

Study of Elements, Antioxidants and Lipid Peroxidation in Hemodialysis Patients*

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Background: During hemodialysis, most of the elements must be kept in a rather narrow physiological range, otherwise life-threatening events may occur. Furthermore, lipid peroxidation in patients may be partly due to the trace element disturbances. It has been mentioned that there are relations between deficiency in trace elements and antioxidant levels. The present study aimed to determine whether there were differences between hemodialysis patients and a healthy group according to selenium, aluminium, malondialdehyde (MDA), reduced form of glutathione (GSH) and superoxide dismutase (SOD) levels.

Methods: The study included 47 hemodialysis patients (hemodialysis group). Blood samples were taken before (pre-hemodialysis) and after (post-hemodialysis) hemodialysis session. The control group included 23 healthy volunteers.

Results: The aluminium, MDA, and SOD levels were lower and reduced form of GSH levels were higher in the control group when compared with the pre-hemodialysis group. MDA and SOD levels were higher in the post-hemodialysis group than in the control group. GSH levels were lower and aluminium levels higher in the pre-versus the post-hemodialysis group.

Conclusions: In order to evaluate the data of antioxidant and oxidant levels, hemodialysis patients are subjected to oxidative stress. Moreover, the study shows that analyzing levels of aluminium may be useful in hemodialysis patients in evaluating elements status.

Key Words: Hemodialysis, antioxidants, oxidants, selenium, aluminium

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Hemodiyaliz Hastalarında Lipit Peroksidasyonu, Antioksidan ve Element Düzeyleri

Giriş ve Amaç: Hemodiyaliz sırasında, birçok element oldukça dar fizyolojik aralık içinde tutulmalıdır ki, aksi durumunda hayati tehlike görülebilir. Bunun yanısıra, bu hastalarda lipid peroksidasyonu, kısmen eser element bozukluklarından dolayı olabilmektedir. Eser elementlerin eksikliği ile antioksidan düzeyleri arasında ilişkinin varlığından söz edilmektedir. Sunulan çalışmanın amacı; selenyum, alüminyum, malondialdehit (MDA), redükte glutatyon (GSH) ve superoksit dismutaz düzeyleri bakımından hemodiyaliz hastaları ile sağlıklı grup arasında farkın olup olmadığını belirlemektir.

Yöntem ve Gereç: "Hemodiyaliz grubu" olarak tanımladığımız grup 47 hemodiyaliz hastasından oluşturulmuştur. Kan numuneleri hemodiyalizden önce (pre-hemodiyaliz) ve sonra (post-hemodiyaliz) olmak üzere toplanmıştır. "Kontrol grubu" 23 sağlıklı gönüllüden oluşmuştur.

Bulgular: Kontrol grubunda, pre-hemodiyaliz grubuna göre alüminyum, MDA, SOD düzeyleri düşük, ve redükte GSH yüksek idi. MDA; SOD düzeyleri post-hemodiyaliz grubunda kontrol grubuna göre yüksekti. Bunun yanısıra, pre-hemodiyaliz grubunda post-hemodiyaliz grubuna göre; GSH düzeyleri düşük, alüminyum düzeyleri yüksekti.

Sonuç: Antioksidan ve oksidan düzeylerine ait veriler değerlendirildiğinde, hemodiyaliz hastaları oksidatif strese maruz kalmaktadır. Bununla birlikte, çalışma alüminyum düzeylerinin analizinin, hemodiyaliz hastalarının element durumlarının değerlendirilmesinde yararlı olabileceğini göstermektedir.

Anahtar Sözcükler: Hemodiyaliz, antioksidan ve oksidanlar, selenyum, alüminyum

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Introduction

During hemodialysis (HD) essential kidney functions, such as the elimination of water and metabolic wastes as well as the correction of the electrolyte and acid/base state, are replaced by the artificial purification system. Elements such as Na^+ , K^+ , Ca^{++} , Mg^+ , Cl^- , and H^+ must be kept in a rather narrow physiological range, otherwise life-threatening events may occur (1). Moreover, renal impairment, anemia and other clinical complications in hemodialysis patients may be commonly related to increased cellular uptake and toxicity of aluminium (2-4), whereas deficiencies may occur more commonly for iron, zinc and possibly selenium and toxicities are possible for copper (4).

Dialysis patients are subjected to an oxidative stress resulting from the dialysis sessions (5,6). Oxidative stress occurs when there is an excessive free radical production and/or low antioxidant defense (7,8), and results in chemical alterations of bio-molecules, which cause structural and functional modifications (7). Lipid peroxidation often occurs in response to oxidative stress, and a great diversity of aldehydes are formed when lipid hydroperoxides break down in biological systems. Some of these aldehydes are highly reactive and may be considered as second toxic messengers, which disseminate and augment initial free radical events. Polyunsaturated fatty acids are oxidized *in vivo* by free radicals and other reactive species. Subsequent degradation of oxidized lipid molecules leads to the formation of several specific metabolites that include aldehydes of variable chain length, such as malondialdehyde (9). Its consequences may be aggravated by the concomitant lowering of the patient's defense system. This event occurs as a result of disturbance in the detoxification enzyme system (e.g. superoxide dismutase, SOD) that detoxifies free radicals (5,6). Selenium and glutathione peroxidase (GSH-Px) play an important role in protecting cell membranes from oxidative damage, and decreased blood selenium and GSH-Px activity are common in chronic renal failure (CRF) (10).

We purposed to determine levels of aluminium and selenium, malondialdehyde (MDA), SOD and reduced form of GSH in hemodialysis patients and healthy volunteers, and in addition to determine whether there are any correlation coefficients of trace elements with antioxidants and oxidants.

Materials and Methods

This study involved 47 hemodialysis patients ("hemodialysis group", 34 female, 13 male) who were treated in the Polyclinic of the Hemodialysis Department of Nephrology in Selcuk University Medical Faculty. The mean age of the patients was 50.26 ± 16.36 years. All patients were dialyzed three times a week and each session was at least four hours. They were dialyzed with polysulfone dialyzing membrane. The duration of dialysis ranged from 2-16 years. Patients with hepatitis B, acute medical events, and aluminium-containing drug usage were excluded from this study.

The control group was composed of 23 healthy volunteers (7 female, 16 male) with a mean age of 39.52 ± 11.54 years. Controls had no medical problems, and no alcohol or cigarette consumption.

Samples Collection: Blood samples were taken from the hemodialysis patients during their regular monthly check-ups. No extra blood samples were taken from the patients for the biochemical parameters investigated in this study. Samples were collected immediately before (pre-hemodialysis) and after (post-hemodialysis) the dialysis sessions.

Samples from the control group were taken after a 10-hour fasting period. The control group was selected from individuals presenting for their routine check-ups. Extra blood samples were not taken.

Blood samples were divided into two tubes (with and without anticoagulant -EDTA). The EDTA anticoagulant samples were centrifuged at 2000 rpm for 10 min at $+4^\circ\text{C}$. Red cells were washed three times with ice-cold normal saline solution. Erythrocyte SOD, reduced form of GSH and plasma MDA were analyzed immediately after taking blood samples.

Serum samples were stored into polyethylene tubes at -85°C to analyze the levels of aluminium and selenium. Elements levels were determined by inductively coupled plasma atomic emission spectrometry (ICP-AES, Varian Australia Pty Ltd, Australia). Plasma MDA and erythrocyte-reduced form of GSH levels were determined respectively according to the method of TBARS (11,12) and Ellmann (13). SOD (catalog no: SD 125) levels were analyzed by using Randox test kits in spectrophotometers (Shimadzu-1601, Japan).

Statistical Analysis: Data were expressed as mean \pm SD and analyzed with SPSS packet program. Student's t-test and Mann-Whitney U test were used to compare the groups. Pearson correlation coefficients were applied to evaluate the relationship between levels of trace elements and the other parameters. The level of statistical significance was set at $P < 0.001$.

Results

Data concerning the hemodialysis and control groups are shown in Table 1.

In pre-hemodialysis patients, the levels of aluminium, MDA, and SOD ($P < 0.001$) were higher and reduced form of GSH levels ($P < 0.001$) were lower than in controls. There were no significant differences in selenium levels between controls and the pre-hemodialysis values (Table 1).

While MDA and SOD levels were higher in patients after hemodialysis than in the control group ($P < 0.001$), we did not determine any significant differences in levels of reduced form of GSH, selenium, or aluminium between the control and post-hemodialysis groups.

Reduced form of GSH levels of post-hemodialysis patients were higher ($P < 0.001$) and of aluminium were lower ($P < 0.001$) than levels before the dialysis session. In those groups, levels of SOD, MDA and selenium were not significantly different.

In the control group, a positive correlation between aluminium and selenium ($r = 0.474$), and in the post-

hemodialysis patients a positive correlation between selenium and aluminium ($r = 0.571$, $P < 0.001$), selenium and MDA ($r = 0.365$, $P < 0.05$), MDA and reduced form of GSH ($r = 0.499$, $P < 0.001$), and reduced form of GSH and SOD ($r = 0.865$, $P < 0.001$) were determined. In addition, a positive correlation between levels of GSH and SOD ($r = 0.420$, $P < 0.01$) was determined in the pre-hemodialysis group (data not shown).

Discussion

Though trace elements occur in very low concentrations in the body, their role in the maintenance of undisturbed biological functions is nonetheless highly important. Dialysis remarkably contributes to an increase in the elemental body burden of many elements. In CRF, the concentrations of trace elements are modified partly as a consequence of endogenous toxicities and of impaired renal function, partly due to dietary restriction and, last but not least, due to therapeutic measures (i.e. conservative and modern instrumental therapy) (14).

Whereas some researchers have concluded that zinc and selenium deficits in uremic patients were correlated with disturbance in the enzyme systems which detoxify free radicals (5), other researchers (15) have mentioned that the essential trace element selenium might take part not only in the direct protection of endothelial cells against the accumulation of aggressive oxygen species, but also in the prevention of the toxic effects of cadmium or in the modulation of the active calcium transport (15).

Table 1. Parameter levels in control and hemodialysis groups ($x \pm SD$).

| Parameters | GROUPS | | | P VALUES | | |
|-------------------------|--------------------|----------------------|-----------------------|----------|--------|--------|
| | Control (A) | Pre-hemodialysis (B) | Post-hemodialysis (C) | A vs B | A vs C | B vs C |
| SOD (U/g Hb) | 995.84 \pm 58.88 | 4127.52 \pm 238.50 | 5360.73 \pm 762.82 | 0.000* | 0.000* | 0.475 |
| GSH (μ mol/g Hb) | 5.30 \pm 0.24 | 4.56 \pm 0.38 | 6.55 \pm 0.51 | 0.001* | 0.211 | 0.000* |
| MDA(nmol/ml) | 6.97 \pm 0.07 | 10.27 \pm 0.18 | 11.05 \pm 0.30 | 0.000* | 0.000* | 0.073 |
| Selenium (μ g/dl) | 26 \pm 1.18 | 26 \pm 0.85 | 24 \pm 1.42 | 0.93 | 0.93 | 0.41 |
| Aluminium (μ g/dl) | 3.1 \pm 0.17 | 4.60 \pm 0.18 | 3.73 \pm 0.18 | 0.000* | 0.051 | 0.001* |

* $P < 0.001$

SOD: Superoxide dismutase. GSH: Reduced form of glutathione. MDA: Malondialdehyde.

In this respect, more careful attention is necessary in analyzing the levels of selenium and zinc in regularly dialyzed uremic patients (14).

Data obtained in the present study concerning selenium levels in control and post-hemodialysis patients are inconsistent with results presented by Richard et al. (5), Turan et al. (15), Zima et al. (16) and Lin et al. (17), but are consistent with those of Bogye et al. (18). Bogye et al. suggested that there was no significant difference between serum selenium concentration before and after HD session. Turan, Zima and Lin (15-17) based on their data suggested that selenium was lost through the pores of polysulfone membrane during HD, which was associated with its protein permeability. On the other hand, Krizek et al. (19) measured the greatest changes in blood selenium levels in post-hemodialysis patients dialyzed with cuprophane membranes. Therefore, the type of dialytic treatment could play a role in determining the effective amounts of the elements which are transferred from the dialysis fluid into the patient (14). Thus, in order to clarify the selenium lost, it may be useful to analyze the levels of selenium in dialysis fluid in addition to that in blood samples.

Vanholder et al. (3) stated that decreased element concentrations are related mainly to nutritional intake, intestinal uptake and altered distribution and, in addition, that protein-bound trace elements may be lost more readily in presence of proteinuria. Increased trace element concentrations can result from excessive homeopathic intake, administration of parenteral fluids or blood contact with contaminated dialysate (3). Furthermore, uremic compounds may be related to accumulation of aluminium. Thus, our results, which show that aluminium levels are higher in HD patients than in the control group, are consistent with those studies (2,3,17,20).

Recent studies have suggested that lipid peroxidation in patients may be due only in part to the trace element disturbances (17) and that there are relations between trace elements deficiency and antioxidant levels. In CRF patients, disturbances in enzymatic mechanisms of free radicals detoxification lead to alteration in the antioxidant system (5,17) and ROS (reactive oxygen species) attack on cell membranes also results in formation of lipid peroxidation products such as MDA (21).

Researchers (8,25) have reported that forming free radicals increased lipid peroxidation in CRF patients and

together with dialysis was associated with an impairment of antioxidant defense and an overproduction of oxidative stress markers. Data that show the levels of MDA in all groups in our study are consistent with those studies (5,8,17,21-26) but not with Seymen et al.'s (27).

Defense mechanisms involve enzymes (which scavenge the intermediates of oxygen reduction), elements such as selenium, copper, zinc (which are present in the enzymes SOD and GSH-Px), and substances such as vitamins E, C and A (which defend against peroxidation of fatty acids) (28). In some studies (23-24,26), SOD levels were higher in pre-hemodialysis patients than in the control group. Thus, our results are consistent with those data, but not with the results of Hasanoğlu et al. (20), Seymen et al. (27) and Chen et al. (29).

Meanwhile, protection from oxidant injury involves numerous enzymatic and nonenzymatic pathways. One of the most important nonenzymatic protective mechanisms involves the glutathione pathway (21). Reduced GSH is one of the most important scavengers of free radicals in the red blood cell membrane (28). In the absence of an efficient GSH-generating system, GSH cannot be maintained in the reduced state when subjected to oxidative stress (21). In this regard, our data concerning reduced form of GSH when comparing controls and pre-hemodialysis patients are similar with the results of the researchers (21,23-26,30). However, our results comparing pre- and post-hemodialysis patient groups regarding reduced form of GSH levels are not similar with the results of the researchers (26). In addition, the study (27) performed on the effect of the different dialytic membranes on antioxidant levels determined no significant differences between pre-and post-hemodialysis groups in total GSH levels when using polysulfone membrane. They suggested that the oxidant/ antioxidant system did not statistically change in polysulfone membrane, while hemodialysis patients dialyzed with the cuprophane membrane demonstrated a statistically significant disturbance in the oxidant/antioxidant enzyme system.

Current studies present varying results regarding the relation between antioxidant, oxidant and element levels in hemodialysis patients. Those differences can be attributed to variation in inclusion criteria to the study (number of subjects, duration of dialysis range), timing of blood sample collections and especially to the dialysis membrane.

Conclusion

Our findings show that levels of aluminium are statistically different between groups and suggest that it may be important to analyze this element. The abnormal metabolism of aluminium may contribute to a part of hemodialysis. Therefore, the study points out a significant correlation between the levels of aluminium and selenium. We think that environmental conditions, dialytic treatment, and diet may affect the trace elements metabolism in HD patients. Analyzing the levels of trace elements not only in the blood samples but also in the dialytic fluids may be more useful to clarify the effects of elements in HD patients.

According to results of antioxidant and oxidant levels in controls and pre-hemodialysis patients, finding decreased levels of reduced form of GSH, and increased MDA and SOD levels in pre-hemodialysis patients should be taken

into consideration. We suggest that HD patients are subjected to oxidative stress, as indicated by increased lipid peroxidation and reduced antioxidant levels. But, when we estimate the levels of SOD, GSH and MDA in pre- and post-hemodialysis patients, it seems the relations of SOD and MDA in hemodialysis patients before and after the dialysis session must be investigated more extensively to clarify the changes in the oxidant/antioxidant defense system during the dialysis session.

Thus, seen from this aspect, the present study is important but not extensive in determining the relation between antioxidant and elements levels in the role of disease physiopathology and in acceptable treatment. We conclude that routine determination of those biochemical parameter levels in hemodialysis patients may contribute in monitoring the disease as well as any deficiency or excess in those parameters.

References

1. Krachler M, Scharfetter H, Wirnsberger G. Kinetics of the metal cations magnesium, calcium, copper, zinc, strontium, barium, and lead in chronic hemodialysis patients. *Clin Nephrol* 2000; 54: 35-44.
2. Neiva TJC, Benedetti AL, Tanaka SMCN, Santos JI, D'Amico EA. Determination of serum aluminum, platelet aggregation and lipid peroxidation in hemodialyzed patients. *Braz J Med Biol Res* 2002; 35: 345-350.
3. Vanholder R, Cornelis R, Dhondt A, Lameire N. The role of trace elements in uraemic toxicity. *Nephrol Dial Transplant* 2002; 17(Suppl 2): 2-8.
4. Kalantar-Zadeh K, Kopple JD. Trace elements and vitamins in maintenance dialysis patients. *Adv Ren Replace Ther* 2003; 10: 170-182.
5. Richard MJ, Arnoud J, Jurkovitz C, Hachache T, Meftabi H, Laporte F et al. Trace elements and lipid peroxidation abnormalities in patients with chronic renal failure. *Nephron* 1991; 57: 10-15.
6. Ghoreshi Z, Jagtap PE, Ahaley SK, Gandhi R. Oxidant-antioxidant status in acute and chronic renal failure. *Indian J Med Sci* 2000; 54: 131-135.
7. Massy ZA, Nguyen-Khoa T. Oxidative stress and chronic renal failure: markers and management. *J Nephrol* 2002; 15: 336-341.
8. Ozden M, Maral H, Akaydin D, Cetinalp P, Kalender B. Erythrocyte glutathione peroxidase activity, plasma malondialdehyde and erythrocyte glutathione levels in hemodialysis and CAPD patients. *Clin Biochem* 2002; 35: 269-273.
9. Esterbauer H, Schaur R J, Zollner H. Chemistry and biochemistry of 4-hydroxynonenal, malonaldehyde and related aldehydes. *Free Radic Biol Med* 1991; 11: 81-128.
10. Adamowicz A, Trafikowska U, Trafikowska A, Zachara B, Manitus J. Effect of erythropoietin therapy and selenium supplementation on selected antioxidant parameters in blood of uremic patients on long-term hemodialysis. *Med Sci Monit* 2002; 8: CR202-205.
11. Draper HH, Hadley M. Malondialdehyde determination as index of lipid peroxidation. *Methods Enzymol* 1990; 186: 421-430.
12. Hammode RMA, Khalil MMM, Salem A. Lipid peroxidation products in pleural fluid for separation of transudates and exudates. *Clin Chem* 1995; 41: 1314-1315.
13. Atroshi F, Sandholm M. Red blood cell glutathione as a marker of milk production in Finn sheep. *Res Vet Sci* 1981; 33: 256-259.
14. Padovese P, Galièni M, Brancaccio D, Pietra R, Fortaner S, Sabbioni E et al. Trace elements in dialysis fluids and assessment of the exposure of patients on regular hemodialysis, hemofiltration and continuous ambulatory peritoneal dialysis. *Nephron* 1992; 61: 442-448.
15. Turan B, Delilbası E, Dalay N, Set S, Afrasyap L, Sayal A. Serum selenium and glutathione-peroxidase activities and their interaction with toxic metals in dialysis and renal transplantation patients. *Biol Trace Element Res* 1992; 33: 95-102.
16. Zima T, Mestek O, Nĕmĕcĕk K, Bártova V, Fialova J, Tesar V et al. Trace elements in hemodialysis and continuous ambulatory peritoneal dialysis patients. *Blood Purif* 1998; 16: 253-260.

17. Lin TH, Chen JG, Liaw J, Juang JG. Trace elements and lipid peroxidation in uremic patients on hemodialysis. *Biol Trace Elem Res* 1996; 51: 277-283.
18. Bogye G, Tompos G, Alftan G. Selenium depletion in hemodialysis patients treated with polysulfone membranes. *Nephron* 2000; 84: 119-123.
19. Krizek M, Senft V, Motan J. Influence of hemodialysis on selenium blood levels. *Sb Lek* 2000; 101: 241-248.
20. Hasanoglu E, Altan N, Sindel S, Ongun CO, Bali M, Altuntas E. The relationship between erythrocyte superoxide dismutase activity and plasma levels of some trace elements (Al, Cu, Zn) of dialysis patients. *Gen Pharmac* 1994; 25: 107-110.
21. Weinstein T, Chagnac A, Korzets A, Boaz M, Ori Y, Herman M et al. Haemolysis in haemodialysis patients: evidence for impaired defense mechanism against oxidative stress. *Nephrol Dial Transplant* 2000; 15: 883-887.
22. Samouilidou E, Graspa E. Effect of dialysis on plasma total antioxidant capacity and lipid peroxidation products in patients with end-stage renal failure. *Blood Purificat* 2003; 21: 209-212.
23. Martin-Mateo MC, Del Canto-Jafiez E, Barrero-Martinez MJ. Oxidative stress and enzyme activity in ambulatory renal patients undergoing continuous peritoneal dialysis. *Ren Fail* 1998; 20: 117-124.
24. Martin-Mateo MC, Sanchez-Portugal M, Iglesias S, de Paula A, Bustamante J. Oxidative stress in chronic renal failure. *Ren Fail* 1999; 21: 155-167.
25. Drai J, Bannier E, Chazot C, Hurot JM, Goederet G, Jean G et al. Oxidants and antioxidants in long-term haemodialysis patients. *Farmacol* 2001; 56: 463-465.
26. Chugh SN, Jain S, Agrawal N, Sharma A. Evaluation of oxidative stress before and after haemodialysis chronic renal failure. *J Assoc Physicians India* 2000; 48: 981-984.
27. Seymen P, Seymen HO, Ozdemir A, Belce A, Gümüştaş K, Türkmen F et al. Cuprophan ve polisülfon dializörlerinin oksidan/antioksidan dengesi üzerine etkileri. *Cerrahpaşa J Med* 2000; 31: 74-81.
28. Usberti M, Lima G, Arisi M, Bufano G, D'Avanzo L, Gazzotti MR. Effect of exogenous reduced glutathione on the survival of red blood cells in hemodialyzed patients. *J Nephron* 1997; 10: 261-265.
29. Chen CK, Liaw JM, Juang JG, Lin TH. Antioxidant enzymes and trace elements in hemodialyzed patients. *Biol Trace Elem Res* 1997; 58: 149-157.
30. Ceballos Picot I, Witko Sarsat V, Merad Boudia M, Nguyen AT, Thevenin M, Jaudon MC et al. Glutathione antioxidant system as a marker of oxidative stress in chronic renal failure. *Free Radical Bio Med* 1996; 21: 845-853.