IU/mL)		Full immune protection (>0.1 IU/mL)	
	n	%	n	%	n	%
Patients	9	23	12	30	19	47
Controls	10	25	10	25	20	50

n: number of patients

nificant difference between patients with Type 1 DM and control subjects in their immune responses against diphtheria vaccine.

It is generally accepted that when more than 30% of a population is unprotected against diphtheria there is a risk of an epidemic (12, 13). We found that 23% of patients and 25% of controls had insufficient antibody levels against diphtheria (<0.01 IU/mL). In contrast, 77% of patients and 75% of normal subjects have partial or full protection antibody levels. These findings pointed a normal antigen-specific T-cell response during primary immunization in children with Type 1 DM. In addition, these findings have shown no epidemic risk in our diabetic patients and controls.

The studies performed in developed countries demonstrate that immunity levels against diphtheria continuously decrease with age in normal subjects (14, 15), but there was no adequate report in diabetic patients. In our study there was no statistically significant correlation between age and DAb levels in diabetic patients, but there was a statistically significantly negative correlation between age and DAb levels in controls.

Bouter et al. (16) claimed that Type 1 DM patients had a significantly lower antibody response to influenza vaccination than healthy controls and the antibody response was independent of the HbA1c level. There was no study which compared metabolic control of diabetes and immunity against diphtheria vaccination. In our study there was no statistically significantly correlation between HbA1c and DAb levels in patients, thus antibody response was independent of the HbA1c levels in Type 1 DM.

Conclusion

Patients with diabetes belong to the high risk group for infections. This study did not detect differences in the humoral immune response (for diphtheria vaccination) in diabetic patients compared to healthy controls. Patients with diabetes responded to diphtheria vaccination in the same way as the healthy population. These results require further investigation in a larger study.

Ethics Committe Approval: Ethics committee approval was not received due to the retrospective nature of the study.

Informed Consent: Written informed consent was obtained from parents of the patients who participated in this study.

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Hasta Onamı: Yazılı hasta onamı bu çalışmaya katılan hastaların ebeveynlerinden alınmıştır.

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