

Evaluation of Thiol/Disulfide Homeostasis and Other Oxidative Stress Markers in Patients Undergoing Hemodialysis

İbrahim Söğüt¹ , Almıla Şenat² , Ayşegül Oğlakçı İlhan³ , Adem Sezen⁴ , Özcan Erel² 

¹Department of Biochemistry, Demiroğlu Bilim University School of Medicine, İstanbul, Turkey

²Department of Clinical Biochemistry, Yıldırım Beyazıt University School of Medicine, Ankara, Turkey

³Eldivan Vocational School of Health Services, Çankırı Karatekin University, Çankırı, Turkey

⁴Vocational School of Health Services, Demiroğlu Bilim University, İstanbul, Turkey

17

Abstract

Objective: To investigate the potential role of thiol/disulphide homeostasis as a novel biomarker of oxidative stress in patients with diabetes and undergoing hemodialysis (HD) and its correlation with other oxidative stress markers.

Materials and Methods: This study included 82 patients with end-stage renal disease undergoing HD for four hours, three times weekly for more than 24 months in the dialysis center. Of the 82 patients, 47 were non-diabetic and 35 were diabetic. Blood samples were collected from the patients before and after the HD sessions. The thiol/disulfide pair tests were performed and total antioxidant status (TAS), total oxidant status (TOS), oxidative stress index (OSI), ischemia-modified albumin (IMA) levels, albumin levels, ceruloplasmin, catalase activity (CAT), and myeloperoxidase (MPO) activity were determined in the serum.

Results: The TAS values in all the patients, both diabetic and non-diabetic, decreased significantly after HD ($p < 0.001$, $p = 0.003$, and $p < 0.001$; respectively). The TOS, albumin, native thiol ($p = 0.001$, $p = 0.007$, $p = 0.001$, respectively), OSI, CAT, ceruloplasmin, IMA, MPO, and total thiol ($p < 0.001$, $p < 0.001$, $p < 0.001$, $p < 0.001$, $p < 0.001$, and $p < 0.001$, respectively) values increased significantly in all the patients after the HD session. The TOS, OSI, CAT, IMA, albumin, MPO, native thiol, and percentages of native/total thiol, ceruloplasmin, and total thiol values ($p = 0.002$, $p = 0.002$, $p = 0.002$, $p = 0.001$, $p = 0.008$, $p = 0.001$, $p = 0.001$, $p = 0.003$, $p = 0.023$, and $p < 0.001$; respectively) increased significantly in patients with diabetes after the HD session.

Conclusion: In this study, we demonstrated the relationship between oxidative stress markers, which play a significant role in the pathogenesis of diabetes, and thiol/disulfide balance undergoing HD patients.

Keywords: Ceruloplasmin, diabetes mellitus, disulfides, renal dialysis, ischemia-modified albumin, sulfhydryl compounds

Corresponding Author: İbrahim Söğüt ✉ ibrahim.sogut@gmail.com

Received: 11.04.2020 **Accepted:** 18.09.2020

Cite this article as: Söğüt İ, Şenat A, Oğlakçı İlhan A, Sezen A, Erel Ö. Evaluation of Thiol/Disulfide Homeostasis and Other Oxidative Stress Markers in Patients Undergoing Hemodialysis. *Turk J Nephrol* 2021; 30(1): 17-24.

INTRODUCTION

Diabetes mellitus (DM), usually called diabetes, is a metabolic illness and is characterized by relative or total insulin deficiency. Today, diabetes is known to be one of the main causes of mortality and morbidity worldwide (1). The relative risk for diabetes around the world is expected to reach 642 million individuals by 2040. Approximately 40% of individuals with diabetes develop chronic kidney disease (CKD) (2). DM is the most common cause of CKD. The kidney plays a role in insulin resistance and gluconeogenesis. When people with CKD have reduced insulin clearance, they become more sensitive to hypoglycemia (3).

CKD is a widespread health issue, and various substances accumulate and/or are deficient in patients with CKD. These patients have an elevated potential of developing oxidative stress related to metabolic disorders, immune deficiency, and persistent inflammation (4). Chronic inflammation is a common condition that develops during the dialysis treatment. Myeloperoxidase (MPO) produced from superoxide anion, hydrogen peroxide, and chlorinated oxidants in activated neutrophils is negatively related to uremia and hemodialysis (HD), and even the dialysis media are considered a model for oxidative stress. It is assumed that HD triggers circulating neutrophils to



develop free oxygen radicals and induce oxidative stress in patients undergoing HD depending on the weakening of the antioxidant system (5).

Oxidative stress caused by an increment in the reactive oxygen species (ROS) and reduction in antioxidant defense system is common in numerous health problems such as CKD (6). ROS activate enzymatic and non-enzymatic antioxidant defense systems. Superoxide dismutase, catalase (CAT), and glutathione peroxidase (GPX) are the enzymatic antioxidants. The non-enzymatic antioxidants are thiols, which contain sulfhydryl groups (-SH). Total extracellular and intracellular thiol levels are composed of thiols that are free, in the form of diminished glutathione (GSH), or bound to proteins (7). Thiols existing in plasma are mostly bound to albumin and other proteins, whereas the remaining thiols are present in the structures of low-molecular-weight thiols, such as cysteine, cysteinyl-glycine, GSH, homocysteine, and γ -glutamyl cysteine (8). Oxidant molecules oxidize the thiol groups of proteins, which form disulfide connective structures. This reaction is reversible, however, and disulphide connective structures can be reduced to thiol groups by maintaining the thiol-disulfide balance (8). Thiol/disulfide homeostasis is the basis for detoxification. The markers of this homeostasis include proportions of native and total thiol/disulfide, disulfide/native thiol, native thiol/total thiol, and disulfide/total thiol (9). Measuring thiols in serum gives an indirect result of the antioxidative protection. Thiols can form disulfide bonds, which can again be reduced to thiols, which results in dynamic thiol/disulfide homeostasis. Dynamic thiol/disulfide homeostasis state is involved in antioxidant protection, detoxification, signal transduction, apoptosis, and cellular signaling mechanisms (8, 9). An abnormal thiol/disulfide homeostasis state is involved in the pathogenesis of different illnesses, including diabetes, cardiovascular maladies, malignant growths, rheumatoid arthritis, CKD, acquired immune deficiency syndrome, Parkinson's and Alzheimer's diseases, multiple sclerosis, and liver disease (7).

Therefore, this study aimed to investigate the possible role of thiol/disulphide homeostasis as a novel biomarker of oxidative stress in patients with diabetes and undergoing HD and its relationship with other oxidative stress markers.

Main Points

- Thiol/disulphide parameters can be used as a novel oxidative stress biomarker in patients with diabetes undergoing hemodialysis.
- Serum thiol/disulfide homeostasis appears to be a valuable marker for oxidative stress. Therefore, oxidative stress markers were found to be high in patients with diabetes undergoing dialysis.
- An abnormal thiol/disulfide homeostasis may play a role in the HD patients.

MATERIALS AND METHODS

Study Design and Patients

Blood samples of 82 patients with end-stage renal disease (ESRD) (41 women and 41 men; mean age, 64.6±14.4 years) undergoing HD for four hours, three times weekly for more than 24 months in Doğan Dialysis Center (İstanbul, Turkey) were used in our study. The blood samples were collected from patients before and after HD sessions on the same day. Serum samples were transported in cold chain (-20°C) to the Biochemistry Department of Yıldırım Beyazıt University School of Medicine where analyses were performed.

The study was conducted after receiving an approval from the Clinical Research Ethical Committee of Demiroğlu Bilim University (Approval Number: December 4, 2018; Approval Date: 2018-17-13), and all the patients gave informed consent. Of the 82 patients, 47 were non-diabetic (26 women and 21 men; mean age, 61.1±16.3 years) and 35 were diabetic (15 women and 20 men; mean age, 69.3±9.8 years). The study group included patients who did not use substances, such as cigarettes and alcohol, and did not have inflammatory and chronic liver disease.

Biochemical Measurements

The thiol/disulfide pair tests in the serum were determined using the method by Erel and Neselioglu (10), which is based on the principle of measuring the reduced thiol groups and existing native thiols ($\mu\text{mol/L}$) for the total thiol ($\mu\text{mol/L}$) amounts that were analyzed with 5, 5'-dithiobis-(2-nitrobenzoic) acid (Merch; Darmstadt, Germany). Disulfide levels ($\mu\text{mol/L}$) were determined as half of the subtraction of native thiol from total thiol levels.

Total antioxidant status (TAS; mmol Trolox eq/L), total oxidant status (TOS; $\mu\text{mol H}_2\text{O}_2 \text{ eq/L}$), and oxidative stress index (OSI; arbitrary unit) were detected using commercial kits (Rel Assay Diagnostics; Gaziantep, Turkey) (11, 12). A dark blue-green colored 2, 2'-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid) (ABTS) radical is reduced to a colorless reduced ABTS form with antioxidants. The change of absorbance at 660 nm is related to the TAS level of the sample. Oxidants existing in the sample oxidize the ferrous ioneo-dianisidine complex to the ferric ion. The ferric ion forms a colored complex with xylenol orange in an acidic medium. The change of absorbance at 530 nm is related to TOS level of the sample. The OSI levels in the sample were detected as the ratio of the TOS level to TAS level.

In our study, the ischemia-modified albumin (IMA; ABSU) level in serum was measured by a method reported by Das et al. (13) on the basis of the spectrophotometric measurement (470 nm) of color production because of the reaction of albumin-cobalt with dithio-threitol. The reaction was performed by adding 50 μL of 0.1% cobalt (II) chloride hexahydrate (CoCl_2 ; Merch; Darmstadt, Germany), 50 μL of 1.5 mg/mL dithiothreitol (Merch; Darmstadt, Germany), and 1 μL of 0.9% sodium chloride solution (Merch; Darmstadt, Germany).

Table 1. Comparison of the values before and after hemodialysis in all patient population (diabetic+non-diabetic)

| Parameters | Before hemodialysis session | After hemodialysis session | p |
|---|-----------------------------|----------------------------|--------|
| TAS (mmol Trolox eq/L) | 2.201±0.410 | 1.884±0.381 | <0.001 |
| TOS (µmol H ₂ O ₂ eq/L) | 1.485 (0.897-2.492) | 2.230 (1.387-3.615) | 0.001 |
| OSI (arbitrary unit) | 0.068 (0.038-0.119) | 0.140 (0.063-0.190) | <0.001 |
| CAT (kU/L) | 40.400 (16.675-72.392) | 72.400 (44.800-120.225) | <0.001 |
| Ceruloplasmin (U/L) | 537.997±122.902 | 599.832±138.367 | <0.001 |
| IMA (mg/dL) | 0.159±0.036 | 0.190±0.058 | <0.001 |
| Albumin (g/dL) | 4.148±1.485 | 4.665±1.423 | 0.007 |
| MPO (U/L) | 76.155 (53.195-115.085) | 114.225 (82.485-162.562) | <0.001 |
| Native thiol (µmol/L) | 225.393±100.752 | 285.853±98.400 | 0.001 |
| Total thiol (µmol/L) | 261.034±103.391 | 328.665±99.610 | <0.001 |
| Disulfide (µmol/L) | 16.425 (13.562-21.162) | 19.125(12.337-24.512) | 0.050 |
| Percentage disulfide/native thiol | 7.600 (5.975-11.625) | 6.500 (4.550-9.900) | 0.031 |
| Percentage disulfide/total thiol | 6.600 (5.275-9.425) | 5.750 (4.175-8.225) | 0.035 |
| Percentage native/total thiol | 86.850 (81.075-89.350) | 88.550 (83.500-91.650) | 0.033 |

TAS: total antioxidant status; TOS: total oxidant status; OSI: oxidative stress index; CAT: catalase; IMA: ischemia-modified albumin; MPO: myeloperoxidase

Serum ceruloplasmin level (U/L) is automated and colorimetric and is based on the enzymatic oxidation of ferrous ion to ferric ion (14). Albumin levels (g/dL) were measured by a clinical biochemistry autoanalyzer (Roche; Mannheim, Germany).

CAT activity was determined according to the Goth's method (15). After incubation of the sample (0.2 mL) in 1.0 mL of 65 mmol per H₂O₂ in 60 mmol/L sodium-potassium phosphate (Merck; Darmstadt, Germany) buffer (pH 7.4) at 37°C for 60 seconds, the enzymatic reaction was halted by addition of 1.0 mL of 32.4 mM ammonium molybdate (Merck; Darmstadt, Germany). The yellow complex formed by molybdate and H₂O₂ was measured at 405 nm. One unit of CAT decomposes 1 µmol of H₂O₂ per minute under these conditions; kU/L was used as the unit for the results.

Serum MPO activity assay was performed per the method of Bradley et al. (16) with some modifications. The method was based on the formation of yellowish orange color formed by oxidation of o-dianisidine (Merck; Darmstadt, Germany) with MPO in the presence of H₂O₂. Color formation was kinetically measured at 460 nm, and 1 unit of MPO was defined as that degrading 1 µmol of H₂O₂ per minute at 25°C, U/L of serum was used to express MPO activity.

The percentage of changes of biochemical parameters before and after HD (that is, Δ parameter) was calculated as the difference between the values before and after the HD sessions divided by the values before HD session and the result multiplied by a hundred.

Statistical Analysis

Statistical analyses were performed using the Statistical Package for the Social Sciences version 22.0 program (IBM Corp.; Armonk, NY, USA). The Shapiro-Wilk test was used to assess the normality of distribution of numerical variables. The normally distributed variables were presented as mean±standard deviation. Median values were used when normal distribution was absent. The student's t test (for the parametric variables) and the Mann-Whitney U test (for the non-parametric variables) were performed to compare the variables of diabetic and non-diabetic groups. The paired t test (for the parametric variables) and the Wilcoxon test (for the non-parametric variables) were performed to compare variables before and after HD session. Non-parametric numerical data were presented as median (minimum-maximum), and p<0.050 was accepted as statistically significant.

RESULTS

Table 1 shows the comparison of parameters before and after HD in all the patients. TOS, OSI, CAT, ceruloplasmin, IMA, albumin, MPO, native thiol, total thiol, and percentage of native/total thiol increased significantly after HD session (p=0.001, p<0.001, p<0.001, p<0.001, p=0.007, p<0.001, p=0.001, p<0.001, and p=0.033, respectively). TAS, percentage of disulfide/native thiol, and percentage of disulfide/total thiol decreased significantly after the HD session (p<0.001, p=0.031, and p=0.035, respectively). The disulfide value increased after the HD session, but the p value was not statistically significant (p=0.050).

Table 2. Comparison of parameters before and after hemodialysis in patients with diabetes

| Parameters | Before hemodialysis session | After hemodialysis session | p |
|--|-----------------------------|----------------------------|--------|
| TAS (mmol Trolox eq/L) | 2.163±0.435 | 1.853±0.356 | 0.003 |
| TOS(μmol H ₂ O ₂ eq/L) | 1.420 (0.890-2.080) | 2.320 (1.240-3.560) | 0.002 |
| OSI (arbitrary unit) | 0.062 (0.037-0.108) | 0.145 (0.064-0.189) | 0.002 |
| CAT (kU/L) | 47.800 (16.100-75.369) | 67.400 (34.900-104.900) | 0.002 |
| Ceruloplasmin (U/L) | 568.450±119.376 | 619.832±123.833 | 0.023 |
| IMA (mg/dL) | 0.153±0.036 | 0.186±0.042 | 0.001 |
| Albumin (g/dL) | 3.964±1.374 | 4.569±1.350 | 0.008 |
| MPO (U/L) | 91.533±40.125 | 125.231±41.909 | 0.001 |
| Native thiol (μmol/L) | 208.200±83.560 | 298.134±90.199 | 0.001 |
| Total thiol (μmol/L) | 245.871±83.636 | 336.186±88.726 | <0.001 |
| Disulfide (μmol/L) | 16.400 (13.850-21.350) | 17.850 (10.700-23.900) | 0.787 |
| Percentage disulfide/native thiol | 9.200 (6.400-12.400) | 5.900 (3.000-9.300) | 0.003 |
| Percentage disulfide/total thiol | 7.700 (5.700-9.900) | 5.300 (2.900-7.800) | 0.003 |
| Percentage native/total thiol | 84.500 (80.100-88.700) | 89.500 (84.400-94.300) | 0.003 |

TAS: total antioxidant status; TOS: total oxidant status; OSI: oxidative stress index; CAT: catalase; IMA: ischemia-modified albumin; MPO: myeloperoxidase

Table 2 shows the comparison of parameters before and after the HD session in patients with DM. TOS, OSI, CAT, ceruloplasmin, IMA, albumin, MPO, native thiol, total thiol, and percentage of native/total thiol increased significantly after the HD session in the diabetic patient population ($p=0.002$, $p=0.002$, $p=0.002$, $p=0.023$, $p=0.001$, $p=0.008$, $p=0.001$, $p=0.001$, $p<0.001$, and $p=0.003$, respectively). TAS, percentage of disulfide/native thiol, and percentage of disulfide/total thiol decreased significantly after HD session in the diabetic patient population ($p=0.003$, $p=0.003$, and $p=0.003$, respectively). However, the disulfide value did not change significantly after the HD session in the diabetic patient population ($p=0.787$).

Table 3 shows the comparison of parameters before and after the HD session in the non-diabetic patient population. OSI, CAT, ceruloplasmin, IMA, MPO, and disulfide increased significantly after the HD session in the non-diabetic patient population ($p=0.019$, $p=0.005$, $p=0.002$, $p=0.003$, $p=0.006$, and $p=0.010$, respectively). TAS decreased significantly after the HD session in the non-diabetic patient population ($p<0.001$). TOS and total thiol increased after HD session, but the p value was not statistically significant ($p=0.066$ and $p=0.054$, respectively). However, albumin, native thiol, percentage of disulfide/native thiol, percentage of disulfide/total thiol, and percentage of native/total thiol did not change significantly after the HD session in non-diabetic patient population ($p>0.050$).

Table 4 shows the comparison of the percentage of changes in biochemical parameters before and after the HD session be-

tween patients with and without DM. There was no significant difference between the 2 groups of patients in terms of the percentage of changes in all biochemical parameters ($p>0.05$).

DISCUSSION

Albumin is the most abundant and important protein in blood plasma, and 585 amino acid residues and one free thiol group are present in human serum albumin. Albumin is an excellent scavenger of oxidants in the human plasma that can inhibit hydroxyl and peroxy radicals (4). Serum albumin is an important parameter for the evaluation of prognosis in patients on chronic HD. Serum albumin levels decrease before the start of HD (17). In our study, albumin levels after HD were significantly higher than those before HD in all the groups. Bhonsle et al. (18) have shown that albumin synthesis and secretion decreases owing to insulin deficiency in patients with DM. A study by Tayeb et al. (19) have shown increase in albumin levels after dialyzer membrane change; therefore, decrease/increase in plasma albumin levels after HD is affected by factors, such as diet, lifestyle, inflammation, disease, drugs, and so on. Furthermore, HD includes parameters, such as vascular access, blood flow rate, dialysis membrane, dialysis content, dialysis flow rate, ultrafiltration (UF) amount, dialysis time-frequency, and anticoagulants used, and each parameter can affect the level of albumin. For example, as the fluid that needs to be removed as well as UF during an HD session increases and the dialysis time decreases, the rate of required fluid removed increases. As UF increases, albumin level increases because of excessive fluid loss. Therefore, it may be thought that UF may

Table 3. Comparison of parameters before and after hemodialysis in patients without diabetes

| Parameters | Before hemodialysis session | After hemodialysis session | p |
|--|-----------------------------|----------------------------|--------|
| TAS (mmol Trolox eq/L) | 2.229±0.393 | 1.908±0.401 | <0.001 |
| TOS(μmol H ₂ O ₂ eq/L) | 1.600 (0.990-2.930) | 2.140 (1.410-3.780) | 0.066 |
| OSI (arbitrary unit) | 0.070 (0.038-0.138) | 0.117 (0.060-0.196) | 0.019 |
| CAT (kU/L) | 36.300 (16.700-71.400) | 81.600 (46.300-128.300) | 0.005 |
| Ceruloplasmin (U/L) | 515.318±121.793 | 584.938±147.809 | 0.002 |
| IMA (mg/dL) | 0.162±0.036 | 0.192±0.067 | 0.003 |
| Albumin (g/dL) | 4.285±1.563 | 4.738±1.486 | 0.124 |
| MPO (U/L) | 63.370 (50.560-113.720) | 108.780 (72.340-165.110) | 0.006 |
| Native thiol (μmol/L) | 238.195±111.003 | 276.708±104.095 | 0.133 |
| Total thiol (μmol/L) | 272.325±115.532 | 323.064±107.600 | 0.054 |
| Disulfide (μmol/L) | 17.050±6.544 | 23.151±14.536 | 0.010 |
| Percentage disulfide/native thiol | 8.289±4.051 | 10.343±10.955 | 0.222 |
| Percentage disulfide/total thiol | 6.911±0.289 | 7.670±5.189 | 0.398 |
| Percentage native/total thiol | 86.174±5.793 | 84.657±10.381 | 0.399 |

TAS: total antioxidant status; TOS: total oxidant status; OSI: oxidative stress index; CAT: catalase; IMA: ischemia-modified albumin; MPO: myeloperoxidase

Table 4. Comparison of the percentage of changes in biochemical parameters before and after hemodialysis between patients with and without diabetes

| Parameters | Diabetic patient group | Non-diabetic patient group | p |
|---|--------------------------|----------------------------|-------|
| TAS (mmol Trolox eq/L) | 28.903 (21.303-33.786) | 23.387 (17.750-34.250) | 0.164 |
| TOS (μmol H ₂ O ₂ eq/L) | 62.914 (32.973-173.239) | 71.154 (36.181-219.697) | 0.456 |
| OSI (arbitrary unit) | 105.747 (53.878-247.350) | 87.170 (38.255-365.503) | 0.848 |
| CAT (kU/L) | 86.742 (31.120-208.549) | 107.328 (59.818-387.853) | 0.140 |
| Ceruloplasmin (U/L) | 19.592 (6.736-28.390) | 26.368 (10.673-43.160) | 0.167 |
| IMA (mg/dL) | 26.207 (7.653-46.452) | 19.328 (6.433-38.235) | 0.426 |
| Albumin (g/dL) | 23.588 (14.681-38.333) | 27.591 (16.602-57.664) | 0.636 |
| MPO (U/L) | 37.170 (22.955-120.293) | 67.451 (46.444-143.109) | 0.275 |
| Native thiol (μmol/L) | 83.508 (44.350-106.313) | 57.491 (44.936-104.537) | 0.318 |
| Total thiol (μmol/L) | 68.102±37.827 | 65.202±35.673 | 0.724 |
| Disulfide (μmol/L) | 26.914 (12.253-63.469) | 42.364 (25.468-76.887) | 0.116 |
| Percentage disulfide/native thiol | 56.522 (31.088-74.328) | 48.649(20.833-95.454) | 0.753 |
| Percentage disulfide/total thiol | 52.349 (24.561-66.129) | 42.373 (20.554-81.481) | 0.729 |
| Percentage native/total thiol | 7.938 (4.808-15.633) | 6.715 (2.941-14.377) | 0.251 |

TAS: total antioxidant status; TOS: total oxidant status; OSI: oxidative stress index; CAT: catalase; IMA: ischemia-modified albumin; MPO: myeloperoxidase

also have an effect on the level of albumin after an HD session. In addition, ongoing research has suggested that the concentration of albumin in patients undergoing HD depends on both nutrition and inflammation (20).

IMA, a relatively new marker, is formed as a consequence of modification of albumin by ROS. IMA has been studied mostly in the context of cardiac ischemia. However, it has been demonstrated that IMA levels also increase in conditions not related to

cardiac ischemia, such as in DM and ESRD as well as HD (21). In this study, an increase in IMA levels after HD session was observed compared with the IMA levels before HD session, and this was statistically significant. The elevation in IMA can be related to the increased oxidative stress rate typically encountered in patients undergoing HD. Oxidative stress is also involved in the pathophysiology of DM. Sadik et al. (22) have found that the serum levels of IMA were significantly higher in patients with DM than in healthy individuals without diabetes. However, conflicting results have also been reported. Although a few studies have reported elevated levels of IMA in patients with diabetes, others have not reported differences between the IMA levels in patients with DM and those in controls (23). In this study, we found significantly higher IMA levels in patients with or without DM after HD session, and this was statistically significant.

22

Patients undergoing HD have shown expanded inflammation and lipid peroxidation. The HD procedure hastens the formation and accumulation of oxidative products through activation of platelets, supplement, and polymorphonuclear (PMN) white platelets (24). MPO is stored in abundant amounts in the primary granulocytes of PMNs and is secreted by activated neutrophils. The increased MPO levels are said to be a result of degranulation of PMN cells, released through the dialyzer membrane. MPO catalyzes the reaction between the chloride ion and hydrogen peroxide to form a mighty oxidant, sodium hypochlorite, as the product. Therefore, every session of HD causes the release of this enzyme, the aggregate impact of which would be significantly progressively harmful. The consequences of this study are in concurrence with those of other studies whose results show a significant increase in MPO after a single HD session (25). The relationship between DM and MPO has been investigated by previous studies (26); however, there are a limited number of studies investigating the relationship between HD and patients with DM and MPO levels. In our study, MPO expression was found to be higher in patients with DM before and after HD than that in patients without DM before and after HD. We also found statistically higher MPO levels in patients with or without DM after an HD session.

Although dialysis gives comfort to patients on HD, long-term dialysis treatment causes numerous manifestations, presumably because of the imbalance between ROS synthesis and breakdown. Materials of the HD membrane may affect both the development of ROS by activating PMN cells and monocytes and the reduction of antioxidant capacity of the body because of the loss of hydrophilic antioxidants during the HD session, the utilization of liposoluble antioxidant enzymes, changes in the lipid part of biological liquids in addition to the deficiency of coenzyme and antioxidant enzyme dysfunction (4). A single session of HD can decrease the levels of antioxidants, such as TAS and arylesterase; as a result, HD causes reduction in the antioxidant (6). In our study, OSI levels were higher after the HD session. According to a study by Gonzales Rico et al. (27), losses of antioxidant enzymes during HD session and utilization of less

biocompatible membranes are the variables that might play a role in the imbalance of the oxidative and antioxidative systems in patients undergoing HD. Each one of these factors increases the oxidative stress in patients undergoing HD.

In this study, we analyzed both TOS and TAS levels in patients with and without DM before the HD session and found significant differences compared with those after the HD session. The TAS levels decreased, whereas TOS levels increased after HD. The most striking finding was that as HD in patients with DM progressed, TOS increased and TAS decreased. The highest TOS levels and the lowest TAS levels were found in patients with DM after the HD session. Parallel to our results, Ruskovska et al. (28) have reported that before the HD session, the patients had significantly higher levels of TAS than those after HD session, and lower concentrations of serum TOS before the HD session than after the HD session were also observed. We also found low TAS levels in patients with DM, both before and after HD session, compared with those in patients without DM. This study is similar to the study by Sarah et al. (29), which reported significantly lower levels of TAS in patients with DM than in controls. They have also suggested that TAS may be used as a sole marker of oxidative stress (30).

In our study, the plasma levels of CAT and ceruloplasmin, as antioxidant enzymes, increased after HD in agreement with the study by Tajbakhsh et al. (31) and Ashok et al. (32). Furthermore, increased CAT and ceruloplasmin levels were observed in both patients with and without DM after an HD session, and this was statistically significant. It may be strongly assumed that HD directly affects the antioxidant status in patients with and without DM.

Disruption of the antioxidant balance by various factors is one of the possible causes of increased morbidity in patients undergoing HD. Abnormal thiol/disulfide levels are associated with oxidative stress (8). In our results, native thiol, total thiol, and disulfide levels after an HD session were found to be higher than those before the HD session. We also compared native thiol and total thiol in patients with and without DM before and after HD sessions. The most striking increase was observed in patients with DM after the HD session, and this was statistically significant. Disulfide levels were increased in non-diabetes group after HD session, and this was also statistically significant. However, in the diabetes group, this was not statistically significant. In our study, the percentage of disulfide/native thiol and percentage of disulfide/total thiol levels were decreased after HD session. Moreover, the percentage of disulfide/native thiol levels and percentage of disulfide/total thiol levels significantly decreased in patients with DM after the HD session, but percentage of disulfide/native thiol levels and percentage of disulfide/total thiol levels increased in patients without DM after the HD session, although this was not statistically significant. Percentage of native/total thiol levels decreased in non-diabetes group after the HD session but percentage of native/total thiol levels

was significantly increased after HD session in the diabetes group. Dynamic thiol/disulfide homeostasis status appears to play important roles in antioxidant protection, detoxification, signal transduction, apoptosis, regulation of enzymatic activity and transcription factors, and cellular signaling mechanisms (33). Oxidative balance in patients undergoing HD and with DM may be improved through HD. There are comparable investigations in the literature demonstrating the impact of HD on antioxidant homeostasis (8).

CONCLUSION

In this study, the oxidative stress, which plays an important role in the pathogenesis of DM in patients undergoing HD (diabetes/non-diabetes), was evaluated with the thiol/disulfide balance and the other oxidative stress markers. Our findings indicated that serum thiol/disulfide homeostasis is an accurate marker of oxidative stress in these patients. Consequently, an abnormal thiol/disulfide homeostasis may play a role in the HD patients.

Ethics Committee Approval: Ethics committee approval was received for this study from the Clinical Research Ethical Committee of Demiroğlu Bilim University (Approval Date: December 4, 2018; Approval Number: 2018-17-13).

Informed Consent: Written informed consent was obtained from the patients who participated in this study.

Author Contributions: Concept - İ.S.; Design - İ.S., A.Ş., O.E.; Supervision - Ö.E.; Resources - İ.S., Ö.E., A.S.; Materials - İ.S., Ö.E., A.Ş.; Data Collection and/or Processing - İ.S., A.S., A.Ş.; Analysis and/or Interpretation - İ.S., A.O.İ.; Literature Search - A.O.İ.; Writing Manuscript - İ.S., A.O.İ.; Critical Review - İ.S., A.S., A.O.İ.

Conflict of Interest: The authors have no conflicts of interest to declare.

Financial Disclosure: The authors declared that this study has received no financial support.

REFERENCES

- Mahmoodnia L, Aghadavod E, Beigrezaei S, Rafieian-Kopaei M. An update on diabetic kidney disease, oxidative stress and antioxidant agents. *J Renal Inj Prev* 2017; 6: 153-7. [\[Crossref\]](#)
- Perkovic V, Agarwal R, Fioretto P, Hemmelgarn BR, Levin A, Thomas MC, et al. Management of patients with diabetes and CKD: Conclusions from a “Kidney Disease: Improving Global Outcomes” (KDIGO) Controversies Conference. *Kidney Int* 2016; 90: 1175-83. [\[Crossref\]](#)
- Unwin N, Whiting D, Gan D, Jacqmain O, Ghyoot G, editors. *IDF Diabetes Atlas*. 4th ed. Brussels: Federation. ID, 2009.
- Kisic B, Miric D, Dragojevic I, Rasic J, Popovic L. Role of myeloperoxidase in patients with chronic kidney disease. *Oxid Med Cell Longev* 2016; 2016: 1069743. [\[Crossref\]](#)
- Nguyen-Khoa T, Massy ZA, De Bandt JP, Kebede M, Salama L, Lambrey G, et al. Oxidative stress and haemodialysis: Role of inflammation and duration of dialysis treatment. *Nephrol Dial Transplant* 2001; 16: 335-40. [\[Crossref\]](#)
- Modaresi A, Nafar M, Sahraei Z. Oxidative stress in chronic kidney disease. *Iran J Kidney Dis* 2015; 9: 165-79.
- Dirican N, Dirican A, Sen O, Aynali A, Atalay S, Bircan HA, et al. Thiol/disulfide homeostasis: A prognostic biomarker for patients with advanced non-small cell lung cancer? *Redox Rep* 2016; 21: 197-203. [\[Crossref\]](#)
- Ayar G, Sahin S, Yazici MU, Neselioglu S, Erel O, Bayrakci US. Effects of hemodialysis on thiol-disulphide homeostasis in critically ill pediatric patients with acute kidney injury. *Biomed Res Int* 2018; 2018: 1898671. [\[Crossref\]](#)
- Elmas B, Karacan M, Dervisoglu P, Kösecik M, İşgüven ŞP, Bal C. Dynamic thiol/disulphide homeostasis as a novel indicator of oxidative stress in obese children and its relationship with inflammatory-cardiovascular markers. *Anatol J Cardiol* 2017; 18: 361-9. [\[Crossref\]](#)
- Erel O, Neselioglu S. A novel and automated assay for thiol/disulphide homeostasis. *Clin Biochem* 2014; 47: 326-32. [\[Crossref\]](#)
- Erel O. A new automated colorimetric method for measuring total oxidant status. *Clin Biochem* 2005; 38: 1103-11. [\[Crossref\]](#)
- Erel O. A novel automated direct measurement method for total antioxidant capacity using a new generation, more stable ABTS radical cation. *Clin Biochem* 2004; 37: 277-85. [\[Crossref\]](#)
- Das M, Cevik Y, Erel O, Çorbacioğlu ŞK. Ischemia-modified albumin levels in the prediction of acute critical neurological findings in carbon monoxide poisoning. *Kaohsiung J Med Sci* 2016; 32: 201-6. [\[Crossref\]](#)
- Erel O. Automated measurement of serum ferroxidase activity. *Clin Chem* 1998; 44: 2313-9. [\[Crossref\]](#)
- Goth L. A simple method for determination of serum catalase activity and revision of reference range. *Clin Chim Acta* 1991; 196: 143-51. [\[Crossref\]](#)
- Bradley PP, Priebe DA, Christensen RD, Rothstein G. Measurement of cutaneous inflammation: Estimation of neutrophil content with an enzyme marker. *J Invest Dermatol* 1982; 78: 206-9. [\[Crossref\]](#)
- Kaysen GA. Serum albumin concentration in dialysis patients: Why does it remain resistant to therapy? *Kidney Int Suppl* 2003; 87: S92-98. [\[Crossref\]](#)
- Bhonsle HS, Korwar AM, Kote SS, Golegaonkar SB, Chougale AD, Shaik ML, et al. Low plasma albumin levels are associated with increased plasma protein glycation and HbA1c in diabetes. *J Proteome Res* 2012; 11: 1391-6. [\[Crossref\]](#)
- Tayeb JS, Provenzano R, El-Ghoroury M, Bellovich K, Khairullah Q, Pieper D, et al. Effect of biocompatibility of hemodialysis membranes on serum albumin levels. *Am J Kidney Dis* 2000; 35: 606-10. [\[Crossref\]](#)
- Kliger AS. Serum albumin measurement in dialysis patients: Should it be a measure of clinical performance? *Adv Ren Replace Ther* 2003; 10: 225-7. [\[Crossref\]](#)
- Montagnana M, Lippi G, Tessitore N, Salvagno GL, Targher G, Gelati M, et al. Effect of hemodialysis on traditional and innovative cardiac markers. *J Clin Lab Anal* 2008; 22: 59-65. [\[Crossref\]](#)
- Sadik I, Yagoub Z, Sayed N, El Nour A, Abide El Hameed M, Sate BA. The level of ischemic modified albumin (IMA) as risk marker for cardiovascular disease (CVD) among some diabetic patients (type II) in Khartoum State-Sudan. *Sud J Med Sc* 2017; 12: 231-9. [\[Crossref\]](#)
- Bhaskhar KU, Devi HN, Bitla AR, Rao Pvn S, Kumar SV, Sachan AB. Ischemia modified albumin levels in patients with diabetic nephropathy. *Turk J Endocrinol Metab* 2018; 22: 145-50. [\[Crossref\]](#)

24. Liakopoulos V, Roumeliotis S, Gorny X, Dounousi E, Mertens PR. Oxidative stress in hemodialysis patients: A review of the literature. *Oxid Med Cell Longev* 2017; 2017: 3081856. [\[Crossref\]](#)
25. Rao AM, Apoorva R, Anand U, Anand CV, Venu G. Effect of hemodialysis on plasma myeloperoxidase activity in end stage renal disease patients. *Indian J Clin Biochem* 2012; 27: 253-8. [\[Crossref\]](#)
26. Unubol M, Yavasoglu I, Kacar F, Guney E, Omurlu IK, Ture M, et al. Relationship between glycemic control and histochemical myeloperoxidase activity in neutrophils in patients with type 2 diabetes. *Diabetol Metab Syndr* 2015; 7: 119. [\[Crossref\]](#)
27. Gonzalez Rico M, Puchades MJ, Garcia Ramon R, Saez G, Tormos MC, Miguel A. [Effect of oxidative stress in patients with chronic renal failure]. *Nefrologia* 2006; 26: 218-25.
28. Ruskovska T, Jansen EH, Antarov R. Evaluation of assays for measurement of serum (anti)oxidants in hemodialysis patients. *Biomed Res Int* 2014; 2014: 843157. [\[Crossref\]](#)
29. Sarah NK, Anaja HP, Akuyam SA, Bakari AG. Serum total antioxidant status in type 2 diabetic Nigerians. *IOSR Journal of Nursing and Health Science* 2014; 3: 61-5. [\[Crossref\]](#)
30. Kiran BSR, Lakshmi TM, Srikumar R, Reddy EP. Total antioxidant status and oxidative stress in diabetes mellitus and metabolic syndrome. *Int J Pharm Sci Rev Res* 2016; 40: 271-7.
31. Tajbakhsh R, Qorbani M, Mehrpour G, Rahimzadeh M, Azimzadeh MM, Mirmiranpour H. Effect of hemodialysis on oxidants and antioxidant factors in chronic renal failure. *Saudi J Kidney Dis Transpl* 2017; 28: 507-16. [\[Crossref\]](#)
32. Ashok KJ, Sajida MP, Joseph S. Plasma ceruloplasmin in chronic renal failure patients undergoing haemodialysis. *J Clin Diagn Res* 2010; 3: 2058-60.
33. Fidan F, Alkan BM, Ugurlu FG, Bozkurt S, Sezer N, Biçer C, et al. Dynamic thiol/disulphide homeostasis in patients with fibromyalgia. *Arch Rheumatol* 2017; 32: 112-7. [\[Crossref\]](#)