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LIPID PEROXIDATION AND THE ANTIOXIDANT CAPACITY OF DIALYSIS PATIENTS: The Effects of a Single Hemodialysis Session with Different Dialysis Membranes

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ABSTRACT

Objective: To estimate lipid peroxidation and the antioxidant defense capacity of dialysis patients and the effects of different types of dialysis membranes on these parameters.

Methods: Fifty-four dialysis patients and 30 healthy controls were included in this study. Ten of the dialysis patients were on continuous ambulatory peritoneal dialysis treatment and the rest were on hemodialysis with either polycarbonate membrane (n=10) or hemophan membrane (n=34). Polycarbonate membranes were switched with a vitamin E-coated dialyzer in the subsequent dialysis session. Total antioxidant status and malondialdehyde levels were studied to determine the antioxidant defense capacity and lipid peroxidation, respectively, before and after the dialysis session.

Results: Plasma total antioxidant status levels were lower (1.51±0.2 mmol/l vs. 1.75±0.20 mmol/l $p<0.05$) and malondialdehyde levels were higher (2.2±1.17 nmol/ml vs. 0.60±0.20 nmol/ml $p<0.05$) in all dialysis patients compared to the control group. After one hemodialysis session, there were no significant alterations in parameters for either type of dialysis membrane.

Conclusion: All dialysis patients have an increased oxidative status. A single hemodialysis session with different dialysis membranes does not seem to significantly change the oxidant or antioxidant levels.

Key Words: antioxidant status, malondialdehyde, hemodialysis, peritoneal dialysis.

DİYALİZ HASTALARINDA LİPİD PEROKSİDASYON VE ANTİOKSİDAN KAPASİTESİ: Farklı Diyaliz Membranları ile Yapılan Tek Bir Diyaliz Seansının Etkileri

ÖZ

Amaç: Bu çalışmanın amacı diyaliz hastalarının lipid peroksidasyon ve antioksidan savunma kapasitelerini tespit etmek ve değişik tipteki diyaliz membranlarının bu parametreler üzerine etkilerini göstermektir.

Metot: Çalışmaya 54 diyaliz hastası ve kontrol grubu olarak 30 sağlıklı birey alındı. 54 diyaliz hastasının 10'u periton diyalizi, 44'ü hemodiyaliz hastasından oluşmaktaydı. Hemodiyaliz hastaları polikarbonat (n=10) ya da hemophan membran (n=34) kullanılarak hemodiyalize alınmaktaydı. Polikarbonat membran kullanılan grupta, sonraki diyaliz seansında membranlar vitamin-E kaplı dializer ile değiştirildi. Antioksidan savunma kapasitesi ve lipid peroksidasyonunu belirlemek için total antioksidan durumu ve malondialdehid seviyeleri diyaliz öncesi ve diyaliz sonrasında çalışıldı.

Sonuçlar: Kontrol grubu ile karşılaştırıldığında tüm diyaliz hastalarında plazma total antioksidan seviyesi daha düşük (1.51±0.2 mmol/L 'ye karşı 1.75±0.20 mmol/L $p<0.05$) ve malondialdehit seviyesi daha yüksek (2.2±1.17 nmol/mL' ye karşı 0.60±0.20nmol/mL $p<0.05$) bulundu. Bir hemodiyaliz seansı sonrasında tüm diyaliz membranları için parametrelerde anlamlı değişiklik tespit edilmedi.

Sonuç: Tüm diyaliz hastaları artmış bir oksidatif duruma sahiptirler. Farklı tipte diyaliz membranları ile yapılan tek bir diyaliz seansı oksidan ve anti-oksidan seviyelerini anlamlı olarak değiştirmiyor gibi gözükmektedir.

Anahtar Kelimeler: Antioksidan Durum, Malondialdehit, Hemodiyaliz, Periton Diyalizi.

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INTRODUCTION

Atherosclerotic cardiovascular diseases are recognized as the major cause of morbidity and mortality in uremic patients. During hemodialysis, complement and leukocyte activation through contact with artificial membranes promotes the production of free radicals, which are known to be involved in the pathogenesis of atherosclerosis (1-3). Oxidative stress, which occurs when there is excessive free radical production or low antioxidant levels, has been reported in patients with chronic renal failure (CRF) treated by hemodialysis or continuous ambulatory peritoneal dialysis (CAPD) (4-6). Khoa et al. have shown that the inflammatory status and duration of dialysis treatment are the most important factors related to oxidative stress in hemodialysis patients (7). In order to decrease membrane bioincompatibility and thereby minimize oxidative stress in hemodialysis patients, more compatible filters have been elaborated. Among the most recent compatible filters, vitamin E-coated membranes have been proposed. Preliminary characterization of vitamin E-coated membranes has shown a decreased activation of polymorphonuclear leukocytes and monocytes, a lower free radical production, and a higher biocompatibility. Lipid peroxidation in plasma and red blood cells was lower after a 30-day period use of this membrane in hemodialysis patients (8). In recent studies, it has been shown that oral vitamin E supplementation or the use of vitamin E-coated membranes results in an increase in the activities of antioxidant enzymes and decreased lipid peroxidation in plasma and erythrocytes (9,10).

The aim of this study was to determine the lipid peroxidation and the antioxidant defense capacity in dialysis patients and the effect of different dialysis membranes on these parameters during one dialysis session. The blood oxidative stress status was classically assessed both by plasma lipid peroxidation products determined as malondialdehyde and by antioxidant defenses. In order to make a global assessment of the antioxidant defenses, we also measured the plasma total antioxidant status before and after dialysis.

METHODS

Fifty-four dialysis patients (female/male: 18/36, mean age: 48±14 years) and 30 healthy controls (female/male: 10/20, mean age: 42±12 years) were included in this study. The hemodialysis patients were dialyzed using bicarbonate-buffered dialysate, three times a week, with each session lasting 4 hours. The underlying etiology of end-stage renal disease included chronic glomerulonephritis (n: 15), chronic pyelonephritis (n: 12), nephrosclerosis (n: 7), obstructive uropathy/reflux nephropathy (n: 4), cystic disease (n: 2), amyloidosis (n: 2), and unknown (n: 12). Smokers, diabetics, the patients with chronic inflammatory conditions or hepatic or respiratory disease, and those receiving antioxidant vitamin or fish-oil supplements were excluded. Patients gave their informed

consent to be included in the study. Ten of the dialysis patients were on CAPD (mean dialysis duration: 18±8 months) (group 1) and the remaining patients were on hemodialysis treatment (mean dialysis duration: 54±10 months) with polycarbonate membrane (10 patients) (group 2) and hemophan membrane (34 patients) (group 3). The patients in group 2 were switched to a vitamin E-coated dialyzer (Excebrane) in the subsequent dialysis session (group 4). Malondialdehyde levels were determined for the detection of antioxidant defense capacity, total antioxidant status and for lipid peroxidation products. Total antioxidant status (TAS) and malondialdehyde (MDA) levels were measured before and after a single dialysis session. Twenty-nine HD patients and 3 CAPD patients were receiving regular subcutaneous erythropoietin. Ten HD patients and 2 CAPD patients were on oral or parenteral iron therapy.

TAS Assay

In hemodialysis patients, the first blood samples were obtained after an overnight fast and immediately prior to the dialysis session. The second samples were taken after the session from each patient. In CAPD patients, the blood samples were taken before exchange in the morning. All blood samples were centrifuged at 5000 xg for 5 min at 4 ºC and then the supernatants were immediately snap frozen and stored at –40 ºC. TAS was measured by ABTS (2,2'–azino-di-(3-ethylbenzthiazoline-6-sulphonic acid) method (Randox, UK). After incubation of ABTS with peroxidase (metmyoglobin) and H2O2, a radical inducer ABTS+ was formed. This reaction produces a stable blue-green color, which can be detected at 600 nm. When antioxidant was added to the medium, the formation of the color was pressed. The reactions were as follows:

$HX\text{-}Fe^{3}+H2O2 \rightarrow x\text{-}[Fe^{4}=O]+H2Ox, \rightarrow ABTS+X\text{-}[Fe^{4}=O] \rightarrow ABTS+ + HX\text{-}Fe^{3}$

$HX\text{-}Fe^{3}$=Metmyoglobin, $\rightarrow x\text{-}[Fe^{4}=O]$ = Ferrylmyoglobin.

MDA Assay

Milton Roy spectronic S-3000 with TBARS (thiobarbituric acid-reactive substances, Sigma) was used to measure MDA levels. For the detection of MDA, 0.5 ml of plasma was mixed with 2.5 ml of 20% trichloroacetic acid in a 10 ml centrifuge tube. Following immediate cooling, 1 ml of 0.62% thiobarbituric acid was added to the mixture. This was heated for 30 min in boiled water. After mixing with 4 ml of n-butyl alcohol, the mixture was centrifuged at 3000 xg for 5 min. Plasma MDA level was detected by calorimeter at 525 and 550 nm. The differences in the two levels of absorbency were calculated as plasma MDA (nmol/kg).

Statistical Analysis

The results are expressed as means ± SD. To determine the significance between different groups, one-way analysis of variance was performed, followed by the Tukey post hoc test. Unpaired Student's t-test was used to compare the results between the patients and the control group. Pre- and post-dialysis results were compared using a paired t-test. P value <0.05 was considered to be significant.

RESULTS

Plasma TAS levels were lower and MDA levels were higher in CAPD and hemodialysis patients compared to the control group (Table 1). There were statistically significant differences between the control group and CAPD patients (p: 0.02 for TAS p: 0.00 for MDA), and hemodialysis patients (p: 0.00 for TAS p: 0.00 for MDA) for these parameters. There were no statistically significant differences between CAPD and hemodialysis patients for TAS and MDA. The mean TAS and MDA levels before and after hemodialysis with different types of membranes are demonstrated in Table 2. We found no differences in terms of TAS or MDA levels between the groups.

DISCUSSION

Table 1: Results of plasma malondialdehyde (MDA) and total antioxidant status (TAS) levels in dialysis patients and healthy subjects.

	Peritoneal dialysis n:10	Hemodialysis n:44	Control group n:30	P
TAS (mmol/l)	1.49±0.08*	1.49±0.03**	1.75±0.07	0.00
MDA (nmol/kg)	2.22±0.60**	2.20±0.15**	0.59±0.06	0.00

*p: 0.02 versus controls, **p: 0.00 versus controls

Table 2: The effects of different dialysis membranes on total antioxidant status (TAS) and malondialdehyde (MDA) levels of hemodialysis patients.

	Group 2	Group 3	Group 4	ANOVA, p
TAS				
Before HD	1.44±0.26	1.52±0.16	1.42±0.22	>0.05
After HD	1.58±0.22*	1.51±0.25*	1.52±0.27*	>0.05
MDA				
Before HD	2.03±0.70	2.24±1.20	2.20±0.97	>0.05
After HD	1.79±0.75*	2.41±1.05*	1.99±0.67*	>0.05

*p>0.05 versus values at the beginning of hemodialysis for MDA and TAS levels

Oxygen radicals are toxic and are thought to be involved in the pathogenesis of a variety of diseases including atherosclerosis, diabetes mellitus, and cancer (5). In recent years, numerous studies have focused on detection of signs of oxidative stress in renal patients. There is good evidence indicating that uremia in general is associated with enhanced oxidative stress, and treatment of uremic patients with hemodialysis or peritoneal dialysis has been suggested to particularly contribute to oxidative stress and reduced antioxidant levels in these patients. Hemodialysis membrane induced activation of macrophages on the surface of dialysis membranes during the dialysis session may result in oxidative stress. Loss or deficiency of antioxidant activity could also contribute to enhanced oxidative stress in uremia (1,11).

Our study demonstrated that patients on dialysis for a long period have high MDA levels and low antioxidant capacity regardless of the structure of the dialysis membranes used. There was no significant effect of a single dialysis session on these parameters. Consequently, oxidant status seems to be unrelated to the structure of membrane, semisynthetic or synthetic. There seems to be no significant difference between peritoneal dialysis and hemodialysis patients. Although the number of cases is small, the high oxidant stress and the low TAS activity in the peritoneal dialysis patients using the natural membrane suggest that the dialysis membrane is not the only factor responsible for the increased oxidative stress in these patients. Regardless of the underlying reason, the use of vitamin E-coated membranes could help to minimize the increased oxidative stress along with the increased risk of atherosclerotic cases in dialysis patients.

Mune et al. have shown that the use of vitamin E coated cellulose membrane dialyzers for 6 months resulted in a significant reduction in low density lipoprotein –MDA (LDL-MDA) and oxidized-LDL compared to the ordinary cellulose membrane dialyzer (12). In order to decrease membrane bioincompatibility and thereby minimize oxidative stress in hemodialysis patients, more compatible filters have been elaborated. Preliminary characterization of vitamin E-coated membranes has shown decreased activation of polymorphonuclear cells and monocytes, lower free radical production, and high biocompatibility. Lipid peroxidation in plasma and red blood cells decreased after a 30-day period of use of this membrane in hemodialysis patients (6).

In our study, TAS was found to be lower in dialysis patients than in the healthy control subjects, and MDA levels were significantly higher in dialysis patients than in the controls. Increased MDA level, which is the consequence of lipid peroxidation, and decreased plasma TAS level, a marker of impaired antioxidant defense mechanism, both provide evidence of exaggerated oxidative stress in dialysis patients. Our findings confirm that oxidative stress is enhanced, free radical formation increases, and antioxidant defense mechanism decreases in dialysis patients. CAPD and hemodialysis treatments seem to have no superiority over each other since both groups had low plasma TAS levels, but high MDA levels. After a single hemodialysis session, plasma MDA and TAS levels did not show any significant change. The same results were obtained using both traditional membranes and the vitamin E-coated membranes. Considering that vitamin E has a strong antioxidant characteristic, it might be expected that the dialysis that is applied in vitamin E-coated membranes could show an antioxidant characteristic in the long term. Studies in which these membranes were used have shown antioxidant characteristics after at least 30 days' use.

In conclusion, we found increased oxidative stress in both CAPD and hemodialysis patients. We could not demonstrate any significant difference in antioxidant capacity between different dialysis membranes during one dialysis session. The long-term results of dialysis with vitamin E-coated membranes are expected to show useful results with regard to the antioxidant effect.

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