

# Influence of Hemodialysis on Lipid Peroxidation, Enzymatic and Non-Enzymatic Antioxidant Capacity in Chronic Renal Failure Patients

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**Background:** Free radical induced damages are thought to be involved in chronic kidney disease (CKD), especially in patients who are on hemodialysis (HD) for prolonged periods. Hemodialysis can influence multiple biochemical factors, several of which are useful, although the rest can be harmful and increase the severity of disease.

**Objectives:** The purpose of this study was to evaluate the effect of the HD membrane polysulfone on oxidative stress markers, by measuring the level of lipid peroxidation and total antioxidant activity (TAC), in the blood of HD patients.

**Patients and Methods:** This study was carried out on 31 HD patients and 31 healthy persons, matched for age and sex, as control group. Blood samples were drawn before and after HD from arteriovenous fistulas, and once from the controls. Superoxide dismutase (SOD), catalase (CAT) and thiobarbituric acid-reactive substance (TBARS) in blood hemolyzate, Glutathione peroxidase (GpX) of whole blood and TAC of plasma were measured, respectively. Then, we investigated the association between TAC of plasma, measured by ferric reducing antioxidant power (FRAP), and lipid peroxidation level with its related parameters, in HD patients.

**Results:** The SOD, GpX and CAT were decreased after HD ( $P < 0.05$ ). Also, FRAP was shown to decrease after HD ( $P < 0.05$ ). However, erythrocyte TBARS levels ( $\mu\text{mol}/\text{gr}$  of Hb) were increased after HD, in comparison with controls, and before HD ( $P < 0.05$ ). There was a significant negative correlation between TBARS and antioxidant indices, such as SOD ( $r = -0.67, P = 0.001$ ), GpX ( $r = -0.76, P = 0.001$ ), CAT ( $r = -0.63, P = 0.001$ ) and FRAP ( $r = -0.84, P = 0.001$ ). The FRAP was significantly and directly correlated with uric acid ( $r = +0.62, P = 0.001$ ), SOD ( $r = +0.72, P = 0.001$ ), GpX ( $r = +0.87, P = 0.001$ ) and CAT ( $r = +0.84, P = 0.001$ ).

**Conclusions:** The results of our study proposed that there is a loss or inactivation of antioxidant factors, coupled with increased lipid peroxidation during the procedure of HD, possibly through the dialyzer membrane, with probable consequences on the severity of illness.

**Keywords:** Renal Dialysis; Lipid Peroxidation; Uric Acid; Superoxide Dismutase; Catalase; Glutathione Peroxidase

## 1. Background

Numerous studies, on the role of reactive oxygen species (ROS), as one of the risk factors, which simultaneously reduce the activity of antioxidant factors in the progression atherosclerosis, cardiovascular disorders (CVD) and end stage renal disease (ESRD), has been conducted (1-3). Hemodialysis (HD) is the most common method used to treat advanced and permanent kidney failure. The mortality and morbidity risks of long-term HD can be relevant to several factors like protein wasting, inflammation, impaired immune responsiveness and, especially, oxidative stress (4). The oxidative stress results from an imbalance between oxidants or pro-oxidants production and antioxidant defense mechanisms (5). Antioxidants can be divided into intracellular and extracellular antioxidants. Intracellular enzymatic antioxidants are superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GpX), which convert specific substrates to less reactive compounds (3). Extracellular antioxidants, such as albumin, bilirubin and uric acid prevent free

radical reaction by collecting pro-oxidants or chelating transition metal ions (6). The ROS, such as the superoxide anion, hydrogen peroxide and hydroxyl radical that are produced massively in oxidative stress conditions, cause depletion of sulfhydryl (-SH) groups and oxidation of multiple molecules, such as membrane lipids, protein and nucleic acid molecules, in the human body, that can cause tissue damage (5, 7, 8). There is a range of defense mechanisms to keep cells from oxidative damage. These include the factors that limit the production of ROS, scavenging of radicals that are formed to prevent the production of secondary radicals in chain reactions and to repair the damage that may however occur (7). Although several studies have suggested an alteration of the oxidative stress phenomenon, by production of ROS on the surface of dialysis membranes, by activation of polymorphonuclear leukocytes in patients undergoing HD (9-11), there are limited reports about the correlation between the status of total antioxidant capacity (TAC) of plasma

and their associated components and lipid peroxidation products.

## 2. Objectives

The aim of this study was to identify oxidant and anti-oxidant markers, before and after HD, using the convectional polysulfone membrane dialyzer, in HD patients, and to compare it with the control group. Therefore, the observed effect on markers of oxidative stress can guide us in the use of dialysis membrane and for making appropriate recommendations for better results, in patients.

## 3. Patients and Methods

### 3.1. Patients Population

This study was carried out on 31 end stage renal disease (ESRD) patients, aged 25 - 74 years, on HD, from the dialysis center at Imam-Khomeini hospital, medical university of Mazandaran, Sari, Iran. They were regularly dialyzed three times a week, at least 4 hours per session, with polysulfone membrane dialyzers (minimum of 6 months on HD, as introduction criterion). In the selection of the ESRD patients, we excluded all those with diabetes, liver, respiratory or malignant disorders, acute infections, cigarette and antioxidant supplement consumption. Thirty-one, age-sex matched, healthy volunteers were selected, as the control group. They had no medical problems and vitamin supplement or cigarette consumption. The study protocol was approved by the local ethics committee and all patients signed an informed consent form.

### 3.2. Laboratory Studies

Blood samples were drawn before and after HD from arteriovenous fistulas from patients, and once from the control subjects, after an overnight fasting, in two separate tubes with heparin. One of tubes was immediately centrifuged at 3,000 rpm, for 15 minutes at 4°C, for separating erythrocytes and plasma. Erythrocytes were washed three times with saline 9% and after centrifugation at 3000 rpm, pellet diluted with cold distilled water (1:4). The lysate of erythrocyte, plasma and whole

blood samples were stored at -80°C, until analysis. Whole blood was used for measurement of GpX and erythrocyte SOD by commercial assay kit (Randox Laboratories Ltd., Crumlin, UK). Catalase activity of erythrocyte was measured at 25°C using the Aebi method (12). The TAC of plasma was measured by the ferric reducing antioxidant power (FRAP) assay (13). Lipid peroxidation in erythrocyte was estimated by measuring thiobarbituric acid reactive substances (TBARS) concentrations, according to the method of Satoh et al. (14). Hemoglobin in erythrocyte lysate was assayed by Drabkin method with zist-chemistry kit. Plasma samples were also used for the measurement of urea, creatinine, uric acid and bilirubin by Pars Azmoon laboratory kits (Pars Azmoon Co., Karaj, Iran), using an auto-analyzer (Prestige 24i, Tokyo Boeki Ltd., Tokyo, Japan).

### 3.3. Statistical Analysis

Data was analyzed by SPSS software version 17.0 (SPSS Inc. Chicago, IL, USA) and expressed as the Mean  $\pm$  SD. Normality of the distributions was checked for each variable, using the Kolmogorov-Smirnov test. An independent sample t-test was used to compare the parameters between patients and controls, and a paired t-test was used to compare biochemical parameters, before and after HD in the patients. Pearson's correlation coefficient was used to determine the correlation between variables. A  $P < 0.05$  was considered statistically significant.

## 4. Results

This study was carried out on 31 patients (female: male, 15:16) and 31 controls (female: male, 14:17). Mean age of case and control groups were  $60.2 \pm 16.3$  and  $58.6 \pm 15.6$  years, respectively. There were no significant differences between two groups, based on the age ( $P = 0.705$ ) and sex ( $P = 0.840$ ).

Table 1 shows biochemical parameters in both HD and control groups. Before dialysis, the levels of blood urea nitrogen (BUN), creatinine and uric acid were much higher in HD patients than controls, and decreased after HD ( $P < 0.001$ ). Inversely, albumin and bilirubin levels were lower than in controls, although after dialysis albumin increased ( $P < 0.05$ ) and bilirubin slightly decreased ( $P = 0.183$ ).

**Table 1.** Biochemical Parameters (Blood Urea Nitrogen, Creatinine, Bilirubin, Albumin, Uric Acid) Values of Patient and Control Groups<sup>a</sup>

Parameters	Hemodialysis		Control Group
	Pre	Post	
Urea, mg/dL	$159.09 \pm 49.05^{b,c}$	$62.64 \pm 34.23^b$	$25.82 \pm 3.78$
Creatinine, mg/dL	$9.76 \pm 3.58^{b,c}$	$3.77 \pm 2.12^b$	$1.04 \pm 0.082$
Bilirubin, mg/dL	$0.31 \pm 0.34^b$	$0.27 \pm 0.029^b$	$0.58 \pm 0.14$
Albumin, g/L	$25.77 \pm 1.96^{b,c}$	$35.25 \pm 2.32^b$	$44.39 \pm 6.3$
Uric acid, mg/dL	$8.37 \pm 0.92^{b,c}$	$4.41 \pm 0.89^b$	$5.26 \pm 1.26$

<sup>a</sup> Data are presented as Mean  $\pm$  SD.

<sup>b</sup>  $P < 0.05$ , before and after hemodialysis versus controls.

<sup>c</sup>  $P < 0.05$ , before versus after hemodialysis.

**Table 2.** Status of Indices of Oxidant and Antioxidant Parameters in Hemodialysis Patients and Control Groups <sup>a,b</sup>

Parameters	Hemodialysis		Control Group
	Pre	Post	
TBARS	1.28 ± 0.19 <sup>c,d</sup>	2.8 ± 0.67 <sup>e</sup>	0.93 ± 0.55
FRAP, μmol/L Fe <sup>2+</sup>	799 ± 21.59 <sup>c,d</sup>	576 ± 22.37 <sup>e</sup>	1022 ± 23.55
SOD, u/mg Hb	1015 ± 16.25 <sup>c,d</sup>	943 ± 18.05 <sup>c</sup>	1392 ± 25.43
GpX, U/mg Hb	18.07 ± 0.49 <sup>c,d</sup>	12.07 ± 0.41 <sup>e</sup>	30.1 ± 0.56
CAT, K/ mg Hb	29.64 ± 2.81 <sup>c,d</sup>	21.75 ± 2.69 <sup>e</sup>	42.8 ± 2.72

<sup>a</sup> Data are presented as Mean ± SD.

<sup>b</sup> Abbreviations: CAT, catalase; FRAP, ferric reducing antioxidant power; GpX, glutathione peroxidase; SOD, superoxide dismutase; TBARS, thiobarbituric acid reactive substances.

<sup>c</sup> P < 0.01.

<sup>d</sup> P < 0.05, before versus after hemodialysis.

<sup>e</sup> P < 0.05, before and after hemodialysis versus controls.

**Table 3.** Correlation of Several Effective Biochemical Parameters After Hemodialysis Session With Ferric Reducing Antioxidant Power and Thiobarbituric Acid-Reactive Substance <sup>a</sup>

Parameters	FRAP		TBARS	
	r Value	P Value	r Value	P Value
Albumin, g/L	+0.25	0.35	-0.13	0.21
Uric acid, mg/dL	+0.62	0.001	-0.41	0.001
Bilirubin, mg/dL	+0.32	0.254	-0.56	0.001
SOD, U/mg Hb	+0.72	0.001	-0.67	0.001
GpX, U/mg Hb	+0.87	0.001	-0.76	0.001
CAT, K/mg Hb	+0.84	0.001	-0.63	0.001

<sup>a</sup> Abbreviations: CAT, catalase; FRAP, ferric reducing antioxidant power; GpX, glutathione peroxidase; SOD, superoxide dismutase; TBARS, thiobarbituric acid reactive substances.

Table 2 shows that the erythrocyte antioxidant enzymes activities, SOD, GpX, CAT were found decreased after HD ( $P < 0.05$ ). Also, FRAP was shown to decrease after HD ( $P < 0.05$ ), while erythrocyte TBARS levels ( $\mu\text{mol}/\text{gr Hb}$ ) were increased after HD, in comparison with controls and before HD ( $P < 0.05$ ).

The coefficients of correlation between several important parameters in post dialysis are given in Table 3. There was a significant negative correlation of TBARS with antioxidant indices, such as SOD ( $r = -0.67$ ,  $P = 0.001$ ), GpX ( $r = -0.76$ ,  $P = 0.001$ ), CAT ( $r = -0.63$ ,  $P = 0.001$ ) and FRAP ( $r = -0.84$ ,  $P = 0.001$ ). The FRAP was significantly and directly correlated with uric acid ( $r = +0.62$ ,  $P = 0.001$ ), SOD ( $r = +0.72$ ,  $P = 0.001$ ), GpX ( $r = +0.87$ ,  $P = 0.001$ ), CAT ( $r = +0.84$ ,  $P = 0.001$ ).

## 5. Discussion

Although HD leads to recovery in several biochemical factors, it can also start a number of harmful atherogenic effects, due to incompatibility of dialyzer components. Production of ROS could be an important damaging outcome of HD.

In this work, we revealed significant elevations in TBARS of erythrocyte concentration a lipid peroxidation marker, along with decrease in TAC, which indicate the pro-

duction of oxidative stress in patients. Oxidative stress is a double edged blade, which occurs in humans, as a major part of host defense mechanisms, although it is abundant in active pathological conditions. In chronic kidney disease (CKD), oxidative stress can play an important role in the pathogenesis of atherosclerosis, anemia and malnutrition. Previous studies have revealed a significant imbalance in the amount of pro-oxidants and antioxidants, in patients with CKD (15, 16). Patients are in a status of chronic inflammation and will trigger production of inflammatory cells, such as polymorphonuclear cells (PMNs) and monocytes (17). These cells will increase the secretion of myeloperoxidase and nicotinamide adenine dinucleotide phosphate oxidase that will also increase the production of ROS and alter the vascular endothelial function, therefore worsening the progression of CKD (18, 19). Loss of antioxidants by dialysis and the use of poor biocompatible membranes are the factors that can be responsible for the oxidative stress in HD patients.

Our study showed that TAC, which is evaluated by FRAP assay, is a valuable tool for understanding the ability of the biological system to prevent oxidative stress. The TAC is significantly lower (21.8%) in pre HD than in controls and also decreases after HD (27.9%), which is in agreement with previous studies (20, 21). The principle of FRAP assay

is the reduction of ferric ions by uric acid (60%), protein (10%), bilirubin (5%), ascorbic acid (15%), alpha-tocopherol (5%) and others (5%) (13). The findings of present study showed that, despite a moderate increase in albumin concentration after dialysis, the antioxidant capacity of plasma decreased. This indicates the effect of variation in the second structure by free radicals (22) that are effective in enhancing the antioxidant properties of molecules like proteins, and can be more valuable in comparison to concentration alone. Since hyperbilirubinemia is not an issue in the patients included in this study and proteins are nondialyzable molecules, we focus on uric acid and its correlations with the total antioxidant status assays, both before and after a HD session.

In our study, the serum uric acid decreased markedly during HD procedure (47%) and has a significant positive correlation with reduction of FRAP ( $r = 0.62$ ,  $P < 0.001$ ). Therefore, removal of uric acid by HD could exacerbate oxidative stress, in patients. This suggests a weakness in the defense system against oxidative stress, with the loss of uric acid. Similar result have been reported by Gerardi (23) and Usberti (24). It is well-known that uric acid has free radical scavenging capability (8). Despite this, uric acid alone cannot be considered sufficient to protect against the enhanced oxidative stress in HD patients, as there are other endogenous compounds contributing to it. In the present study, there was significant difference in the GpX, SOD, and CAT activities, before and after HD. After HD, the activity of enzymes like GpX, SOD and CAT were significantly reduced. The current results showed that HD with polysulfone membrane caused oxidative stress. Similar reports showed that one important cause of elevated oxidative stress in chronic HD patients might be the kind of dialysis membrane used (20, 25). In the present study, the highest average erythrocyte concentrations of TBARS were found after HD. Moreover, a negative correlation was observed between the concentrations of TBARS with the antioxidant parameters. This indicates severe damage to the antioxidant system, which is unable to combat oxidative stress. It is a result of chronically elevated ROS and impaired antioxidant defense system, which are probably related to increased utilization of antioxidants for the reason of increased lipid peroxidation. Our findings were comparable with those of Yavuz et al. (25) and Ogunro et al. (20), who analyzed the effects of dialyzer membranes on the malonyl dialdehyde levels post hemodialysis.

The results of this study have shown a significant decrease in activity of SOD, CAT and GpX in erythrocytes and other antioxidants, after dialysis. The decrease in SOD activity seems to result from the effect of increased oxygen-derived free radicals. Hence, lower superoxide levels induce SOD activity, whereas higher levels inhibit it. Furthermore, depressed CAT and GpX activity may be associated with an enhanced protective mechanism against oxidative stress in the initiation and progression of HD or may be related to the loss of these antioxidants by di-

alysis membrane, uremic condition or dialysis procedure (increase of lipid peroxidation). This condition may play an important role in the progression of premature cardiovascular disease in HD patients. In conclusion, the results of our study propose that there is loss or inactivity of antioxidant factors and increased lipid peroxidation during the procedure of HD, possibly through the dialyzer membrane, which probably increases the severity of illness in the future.

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## Authors' Contributions

Mehryar Zargari designed the study, assay samples, analyzed the data and edited the manuscript. Omid Sedighi referred patients to laboratory of Imam Khomeini Hospital.

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