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Changes in thiol/disulfide homeostasis in patients with chronic kidney disease

Ibrahim Solak¹, Ibrahim Guney², Seher Mercan¹, Ozcan Erel³, Salim Neselioglu³,
Cigdem Damla Cetinkaya⁴, Mehmet Ali Eryilmaz⁵

¹University of Health Sciences, Training and Research Hospital, Department of Family Medicine, Konya, Turkey

²University of Health Sciences, Training and Research Hospital, Department of Nephrology, Konya, Turkey

³Yildirim Beyazıt University, Ankara Atatürk Training and Research Hospital, Department of Biochemistry, Ankara, Turkey

⁴University of Health Sciences, Training and Research Hospital, Department of Biochemistry, Konya, Turkey

⁵University of Health Sciences, Training and Research Hospital, Department of General Surgery, Konya, Turkey

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Abstract

Thiol/disulfide homeostasis (TDH) is a new marker of oxidative stress. In this study, we would like to determine the changes in TDH in hemodialysis (HD) patients with chronic kidney disease (CKD). This cross-sectional study was conducted in the Nephrology Clinic of Konya Training and Research Hospital. A total of 197 individuals including 75 HD patients, 41 end stage renal disease (ESRD) patients (having stage 3-5 CKD but not receiving hemodialysis), and 81 healthy controls were enrolled in the study. Serum native thiol, total thiol, and disulfide levels were measured with a new method developed by Erel and Neselioglu. It was determined that there was a statistically significant difference in the mean age, body mass index (BMI), modification of diet in renal disease (MDRD), and creatinine level between the three groups ($p < 0.001$). It was shown that there was a statistically significant difference in native thiol and total thiol levels and disulfide/native thiol, disulfide/total thiol, and native thiol/total thiol ratios between the three groups, whereas there was no statistically significant difference in disulfide level between the three groups. Consequently, our study found that TDH shifted more towards the left (disulfide side) and oxidative stress was higher in both HD (markedly) and CKD groups when compared to the control group.

Keywords: Hemodialysis, chronic kidney disease, oxidative stress, thiol/disulfide homeostasis

Introduction

Oxidative stress is defined as a disturbance in the prooxidants–antioxidants balance in favor of the former, thus leading to a potential damage to the cells and organs [1].

The most common comorbid conditions during hemodialysis (HD) treatment are infections, cardiovascular diseases, amyloidosis (due to β 2-microglobulin accumulation), and malnutrition. These diseases are considered to be side effects of long-term HD. Oxidative stress and microinflammation are accepted as the main causes of these comorbid conditions. There are two main reasons for increased oxidative stress in HD patients. The first reason is that free radical production occurs for improving the biocompatibility of HD treatment. The second reason is that antioxidant levels decrease [2]. Oxidative stress significantly contributes to the morbidity and mortality of HD patients [3,4]

Oxidative stress is present in the early stages of chronic kidney disease (CKD) and increases in more advanced stages of CKD. Oxidative stress plays a central role in the pathophysiology of uremia and cardiovascular complications of CKD. However, the stage where oxidative stress begins to develop during the progression of CKD is unknown [5,6].

Thiol groups are important antioxidants. Intracellular thiols, including glutathione and thioredoxin present in millimolar concentrations within cells, are very important for preserving a highly reduced intracellular environment. Extracellular thiols also constitute an important component of antioxidant defense. The plasma protein-reduced thiols (located primarily on albumin) are depleted in HD patients and are thus not able to participate in antioxidant defense [7].

Previous studies have used various parameters to determine oxidative stress and to assess its severity in HD and CKD patients [8-10]. Thiol/disulfide homeostasis (TDH) is a marker of oxidative stress. In many inflammatory diseases, increased production of proinflammatory cytokines is associated with increased levels of oxidative stress mediators [11,12].

*Corresponding Author: Ibrahim Solak, University of Health Sciences, Training and Research Hospital, Department of Family Medicine, Konya, Turkey
E-mail: isolaktr@yahoo.com

TDH has vital importance. One side of this double-sided balance has been measurable since 1979, but today a new method, developed by Erel and Neselioglu, can measure levels of both variables separately and additively and can evaluate both individually and holistically.

The aim of this study was to determine the changes in TDH in HD and CKD patients.

Material and Methods

This cross-sectional study was conducted in the Nephrology Clinic of Konya Training and Research Hospital. The study was approved by the Ethics Committee of KTO Karatay University Faculty of Medicine (2017/002). All individuals were informed about the study design. Participants who agreed to take part in the study gave written informed consent in accordance with the World Medical Association's Declaration of Helsinki.

Those who were not between the ages of 18 and 65 years old, who did not smoke cigarettes, who had cardiovascular diseases (such as acute coronary syndrome, myocarditis, left ventricular dysfunction, and heart failure), who had chronic inflammatory autoimmune diseases, who received antioxidant drugs (such as angiotensin-converting enzyme inhibitors, beta-blockers with antioxidant properties, and antioxidant vitamin preparations), who were diagnosed with diabetes mellitus, who had chronic liver failure or acute renal failure, who were diagnosed with cancer, and who had Parkinson's disease or Alzheimer's disease were excluded from the study [13]. Patients who received HD treatment for at least 3 months were included in the HD group. Patients who had predialysis stage 3-5 CKD were included in the CKD group.

The abbreviated MDRD estimate [14] of kidney function was calculated as $175 \times \text{plasma creatinine}^{-1.154} \times \text{age}^{-0.203}$ ($\times 0.742$ if female; $\times 1.21$ if black).

The body mass index (BMI) was calculated by dividing weight in kilograms by the square of height in meters ($\text{BMI} = \text{kg}/\text{m}^2$).

Laboratory Analysis

Blood samples were taken from the patients and the controls into serum separator tubes. Sera were obtained by centrifugation at $1500 \times g$ for 10 min. The samples were immediately put in the freezer at -80°C . The same process was applied to all specimens. When the study was completed, the samples were sent to the

biochemistry laboratory of Ankara Ataturk Training and Research Hospital to measure thiol/disulfide homeostasis parameters.

Total thiol (-SH+-S-S-) consists of reduced and native thiols. Thiol/disulfide homeostasis tests were performed using a novel automatic and spectrophotometric method developed by Erel and Neselioglu. The principle of the thiol/disulfide measurement method is the reduction of dynamic disulfide bonds (-S-S-) to functional thiol groups (-SH) by a reductant solution (10 μL), sodium borohydride (NaBH_4). The unused NaBH_4 remnants are completely removed by formaldehyde. So this prevents further reduction of 5,5'-dithiobis-2-nitrobenzoic acid (DTNB) as well as any disulfide bonds resulting from the reaction with DTNB. Total thiol content in the samples were determined by using a modified Ellman reagent after the reaction with DTNB. The disulfide parameter is a value which can be calculated automatically as half of the native thiol content and total thiol content. After the determination of the main parameters (native thiol, total thiol, and disulfide values), disulfide/total thiol percent ratios, disulfide/native thiol percent ratios, and native thiol/total thiol percent ratios were calculated [15].

Statistical methods

Statistical analyzes were performed with SPSS software V21.0 (Statistical Package for the Social Sciences, IBM Corp., Armonk, NY). Numbers, percentages, means, and standard deviations were used for data presentation. The Shapiro-Wilk test was used to examine if the data were normally distributed. The Paired samples t-test was used if the data were normally distributed. The Wilcoxon signed-rank test and Kruskal-Wallis test were used if the data were not normally distributed. All analyzes were performed as two-sided hypotheses with a 5% significance level and a 95% confidence interval.

Results

A total of 197 individuals including 75 HD patients, 41 ESRD patients (having stage 3-5 CKD but not receiving hemodialysis), and 81 healthy controls were enrolled in the study. The sociodemographic features of the participants are shown in Table 1. It was determined that there was a statistically significant difference in the mean age, BMI, MDRD, and creatinine level between the three groups ($p < 0.001$).

Plasma levels of TDH parameters in the three groups are shown in Table 2.

Table 1. Sociodemographic and laboratory features of three groups

		HD n=75(%)	CKD n=41(%)	Control n=81(%)	P
Age	Female	40(53.3)	34(82.9)	57(70.4)	0.003
	Male	35(46.7)	7(17.1)	24(29.6)	
	Mean \pm SD		Mean \pm SD	Mean \pm SD	
Age (year)		44.75 \pm 12.54	48.82 \pm 10.54	46.71 \pm 8.95	<0.001
BMI (kg/m ²)		24.39 \pm 4.73	29.76 \pm 5.66	31.19 \pm 6.42	<0.001
Creatinine (mg/dL)		7.22 \pm 2.16	2.78 \pm 1.26	0.90 \pm 0.11	<0.001
MDRD(mL/min/1.73 m ²)		8.44 \pm 2.96	25.35 \pm 11.98	91.56 \pm 10.07	<0.001

Table 2. Plasma thiol/disulfide levels of three groups

	HD n=94 Mean±SD	CKD n=67 Mean±SD	Control n=102 Mean±SD	P
Native thiol (µmol/L)	245.65±52.13	247.76±40.86	375.09±63.18	<0.001
Total thiol (µmol/L)	274.34±58.00	270.82±45.38	403.02±63.04	<0.001
Disulfide (µmol/L)	14.34±11.02	11.52±6.68	13.96±6.62	0.155
Disulfide/native thiol (%)	6.08±5.34	4.69±2.68	3.87±2.05	0.007
Disulfide/total thiol (%)	5.09±3.47	4.18±2.16	3.52±1.70	0.007
Native thiol/total thiol (%)	89.80±6.95	91.62±4.33	92.94±3.41	0.007

Native thiol level was found to be highest in the control group and to be lowest in the HD group. There was a statistically significant difference in native thiol level between the three groups ($p<0.001$).

Total thiol level was found to be highest in the control group and to be lowest in the HD group. There was a statistically significant difference in total thiol level between the three groups ($p<0.001$).

Disulfide level was found to be highest in the HD group and to be lowest in the CKD group. There was no statistically significant difference in disulfide level between the three groups ($p=0.155$).

Disulfide/native thiol ratio was found to be highest in the HD group and to be lowest in the control group. There was a statistically significant difference in disulfide/native thiol ratio between the three groups ($p=0.007$).

Disulfide/total thiol ratio was found to be highest in the HD group and to be lowest in the control group. There was a statistically significant difference in disulfide/total thiol ratio between the three groups ($p=0.007$).

Native thiol/total thiol ratio was found to be highest in the control group and to be lowest in the HD group. There was a statistically significant difference in native thiol/total thiol ratio between the three groups ($p=0.007$).

Discussion

Patients with uremia due to renal failure are at a higher risk of morbidity and mortality than nonuremic patients. Many factors are responsible for this increased risk. Oxidative stress and formation of reactive oxygen species seem to play an important role. Both experimental and clinical studies have demonstrated that there is an increase in oxidation state due to disease progression and that it relates to clinical complications in the context of different renal injury models [2]. In this study, we tried to show the changes in oxidative stress in HD and CKD patients by TDH.

As a member of the antioxidant cascade, thiol group eliminates reactive oxygen species (ROS), and thus the measurement of total thiol level can be used to assess oxidative status [16]. Ates et al. [17] determined that total thiol level was lower in CKD patients than in healthy controls. In our study, total thiol level was found to be lowest in the HD group, followed respectively by the CKD and control groups. There was a statistically significant difference in total thiol level between the three groups.

Native thiol capacity was shown to decrease linearly by application of oxidation process [15]. Previous studies revealed that serum thiol levels decreased in CKD patients [18, 19]. This decrease can be due to two reasons: decreased protein uptake and loss of amino acids through proteinuria (1) and transformation of thiols to disulfides by oxidation (2) [17]. Ates et al. [17] determined that native thiol level was lower in CKD patients than in healthy controls. In our study, native thiol level was found to be lowest in the HD group, followed respectively by the CKD and control groups. There was a statistically significant difference in native thiol level between the three groups.

Ates et al. [17] determined that disulfide level was lower in CKD patients than in healthy controls. In our study, disulfide level was found to be lowest in the CKD group, followed respectively by the control and HD groups. However, there was no statistically significant difference in disulfide level between the three groups.

The thiol–disulfide equilibrium provides rapid and dynamic regulation, generates redox signaling, and occupies a central place as a target of oxidative stress. This feature makes serum disulfide/thiol ratio useful as a clinical measure of oxidative stress [15]. Ates et al. [17] found that there was no statistically significant difference in disulfide/native thiol, disulfide/total thiol, and native thiol/total thiol ratios between the CKD and healthy control groups. In our study, disulfide/native thiol and disulfide/total thiol ratios were found to be highest in the HD group, followed respectively by the CKD and control groups. There was a statistically significant difference in disulfide/native thiol and disulfide/total thiol ratios between the three groups. In our study, native thiol/total thiol ratio was found to be lowest in the HD group, followed respectively by the CKD and control groups. However, there was no statistically significant difference in native thiol/total thiol ratio between the three groups.

Ateş et al. [20] reported that there was a positive correlation between oxidative stress and age and that native thiol level decreased with age. Babaoglu et al. [21] also indicated that there was a negative correlation between age and total and native thiol levels. This information shows us that oxidative stress may increase with age and that thiol/disulfide homeostasis may shift towards disulfide side. In our study, there was a statistically significant difference in the mean age between the three groups. Although the HD group had the lowest median age, oxidative stress was found to be highest in this group.

Studies have revealed that adipose tissue formation is associated with increased levels of inflammation and oxidative stress and antioxidant levels decreased in obese individuals [22]. Sögüt et al. [23] reported that TDH shifted more towards disulfide side (decreased total and native thiol levels, increased disulfide level) in obese individuals when compared to normal-weight individuals. In our study, the fact that the control group had highest BMI could explain that disulfide levels did not differ between the three groups. If there was no difference between in BMI between the three groups in our study, the other groups would have higher levels of oxidative stress when compared to the control group.

Conclusion

Consequently, our study found that TDH shifted more towards the left (disulfide side) and oxidative stress was higher in both HD (markedly) and CKD groups when compared to the control group.

More comprehensive studies will show that different strategies to reduce oxidative stress in HD and CKD patients can prevent the development of additional diseases due to oxidative stress in these patient groups.

Competing interests

The authors declare that they have no competing interest.

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Ethical approval

The study was approved by the Ethics Committee of KTO Karatay University Faculty of Medicine (2017/002).

Ibrahim Solak ORCID: 0000-0001-5311-0631

Ibrahim Guney ORCID: 0000-0002-1646-2811

Seher Mercan ORCID: 0000-0002-4066-2928

Ozcan Erel ORCID: 0000-0002-2996-3236}

Salim Neselioglu ORCID: 0000-0002-0974-5717

Cigdem Damla Cetinkaya ORCID: 0000-0002-6052-4645

Mehmet Ali Eryilmaz ORCID: 0000-0002-5280-3943

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