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Tetanus antitoxin levels and cutaneous anergy in hemodialysis patients in two university hospitals in Iran

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ABSTRACT

Background: The global incidence of tetanus is about 1 million cases annually. Tetanus antibody values decrease with age. Some patients with humoral immune deficiencies may not respond adequately to tetanus toxoid vaccination. The incidence of infectious disease is increased in patients with chronic renal failure. The purpose of this study was to determine tetanus antitoxin level and cutaneous anergy test in hemodialysis patients.

Materials and methods: A cross sectional study was performed on 44 hemodialysis patients who had been on dialysis thrice a week for at least 2 months. Quantitation of tetanus-specific antibodies was achieved by ELISA technique. Then, for Manteaux test 0.1ml of 1/10 saline diluted solution of tetanus and diphtheria toxoid was injected intradermally to the volar surface of the shunt-free arm. Induration was recorded 48-72h and 7-9 days after the injection.

Results: Twenty-eight (64%) patients had induration ≤ 5 mm in 48-72h, classified as non-responsive, whereas 16 (36%) had induration ≥ 5 mm that was classified as positive test (NS). There was no significant correlation between age, sex, duration of dialysis, frequencies of dialysis, history of tetanus-diphtheria vaccination and cutaneous anergy test. Of 44 patients, 34(77.3%) had antibody level of < 0.01 IU/ml, 8 (18.2%) between 0.01-0.1 IU/ml and 2 (4.5%) had an antibody level of ≥ 0.1 IU/ml. There was no significant correlation between age, sex, duration of dialysis, frequencies of dialysis, history of tetanus-diphtheria vaccination, and tetanus antitoxin levels. There was a significant difference between induration size of anergy test results recorded on two separate observations (48-72h and 7-9 days after the test) ($p < 0.05$).

Conclusion: Our results indicate that immunization history was not consistent with protective antibody level, so monitoring immunization status and administering the tetanus vaccine in hemodialysis patients are required.

Keywords: Anergy test, Anti-tetanus antibody, Hemodialysis.

(Iranian Journal of Clinical Infectious Diseases 2006;1(1):31-34).

INTRODUCTION

It is well known that the global incidence of tetanus is about 1 million cases annually (1). The majority of repeated cases are in patients older than

60 years of age; this is one of the several indicators that waning immunity is an important risk factor (1). Several studies suggest that tetanus antibody values decrease with age (2-5). Some patients with humoral immune deficiencies may not respond adequately to tetanus toxoid vaccination (1). The incidence of infectious disease is increased in patients with chronic renal failure (6) that could be

Received: 29 May 2005 Accepted: 7 September 2005

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explained by impaired cell stimulation by antigen presenting cells. Little is known about cutaneous anergy test and antitoxin levels to tetanus toxoid in long-term dialysis patients. The purpose of this study was to determine tetanus antitoxin level and cutaneous anergy test in hemodialysis patients. Therefore, we measured the levels of antitetanus antibodies and performed cutaneous anergy test in hemodialysis patients.

PATIENTS and METHODS

This was a descriptive study conducted in two academic hospitals of Shaheed Beheshti University of Medical Science in Tehran. The hemodialysis wards of Shaheed Modarres and Ayatollah Taleghani hospitals were chosen. Forty-four patients who had been on dialysis thrice a week for at least 2 months, were enrolled. Patients suffering from any other kind of immunodeficiency such as HIV infection were excluded. All patients were asked to complete an informed consent. Demographic data such as age, sex, and the initial data regarding the history of tetanus-diphtheria vaccination, duration and frequencies of dialysis were gathered by a questionnaire.

To determine serum tetanus antibodies and anergy test, serum samples of patients were obtained and stored at 2-8°C for 48h or stored at -20°C for longer duration. Quantitation of tetanus-specific antibodies was achieved by ELISA technique developed by IBL kit (Hamburg, Germany). The results were expressed as IU/ml. An IgG antibody level of ≥ 0.1 IU/ml was considered protective. An IgG antibody level of ≤ 0.01 IU/ml was considered not to be protective and values of $0.01 < \text{IgG} < 0.1$ was defined as poor protection.

Then, using Manteaux technique 0.1ml of 1/10 saline diluted solution of tetanus and diphtheria toxoid was injected intradermally to the volar surface of the shunt-free arm using an insulin syringe. Induration was recorded 48-72h and 7-9

days after the injection. To determine the size of induration, an average was estimated (the sum of the longest diameter divided by two). The ball-point pen technique (7) was used for this purpose. Induration of $< 5\text{mm}$ was considered unresponsive and induration of $\geq 5\text{mm}$ was considered a positive test.

Statistical analysis was performed by SPSS software (version 11.5, SPSS Inc., USA).

RESULTS

We used ELISA technique and Manteux test to determine serum tetanus antibody and cutaneous anergy test to tetanus toxoid in hemodialysis patients. The study population included 24 males and 20 females with the mean age (\pm standard deviation) of 56.4 ± 15.5 years (range, 19-84 years). Of 44 subjects carried the skin anergy test, 28(64%) had induration less than 5mm in 48-72h, classified as non-responsive. Sixteen (36%) had induration $\geq 5\text{mm}$ in 48-72h which were classified as positive test. In non-responsive patients, the mean duration of dialysis was 54.7 ± 6.7 whereas in subjects with positive test it was 50.9 ± 6.7 . The slight difference did not reach a significant level. Antibody level ≤ 0.01 IU/ml was reported in 34(77.3%) subjects, however, 8(18.2%) had an antibody level between 0.01-0.1 IU/ml (poor protection) and 2(4.5%) were protective against the disease.

Insignificant correlation between age, sex, duration of dialysis, frequencies of dialysis, and history of tetanus-diphtheria vaccination were found not only with condition of cutaneous anergy test but also with tetanus antitoxin levels. The mean induration size was 4.0 ± 2.8 and 4.7 ± 3.4 after 48-72h and 7-9 days, respectively. However, there was a significant difference between induration sizes of anergy test results recorded on two separate observations with paired t test ($p < 0.05$).

DISCUSSION

Our results showed that 64% of the hemodialysis patients had non-responsive cutaneous anergy test and there was no significant correlation between age and induration of anergy test. This finding supports the result of Woeltij and colleagues (8), but is in disagreement with the results of Fang and associates (10).

Results have revealed that the majority (77.3%) of the hemodialysis patients had a non-protective level of IgG against tetanus and they were susceptible to tetanus. Redwan et al found a protective level of IgG against tetanus in most of their patients (68.3%) (4). This could be in part explained by larger sample size and healthy status of their subjects (4).

Our study showed that 18.2% of hemodialysis patients had a poor protective level of IgG and 4.5% had a protective level of IgG against tetanus. The protection level found in our study is less than that of Ozturk (3) and Kruger (6). Ozturk reported a protection level of 23.3% among ≥ 40 years old adults without dialysis (3), however, ACIP and AAP recommended vaccination with tetanus-diphtheria toxoid in chronic dialysis patients (9). In another study by Kruger, a sufficient protection against tetanus was reported in 44% of hemodialysis patients (6).

In our study, there was no significant correlation between age and induration in cutaneous anergy test. This finding is in agreement with Woeltij (8) and Ozturk (3) studies.

Redwan has suggested a significant correlation between male gender and non-protective levels of IgG (4), however, we have not found a significant correlation between sex and antitetanus antibody level. There was no significant correlation between duration of dialysis and antitetanus antibody level as well as induration of anergy test. It seems as if impaired cellular and humoral immunity in patients with chronic renal failure are not correlated with the duration of dialysis.

There was no significant correlation between history of tetanus-diphtheria vaccination and anergy response or even antitetanus antibody level. This is in disagreement with the results of Ozturk study (3). Furthermore, we did not find significant correlation between history of tetanus-diphtheria vaccination and protective antitoxin level. This finding clearly demonstrates the need for monitoring of antibody levels after immunization against tetanus in hemodialysis patients.

There was a significant difference between mean induration size of anergy test result recorded after 48-72h and on 7th-9th days. It is important to add the 7th-9th day recording in order to determine the real size of induration.

The results of this study indicate that immunization history was not consistent with protective antibody level, so monitoring immunization status and administering the tetanus vaccine in hemodialysis patients are required.

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