Evaluation of Oxidative Damage During Hemodialysis with Different Dialysis Membranes

FARKLI DİALİZ MEMBRANLARI İLE HEMODİYALİZDE OKSİDATİF HASARIN DEĞERLENDİRİLMESİ

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Summary

Purpose:Oxidative/antioxidative balance is changed in patients undergoing chronic hemodialysis. The aim of this study was to evaluate the lipid peroxidation and antioxidative enzyme activities for different membranes in patients with chronic renal failure, before and after hemodialysis.

Materials and Methods: Serum MDA levels and erythrocyte GSH-Px, SOD activities were measured from 76 patients that underwent hemodialysis with different dialysing membranes (polysulfone, hemophane, cellulose). Statistical analysis was performed by using Student's t test.

Results:There was a statistically significant difference between the MDA levels determined before and after the hemodialysis, MDA levels decreased significantly after hemodialysis ($p<0.005$). Similarly, there was also an increase in the GSH-Px (pO.001) and SOD ($p<0,05$) enzyme activities after hemodialysis when compared to the levels determined before hemodialysis. In hemodialysis with 3 different membrane type; hemodialysis with polysulfone membrane induced significant changes in plasma MDA ($p<0.005$). Erythrocyte SOD and GSH-Px activities increased insignificantly in hemodialysis with 3 different membrane type.

Conclusion: Our results suggest that use of polysulfone membranes for hemodialysis prevents the formation of free radicals and consequently increases the antioxidant enzyme activities.

Key Words: Antioxidative enzymes, Dialysis membrane, Lipid peroxidation

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Özet

Amaç: Kronik hemodiyaliz tedavisi gören hastalarda oksidatif/antioksidatif denge değişir. Bu çalışmanın amacı; kronik renal yetmezliği olan hastalarda diyaliz öncesi ve sonrası lipid peroksidasyonu ve antioksidan enzim aktivitelerini farklı membranlar kullanarak değerlendirmektir.

Materyal ve Metod: Serum MDA düzeyleri ve eritrosit GSH-Px ve SOD aktiviteleri farklı diyaliz membranları (polisülfon, hemofan, selüloz) ile diyalize giren 76 hastada ölçülmüştür. İstatistiksel analizler Student's t testi ile yapılmıştır.

Bulgular: Hemodializ öncesi ve sonrasında ölçülen MDA düzeyleri bakımından anlamlı bir farklılık bulunmuş olup MDA düzeyleri hemodiyaliz sonrasında azalmıştır ($p<0,005$). Benzer şekilde antioksidan enzimlerden GSH-Px ($p<0,001$) ve SOD aktiviteleri ($p<0,05$) hemodiyaliz öncesi ile karşılaştırıldığında diyaliz sonrasında anlamlı olarak artmıştır 3 farklı membran ile hemodiyaliz yapıldığında, polisülfon membran ile diyaliz plazma MDA düzeylerinde istatistiksel olarak anlamlı bir değişime neden olur ($p<0.005$). Eritrosit antioksidan enzim aktiviteleri 3 farklı membran tipi ile hemodiyaliz sırasında istatistiksel açıdan anlamlı olmayan bir artış göstermiştir.

Sonuç:Sonuçlarımız, hemodiyaliz sırasında serbest radikal oluşumunu önlemek ve antioksidan aktiviteyi artırmak için polisülfon membran kullanılmasının daha uygun olduğunu göstermiştir.

Anahtar Kelimeler: Antioksidatif enzimler, Dializ membranı, Lipid peroksidasyonu

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Chronic renal failure is accompanied by a complex pathology. Some manifestations such as accelerated aging, cataract, atherosclerosis, impaired red blood cell deformability, increased hemolysis and platelet dysfunction may be related to the hyperproduction of free radicals. Healthy organisms are protected by several defence mechanisms against oxygen free radicals generated by exogenous agents or by endogenous metabolic factors. These defence mechanisms include scavenger enzymes (e.g. superoxide dismutase, glutathione peroxidase) (1,2).

Hemodialysis used to compensate deficient renal function in patients with chronic renal failure, is not totally harmless for blood cells. It has been reported that dialysis membranes used in hemodialysis alter the oxidative metabolism of polymorphonuclear leukocytes. The mobilization of the NADPH oxidase system of these cells and the overproduction of NAD lead to the formation of hydroxy l radicals and superoxide anions. These radicals, with a very short half life are eliminated by metalloenzymes: superoxide dismutase and glutathione peroxidase (3). In this study the changes of oxidative metabolism and membrane biocompatibility were evaluated during hemodialysis.

Material and Methods

Collection of samples: Seventy- six patients (37 men and 39 women, ages 25-65 years, median 45) receiving regular hemodialysis for end stage renal failure were recruited. Patients with diabetes, chronic respiratory insufficiency, ischemic heart disease, hepatic disorders or intercurrent infection were excluded from the study. A11 patients had been on regular hemodialysis for at least 2 months and several groups of patients underwent dialysis twice weekly with different dialyzing membranes (polysulfone, n: 26, hemophane, n: 32, cellulose asetate, n: 18). Serum creatinine levels and hemoglobin concentrations of patients were found to be 11,0 ± 2.6 mg/dl and 7.8+ 1.8 g/dl, respectively . Venous blood samples were taken immediately before and after hemodialysis. Blood samples were taken into tubes containing K_3-EDTA and samples were immediately centrifuged at 1500 x g for 5 minutes to separate plasma and erythrocytes at 4 °C. Erythrocytes were washed three times with cold isotonic saline (%0.9 NaCl), and then diluted with saline to the orginal blood volume. Erythrocyte samples were stored at -70 °C for measurement of GSH-Px and SOD activities.

Measurement of MDA levels: Plasma lipid peroxide levels were measured colorimetrically by the thiobarbituric acid method which was modified from the methods of Satoh (4) and Yagi (5). MDA results were expressed as nanomoles per milliliter.

Measurement of erythrocyte GSH-Px levels: The enzyme activity was measured using the method of Paglia and Valentina in which GSH-Px activity was coupled with the oxidation of NADPH by glutathione reductase (6). The oxidation of NADPH was followed spectrophotometrically at 340 nm and at 37 °C. The activity was calculated from the slope of the lines as micromoles of oxidized NADPH per minute. GSH-Px activities were expressed as unit per liter.

Measurement of erythrocyte SOD levels: The enzyme activity was measured with a RANSOD Kit (Randox Laboratories Ltd, UK). SOD activities were expressed as unit per milliliter.

Statistical analysis was performed by Student's t test for a group comparison and the results were expressed as mean ± SD. Differences at the $p<0.005$ and pO.OOl levels were considered to be statistically significant.

Results

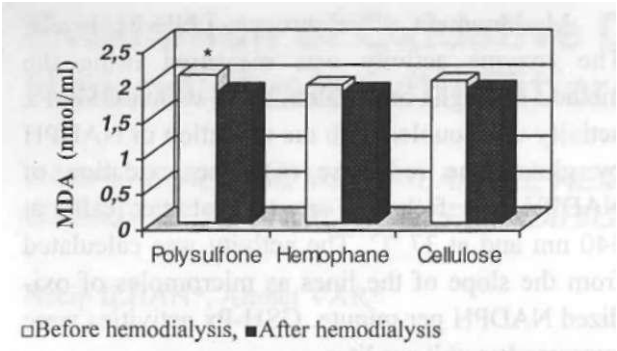
Mean plasma MDA levels and erythrocyte GSH-Px , SOD activities before and after hemodialysis are shown in Table 1. There was statistically significant difference between the levels of each parameter before hemodialysis and after hemodialysis ($p<0.005$, pO.OOl, $p<0.05$, respectively). Mean plasma MDA levels before hemodialysis were significantly higher than the values after hemodialysis ($p<0.005$). Conversely erythrocyte GSH-Px and SOD activities were significantly lower before hemodialysis when compared with the after hemodialysis ($p<0.001$, $p<0.05$).

Plasma MDA levels significantly decreased

Table 1. Plasma MDA levels and erythrocyte GSH-Px, SOD activities

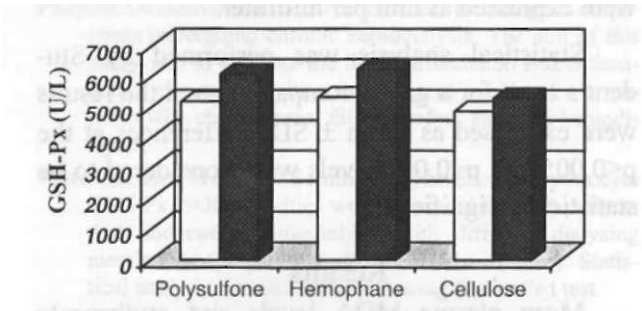
	MDA (nmol/ml)	GSH-Px (U/L)	SOD (U7 ml)
Before Hemodialysis	2,13 ±0.58* (n : 76)	5064 ± 1252** (n : 76)	120.3152.7*** (n : 76)
After Hemodialysis	1,84± 0.66 (n :76)	594811252 (n : 76)	138.3163.9 (n : 76)

*p<0.005, ** pO.OOl , *** p<0,05



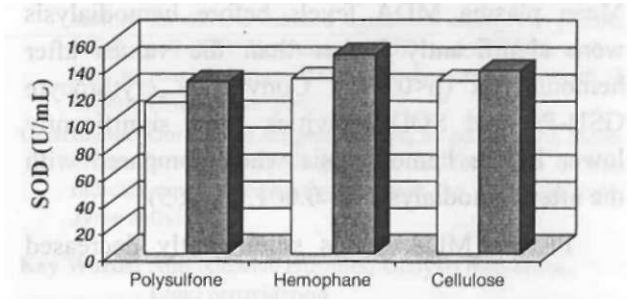
□Before hemodialysis, ■After hemodialysis

Figure 1. Effect of hemodialysis membranes on MDA levels.
* $p<0.005$ compared to before-HD



□Before hemodialysis, ■After hemodialysis

Figure 2. Effect of hemodialysis membranes on erythrocyte GSH-Px activities.



□Before hemodialysis, ■After hemodialysis

Figure 3. Effect of hemodialysis membranes on erythrocyte SOD activities.

($p<0.005$) during hemodialysis with the polysulfone membranes while no significant changes in the plasma MDA levels were observed with the hemophane and cellulose asetate membranes (Figure 1). Erythrocyte GSH-Px and SOD activities were found to be higher during hemodialysis with the different dialysis membranes in relation to predialysis value while the changes in the enzyme activities were insignificant (Figure 2 and 3).

Discussion

Chronic renal failure is a slowly progressing disease that leads to continuous loss of renal functions and glomerular filtration rate as well as enhancing the excessive production of free radicals and the development of atherosclerosis, aging, cardiac disease and cataract formation. In the present study, when comparing the MDA levels in patients with chronic renal failure before and after hemodialysis, a significant decrease was detected in MDA levels after hemodialysis. The decrease in MDA levels was related to the decreased complications of chronic renal failure following hemodialysis and this finding was consistent with the results of similar investigations (7,8).

In this study, plasma MDA level was compared in the dialysis with different membrane types and as shown in Figure 1, hemodialysis performed by polysulfone membrane was found to be more effective than the other two membrane types. Consequently, plasma MDA levels significantly decreased following hemodialysis with polysulfone membrane. Several studies revealed that the level of MDA was found to be increased post hemodialysis with cuprophane membrane (9) Lucchi et al (10), investigated the level of conjugated fatty acids after dialysis with different membranes. It was reported that conjugated fatty acid levels were found to be increased in cuprophane, cellulose diacetate and hemophane membranes when compared to polysulfone, polyacrylonitrile and polyamide membranes. In another study performed by the same investigator, it was shown that hemodialysis with cellulose membranes increased neutrophil activation and the rate of formation of SOR (11). The results of our study showed positive correlation with the other studies.

The activity of erythrocyte GSH-Px, an antioxidant enzyme was found to be increased after dialysis in patients with chronic renal failure. Kose et al (7) and Zima et al (8) found this activity to be

significantly increased post dialysis when it was compared to pre-dialysis and showed a positive correlation between hemoglobin and GSH-Px. In this study, we think that GSH-Px activities were related to the anemia that is secondary to chronic renal failure. In other studies performed by Canestrari et al (12) and Avissar et al (13) , it was revealed that erythrocyte GSH-Px activity had increased whereas plasma GSH-Px activity was found to be decreased. In hemodialysis performed by hemophane, cellulose diacetate and polysulfone membranes, the activity of erythrocyte GSH-Px was found to be higher in post hemodialysis when compared to the levels determined pre-hemodialysis (Figure 2). In a study performed by Eiselt (14), erythrocyte GSH-Px and SOD activities were found to be decreased in patients undergoing hemodialysis with polysulfone membrane. This was not consistent with our findings.

In the present study, when comparing the SOD activities to pre-hemodialysis levels it was found to be increased following hemodialysis. Swirski et al (15) and Steiner et al (16) reported decreased levels of SOD in patients with chronic renal failure. However, Durak et al (17) reported no change in SOD activity of patients with renal failure in comparison to control group but found significant decrease in the activity of this enzyme when hemodialysis or peritoneal dialysis was performed. Canestrari et al (18) reported contradictory finding in which they found increased SOD enzyme activity in hemodialysis patients and determined it to be decreased to normal values after hemodialysis. In the present study, different membranes have been used for hemodialysis and it was found that the level of SOD enzyme non significantly increased after hemodialysis in polysulfone, hemophane and cellulose acetate membranes. In another study performed by Luciak (19), erythrocyte SOD activity was found to be decreased with Cuprophane membrane whereas, it was found not to be changed when the hemodialysis was performed by polysulfone and polyacrylonitrile membranes. Our results show that hemodialysis with polysulfone membrane did not induce significant changes in erythrocyte GSH-Px and SOD activities except for plasma malondialdehyde levels. The membranes used in hemodialysis are affected by plasma proteins which have different molecular weights. These proteins change the reactivity of the dialysis membranes, prevent membrane-cell interaction and neutrophil activation is observed in respect to the amount of absorbed proteins. Finally the production of free oxygen radicals are increased. For this reason, the membranes used in hemodialysis of patients with chronic renal failure are considered to be an important factor in the morbidity and mortality of this procedure. As a result, to prevent oxidative stress it was concluded that using biocompatible membranes such as polysulfone which has high flux and synthetic properties would be more suitable than the cellulose membranes.

REFERENCES

1. Haklar G, Yeğenağa I, Yalçın AS. Evaluation of oxidant stress in chronic hemodialysis patients: use of different parameters. Clinical Chimica Acta 1995; 234: 109-14.
2. Schmidtmann S, Müller M, Von Baehr R, Precht K. Change of antioxidative homeostasis in patients on chronic hemodialysis.Nephrology Dialysis Transplantation 1991; 3: 71-4.
3. Giardini O, Galluchi MT, Lubrano R, Tcnore GR, Bandino D, Silvi 1, Ruberto U, Casciani CU. Evidence of red blood cell membrane lipid peroxidation in haemodialysis patients. Nephron 1984;36:235-7.
4. Satoh K. Serum Lipid Peroxide in cerebrovascular disorders determined by a new colorimetric method. Clinica Chimica Acta 1978; 90: 37-43.
5. Yagi K. Assay of blood plasma or serum for lipid peroxide level and its clinical significance. Methods in Enzymology 1984;105:328-31.
6. Paglia DE, Valentine WN. Studies on the quantitative and qualitative characterisation of erythrocyte glutathione peroxidase. Journal Laboratory & Clinical Medicine 1967; 70: 158-68.
7. Köse K, Doğan P, Gündüz Z, Düşünsel R, Utaş C. Oxidative stress in hemodialyzed patients and the long-term effects of dialyzer reuse practice. Clinical Biochemistry 1997; 30 (8):601-6.
8. Zima T, Stipek S, Crkovska J, Nemecek K, Platenik J, Bartova V, Tesar V. Antioxidant enzymes-superoxide dismutase-in haemodialyzed patients. Blood Purification 1996; 14: 257-61.
9. Zima T, Haragsim L, Stipek S, Bartova B et al.(1993). Lipid peroxidation on dialysis membranes. Biochemistry and Molecular Biology International 1993; 29 (3):531-7.
10. Lucchi L, Banni S, Botti B, Cappelli G, Medici G, Melis MP, Tomasi A, Vannini V, Lusvarghi E. Conjugated diene fatty acids in patients with chronic renal failure: evidence of increased lipid peroxidation? Nephron 1993; 6: 401-9.

11. Lucchi L, Bergamini S, Bolti B, Rapana R, Ciuffreda A, Ruggiero P, Ballestri M, Tomasi A, Albertazzi A. influence of different hemodialysis membranes on red blood cell susceptibility to oxidative stress. Artificial Organs 2000; 24(1): 1-6.

12. Canestrari F, Buoncristiani U, Galli F, Giorgini A, Albertini MC, Carobi C, Pascucci M, Bossu M. Redox state, antioxidative activity and lipid peroxidation in erythrocytes and plasma of chronic ambulatory peritoneal dialysis patients. Clinica Chimica Acta 1995; 234: 127-36.

13. Avıssar N, Ornt D, Yağıl Y, Horowitz S, Richard H, Watkins E, Kerl A.Takahashi K, Palmer IS, Cohen HJ. Human kidney proximal tubules are the main source of plasma glutathione peroxidase. American Physiological Society 1994; 367-75.

14. Eiselt J, Racek J, Holecek V, Krejcova 1, Opatrny K. Antioxidants and malondialdehyde during hemodialysis with cellulose diacetate and polysulfone membranes. Cas Lek Cesk 1996; 135(21): 691-4.

15. Swirski R, Mashiac E, Kristal B, Shkolnik T, Shasha S. Antioxidant enzymes activity in polymorphonuclear leukocytes in chronic renal failure. Nephron 1995 ; 71 :176-9.

lö. Steiner M, von Appen K, Klinkmann H, Ernst B. Superoxide dismutase activity and lipid peroxidation products in patients with chronic renal failure on maintenance hemodialysis. Nephrology Dialysis Transplantation 1992; Letters, 368-9.

17. Durak î, Akyol Ô, Basesme E, Canbolat O, Kavutcu M. Reduced erythrocyte defense mechanisms against free radical toxicity in patients with chronic renal failure. Nephron 1994; 66: 76-80.

18. Canestrari F, Galli F, Giorgini A, Albertini MC, Galiotta P, Pascucci M, Bossu M. Erythrocyte redox state in uremic anaemia: effects of hemodialysis and relevance of glutathione metabolism. Acta Haematologica. 1994; 91: 187-93.

19. Luciak M, Trznadel K Free oxygen species metabolism during hemodialysis with different membranes. Nephrology Dialysis Transplantation 1991; 3: 66-70.

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