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Erythrocytic Reduced/Oxidized Glutathione and Serum Thiol/Disulfide Homeostasis in Patients with Opioid Use Disorder

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ABSTRACT

Background: This study aimed to evaluate oxidative damage by measuring erythrocytic reduced/oxidized glutathione as an intracellular thiol pool and serum thiol/disulfide homeostasis as an extracellular thiol pool in patients with opioid use disorder.

Methods: In this prospective cross-sectional study, 33 male patients diagnosed with opioid use disorder and 30 healthy male controls were included. Sociodemographic characteristics and psychometric analyzes were performed and addiction characteristics (duration and amount of heroin use, usage methods) were recorded. For the evaluation of oxidative balance, intracellular reduced-oxidized glutathione (reduced glutathione and oxidized glutathione), and extracellular thiol-disulfide (native thiol and disulfide) levels were measured.

Results: There was a decrease in reduced glutathione and native thiol levels and an increase in GSSG and SS levels. Similarly, while oxidized/reduced glutathione, oxidized/total glutathione%, and disulfide/native thiol % ratios increased, the ratio of reduced glutathione/total glutathione% and native thiol/total thiol% decreased. Moreover, a positive correlation was found between the level of both intracellular and extracellular oxidant molecules and the duration and amount of opioid use.

Conclusion: Impaired intracellular reduced glutathione/oxidized glutathione and extracellular disulfide/native thiol homeostasis were found in patients with opioid use disorder. The intracellular and extracellular oxidative stress may cause complications related to chronic opioid use.

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INTRODUCTION

Opioid use disorder (OUD) is the chronic use of opioids that causes clinically significant impairment in the person's life. It affects over 16 million people worldwide and causes important clinical morbidities and mortality.[1] Chronic exposure to opioids could induce inflammatory processes, alter the oxidative balance, and thus lead to neuronal dysfunction.[2]

Reactive oxygen species (ROS) are generated during mitochondrial oxidative metabolism as well as in exposure to external stress and agents. [3] The oxidative effects of ROS can be neutralized by extracellular and intracellular antioxidant enzymes and molecules. Thiols (–SH) are important nonenzymatic groups that are located at the main redox control systems in biological signaling. The thiol groups are highly sensitive to oxidation and form covalent disulfide bonds (–S–S–) between the 2 thiol groups as a result of redox reactions. This reaction is reversible and could again be reduced to –SHs, and dynamic thiol-disulfide homeostasis is maintained in this way. Dynamic thiol-disulfide homeostasis is associated with important biochemical processes including detoxification, protein functioning, cellular signaling, transcription, enzyme regulation, and apoptosis.[4]

Thiol groups are found both extracellularly and intracellularly. They are mainly carried by cysteine residues of albumin and other proteins and constitute the extracellular thiol pool which is represented as serum thiol-disulfide (SH/SS) homeostasis.[5] On the other hand, glutathione is an important molecule that constitutes the main intracellular thiol pool. It can be found in oxidized

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(GSSG) and reduced (GSH) forms as a result of the redox reactions of the SH groups it carries. Reduced/oxidized glutathione (GSSG/GSH) homeostasis represents the intracellular thiol state.[6] Various disorders may affect intracellular or extracellular oxidative balance in different ways. For this reason, the assessment of both intracellular and extracellular oxidative status together allows a more integrative approach to understand the underlying pathophysiology of the diseases.

Illicit drug use is accepted to induce oxidative stress by increasing the formation of ROS and reducing the activity of the antioxidant system. Opioids are well-known substances that increase oxidative stress in patients with OUD.[7] This oxidative imbalance could result in inflammation, cellular loss, and neuronal dysfunction. Although there are some studies in the literature examining either only intracellular glutathione levels[8] or only extracellular thiol-disulfide homeostasis in opioid users[9], there is no study investigating both intracellular and extracellular thiol-disulfide status together.

Evaluating these two basic homeostatic systems, which contain the most important intracellular and extracellular antioxidant thiol pools, will allow a better understanding of the oxidative balance in patients with OUD. Therefore this study aimed to investigate both erythrocytic reduced/oxidized glutathione and serum thiol/disulfide homeostasis in patients with OUD.

MATERIAL AND METHODS

Subjects

The patient group comprised 33 males who were between 18 and 65 years old, admitted to the psychiatry department for treatment, and had the diagnosis of OUD according to the Diagnostic and Statistical Manual of Mental Disorders-5 (DSM-5) criteria. *A priori* power analysis was performed for the determination of the sample size considering the results from a previous study[9] and calculated as 56 by taking impact size 0.8, $a = 0.05$, and statistical power of 0.90. The inclusion criteria were using heroin for at least 3 months, no other addictive substance use (excluding tobacco), and no other known physical or psychiatric disorders (e.g., mood disorders, anxiety disorders, neurodevelopmental disorders, or psychotic disorders). Subjects having used other substances (alcohol, psychostimulants, ecstasy, or cannabinoids) in the past 3 months or chronically, having a history of acute or chronic infection (e.g., influenza, hepatitis B or C, HIV), chronic physical illness (e.g., liver disease, kidney diseases, or metabolic diseases), using a chronic medication, taking maintenance therapy for addiction (buprenorphine + naloxone or naltrexone), or body mass index (BMI) >30 were excluded from the study.

MAIN POINTS

- Intracellular and extracellular thiol/disulfide homeostasis, which is the main oxidative balance pool in the body, is disturbed towards oxidants in opioid users.
- There is a positive correlation between the level of both intracellular and extracellular oxidant molecules and the duration and amount of opioid use.
- Interventions to restore both intracellular and extracellular antioxidant balance may be important in preventing complications in patients using opioids.

The control group was constituted of 30 healthy males who were matched for age, BMI, and other sociodemographic features, and had no known medical diseases or psychiatric disorders. They were evaluated by a psychiatrist through psychiatric interviews and mental state examinations. Alcohol or substance users and those who had a history of acute infection or trauma in the last 3 months were not included in the study as healthy controls.

The patients' duration of heroin usage, usage methods (intranasal (foil), intravenous (i.v.), or both), and the amount of daily heroin use for the last 3 months (grams) were recorded. In the clinical assessment, the sociodemographic data were collected with a form prepared by the researchers. In the clinical assessment, The Beck Depression Inventory (BDI) and Beck Anxiety Inventory (BAI) were administered to both patient and control groups. The BDI is a 17-item self-report scale and the BAI is a 21-item Likert-type self-report scale (0-3 points) that evaluates depression and anxiety symptoms, and also symptom severity.[10,11] The Turkish validity and reliability studies of both scales were performed. In the reliability analysis conducted with the clinical samples, the Cronbach-alpha coefficients were calculated as 0.89 for BDI and 0.93 for BAI.[12,13]

The study was conducted following the Helsinki Declaration and the study protocol was approved by the Ethics Committee of Istanbul Bilgi University (no:2021-40034-44), and written informed consent was obtained from all participants before study participation.

Blood Samples

Whole blood samples were taken from all participants via venipuncture into tubes following 8 hours of fasting. The patients had opioid positivity in the urine drug test when the blood samples were taken, but the blood level of the opioids could not be measured.

To evaluate intracellular GSH/GSSG homeostasis, blood samples were drawn into tubes treated with ethylene diamine tetra-acetic acid (EDTA) and centrifuged at 1500 g for 10 minutes. After centrifugation, the plasma fraction was discarded. Then, the samples in EDTA tubes were washed 3 times in 0.9% NaCl and lysed with distilled water. To precipitate proteins, 3 parts of erythrocyte lysate and 1 part of 20% w/v trichloroacetic acid (TCA) solution were used. Obtained supernatant fractions were used to evaluate intracellular reduced/oxidized glutathione analysis.

Additionally, serum samples were obtained after centrifugation at 4000 g and used for evaluating extracellular thiol-disulfide homeostasis. All of the serum samples and supernatants were stored at -80°C until being used for analysis. All biochemical analysis was conducted in a Siemens Advia 1800 chemistry analyzer and a Siemens Advia Centaur XPT immunoassay device (Erlangen, Germany).

Measurement of Intracellular Reduced and Oxidized Glutathione

Intracellular GSH and GSSG levels (or by another name erythrocytic thiol-disulfide homeostasis) were measured by a new method.[14] To measure GSH levels in samples, the modified Ellman method was used.[15] GSSG in the sample was reduced with a solution containing sodium borohydride ($NaBH_4$) and NaOH to form GSH. To measure total glutathione (GSH+GSSG) levels, GSH measurement steps were repeated. The measurement was performed according to GSH reducing the 5,5'-dithiobis-(2-nitrobe nzoic) acid (DTNB) molecules to 2-nitro-5-benzoic acid which has an absorbance at 412 nm spectrophotomet rically. The GSH content was subtracted from the total glutathione (GSH+GSSG) content and divided by 2 to calculate the GSSG amount. The tests of erythrocytic thiol-disulfide homeostasis were standardized by proportion to hemoglobin. An Advia 2120i Hematology System (Siemens, Germany) was used to assess hemoglobin levels in the samples. Results were expressed as µmol/L. In addition, the oxidized/reduced glutathione ratio (GSSG/GSH%), oxidized/total glutathione ratio (GSSG/GSH+GSSG%), and reduced/total glutathione ratio (GSH/GSH+GSSG%) were calculated.

Measurement of Extracellular Thiol-Disulfide Homeostasis

Extracellular SH-SS homeostasis parameters were assessed by an automated spectrophotometric method described by Erel and Neselioglu[16] in the serum samples. Reducible SS bonds were first reduced by $NaBH_4$ to form free functional SH groups. After the reduction step, total SH levels were determined again using DTNB. Free SH groups converted the DTNB molecules to 5-thionitro-benzoic acid with the spectrophotometric absorbance at 412 nm. After that, the amount of SS is determined by half of the difference between serum total and native SH levels. Serum SH, SS, and SH+SS levels were expressed as µmol/L. Additionally, disulfide/native thiol% (SS/SH%), disulfide/total thiol% (SS/SH+SS%), and native thiol/total thiol% (SH/SH+SS%) ratios were calculated.

Statistical Analysis

The Statistical Package for the Social Sciences (SPSS) version 23.0 (IBM SPSS Corp.; Armonk, NY, USA) was used to analyze the study data. The Kolmogorov-Smirnov test was used to assess the distribution of the variables. Continuous variables were analyzed with the independent sample *t*-test or Mann-Whitney *U*-test for comparison of the 2 groups. Number of the cases (percentage %), mean ± SD, or median and 25th-75th percentiles (minimum-maximum) were reported. Pearson's and Spearman's correlation tests were used to evaluate the correlation between the variables.

The impact of independent variables including age, BMI, history of smoking, BDI, and BAI as also patients' characteristics on extra thiol/disulfide and intracellular reduced/oxidized glutathione homeostasis as dependent variables. We performed a multiple regression by using enter method. In this method, all independent variables were entered into the equation at the same time. The multiple regression model was shown as the estimation of coefficients (β) and 95% confidence interval (CI). The statistical significance level was accepted as $P < .05$.

RESULTS

Characteristics of Participants

A total of 33 patients with OUD and 30 healthy controls were included in this study. The substance use profile of the patient group and the demographic characteristics of both patients and the control group are shown in Table 1.

There was no significant difference in age, educational status, BMI, and hemoglobin levels between the groups ($P=.289$, $P=.983$, $P=.541$, $P=.149$, respectively). Smoking (pack-year) was significantly higher in the patient group than in the control group ($P=.012$).

The BDI and BAI scores were significantly higher in the patient group compared to the control group (Table 1).

Oxidative Stress Parameters

Intracellular reduced/oxidized glutathione and extracellular thiol/disulfide parameters are given in Table 2. In the evaluation of intracellular reduced/oxidized glutathione homeostasis, while GSH levels and GSH/total glutathione% ratios were statistically significantly decreased in the patient group than the control group ($P=.014$ and $P < .001$, respectively), GSSG levels GSSG/GSH% and GSSG/total glutathione% ratios were significantly higher in the patient group compared to in the healthy controls ($P < .001$). Total glutathione level was lower in the patient group, but the difference between both groups was statistically insignificant ($P=.27$).

When the extracellular oxidative stress parameters were compared between the groups, SH levels, SS/SH+SS%, and SH/SH+SS% ratios were statistically significantly lower in the patient group than in the control group ($P < .05$). Total thiol levels were higher in the patient group than in the healthy controls, but the difference

Table 1. General Characteristics of Patient and Control Groups

	Patients with OUD (n = 33)	Control Group (n = 30)	*P*
Age (years)	27.3 ± 4.52	26.8 ± 8.85	.289
Educational status (years)	8 (8-12)	8 (5-12)	.983
BMI (kg/m^2)	22.9 ± 2.26	23.6 ± 2.46	.541
Duration of opioid use (years)	6 (4-10)	0	NA
Amount of daily opioid use (g/day)	2 (2-3)	0	NA
Method of opioid use			
Intranasal (foil)	18 (55%)	0	
Intravenous (i.v.)	6 (18%)	0	NA
Both intranasal and i.v.	9 (27%)	0	
Smoking (pack-year)	10 (5-14.5)	5 (0-8.6)	**.012**
BDI score	13.8 ± 4.77	7.8 ± 3.64	**<.001**
BAI score	12 ± 4.49	8.36 ± 4.82	**.033**

Values are given as a mean ± SD, number (percentage), or median (IQR; 25th-75th percentile).
BAI, Beck Anxiety Inventory; BDI, Beck Depression Inventory; BMI, body mass index; IQR, interquartile range; OUD, opioid use disorder; NA, not applicable.

was not statistically significant (*P* = .056). On the other hand, the SS levels and SS/SH% ratio were significantly increased in the patient group compared to the control group (*P* < .05).

When the groups were compared according to opioid use methods, no significant difference was found between the 3 patient groups in terms of intracellular and extracellular oxidative stress parameters (*P* > .05 for all).

Multiple linear regression analysis was performed to predict the possible effects of demographic (age, BMI, and history of smoking) and clinical characteristics (BDI and BAI) on intracellular and extracellular oxidative stress parameters. It was found that BMI, smoking, and BAI scores did not have any effect on intracellular and extracellular oxidative stress parameters (*P* > .05 for all). On the other hand, BDI scores and the age of the patients had a statistically significant effect on disulfide levels (*P* = .034 and *P* < .001, respectively) (Table 3).

The correlation analysis between the duration and amount of opioid use and oxidative stress parameters is shown in Table 4. It was found that SS, SS/SH%, and SS/SH+SS% as extracellular parameters and GSSG, GSSG/GSH%, and GSSG/GSH+GSSG% as intracellular parameters were positively correlated with duration and amount of opioid use. On the other hand, SH, SH/SH+SS%, and GSH/GSH+GSSG% levels were negatively correlated with duration and amount of opioid use.

DISCUSSION

Our study showed that there is both intracellular and extracellular oxidative imbalance in patients with OUD. While there was a decrease in the levels of intracellular GSH and extracellular SH, an increase was found in the levels of GSSG and SS. Similarly, while the ratio of both intracellular and extracellular oxidant molecules in the oxidative balance increased, the ratio of antioxidants decreased. Moreover, a positive correlation was found between the level of both intracellular and extracellular oxidant molecules and the duration and amount of opioid

Table 2. Intracellular and Extracellular Oxidative Parameters of Patient and Control Groups

	Patients with OUD (n = 33)	Control Group (n = 30)	*P*
Intracellular glutathione parameters			
Reduced glutathione (GSH) (µmol/L)	63.73 ± 15.35	72.35 ± 11.21	**.014**
Total glutathione (GSH+GSSG) (µmol/L)	76.87 ± 16.45	80.96 ± 12.12	.271
Oxidized glutathione (GSSG) (µmol/L)	6.57 ± 2.26	4.3 ± 1.53	**<.001**
GSSG/GSH ratio (%)	10.76 ± 4.56	6.04 ± 2.22	**<.001**
GSSG/GSH+GSSG ratio (%)	8.65 ± 2.81	5.32 ± 1.74	**<.001**
GSH/GSH+GSSG ratio (%)	82.72 ± 5.63	89.35 ± 3.48	**<.001**
Extracellular thiol-disulfide parameters			
Native thiol (SH) (µmol/L)	369.9 ± 22.87	389.9 ± 40.41	**.050**
Total thiol (SH+SS) (µmol/L)	426.25 ± 26.08	416.48 ± 38.22	.056
Disulfide (SS) (µmol/L)	28.83 ± 7.61	13.31 ± 5.3	**.012**
SS/SH ratio (%)	7.86 ± 2.25	3.5 ± 1.46	**<.001**
SS/SH+SS ratio (%)	6.70 ± 1.65	3.22 ± 1.26	**<.001**
SH/SH+SS ratio (%)	86.53 ± 3.34	93.53 ± 2.53	**.018**

Values are given as a mean ± SD or median (IQR 25th-75th percentile).
GSH/GSH+GSSG %, reduced glutathione/total glutathione%; GSSG/GSH %, oxidized glutathione/reduced glutathione %; GSSG/GSH+GSSG%, oxidized glutathione/total glutathione%; SS/SH, disulfide/ native thiol%; SS/SH+SS, disulfide/total thiol%; SH/SH+SS, native thiol/total thiol%; IQR, interquartile range; OUD, opioid use disorder.

Table 3. Multiple Linear Regression Analysis to Predict the Possible Effect of Demographic and Clinical Characteristics on Extracellular Thiol/Disulfide Parameters

	Disulfide (SS)		*P*
	St. B	95% CI	
Age (years)	-0.415	(-0.766-0.239)	**<.001**
BDI score	0.329	(0.051-1.22)	**.034**

Adjusted *R* square was for disulfide (R^2 = 0.321; *P* < .001).
BDI, Beck Depression Inventory.

use. To the best of our knowledge, this is the first study to demonstrate the status of the thiol pool in opioid users by investigating both intracellular oxidized-reduced glutathione and extracellular thiol-disulfide homeostasis together.

Patients with OUD encounter several physical, neurological, and psychiatric problems. Both poor psychosocial factors and the strong pharmacodynamic effects of opioids could induce cellular damage and cause central nervous system toxicity.[17] Adverse environmental factors such as inadequate nutritional intake, recurrent systemic infections, chronic stress, and inappropriate living conditions could trigger inflammation and oxidative stress.[18] In addition to these conditions, chronic opioid exposure has direct neurotoxic and inflammatory effects.

Table 4. Correlation Analysis Between Opioid Use Characteristics and Intracellular and Extracellular Oxidative Parameters

	Duration of Opioid Use	Amount of Daily Opioid Use
SH	*r* = -0.351	*r* = -0.214
	***P* = .005**	***P* = .092**
SS	*r* = 0.535	*r* = 0.679
	***P* < .001**	***P* < .001**
SH + SS	*r* = -0.48	*r* = 0.18
	***P* = .715**	***P* = .166**
SS/SH	*r* = 0.565	*r* = 0.668
	***P* < .001**	***P* < .001**
SS/SH + SS	*r* = 0.570	*r* = 0.499
	***P* < .001**	***P* < .001**
SH/SH + SS	*r* = -0.568	*r* = -0.669
	***P* < .001**	***P* < .001**
GSH	*r* = -0.220	*r* = -0.191
	***P* = .084**	***P* = .134**
GSSG	*r* = 0.286	*r* = 0.447
	***P* = .023**	***P* < .001**
GSH + GSSG	*r* = -0.124	*r* = -0.047
	***P* = .331**	***P* = .714**
GSSG/GSH	*r* = 0.283	*r* = 0.421
	***P* = .023**	***P* = .001**
GSSG/GSH + GSSG	*r* = 0.323	*r* = 0.453
	***P* = .009**	***P* < 0.001**
GSH/GSH + GSSG	*r* = -0.322	*r* = 0.450
	***P* = .010**	***P* < 0.001**

GSH, reduced glutathione; GSH + GSSG = total glutathione; GSSG, oxidized glutathione; GSSG/GSH, oxidized/reduced glutathione%; GSSG/GSH + GSSG, oxidized/total glutathione%; GSH/GSH + GSSG, reduced/total glutathione%; SH, native thiol; SS, disulfide; SH + SS, total thiol; SS/SH, disulfide/ native thiol%; SS/SH + SS, disulfide/total thiol%; SH/SH + SS, native thiol/total thiol%.

Human studies have also shown the relationship between opioid use and oxidative imbalance. As a result of the examination of postmortem brain samples of people with OUD, a decrease in the levels of SOD, an antioxidant enzyme, and an increase in the levels of malondialdehyde (MDA), a marker of lipid peroxidation, were found.[23] In another study, lower SOD and catalase activities, and higher TNF-α and MMP-9, a member of matrix metalloproteinases and involved degradation of the extracellular matrix, levels were found in patients with OUD, and this oxidative imbalance was improved by methadone treatment.[8]

In addition to these oxidative stress markers, there are limited studies evaluating the thiol pool in patients with OUD. In a study investigating the extracellular thiol-disulfide balance in men with OUD, it was revealed that the thiol-disulfide balance was impaired, with a shift to an increase in disulfide formation. However, unlike our study, the participants in this study were using buprenorphine-naloxone for addiction treatment.[9] Our study showed that the thiol-disulfide imbalance was similar in OUD patients who did not receive treatment and eliminated the confounding effect of treatment. Moreover, it also revealed that serum disulfide levels and the disulfide/thiol ratio were positively correlated with duration and amount of opioid use.

In our study, it was shown that age, BMI, smoking, and anxiety levels did not have any effect on extracellular thiol/disulfide homeostasis in patients with OUD. On the other hand, it was found that depression scores of patients had a statistical effect only on disulfide levels. There are findings in the literature that mood disorders have some effects on oxidative balance either through decreasing antioxidants or increasing oxidants.[24] Our study suggested that depressive symptoms may contribute to the extracellular oxidant load in patients with OUD. Comorbid mood disorder is common in patients with substance use disorder.[25] Opioid use disorder patients included in our

study had subthreshold or mild depressive symptoms. Considering that both disorders can induce oxidative stress, it can be predicted that the oxidative load may increase even more in patients with OUD. Additionally, interventions aiming to decrease oxidative load may reduce both mood symptoms and substance-related problems, and enhance patients' overall outcomes.

Some researchers studied intracellular GSH levels. Although different animal models exposed to opioids showed decreased GSH levels in the central nervous system, there are conflicting results in human studies. While a postmortem study revealed decreased GSH levels in the cerebral cortex, hippocampus, brain stem, and white matter of opioid users,[26] some human studies could not show any difference in GSH levels.[27] It has been suggested that the discrepancies in these results may be due to the differences in the samples of the postmortem study. However, in a study conducted with OUD, no difference was found in GSH and MDA levels between opioid users and healthy controls.[8] It was proposed that GSH levels could be influenced by several compensatory mechanisms. On the other hand, intracellular thiol levels were measured together with oxidized and reduced GSH levels in our study, and a decrease in GSH levels and an increase in GSSG levels and GSSG/GSH ratio were detected. Reduced glutathione is known to be one of the most important intracellular nonenzymatic antioxidants, and it has been shown to play a role in cell defense, similar to the thiol-disulfide reactions in the extracellular environment in OUD.

Opioids are known to induce dopamine release in the central nervous system. Excessive dopamine is degraded by the enzyme called monoamine oxidase B and as a result, ROS are formed. Increases in dopamine metabolization and production of ROS induced by opioids have cytotoxic effects with the disruption of redox balance and have stressed the protective role of GSH.[28]

Glutamate is the primary excitatory neurotransmitter in the brain. It is known that levels of glutamate and its *N*-methyl-D-aspartate (NMDA) receptor increase in individuals with chronic opioid use. Excessive NMDA receptor levels cause oxidative stress and can induce cyclooxygenase-2-related inflammation, apoptosis, and neurotoxicity in the brain.[29] It has also been shown that opioids can directly induce superoxide formation and contribute more to oxidative stress.[30]

Oxidative stress induced by opioids causes damage to other organs as well as neurotoxicity. It was shown that opioid use was associated with cardiac pathologies. Chronic opioid exposure could cause cardiotoxicity and induce myocardial fibrosis.[31] It was also proven that opioids could induce dose-dependent glomerulosclerosis and cause nephrotoxicity by inducing oxidative load.[29] Disruptions in thiol-disulfide homeostasis, which are generally present in serum and cells, may also lead to other medical diseases.

The study has some limitations. The study had a cross-sectional design with a limited number of participants all of whom were male. Since a sufficient number of female patients could not be reached for statistical evaluation, the study was conducted only with male patients. The other reason for the limited number of participants was due to the inclusion of patients who did not use additional substances other than smoking or were not under treatment (such as buprenorphine-naloxone or naltrexone). Another limitation of our study is that there is no comparison with other intracellular or extracellular markers that show oxidant and antioxidant capacity (such as SOD, MDA, catalase, lipid peroxidation, or total oxidant and antioxidant capacity). Although the patients were using active opioids, their relationship with oxidative parameters could not be evaluated since their blood opioid blood levels could not be measured.

In conclusion, the disturbances in intracellular GSH/GSSG and serum SH/SS balances may play an important role in oxidative stress-related cell and organ damage in patients with OUD. Oxidative load in both intracellular and extracellular areas may contribute to systemic complications related to opioid use. Therefore, an approach to the evaluation of both intracellular GSSG/GSH balance and extracellular SH/SS homeostasis together can provide better maintenance and help control complications due to opioid use.

Ethics Committee Approval: This study was approved by Ethics committee of Istanbul Bilgi University (Approval No: 2021-40034-44, Date: July 12, 2021).

Informed Consent: Written informed consent was obtained from the patients who agreed to take part in the study.

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