Hemodiyaliz Hastalarının Polimorfonükleer Lökositlerinin Oksidatif Stresi Üzerine Farklı Diyalizör Membranlarının Etkileri

Effect of Different Dialyzer Membranes on the Oxidative Burst Status of Polymorphonuclear Leukocytes in Hemodialysis Patients

Cem İlgen[1], Fatih Bulucu[1], Levent Yamanel[1], İzzet Yavuz[2], Volkan Inal[1], Ahmet Aydın[3], Abdülgaffar Vural[2]

[1]Department of Internal Medicine, Gülhane Military Medical Academy, Ankara
[1]Department of Nephrology, Gülhane Military Medical Academy, Ankara
[1]Department of Pharmaceutical Sciences, Gülhane Military Medical Academy, Ankara

ÖZET

Bu çalışmada, hemodiyaliz tedavisi gören son dönem böbrek yetmezliği hastalarında farklı diyaliz membranı (kuprofan ve polisülfon) kullanımının polimorfonükleer lökositlerde (PMNL) hücre içi antioksidan enzim aktiviteleri üzerindeki etkilerinin ve anormalliklerinin araştırılması amaçlanmıştır. Çalışmaya hemodiyaliz tedavisi gören 15 son dönem böbrek yetmezliği hastası dahil edilmiş ve birbirini takip eden iki hemodiyaliz seansında bir kez kuprofan (C) ve bir kez de polisülfon (P) membran ile diyalize alınmışlardır.

Her bir hemodiyaliz öncesi ve sonrası PMNL süperoksit dismutaz (SOD) ve glutatyon peroksidaz (GSH-Px) aktiviteleri saptanmıştır. Ayrıca karşılaştırmalar için 15 sağlıklı bireyden bir kontrol grubu oluşturulmuştur. Hastaların hemodiyaliz öncesi PMNL SOD (p=0.285 P diyalizi, p=0.512 C diyalizi) ve GSH-Px (p=0.285 P diyalizi, p=0.486 C diyalizi) aktiviteleri kontrol grubundan farklı bulunmamıştır. Benzer olarak, hastaların hemodiyaliz sonrası PMNL SOD (p=0.838 P diyalizi, p=0.744 C diyalizi) ve GSH-Px (p=0.512 P diyalizi, p=0.870 C diyalizi) aktiviteleri de kontrol grubundan farklı bulunmamıştır. Her iki tür membran ile uygulanan hemodiyaliz PMNL SOD (p=0.460 P diyalizi, p=0.532 C diyalizi) ve GSH-Px (p=0.773 P diyalizi, p=0.570 C diyalizi) aktivitelerinde istatistiksel olarak anlamlı bir değişikliğe neden olmamıştır. Sonuç olarak, son dönem böbrek yetmezlikli, hemodiyaliz tedavisi gören hastalar ve kontroller arasında PMNL oksidatif stresi açısından bir fark yoktur. Ayrıca, hemodiyaliz hastalarında sentetik (polisülfon) ve selüloz (kuprofan) membran kullanımı PMNL SOD ve GSH-Px enzim aktiviteleri üzerinde farklılığa neden olmamaktadır.

Anahtar sözcükler: nötrofil, oksidatif stres, hemodiyaliz hastaları

Yazışma adresi: Dr. Fatih Bulucu
Ataç Sokak 52/14
TR-06420 Kızılay, Ankara, Turkey
Tel: 0 (312) 304 40 03
Faks: 0 (312) 323 49 23
E-posta: fbulucu@yahoo.com

ABSTRACT

In the present study, it was aimed to investigate whether there is an abnormality in intracellular antioxidant enzyme activities of polymorphonuclear leukocytes (PMNL) of end-stage renal disease patients who had been treated with hemodialysis and different types of dialyzer membranes (cuprophane and polysulphone) affect these enzyme activities. Fifteen end-stage renal disease patients undergoing hemodialysis treatment were enrolled into the study and they were dialyzed with polysulphone (P) and cuprophane (C) membranes in two consecutive hemodialysis sessions. Superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) activities in PMNLs before and after each hemodialysis session were determined. A control group was also consisted of 15 healthy subjects to perform the comparisons. Predialysis PMNL SOD (p=0.285 for P dialysis, p=0.512 for C dialysis) and GSH-Px (p=0.285 for P dialysis, p=0.486 for C dialysis) activities of the patients were not different from those of the controls. Similarly, postdialysis PMNL SOD (p=0.838 for P dialysis, p=0.744 for C dialysis) and GSH-Px (p=0.512 for P dialysis, p=0.870 for C dialysis) activities of the patients did not show difference from the data of the controls. Hemodialysis with both types of the membranes did not cause a statistically significant change in PMNL SOD (p=0.460 for P dialysis, p=0.532 for C dialysis) and GSH-Px (p=0.773 for P dialysis, p=0.570 for C dialysis) activities. In conclusion, there is no difference between end-stage renal disease patients under the hemodialysis treatment and the controls with respect to PMNL oxidative stress status. Furthermore, it also seems that both synthetic (polysulphone) and cellulose (cuprophane) membranes have no effect on SOD and GSH-Px enzyme activities in the PMNLs of the hemodialysis patients.

Keywords: neutrophils, oxidative burst, hemodialysis patients

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Introduction

The incidence of bacterial infections has been reported to increase in chronic renal failure patients treated with hemodialysis (HD) and continuous ambulatory peritoneal dialysis (CAPD) (1-3). Although advances have been achieved in the treatment of chronic renal failure, increased risk of infection in these patients suggests a defect of defense mechanisms in the host. Studies investigating the mechanisms responsible for this condition have been continued and reduced functional capacity of the phagocytic leukocytes have been implicated as the disorder in defense mechanisms of these patients (1,4). The results of the studies on phagocytic and oxidative burst activities of the granulocytes in the uremic patients are in conflict. There are many studies reporting decreased (4-11), increased (12-18) and unchanged (19-22) oxidative activity in the PMNLs of the uremic patients. In the present study, oxidative burst status of PMNLs in HD patients and the effect of HD treatments, performed with two different membrane types in two consecutive sessions, on PMNL oxidative metabolism in these patients were investigated.

Materials and Methods

Fifteen (12 males and 3 females) chronic renal failure patients who had been treated with HD were taken into the study. Their ages ranged between 25-67 years and the mean was 42.8 ± 14.5 years. Mean HD duration was 27.7 ± 21.9 months (range 6-74 months). None of them had an infectious disease or a systemic disease at the time of the study. They were not under treatment with antibiotics, steroids or any immunosuppressive drug. The control group was consisted of 15 healthy persons (12 males and 3 females). The mean age of the controls was 34.0 ± 5.7 years (range 24-48).

The patients had been treated with HD for 4 hours in each dialysis session, thrice weekly. According to the study design, the patients were hemodialyzed with polysulphone (Hemoflow F5, Fresenius Polysulphone, UF:4.0, 1.2 m^2, Germany) and cuprophane (Baxter CF Capiller Flow Dialyzer Model 15.11, 1.2 m^2, USA) membranes in their two scheduled consecutive HD sessions, respectively. Just before and 1 hour after every HD session, 10 ml venous blood samples from the patients were collected into the heparinized tubes (Lithium heparin, 454029/A020102 Greiner bio-one). Venous blood samples from the patients were diluted with 3 ml phosphate buffer saline (PBS) and 7 ml Histopaque (d: 1.119-1, Histopaque, Sigma Diagnostics, Inc. St. Louis, MO 63178, USA) were added. The mixture was centrifuged at 500 g for 30 minutes at room temperature. Following the centrifugation, there were several layers in the mixture. These were erythrocyte and platelets, Histopaque, mononuclear cells, PBS, PMNLs and plasma layers, respectively, from bottom to top in each tube. The PMNL layer was aspirated with a capillary Pasteur pipette and PMNLs were diluted with 2 ml Hank's solution (HBSS, Biological Industries, Beit Haemeks, Israel). This solution was centrifuged at 250 g for 5 minutes and the supernatant was removed by aspirating. The cell layer at the bottom of the tube was diluted with 2 ml HBSS and centrifuged at 250 g for 5 minutes. The latter was repeated one more time. Subsequently, in order to remove the red blood cells completely, 3 ml lysing solution (Lysing Solution, San Jose, CA 95131, USA) was added and the mixture was incubated for 10 minutes. After the incubation period, this solution was recentrifuged at 250 g for 5 minutes and the final PMNL layer was resuspended by diluting it with foetal calf serum (Biomedical Industries, UK) and Hank's solution. Isolated PMNLs in a suspension were counted in a Coulter counter. PMNLs were isolated from the whole blood in one hour. The same procedure was also performed in venous blood samples of the controls. Isolated PMNL suspensions were stored at -80°C until the assay.

The samples from the patients and the controls were studied for their superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) activities in the same batch. PMNL suspension was homogenized in a glass homogenizator with 50 mM potassium phosphate buffer (pH 7.8) cooled with ice. Then, the solution was centrifuged at 1000 g for 10 minutes at +4°C to prepare the cell lysate. GSH-Px activities in PMNL lysate were measured by the method of Aydın et al (23). Reaction mixture was 50 mmol/L Tris buffer, pH 7.6 containing 1 mmol/L of Na_2 EDTA, 2 mmol/L of reduced glutathione (GSH), 0.2 mmol/L of NADPH, 4 mmol/L of sodium azide and 1000 U of glutathione reductase (GR). Fifty microlitre of lysate and 950 µL of reaction mixture were mixed and incubated for 5 minutes at 37°C. Then, the reaction was initiated with 8.8 mmol/L H_2O_2 and the decrease in NADPH absorbance at 340 nm was followed

for 3 minutes. Enzyme activities were reported as U/μL in PMNL lysate. CuZn-SOD activity in PMNL lysate was also measured by the method described by Aydın et al (23). PMNL lysate samples were diluted with 10 mM phospate buffer pH 7.0 about 400 fold. Twenty-five microlitre diluted PMNL lysate samples were mixed with 850 μL substrate solution containing 0.05 mmol/L xanthine and 0.0025 mmol/L 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyl tetrazolium chloride (INT) in a buffer solution containing 50 mmol/L CAPS and 0.94 mmol/L EDTA pH 10.2. Then, 125 μL xanthine oxidase (80 U/L) was added to the mixture and absorbance increase was followed at 505 nm for 3 minutes against air. Twenty-five microlitre phosphate buffer or 25 μL various standard concentrations in place of sample were used as blank and standard determinations. CuZn-SOD activity was expressed as U/μL. Subsequently, enzyme activities in lysate samples were calculated for 10^7 PMNLs and the comparisons were performed. The enzyme activities in pre- and postdialysis PMNLs of the patients in both hemodialysis sessions were compared with those of the healthy controls. Then, pre- and postdialysis PMNL enzyme activities of the patients were also compared for each HD session performed with different types of the membranes.

Statistical analyses were done by SPSS (Statistical Package for the Social Sciences Program) statistical program. The enzyme activity results were expressed as mean ± standard error. Fisher's exact (for the comparison gender between the patients and the controls), Mann-Whitney U (for the comparisons between the patients and the controls) and Wilcoxon (for the pre- and postdialysis enzyme activity comparisons) tests were used in the comparisons. P values less than 0.05 were accepted statistically significant.

Results

The characteristics of the patients and the controls are shown in Table I.

There was no difference between the patients and the controls with respect to gender (x^2 = 0.000, p=1.000) and age (p= 0.137).

Table II demonstrates the PMNL SOD and GSH-sand the controls.

Predialysis PMNL SOD activities of the patients were not significantly different from those of the controls (p=0.285 for polysulphone dialysis, p=0.512 for cuprophane dialysis). The difference between the PMNL SOD activities of the patients and controls after the HD sessions was not statistically significant either (p=0.838 for polysulphone dialysis, p=0.744 for cuprophane dialysis). Pre- (p=0.389 for polysulphone dialysis, p=0.486 for cuprophane dialysis) and postdialysis (p=0.512 for polysulphone dialysis, p=0.870 for cuprophane dialysis) PMNL GSH-Px activities of the patients and

Table I. The characteristics of the patients and the controls

	Patients	Controls
n	15	15
Female/male (n)	3/12	3/12
Mean age (years)*	42.8 ± 14.5	34.0 ± 5.7
HD duration (months)*	27.7 ± 21.9	

*Mean ± standard deviation

Table II. PMNL SOD and GSH-Px activities of the patients (pre-and postdialysis) and the controls.

	Patients (n=15)				Controls (n=15)
	Polysulphone		Cuprophane		
	Predialysis	Postdialysis	Predialysis	Postdialysis	
SOD (U/10^7PMNL)	85.79 ± 25.60	60.42 ± 21.32	84.73 ± 29.73	52.50 ± 15.02	59.64 ± 26.75
GSH-Px (U/10^7PMNL)	25.08 ± 7.31	18.81 ± 3.47	27.04 ± 7.06	22.47 ± 6.89	17.42 ± 4.19

those of the controls did not show a statistically significant difference. Although HD seemed to lead a reduction in PMNL SOD (p=0.460 for polysulphone dialysis, p=0.532 for cuprophane dialysis) and GSH-Px (p=0.773 for polysulphone dialysis, p=0.570 for cuprophane dialysis) activities of the patients, the differences were not statistically significant.

Discussion

An increased PMNL oxidative activity in HD patients has been reported in some studies (14,15, 17,18). Enhanced predialytic PMNL H_2O_2 production in HD patients was observed by Jacobs et al (14). Another group (15) also suggested that PMNLs from regular dialysis patients have an increased reactive oxygen metabolite production in the resting state that may cause cell and tissue damage. Increased SOD but decreased GSH-Px activities in granulocytes of HD patients before dialysis session was reported in a study (17); however, after dialysis, SOD and GSH-Px activities were significantly induced by HD in this study. In another study (18), PMNLs from HD patients exhibited increased baseline H_2O_2 production compared with PMNLs from the normal subjects. On the other hand, a number of studies (6,9,11,24) indicated a decrease in PMNL oxidative activity in HD patients. Two studies (6,24) reported decreased PMNL superoxide anion generation in HD patients and superoxide anion generation by these cells decreased as the time of HD advanced. In the study of Swirski et al (9), the activity of SOD and GSH-Px was reduced in HD patients. Although unstimulated enzyme activity was similar to that of control in a study (11), activity after stimulation was significantly lower than the control. Based upon these results, they concluded that neutrophils in dialysis patients have diminished intracellular oxidative burst. In the study we present here, pre- and post-hemodialysis PMNL SOD and GSH-Px activities of the regular HD patients were compared to those of the controls and no statistically significant difference was found. The indirect evidences of an unchanged oxidative burst status in HD patients when compared with normal subjects in our study are in agreement with the data of Paul et al (19). In their study, resting values of PMNL superoxide anion production and oxygen consumption in HD patients were not significantly different from values for PMNLs from normal subjects, before and after the 1st, 4th and 10th dialysis sessions.

In the present study, PMNL SOD and GSH-PX activities of the regular HD patients showed statistically nonsignificant increases between two consecutive HD sessions performed with different dialyzer membranes on alternate days. This tendency is believed to support the data of some authors (14,15, 17,18), which suggest an increased PMNL oxidative burst activity in HD patients. Two authors (13,18) implicated serum factor(s) capable of reversibly enhancing PMNL oxidative metabolism in sera of chronic renal failure patients. It can be speculated that if the interval between the two consecutive HD sessions were longer in our study, it would be possible to observe the significantly increased predialysis enzyme activites in HD patients compared to those of the controls.

Although it has been suggested that granulocytes are activated on the surface of dialyzer membranes and generate free radicals (17), the data of us and those of some others (6,14,19) do not support this view. Our data yielded nonsignificant reductions in the PMNL SOD and GSH-PX activities of the patients following dialysis sessions. Dialysis with cuprophane membrane lowered PMNL SOD activities more effectively (38.1% vs 29.5%) while polysulphone membrane seemed superior to reduce the PMNL GSH-PX activities (25.0% vs 17.0%). However, these reductions did not achieve a statistical significance. In the same manner, some authors (14,19,6) also reported that HD was not effective to alter the predialysis oxidative burst status of the HD patients. Jacobs (14) observed that induced complement activation in the initiation of HD session did not cause further increase in PMNL oxidative burst activity of HD patients when compared to predialysis values. However, in another study (17), it was found that HD performed with low-flux polysulphone membranes significantly induced PMNL SOD and GSH-Px activities. In conclusion, there is no difference between regular hemodialysis patients and the controls with respect to PMNL oxidative burst status. Furthermore, polysulphone and cuprophane dialyzer membranes do not cause a change in PMNL oxidative burst status of hemodialysis patients and activities and both types of the membranes also do not seem to be different from each other in regard of their effects on PMNL SOD and GSH-Px enzyme in these patients.

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