

Effects of Serum Selenium Level on Cell-Mediated Immunity and on Antibody Response to Multivalent Influenza Vaccine in Hemodialysis Patients

Hemodiyaliz Hastalarında Serum Selenyum Düzeyinin Hücre Aracılı Bağışıklığa ve Çok Değerlikli İnfluenza Aşısına Karşı Gelişen Antikor Yanıtına Etkileri

ABSTRACT

OBJECTIVE: End-stage renal disease (ESRD) patients are more prone to serious influenza virus infection than healthy subjects. Selenium (Se) play an important role in cellular and humoral immunity and serum Se levels were lower in hemodialysis patients. Studies have demonstrated that Se deficiency results in less reboust immune responses to vaccination and infections. We aimed to investigate the effect of serum Se levels on immune parameters and antibody response to multivalent influenza vaccine (MIV) in HD patients.

MATERIAL and METHODS: Twenty-six HD patients (Group 1) and 11 healthy subjects (Group 2) were vaccinated with a trivalent inactivated MIV. In both groups, serum Se levels, CD3, CD4, CD4/CD8 ratio, CD3+HLA-DR+ cell percentages and antibody response to MIV were determined before and 1 month after the vaccination.

RESULTS: There were statistically significant differences between Group 1 and Group 2 in terms of baseline serum Se levels, CD8, CD4/CD8 ratio, and CD3+HLA-DR cell percentages. One month after the vaccination, no significant changes were observed in any of the parameters except antibody titers with to baseline levels.

CONCLUSIONS: We did not observe any difference in terms of Se levels and the immune parameters mentioned above before and 1 month after MIV vaccination in HD patients. Further studies investigating the link between Se status and clinical outcomes are needed in dialysis patients.

KEY WORDS: Selenium, Hemodialysis, Cellular immunity

ÖZ

AMAÇ: Son dönem böbrek yetmezliği (SDBY) olan hastalar sağlıklı bireylere oranla ciddi influenza virüs enfeksiyonuna daha yatkındırlar. Selenyum (Se) hücrese ve hümoreal bağışıklıkta önemli bir rol oynamaktadır ve hemodiyaliz hastalarında serum Se düzeyleri düşüktür. Yapılan çalışmalarda Se eksikliği olanlarda aşılar ve enfeksiyon hastalıklarına yeterli bağışıklık yanıtının oluşmadığı gösterilmiştir. Çalışmamızda SDBY hastalarında serum Se düzeyinin bağışıklık göstergeleri ve çok değerlikli influenza aşısına (MIV) antikor yanıtı üzerine etkisini göstermeyi hedefledik.

GEREÇ ve YÖNTEMLER: 26 hemodiyaliz hastası (grup 1) ve 11 sağlıklı kontrol (grup 2) trivalan MIV ile aşılandı. Aşı öncesi ve 1 ay sonrasında serum Se, CD3, CD4, CD4/ CD8 oranı, CD3+HLA-DR+ hücre oranları değerlendirildi.

BULGULAR: Grup 1'deki hastaların grup 2'deki hastalara göre bazal serum Se düzeyleri, CD4/CD8 oranı düşük, CD8 ve CD3+HLA-DR hücre yüzdeleri ise yüksek bulundu. Her iki grubta MIV aşılanma sonrasında antikor düzeyleri dışında Se düzeyleri, CD8, CD4/CD8 oranı ve CD3+HLA-DR hücre yüzdelerinde anlamlı bir fark bulunmadı.

SONUÇ: Hemodiyaliz hastalarında, MIV aşısı öncesi ve 1 ay sonrasında serum Se ve bağışıklık göstergeleri üzerinde herhangi bir fark saptamadık. Hemodiyaliz hasta grubunda Se düzeyi ile klinik sonuçları değerlendiren çalışmalara gereksinim vardır.

ANAHTAR SÖZCÜKLER: Selenyum, Hemodiyaliz, Hücrese immünite

Kültigin TÜRKMEN¹
Tevfik ECDER²
Süleyman TÜRK¹

1 Selcuk University Faculty of Medicine,
Department of Nephrology,
Konya, Turkey

2 Istanbul University Faculty of
Medicine, Department of Nephrology,
Istanbul, Turkey

Received : 15.06.2011

Accepted : 02.11.2011

Correspondence Address:

Kültigin TÜRKMEN
Selcuk Üniversitesi Tıp Fakültesi,
Nefroloji Bilim Dalı, Konya, Turkey
Phone : +90 332 223 67 88
E-mail : mdkt2010@yahoo.com

INTRODUCTION

End-stage renal disease (ESRD) patients are more prone to serious influenza virus infection than healthy subjects (1). Among trace elements, zinc and selenium (Se) play an important role in cellular and humoral immunity (2-4). Data from experimental and human studies have demonstrated that Se deficiency results in less robust immune responses to vaccination and infections compared with Se-adequate controls (5-6). The production of specific antibodies is depressed in selenium-deficient animals and restored by selenium supplementation (4). It appears to affect non-specific immune indices, humoral immunity, cellular immunity, and cytotoxicity (3).

Infections, especially acute forms, contribute substantially to increased morbidity and mortality in patients with ESRD (7). Uremia was found to be associated with impairment of immune system especially cellular immunity (8). It is also reported that chronic kidney disease (CKD) patients present decreased percentages of peripheral CD (cluster differentiation) -4⁺ and CD-8⁺ T lymphocytes and B lymphocytes (9-10). In patients with CKD, serum Se levels have been found to be lower than healthy subjects (11). Data on the effects of Se levels on immune system in CKD patients receiving hemodialysis (HD) is inadequate. We therefore aimed to investigate the effect of serum Se levels on immune parameters and antibody response to multivalent influenza vaccine (MIV) in HD patients.

MATERIAL and METHODS

Twenty-six HD patients (Group 1) and 11 healthy subjects were vaccinated with a trivalent, split, inactivated MIV (Vaxigrip, Pasteur- Merieux) intramuscularly in the deltoid region. The vaccine contained 15 microgram of each the following strains: A/ Johannesburg/33/94 (H3N2) , A/Texas /39/91(H1N1) and B/Harbin/07/94. Hemodialysis patients were on a regular HD session thrice a week for at least 10 months before the beginning of the study.

Group 1 (15 females, 11 males with a mean age of 42±14 years) patients who had been receiving HD treatment for 66.3±8.1 months and group 2 patients (5 females, 6 males with a mean age of 39±7 years) were enrolled in the study.

Serum Se levels, CD3, CD4, CD4/ CD8 ratio, CD3+HLA-DR+ cell percentages and antibody response to subgroups of MIV were determined before and 1 month after the vaccination in all cases.

Blood samples were collected before the dialysis sessions and centrifuged at 3000/min for 5 min. Serum samples were placed in Se-free propylene tubes and stored at -20 °C. Heparinised whole blood was also drawn for flow-cytometric analysis.

Selenium determination was performed by atomic absorption spectrophotometry (Varian, GTA-96 model, graphite tube atomizer)

Lymphocyte staining was performed as follows: 100 ml heparinised anticoagulated whole blood was incubated with various two-colour combinations of monoclonal antibodies produced by Becton Dickinson (USA). After staining for 10 min at room temperature, the erythrocytes were lysed using FACSlyse solution (Becton Dickinson). The samples were then centrifuged at 850 rpm for 5 min and the supernatant was removed.

The cell pellets were washed with phosphate buffered saline (PBS) and fixed in a 0.5 ml suspension of 1% formaldehyde in PBS. Cells were protected from light, at +4 °C until the flow cytometric analysis analysis was performed.

The immunophenotypic analysis of the cells was performed using FACScan flow cytometer (Becker Dickinson, USA) equipped with a 15 mW air cooled argon-ion laser. A minimum of 1000 events were collected on each sample. Data analysis was performed using lysis II software (Becton Dickinson). Gating was performed using 90° right angle scatter. The immunofluorescence signals were amplified on a logarithmic scale.

The human sera were studied for influenza virus antibodies at dilutions of 1/10-1/320 using standard microtiter techniques. The influenza A (A/Johannesburg/33/94 H3N2, A/Texas/36/91 H1N1) and B (Harbin/07/94) virus preparations were used as antigens.

Statistical Analysis

Statistical analysis was performed with unpaired t-test, Student's t-test and correlation test. Data were presented as means±SD. Significance was defined as p<0.05.

RESULTS

The results can be summarized as follows: 1) There were statistically significant differences between Group 1 and Group 2 in terms of baseline serum Se levels, CD8, CD4/CD8 ratio, and CD3+HLA-DR cell percentages (Table I). 2) On the other hand, baseline CD3, CD4, CD19 and HLA-DR+, cell percentages were not different between Group 1 and 2. 3) There were no statistically significant correlations between baseline serum Se levels and immune parameters (CD3, CD4, CD8, CD19, HLA-DR +, CD3+HLA-DR cell percentages and CD4/CD8 ratio) in either group. 4) One month after the vaccination, no significant changes were observed in any of the parameters except antibody titers with regard to baseline levels (Table II). 5) There were no statistically significant correlations between Se levels and immune parameters (CD3, CD4, CD8, CD19, HLA-DR +, CD3+HLA-DR cell percentages, CD4/CD8 ratio and antibody response to MIV) in either group 1 month after the vaccination.

DISCUSSION

There are five main findings in the present study and they can be summarized as follows: 1) There were statistically significant differences between Group 1 and Group 2 in terms of baseline

Table I: Baseline serum Se levels, CD3, CD4, CD4/ CD8 ratio, CD3+HLA-DR+ cell percentages of (Group1) and healthy Subjects (Group 2)

Parameters	Group 1 (HD patients) (n=26) (Mean±SD)	Group 2 (Healthy Subjects) (n=11) (Mean±SD)	P value
CD3 (%)	74.8±7.5	72±5.4	NS
CD4 (%)	43.9±6.8	47±2.9	NS
CD8 (%)	32.6±5.3	27.9±6.9	P<0.01
CD4/CD8 ratio (%)	1.3±0.4	1.82±0.6	P<0.01
CD19 (%)	8.3±5.0	11.8±0.6	NS
HLA-DR + (%)	8.9±4.9	12.8±3.6	NS
CD3+HLA-DR cell (%)	8.8±5.7	4.3±1.9	P<0.01
Selenium (mcg/L)	59±6.5	80±8.1	P<0.001

Table II: Serum Se levels, CD3, CD4, CD4/ CD8 ratio, CD3+HLA-DR+ cell percentages of (Group1) and Healthy Subjects (Group 2) 1 month after vaccination.

Parameters	Group 1 (HD patients) (n=26) (Mean±SD)	Group 2 (Healthy Subjects) (n=11) (Mean±SD)	P value
CD3 (%)	73.4±8.3	71.5±5.8	NS
CD4 (%)	41.7±7.0	46.6±4.1	NS
CD8 (%)	33.15±6.14	27.1±7.5	P<0.01
CD4/CD8 ratio (%)	1.3±0.37	1.90±0.8	P<0.01
CD19 (%)	9.6±4.5	11.5±4.5	NS
HLA-DR + (%)	9.0±4.0	12.4±4.3	NS
CD3+HLA-DR cell (%)	8.5±5.0	4.3±1.85	P<0.01
H3N2 Ab	54.6±30.2	254.5±93.4	P<0.0001
H1N1Ab	39.2±20	247.2±45.4	P<0.0001
Harbin 07/94 Ab	21.5±10.1	76.4±45.4	P<0.0001
Selenium (mcg/L)	55.2±5.6	74.85±9.3	P<0.001

Abbreviations: Influenza Stain A/ Johannesburg/33/94 (H3N2), Influenza Stain A/Texas /39/91(H1N1) and Influenza Stain B/ Harbin/07/94. Ab: antibody

serum Se levels, CD8, CD4/CD8 ratio, and CD3+HLA-DR cell percentages. 2) Baseline CD3, CD4, CD19 and HLA-DR+ cell percentages were not different between Group 1 and 2. 3) There were no statistically significant correlations between baseline serum Se levels and immune parameters (CD3, CD4, CD8, CD19, HLA-DR +, CD3+HLA-DR cell percentages and CD4/CD8 ratio) in either group. 4) One month after the vaccination,

no significant changes were observed in any parameter except antibody titers when compared to baseline levels. 5) There were no statistically significant correlations between Se levels and immune parameters (CD3, CD4, CD8, CD19, HLA-DR +, CD3+HLA-DR cell percentages, and CD4/CD8 ratio and antibody) in either group 1 month after the vaccination.

Selenium is normally present in multiple proteins as a constituent of amino acids. Although the major function of Se is antioxidant activity as a part of glutathione peroxidase, it also protects against the toxicity of heavy metals such as mercury (12). The bioavailability of Se is high and approximately 50% of Se is absorbed in the small intestine, independent of serum Se levels (13). Experimental and clinical studies have shown that severe Se deficiency leads to coronary artery disease (CAD), cardiomyopathy and sudden cardiac death (14-16). End-stage renal disease (ESRD) patients were found to be more prone to oxidative stress that could be associated with increased cardiovascular (CV) mortality (17). Mild Se deficiency also appears to increase susceptibility to oxidant stress (18), risk of infections (4), and uremic cardiomyopathy (11), thus contributing to the increased risk of CVD in ESRD patients.

In the present study, we found that serum Se levels were significantly lower in HD patients when compared with healthy subjects. This result was consistent with those obtained from previous studies (19-20).

In the literature, there are conflicting results about CD3+ cell percentages in ESRD patients. Caruana et al. (21) reported that hemodialysis patients had significantly lower percentages of CD3+ cells when compared with healthy subjects. However, Raska et al. (22) showed that the percentages of CD 3+cells was not significantly affected in HD patients. In a recent study, no changes were seen in the percentages of CD3+ T lymphocytes between hemodialysis, peritoneal dialysis and healthy subjects (10). In the present study, we found statistically significant differences between Group 1 and Group 2 in terms of CD3+HLA-DR cell percentages. These different results of CD3+ cell percentages in different studies could be secondary to the measurement time of the parameter (before or after dialysis).

In previous studies, CD 8+cell percentages were found to be higher and CD4/CD8 ratio were found to be lower in HD patients when compared to healthy subjects (22-23). In the present study, CD 8+ cells were higher and CD4/CD8 ratio were lower in HD patients than healthy subjects. However, there were no significant changes in all of the parameters except antibody titers one month after the vaccination when compared to baseline parameters.

Although the decreased response to vaccination is mainly associated with uremia-related immune dysfunction, there are many other factors including endotoxin presence in the dialysis water, access related infections, malnutrition and trace element deficiency in the pathogenesis of decreased adaptive and innate immune defence in patients with ESRD (8, 24). In our study, there were no statistically significant correlations between Se levels and immune parameters (CD3, CD4, CD8, CD19, HLA-DR +, CD3+HLA-DR cell percentages, CD4/CD8 ratio and antibody) in both groups 1 month after the vaccination.

Our study have some limitation. First, the sample size is relatively small. Second, supplemental Se effect on immune system and antibody response should be determined.

In conclusion, we did not observe any difference in terms of Se levels and immune parameter mentioned above before and 1 month after MIV vaccination. Further studies investigating the link between Se status and clinical outcomes were needed in dialysis patients, in whom the risks of infection and CVD are dramatically elevated compared with people who have normal kidney function.

REFERENCES

1. Cappel R, Van Beers D, Liesnard C, Dratwa M: Impaired humoral and cell-mediated immune responses in dialyzed patients after influenza vaccination. *Nephron* 1983; 33 (1): 21-25
2. Turk S, Bozfakioglu S, Ecder ST, Kahraman T, Gurel N, Erkok R, Aysuna N, Türkmen A, Bekiroğlu N, Ark E: Effects of zinc supplementation on the immune system and on antibody response to multivalent influenza vaccine in hemodialysis patients. *Int J Artif Organs* 1998; 21 (5): 274-278
3. Carlson BA, Yoo MH, Shrimali RK, Irons R, Gladyshev VN, Hatfield DL, Park JM: Role of selenium-containing proteins in T-cell and macrophage function. *Proc Nutr Soc* 2010; 69 (3): 300-310
4. Field CJ, Johnson IR, Schley PD: Nutrients and their role in host resistance to infection. *J Leukoc Biol* 2002; 71 (1): 16-32
5. Petrie HT, Klassen LW, Klassen PS, O'Dell JR, Kay HD: Selenium and the immune response: 2. Enhancement of murine cytotoxic T-lymphocyte and natural killer cell cytotoxicity in vivo. *J Leukoc Biol* 1989; 45 (3): 215-220
6. Roy M, Kiremidjian-Schumacher L, Wishe HI, Cohen MW, Stotzky G: Selenium and immune cell functions. II. Effect on lymphocyte-mediated cytotoxicity. *Proc Soc Exp Biol Med* 1990; 193 (2): 143-148
7. Dalrymple LS, Go AS: Epidemiology of acute infections among patients with chronic kidney disease. *Clin J Am Soc Nephrol* 2008; 3 (5): 1487-1493 PMID: 18650409
8. Hauser AB, Stinghen AE, Kato S, Bucharles S, Aita C, Yuzawa Y, Pecoits-Filho R: Characteristics and causes of immune dysfunction related to uremia and dialysis. *Perit Dial Int* 2008; 28 (3): 183-187
9. Ueki Y, Nagata M, Miyake S, Tominaga Y: Lymphocyte subsets in hemodialysis patients treated with recombinant human erythropoietin. *J Clin Immunol* 1993; 13 (4): 279-287
10. Lisowska KA, Debska-Slizien A, Jasiulewicz A, Heleniak Z, Bryl E, Witkowski JM: Hemodialysis affects phenotype and proliferation of CD4-Positive T Lymphocytes. *J Clin Immunol* 2011, DOI 10.1007/s10875-011-9603-x

11. Girelli D, Olivieri O, Stanzial AM, Azzini M, Lupo A, Bernich P, Menini C, Gammaro L, Corrocher R: Low platelet glutathione peroxidase activity and serum selenium concentration in patients with chronic renal failure: Relations to dialysis treatments, diet and cardiovascular complications. *Clin Sci (Lond)* 1993; 84 (6): 611-617
12. Rucker D, Thadhani R, Tonelli M: Trace element status in hemodialysis patients. *Semin Dial* 2010; 23 (4): 389-395
13. Levander OA, Whanger PD: Deliberations and evaluations of the approaches, endpoints and paradigms for selenium and iodine dietary recommendations. *J Nutr* 1996; 126(9 Suppl): 2427-2434
14. Moore JA, Noiva R, Wells IC: Selenium concentrations in plasma of patients with arteriographically defined coronary atherosclerosis. *Clin Chem* 1984; 30 (7): 1171-1173
15. Ge K, Xue A, Bai J, Wang S: Keshan disease-an endemic cardiomyopathy in China. *Virchows Arch A Pathol Anat Histopathol* 1983; 401 (1): 1-15
16. Salonen JT, Alfthan G, Huttunen JK, Pikkarainen J, Puska P: Association between cardiovascular death and myocardial infarction and serum selenium in a matched-pair longitudinal study. *Lancet* 1982; 2 (8291): 175-179
17. Stenvinkel P: Interactions between inflammation, oxidative stress, and endothelial dysfunction in end-stage renal disease. *J Ren Nutr* 2003; 13 (2): 144-148
18. Klotz LO, Kroncke KD, Buchczyk DP, Sies H: Role of copper, zinc, selenium and tellurium in the cellular defense against oxidative and nitrosative stress. *J Nutr* 2003; 133 (5 Suppl 1): 1448-1451
19. Zachara BA, Salak A, Koterska D, Manitius J, Wasowicz W: Selenium and glutathione peroxidases in blood of patients with different stages of chronic renal failure. *J Trace Elem Med Biol* 2004; 17 (4): 291-299
20. Zachara BA, Adamowicz A, Trafikowska U, Trafikowska A, Manitius J, Nartowicz E: Selenium and glutathione levels, and glutathione peroxidase activities in blood components of uremic patients on hemodialysis supplemented with selenium and treated with erythropoietin. *J Trace Elem Med Biol* 2001; 15 (4): 201-208
21. Caruana RJ, Leffell MS, Lobel SA, Campbell HT, Cheek PL: Chronic T-lymphocyte activation in chronic renal failure: A study of hemodialysis, CAPD and pre-dialysis patients. *Int J Artif Organs* 1992; 15(2): 93-98
22. Raska K, Jr, Raskova J, Shea SM, Frankel RM, Wood RH, Lifter J, Ghobrial I, Eisinger RP, Homer L: T cell subsets and cellular immunity in end-stage renal disease. *Am J Med* 1983; 75 (5): 734-740
23. Angelini D, Carlini A, Giusti R, Grassi R, Mei E, Fiorini I, Mazzotta L, Antonelli A: Parathyroid hormone and T-cellular immunity in uremic patients in replacement dialytic therapy. *Artif Organs* 1993; 17 (2): 73-75
24. Girndt M, Sester M, Sester U, Kaul H, Kohler H: Molecular aspects of T- and B-cell function in uremia. *Kidney Int Suppl* 2001; 78: 206-211