

Evaluation of thiol/disulfide homeostasis in patients with a first episode of major depressive disorder

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Abstract

Objective: The aim of this study was to investigate the role of dynamic thiol-disulfide homeostasis as a new oxidative stress parameter in patients with major depressive disorder (MDD).

Material and Methods: Sixty-three patients with their first episode of untreated MDD, and 61 healthy volunteers were included in the study. Serum thiol/disulfide levels were measured in fasting blood samples. The data were compared between the two groups.

Results: No significant difference was observed between the two groups in terms of age, gender distribution, or body mass index. Plasma native and total thiol levels were lower in the MDD group compared to those in the controls ($p=0.004$, $p=0.001$). No significant differences were observed between the groups in terms of disulfide, the disulfide/native thiol, the disulfide/total thiol or the native thiol/total thiol ratio ($p>0.05$). No relationship was detected between these parameters.

Conclusion: As far as we know, this is the first study to evaluate changes in thiol/disulfide homeostasis in male and female patients with MDD. Our data show that thiol levels decrease during the first episode of untreated depression. Thiol/disulfide homeostasis may be useful as a biomarker for depression after long-term follow-up and treatment studies.

Keywords: major depressive disorder, oxidative stress, thiol/disulfide homeostasis.

Introduction

Major depressive disorder (MDD) is a recurrent and chronic disorder with a high mortality rate (1). The incidence rates of MDD in a lifetime are 10–25% in women and 5–12% in men. According to data of the World Health Organization, unipolar major depression constitutes 36% of all psychiatric disorders (2). It is predicted that depressive diseases will be the second major cause of labor-power loss in the entire world by the year 2020 (3), suggesting that MDD is a growing important public health issue (4).

Environmental and genetic factors as well as neurotransmitters and neuroendocrine system disorders play important roles in the pathogenesis of MDD (5).

Oxidative stress is defined as the altered balance between oxidant and antioxidant mechanisms. Oxidant end products generated by oxidative stress damage lipids, proteins, and nucleic acids (6). A number of studies have researched the role of oxidative metabolism in MDD (7,8).

Cumurcu et al. reported that serum total oxidant status (TOS) and the oxidative stress index (OSI) levels are high and total antioxidant capacity level is low in patients with MDD, whereas the total antioxidant level increases and TOS and OSI levels decrease after antidepressant treatment (9).

Thiols are a group of sulfur-containing organic compounds that combine with amino acids and proteins to play important roles in biological systems (10). Thiols are antioxidants that neutralize reactive oxygen types enzymatically and non-enzymatically. Major thiols in the plasma include albumin thiols, protein thiols, and thiols with low molecular weights, such as cysteine, homocysteine, and glutathione. Thiols react with oxidant molecules to form disulfide bonds. Therefore, active thiol/disulfide homeostasis (TDH) is necessary for regulating detoxification, apoptosis, and enzymatic reactions (11,12,13).

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Previous studies have shown that TDH is related to the etiopathogenesis of diseases, such as Parkinson's disease, diabetes, Alzheimer's disease, cardiovascular disease, malignancy and multiple sclerosis (14,15,16,17).

The brain is sensitive to changes in oxidative mechanisms, and neurodegenerative changes have been detected in patients with neuropsychiatric diseases, suggesting that oxidative damage may be involved in the etiology of neuropsychiatric disorders (18,19). Oxidants interact with membrane-bound proteins and block neurotransmitter reuptake causing psychiatric disorders (20). Thiol and disulfide take part in the dysregulation of the oxidative stress system, and their role has been reported in various neuropsychiatric disorders (21,22,23,24,25). The purpose of this study was to compare dynamic TDH in untreated patients with a first episode of MDD with healthy controls. This is the first study to consider TDH in male and female patients with MDD together.

Material and Methods

Subjects

Between April 2017 and February 2018, 66 patients who accepted the study were recruited from 74 patients with a first episode of untreated MDD aged 18–65 years and diagnosed according to the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition criteria who applied to the psychiatric outpatient clinic. Since the inadequate sample of 3 patients excluded from study. Sixty-three patients (34 women and 29 men) and 61 healthy volunteers (32 women, 29 men) were included. All 124 participants, who were admitted to the psychiatric clinic of Bozok University Medical School, were included in the study. This study was approved by the Bozok University Ethics Committee (2017-12/04), and written informed consent was obtained from all participants. Subjects with comorbid psychiatric disorders, systemic or metabolic disease, obesity, smokers, alcohol and substance use disorders, and pregnant and lactating women were excluded from the study. Severity of MDD in the patients was evaluated by the Hamilton Depression Rating Scale (HDRS). The patients were separated into three groups according to this scale as mild, moderate, and severe MDD (Table 1).

Hamilton Depression Rating Scale

The original version of the (Hamilton Depression Rating Scale) HDRS contains 17 items, designed by Hamilton (1960), each scored from 0 to 4 for a maximum total score of 53 (26). The Turkish version of the scale has been validated and is reliable (27). In our study, all patients were evaluated with the 17 item version.

Biochemical tests

Blood Samples were collected in 10 ml serum separator tubes and centrifuged at 1300g for 10 min and stored at –800C until time of analysis. For serum thiol/ disulfide homeostasis measured by, a novel fully automated colorimetric method described by Erel and Neselioglu (28),

(used modified Elman reagents for thiol measurement) First, for total thiol determination, NaHB₄, a reducing agent was added into serum. This operation causes reduction of dynamic disulphide bonds and generates free thiol groups.

Remaining reductants were eliminated by extraction with 110 ml 6.715 mM formaldehyde and 10.0 mM EDTA in tris buffer 100 mM (pH 8.2). For native thiol detection, 10 ml sample was treated with 10 ml, 10 mM sodium chloride in 50% methanol– water solution (v/v; R10) and 110 ml 6.715 mM formaldehyde and 10.0 mM EDTA in Tris buffer 100 mM (pH 8.2) and DTNB solution was added. The primary wavelength is 415 nm, and the secondary wavelength is 700 nm (optionally bichromatic).

Statistical Analysis

The data were analyzed with SPSS v18 software (SPSS Inc., Chicago, IL, USA). Kolmogorov-Smirnov test was employed to check the compliance of the continuous variables to a normal distribution. Student's t-test was applied because distribution of age, native thiol, total thiol and disulphide were normally. Correlations between the groups were identified by Pearson's and Spearman's rho tests. A p-value < 0.05 was deemed to be significant for all tests.

Results

A total of 63 patients (34 females and 29 males) and 61 healthy controls (32 females and 29 males) were evaluated in this study. The mean age of patients was 39.02 ± 12.11 years, and the mean age of the control group was 38.43 ± 9.84 years. No statistically significant differences were observed in terms of age or gender. The demographic characteristics of the patients with MDD and the control group are summarized in Table 1.

The median total thiol level was 378.76 ± 64.45 mmol/l and that of native thiol was 342.98 ± 59.14 mmol/l in the MDD group. The median total thiol level was 415.24 ± 60.39 mmol/l and native thiol level was 373.24 ± 54.71 mmol/l in the healthy control group . Plasma native thiol and total thiol levels were lower in patients than controls (p: 0.004, p: 0.001) (Figure 1, 2).

The median disulphide level was 17.88 ± 8.47 mmol/l in the MDD group and 21.00 ± 11.35 mmol/l in the healthy control group (Figure 3). The differences observed between the two groups in terms of disulfide, the disulfide/native thiol ratio, the disulfide/total thiol ratio, or the native thiol/total thiol ratio were insignificant. The serum TDH parameters of the MDD and control groups are summarized in Table 2.

The thiol/disulfide levels compared between male and female patient groups and also with healthy controls, No statistically significant differences were observed (p>0.05).

No statistically significant correlations were detected between the patient's parameters and disease severity as determined by HDRS in the untreated patients with MDD.

Table 1. Demographic characteristics of the patients with MDD and the control.

| | MDD (n=63) | Controls (n=61) | p-value |
|-----------------|---------------|-----------------|---------|
| Female/Male | 34/29 | 32/29 | >0.05 |
| Age (Mean ± SD) | 39.02 ± 12.11 | 38.43 ± 9.84 | >0.05 |
| BMI (Mean ± SD) | 25.4 ± 4.1 | 25.1 ± 3.9 | >0.05 |
| HDRS mild | 6 (9.5%) | | |
| HDRS moderate | 25 (39.7%) | | |
| HDRS severe | 32 (50.8%) | | |

BMI:body mass index; HDRS:Hamilton Depression Rating Scale; MDD:major depressive disorder; SD: standard deviation

Table 2. Serum thiol/disulfide homeostatic parameters in patients with major depressive disorder and healthy controls.

| | MDD (n = 63) Mean ± SD | Controls (n = 60) Mean ± SD | p-value |
|--------------------------|---------------------------|--------------------------------|---------|
| Native thiol (µmol/l) | 342.98 ± 59.14 | 373.24 ± 24 | 0.004* |
| Total thiol (µmol/l) | 378.76 ± 76 | 415.24 ± 60.39 | 0.001* |
| Disulfide (µmol/l) | 17.88 ± 8.47 | 21 ± 11.35 | 0.086 |
| Disulfide/Native thiol | 5.27 ± 2.53 | 5.71 ± 3.23 | 0.405 |
| Disulfide/Total thiol | 4.68 ± 2.09 | 4.98 ± 2.50 | 0.46 |
| Native thiol/Total thiol | 90.63 ± 4.18 | 90.02 ± 5.01 | 0.46 |

MDD:major depressive disorder ; SD:standard deviation; *p<0.01; **p<0.001

Table 3. Correlations between HDRS and other parameters

| HDRS | r | p-value |
|-----------------------|--------|---------|
| Native thiol (µmol/l) | -0.146 | 0.10 |
| Total thiol (µmol/l) | -0.172 | 0.057 |
| Disulfide (µmol/l) | -0.127 | 0.16 |

HDRS: Hamilton Depression Rating Scale

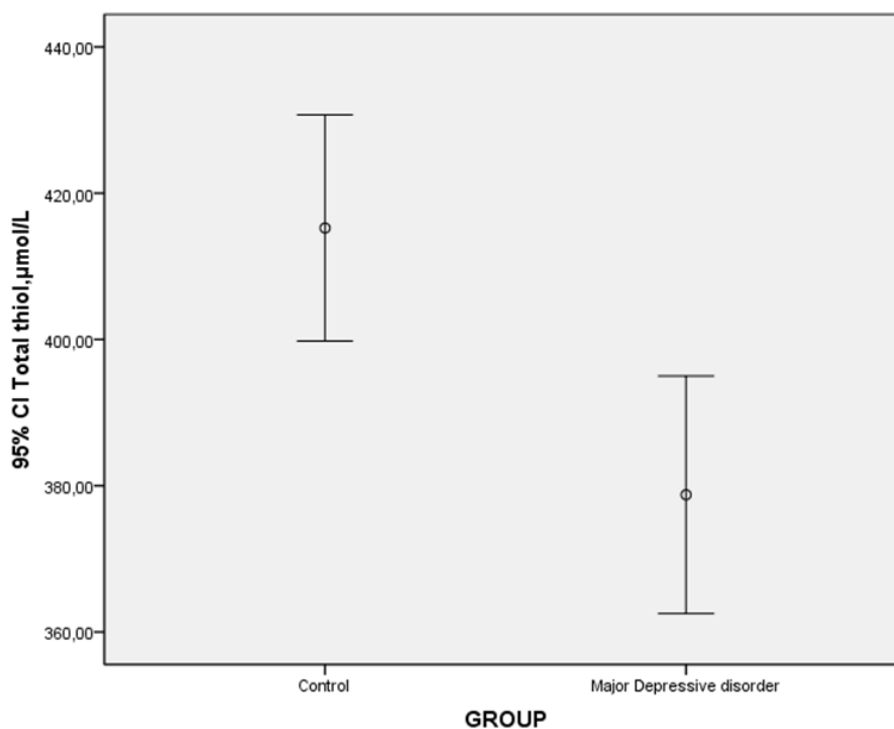


Figure 1: Total thiol levels and 95% confidence intervals (CIs) in patients with major depressive disorder and controls.

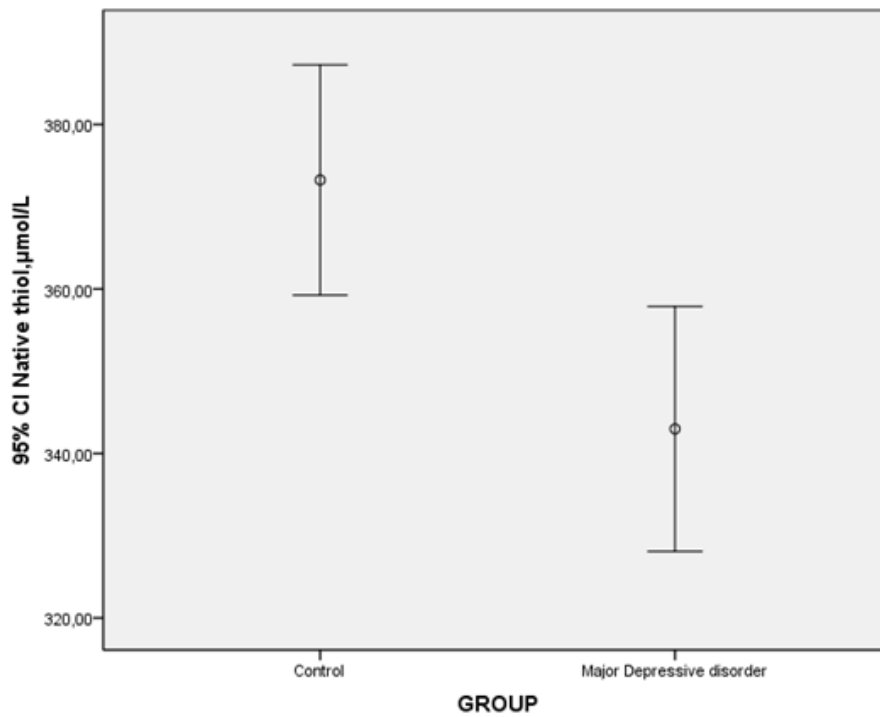


Figure 2: Native thiol levels and 95% confidence intervals (CIs) in patients with major depressive disorder and controls.

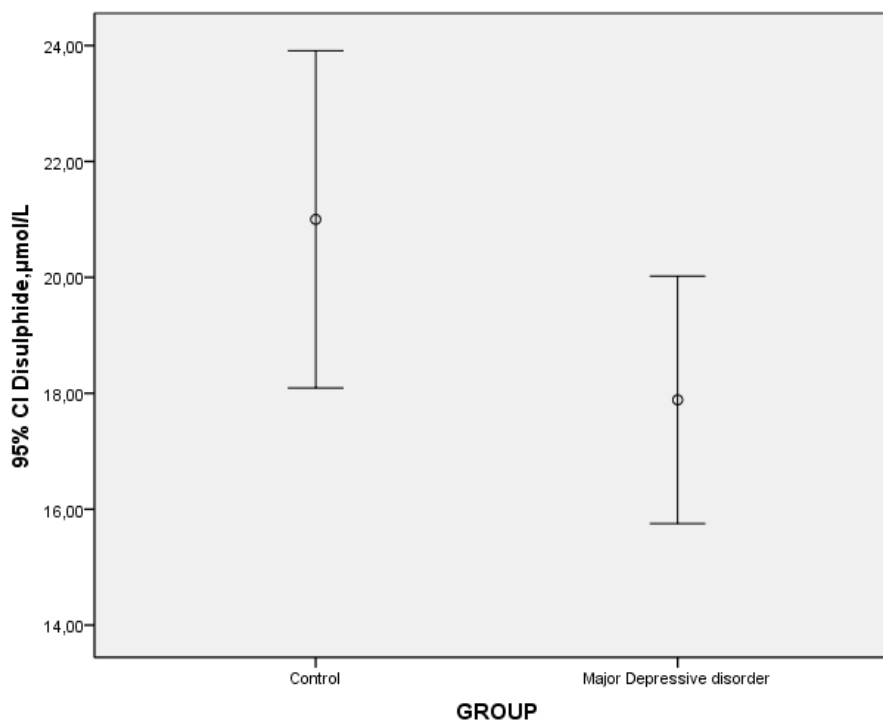


Figure 3: Disulfide levels and 95% confidence intervals (CIs) in patients with major depressive disorder and controls.

Discussion

TDH in patients with a first episode of untreated MDD was evaluated in this study. Plasma native and total thiol levels of patients with MDD were lower than those in the control group.

No statistically significant differences were observed in disulfide levels, disulfide/native thiol, disulfide/total thiol or native thiol/total thiol ratios between the patient and control groups.

No statistical correlation was found between any of these parameters and the HDRS score, which evaluates disease severity (Table 3). Similar to many other psychiatric disorders, the etiology and pathogenesis of depression is not fully understood; however, environmental, genetic, and stress-related factors are known to take part in the etiology. Oxidative system deficits are also thought to be part of the etiology of depression and other psychiatric disorders (9,22,23,24,25). Meta-analysis results have shown that oxidative stress plays a role in depression, and that antidepressant treatment mediates the regulation of oxidative stress/antioxidant balance (29). Thiols are a relatively new parameter with antioxidant properties. Thiols have been used to evaluate balance in the oxidative system of patients with different psychiatric disorders. Thiols enter the oxidative reaction through oxidants and form a covalent disulfide bond. The disulfide bonds can be reduced to thiol groups; thus, maintaining dynamic TDH (30). Thiols play a major role in reactions against oxidative metabolism, apoptosis, signal transmission, protein synthesis, cell growth and proliferation, immunoregulation, and metabolism of xenobiotics (31). Erel and Neselioglu (2014) reported that increases in thiol and decreases in disulfide levels are associated with a proliferative diseases such as malignancies and multiple myeloma, whereas lower thiol levels and higher disulfide levels are associated with degenerative diseases, such as obesity, diabetes and smoking-related diseases (28). Total and native thiol levels are significantly lower in patients with Parkinson's disease (32). Once serum thiol levels decrease, their antioxidant power decreases as well. Oxidative stress is believed to take place in the pathology of neuropsychiatric disorders (33). Unbalanced oxidative metabolism has been increasingly reported in patients with schizophrenia, bipolar disorders, MDD, and anxiety disorders (23,34,35,36).

All thiol levels are lower and disulfide levels are higher in patients with schizophrenia, which is a chronic psychiatric disease. This has been explained by a shift to the disulfide bond side of the equilibrium after reduction of thiols. Lower total thiol levels are also related to nutritional insufficiency. TDH parameters are correlated with disease severity (22). Changes in the oxidative system in patients with schizophrenia have also been reported previously (36). Total and native thiol levels are lower in patients with Parkinson's disease, which is a chronic degenerative disorder. A negative correlation has been detected between these parameters and disease duration (32).

Oxidative stress is higher and antioxidant status is lowered in patients with generalized anxiety disorder (GAD) (37). Total and native thiol levels are similar to controls, while disulfide levels are higher in patients with GAD than those in controls. No correlation has been observed between these parameters and disease severity (23). Similarly, no correlation has been reported between oxidative stress parameters and the severity of panic disorder (38). Oxidative stress findings are controversial in patients with an anxiety disorder (39).

Serum thiol levels are higher during the period of mania in patients with bipolar disorder compared to those in remission and a control group. No statistically significant

difference was found between serum disulfide, disulfide/total thiol, or native thiol/total thiol ratios in manic, remission, and control group subjects. The reduction in total thiol levels in the manic and remission periods, without a change in disulfide levels, is associated with a decrease in thiol levels. Disulfide levels do not increase despite a decrease in thiol levels associated with insufficient nutrient intake and increased consumption to synthesize substances, such as pheomelanin and neuromelanin, rather than converting thiol to disulfide (25).

It was shown in the previous studies that the oxidative stress system is impaired in patients with depression and is improved by antidepressant treatment (9). Baykan et al. (2018) showed that native thiol levels in female patients with MDD are elevated and disulfide levels are lower, whereas total thiol levels are not different. That study concluded that depression is related to an increase in oxidative stress but a shift occurs in the reductive side in female patients. This was explained by the possible involvement of TDH in compensatory mechanisms of oxidative stress, which caused an increase in thiol, but the mechanisms of action were not clarified (24). That study was the first to evaluate thiol and disulfide together but it was conducted only in female subjects. Our results revealed that plasma native and total thiol levels decreased and disulfide level increased in MDD compared to those in the control group. The reasons why our results are different from those of Baykan et al. can be summarized as follows. Thiol can be affected by nutrition, body mass index, smoking status, and sleep routine. Another reason may be disease duration; 38.9% of all subjects in the study of Baykan et al. were second episode patients. Our study group was composed solely of first episode patients. Recurrent attacks in depression are known to cause neurobiological changes. A first episode patient with a short duration of disease may have lower thiol levels but not higher disulfide levels. A relationship between disease duration and changes in the oxidative system has been shown in some disorders (22,40). However, few studies have shown a relationship between disease duration and changes in the oxidative system of patients with MDD. Another reason for the low thiol levels in our study may be changes in nutrition and appetite patterns of patients with MDD. Erzin et al. (2018) suggested that low thiol levels during mania and formal periods may be due to dietary habits (25).

No statistical correlation was found between the HDRS score and thiol and disulfide levels in our study. In concordance with our results, no correlations have been reported between disease severity and oxidative parameters in patients with bipolar affective disorder, GAD, or a panic disorder (23,25,38).

Conclusion

In summary, there is no clear evidence on whether oxidative stress causes psychiatric disorders or psychiatric disorders cause disruptions in the oxidative balance. Oxidative parameters are found in different systems and are released in the body from various organs. In addition, MDD cases generally present with co-morbidities, such as other

psychiatric disorders, including drug or alcohol abuse. Lifestyle, nutritional habits, smoking status and pre-disease antioxidant activities are known to affect these results. Oxidative parameters are affected by numerous factors but a deficit occurs in this system in patients with depression according to other studies. For a parameter to be used as a biomarker, the disease period must be routinely followed, parameters of treated and untreated patients should be compared, different phases of the disease must be evaluated (active, remission etc.), and differences in subtypes of the disease must be detected. However, like many other psychiatric disorders, no biological marker has been used routinely in patients with MDD in clinical practice. Although deficits of oxidative balance in patients with MDD are insufficient for identifying the etiopathology of the disease, data obtained from long-term follow-up studies of patients in different phases of the disease may bring us candidate biomarkers to be used in these patients.

Limitations of this study are the relatively small number of patients, the lack of a cross-sectional design, difficulties recording nutritional status, and the lack of information about MDD, such as the duration of disease and depressive symptomatology (e.g. suicidal ideation, lack of appetite, sleep disturbances).

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Author's Contributions: **ÖK, YH:** Research concept and design; data collecting, **CB, ME:** Biochemical analysis and interpretation of data. **ÖK:** Preparation of article, and Revisions. All authors approved the final version of the manuscript

Ethical issues: All Authors declare, Originality and ethical approval of research. Responsibilities of research, responsibilities against local ethics commission are under the Authors responsibilities.

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