Thiol-disulphide Homeostasis in Patients with Schizophrenia: The Potential Biomarkers of Oxidative Stress in Acute Exacerbation of Schizophrenia

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Objective: Recent evidence suggests that oxidative stress contributes to the pathophysiology of schizophrenia. This study aimed to compare thiol-disulphide homeostasis in acute and stable phases of schizophrenia for the first time.
Methods: Among the patients with schizophrenia, 61 in the acute-phase and 61 in the stable phase of their illness were enrolled in the study. Native thiol (NT), total thiol (TT), disulphide (SS), disulphide/native thiol, disulphide/total thiol, and native thiol/total thiol for thiol-disulphide homeostasis were compared between the groups. The Brief Psychiatric Rating Scale (BPRS), Scale for the Assessment of Positive/Negative Symptoms (SAPS/SANS), Clinical Global Impression-Severity Scale (CGI-S), Barnes Akathisia Rating Scale, and Simpson-Angus Scale were used to assess symptoms.
Results: After controlling for age, sex, body mass index, and smoking status there were significant differences in NT, TT, SS/NT, SS/TT, and NT/TT, but not SS. Thiol/disulphide homeostasis has shifted in favour of the oxidative side in patients with acute-phase compared to that in stable schizophrenia. BPRS, SAPS, and CGI-S scores were significantly correlated with all six thiol-disulphide parameters, but not SANS, when controlling for age and sex. Significant receiver operating characteristic (ROC) curves were obtained for all thiol-disulphide homeostasis parameters. Discriminant analysis was found to be statistically significant in discriminating between groups.
Conclusion: These results show that oxidative status increases thiol-disulphide homeostasis in patients with acute-phase schizophrenia compared to those with stable schizophrenia. These findings suggest that thiol-disulphide parameters can be used as biomarkers for the acute exacerbation of schizophrenia.

KEY WORDS: Schizophrenia; Biomarkers; Inflammation; Thiol; Disulfide; Oxidative stress.

INTRODUCTION

Schizophrenia (SCZ) is a chronic and debilitating disorder that manifests as abnormal mental processes and disturbed behaviours. However, its etiopathogenesis is not yet clearly understood. The heterogeneity of SCZ is associated with many causal biological pathways. To improve the clinical outcomes and disease prognosis, a better understanding of the biological pathways underlying SCZ has become a priority. It has been reported that oxidative stress is involved in those biological pathways [1]. It has been hypothesized that oxidative stress may play a role in neuroprogression through DNA damage and neuro-inflammatory pathways in SCZ [2,3]. Oxidative stress is an imbalance between free radicals and antioxidant defences, resulting in increased levels of reactive oxygen species, such as superoxide, hydroxyl radicals, peroxides, and singlet oxygen. Oxidative stress targets proteins, lipids, and DNA, causing cellular dysfunction, apoptosis, and disruption of cell signalling cascades in the brain [4,5].

Many meta-analyses and reviews have revealed that oxidative damage is present in SCZ [6-8]. It has been shown that there is free-radical damage due to impaired

anti-oxidant defence systems in SCZ. It has been reported that malondialdehyde and nitric oxide levels, which are indicators of oxidative stress, are increased in schizophrenia [9]. Furthermore, the level of antioxidant defence enzymes (i.e., superoxide dismutase, catalase, glutathione peroxidase, and paraoxonase) in patients with SCZ has been lower than in healthy subjects [10-12]. It has been reported that oxidative stress marker levels are affected by the illness phase (acute vs. stable) and may be state markers of acute exacerbation [7,13]. A meta-analysis emphasized that antioxidant capacity decreases even in individuals with first-episode psychosis [14]. Many studies have shown that oxidative stress marker levels are associated with positive, negative, and general psychopathology and functioning [15,16]. However, there are also studies in which no relationship between symptoms and oxidative stress has been reported [1]. The reasons for inconsistent results include the use of antipsychotic medication, variable illness severity, the number of psychotic episodes, and the source of the material [1]. Several additional confounding factors may influence the results, such as smoking and obesity [17,18].

Thiols are organic compounds containing a sulfhydryl group that are essential antioxidant components [19]. Thiol groups also play a role in various metabolic processes such as protein synthesis, cell proliferation and growth, signal transduction, apoptosis, and immune regulation [20]. Thiols are present in albumin, glutathione, cysteine, homocysteine, gamma-glutamylcysteine, and free thiols can also circulate in the plasma [21]. Thiols are good reductants and can undergo oxidation reactions to form a broad range of products, such as reversible disulphide bonds (in short, SS). Under oxidative stress conditions, oxidation of cysteine residues can lead to the reversible formation of mixed disulphide bonds between protein thiol groups and low-molecular-mass thiols. The formed disulphide bonds can again be reduced to thiol groups by enzymes such as thioredoxin and glutaredoxin. Thus, dynamic thiol–disulphide homeostasis is maintained [22,23]. There was no automated colorimetric method for measuring plasma/serum dynamic disulphide levels. Therefore, only the thiol level could be calculated. Dynamic thiol-disulphide homeostasis can be determined using the automatic spectrometric method developed recently by Erel and Neselioglu [24]. This method has been used to investigate dynamic thiol-disulphide homeostasis

in several mental disorders [25-28].

In this study, we aimed to investigate whether thiol-disulphide homeostasis is a possible marker of acute exacerbation for SCZ. For this purpose, thiol-disulphide homeostasis in the acute and stable phases of SCZ was compared by using the automatic method. All previous studies evaluating thiol-disulphide homeostasis in SCZ have been compared with healthy controls [29-32]. However, it remains unclear whether there is a difference between thiol-disulphide homeostasis in patients with acute-phase vs. stable SCZ. Thiol-disulphide homeostasis may be restored by repairing the oxidative balance by increasing antioxidant defence mechanisms during the remission of SCZ. The other aim of this study was to examine the impact of symptom severity and disorder-related predictors on thiol-disulphide homeostasis. To our knowledge, this is the first study to compare thiol-disulphide homeostasis in patients with acute-phase and stable-phase SCZ. Another aim of this study was to examine the relationship between thiol-disulphide homeostasis and symptom severity and disease-related factors.

METHODS

Study Design and Sampling

We conducted a cross-sectional survey of inpatients and outpatients diagnosed with SCZ based on the Diagnostic and Statistical Manual of Mental Disorders fifth edition (DSM-5). A total of 122 patients diagnosed with SCZ, 61 in the acute phase and 61 in the stable phase, followed by the Department of Psychiatry at Ankara City Hospital, Ankara, Turkey, were included in the study. Two clinical psychiatrists assessed the patients' current mental status and medical and psychiatric history. An important issue is the potential confounding factors that affect the oxidative/anti-oxidative status of patients, such as smoking, body mass index (BMI), use of regular anti-inflammatory agents, and current active physical diseases [33]. It is recommended that these parameters, which may affect the oxidative/antioxidative status, be controlled, but few studies have considered this [34]. To avoid inconsistent results, we either controlled or excluded circumstances that could potentially affect thiol-disulphide homeostasis.

Patients were evaluated according to the inclusion and exclusion criteria. The inclusion criteria were a diagnosis of schizophrenia and an age between 18 and 65 years.

The exclusion criteria were as follows: < 18 years of age, a diagnosis of bipolar or schizoaffective disorders at any time, a diagnosis of alcohol and substance use disorders, history of major surgeries, hepatic or renal failure, malignancy, autoimmune diseases, active infection, active inflammatory or collagen tissue disease, and treatment with anti-inflammatory, antioxidant, or immunosuppressive medications. Demographic characteristics (age, sex, occupation, marital status, education level), clinical information (duration of the disorder, age at onset of the disorder, number of hospitalizations, history of electroconvulsive therapy (ECT) or clozapine use), mental disorders in first-degree relatives, cigarettes consumption, and BMI were recorded.

The study was conducted according to the Declaration of Helsinki and approved by the Ankara City Hospital Ethics Committee (decision number E2-22-2952). The study methods were compliant with the STROBE checklist.

Measurement Tools and Outcomes

The Turkish version of the Brief Psychiatric Rating Scale (BPRS), the Scale for the Assessment of Positive Symptoms (SAPS), the Scale for the Assessment of Negative Symptoms (SANS), the Clinical Global Impression-Severity Scale (CGI-S), the Barnes Akathisia Rating Scale (BARS), and the Simpson-Angus Scale (SAS) were used to assess psychotic and extrapyramidal symptoms severity [35-37]. Furthermore, thiol-disulphide homeostasis parameters were measured in the blood samples of patients.

The BPRS is the most widely used scale for measuring psychiatric symptoms [38]. It is an 18-item scale that a researcher can use to assess depression, anxiety, mannerism-posturing, hallucinatory behaviour, and hostility. Items are rated on a 7 points scale, where one is not present and seven is extremely severe. The item scores can vary between the range of 18−126 points.

The SAPS and SANS are among the most valuable scales for evaluating SCZ symptoms. The SAPS measures positive symptoms on a 34-item, 6-point scale. Items are listed under hallucinations, delusions, bizarre behaviour, and positive formal thought disorders. In contrast, the SANS measures negative symptoms on a 25-item, 6-point scale. Items are listed under affective blunting, alogia, avolition/apathy, anhedonia/asociality, and attention [39]. A total score is obtained by summing the item scores, varying between 0−170 points in the SAPS and 0−150

points in the SANS.

The CGI-S is designed to assess the global severity of disorders [40]. It is rated on the following seven-point scale: 1 = normal, not at all ill; 2 = borderline mentally ill; 3 = mildly ill; 4 = moderately ill; 5 = markedly ill; 6 = severely ill; and 7 = among the most extremely ill patients. This rating is based on observed and reported symptoms, behaviour, and function in the past seven days.

The BARS are used to evaluate drug-induced akathisia [41]. It consists of one objective and two subjective items rated on a 4-point scale, and a global clinical assessment item rated on a 6-point scale (totally scored 0−14). The subjective items included awareness of restlessness and distress related to restlessness. The SAS is a 10-item rating scale widely used to assess drug-induced parkinsonism in clinical practice and research settings [42]. The SAS score is between 0 and 4 for each item (totally scored 0−40), and a high score indicates a high level of parkinsonism.

We approved the remission (stable phase) criteria for SCZ as presented by Lieberman *et al.* [43]. These include the following conditions: five BPRS psychosis items (conceptual disorganisation, mannerisms, suspiciousness, hallucinations, unusual thought content) must be rated as not worse than "mild" (3 points or lower), and the overall severity according to the CGI-S not worse than "mild" (3 points or lower). Moreover, a patient who had not been hospitalised for more than three months, had received the same treatment dose in the last three months, and had achieved at least 50% improvement with treatment was considered to be in the stable phase of schizophrenia. We also defined the acute phase of schizophrenia as the presence of severe positive symptoms (such as delusions, hallucinations, disorganised thinking, and speech) (CGI ≥ 5) or general psychopathology and psychosis severe enough to require hospitalization. Patients with acute-phase schizophrenia experience first-episode psychosis or relapse.

Blood samples were obtained from the antecubital veins between 08:00−12:00 am. All participants fasted for 12 hours. The blood samples were left for 30 minutes at room temperature (22°C) to activate the clotting process, followed by centrifuges at 1,500 g for x15 minutes. Serum samples were aliquoted and stored at −80°C. The samples were then transferred to the biochemistry laboratory for analysis. Thiol/disulphide homeostasis tests were measured by an automated spectrophotometric method described by Erel and Neselioglu [24]. Briefly, di-

sulphide bonds were first reduced with sodium borohydride to form free functional thiol groups. Unused reductant sodium borohydride was consumed and removed with formaldehyde to prevent the reduction of DTNB (5,5'-dithiobis-(2-nitrobenzoic) acid). All thiol groups, including reduced and native thiol groups, were determined after the reaction with DTNB. Half of the difference between the total thiols and native thiols provided a dynamic disulphide amount. After determining native (NT) and total thiols (TT), disulphide amounts (SS), disulphide/total thiol percent ratios (SS/TT), disulphide/native thiol percent ratios (SS/NT), and native thiol/total thiol percent ratios (NT/TT) were calculated. These results are presented as micromole/liter (μmol/L) ratio and percentage (%).

In addition, waist circumference, high-density lipoprotein (HDL), triglyceride, blood pressure, and blood glucose values were also measured to determine whether the patients met the diagnosis of metabolic syndrome. Serum iron and ferritin levels were measured additionally.

Statistics

The sample size was determined to be 30 observations for each group, based on $\alpha = 0.05$, $\beta = 0.20$, and the actual power was 0.823. Statistical analyses were performed using SPSS 28.0 (IBM Corporation) software, and graphics were drawn using GraphPad Prism 9.2.0 software (Dotmatics). The significance level was set at $\alpha = 0.05$; all tests were 2-tailed. Continuous variables were reported as means, standard deviations (SD), medians, and interquartile ranges (IQR). Categorical variables are reported as frequencies and percentages. Continuous variables were checked using skewness, kurtosis, and histogram plots to determine their distribution characteristics. Parametric tests were selected for normally distributed variables, and non-parametric tests were chosen for skewed variables. The groups were compared to determine whether there were any statistical differences between the groups for any variable. Differences in sociodemographic and clinical characteristics between the two groups were also compared using the chi-square test, Student's t test, or Mann–Whitney test, where appropriate.

To investigate the differences in NT, TT, SS, SS/NT, SS/TT, and NT/TT levels in patients with acute-phase and stable SCZ, we performed multiple analyses of covariance (MANCOVA) to provide the overall p value, considering these six thiol-disulphide parameters as multiple continuous dependent variables. They may have interactive effects among them, which could cause type I errors without this initial test. In this MANCOVA model, levels of thiol-disulphide parameters were identified as dependent variables, with diagnosis (acute-phase vs. stable phase SCZ) set as a fixed factor and age, sex, BMI, duration of the disorder, and smoking status as covariates. Subsequently, an analysis of covariance (ANCOVA) was performed with each of the six thiol-disulphide parameters as the dependent variable, with diagnosis as an independent variable, adjusting for age, sex, BMI, and smoking as covariates. Partial correlation analyses or Spearman's test were conducted to assess the relationship between mental health symptoms and thiol-disulphide homeostasis parameters depending on whether the assumptions of parametric testing were fulfilled. In the partial correlation, we controlled for the effects of age and sex variables. Multiple regression analysis with a stepwise method was used to examine the relationship between clinical scale scores, thiol-disulphide parameters, and some clinical variables.

Receiver operating characteristic (ROC) curve analysis was used to determine the optimum cut-off levels of NT, SS, TT, SS/NT, SS/TT, and NT/TT. A significant area under the curve (AUC) with 95% confidence intervals ($p < 0.05$ compared to the reference AUC) was used as an indicator of the ability of each variable to differentiate between patients in the acute and stable phases. We considered the AUC at least 0.7 as satisfactory, and the null hypothesis value was set at 0.5. Responsiveness was described in terms of sensitivity and specificity using the ROC curve. Values for sensitivity and false-positive rates (1−specificity) are plotted on the y- and x-axes. Finally, stepwise discriminant analyses using the Wilks' lambda method were performed to distinguish patients with acute-phase from those with stable phase SCZ. Six thiol-disulphide parameters were obtained as independent variables. The criterion for inclusion/exclusion of the p value was 0.05. Canonical correlation and sensitivity-specificity values were also calculated in this discriminant analysis.

RESULTS

Demographic and Clinical Characteristics of the Participants

The sociodemographic and clinical characteristics of

the study are presented in Table 1. Age, sex, and education were matched between the groups (all $p > 0.05$). There were no significant differences between the two groups in terms of marital status, working status, duration of the disorder, age at onset of the disorder, number of hospitalizations, the prevalence of metabolic syndrome, smoking status, and BMI (all $p > 0.05$). As expected, patients with acute-phase SCZ had higher BPRS, SAPS, SANS, and CGI-S scores (all $p < 0.05$). While there was no significant difference between the two groups in the BARS scores ($p = 0.142$), a significant difference was found in the SAS scale ($p = 0.44$). All patients were administered antipsychotic medications. There were no statistically significant differences between the two groups regarding waist circumference, HDL, triglyceride, blood pressure, blood glucose, and serum iron levels (all $p > 0.05$). However, serum ferritin levels were lower in patients with acute-phase SCZ ($p = 0.038$) than in those with stable-phase SCZ, which was expected.

Levels of Thiol-disulphide Parameters in Patients with Schizophrenia

MANCOVA showed that the effect of the disorder phase was significant (Wilks' lambda F = 4.519, $p = 0.001$, $\eta_p^2 =$

0.361). ANCOVA was conducted to examine the differences in each parameter (NT, TT, SS, SS/TT, SS/NT, and NT/TT) between patients in the acute and stable phases. As shown in Table 2 and Figure 1, there were significant differences in NT, TT, SS/NT, SS/TT, and NT/TT (F = 15.02, $p < 0.001$; F = 9.54, $p = 0.003$; F = 5.67, $p = 0.021$; F = 5.72, $p = 0.02$; and F = 4.43, $p = 0.04$, respectively). However, there was a non-significant trend (F = 3.75, $p = 0.058$) between the two groups in terms of SS levels, after controlling for age, sex, BMI, and smoking status.

Associations between Thiol-disulphide Parameters and Clinical Scores

We performed partial correlations between BPRS, SAPS, SANS, CGI-S scores, and thiol-disulphide parameters after controlling for age and sex. Table 3 presents a partial correlation matrix. The BPRS, SAPS, and CGI-S scores were significantly correlated with all six thiol-disulphide parameters (all $p < 0.05$), but not SANS with all parameters when controlling for age and sex. Spearman's correlation was also tested between BARS, SAS, and thiol-disulphide parameters. The SAS scores were significantly correlated with NT (r = -0.256, $p = 0.006$) and TT (r = -0.262, $p =$

Table 1. Demographic and clinical characteristics of the patients

Characteristics	Acute SCZ (n = 61)	Stable SCZ (n = 61)	Statistic	p value
Age (yr)	37.28 ± 12.05	40.07 ± 12.90	1.23	0.220
	Range = 19−63	Range = 18−65		
Sex (male)	40 (65.6)	35 (57.4)	0.87	0.457
Education (yr)	10.5 ± 3.28	11.11 ± 2.92	0.59	0.557
Marital status (unmarried[a])	51 (83.61)	42 (68.85)	3.98	0.264
Working status (working)	16 (26.2)	21 (34.4)	3.05	0.803
Age at disorder onset (yr)	25.03 ± 8.32	27.18 ± 10.26	1.27	0.206
Duration of the disorder (yr)	12.37 ± 9.93	12.42 ± 10.45	0.03	0.978
Number of hospitalisation	2.4 ± 1.99	2.52 ± 2.57	0.29	0.766
Active smoking (n)	25 (41.0)	35 (57.4)	0.83	0.363
BMI	25.35 ± 4.51	26.67 ± 4.38	1.54	0.126
Metabolic syndrome (n)	13 (21.3)	16 (26.2)	0.29	0.591
BPRS score	33.37 ± 9.61	16.28 ± 5.65	11.64	< 0.001
SAPS total score	41.32 ± 15.69	15.22 ± 9.30	11.12	< 0.001
SANS total score	41.98 ± 19.33	27.45 ± 16.48	4.34	< 0.001
CGI-S score	5.50 ± 0.80	3.37 ± 0.75	15.08	< 0.001
SAS score	1 (0−3)	0 (0−1)	1.47	0.441
BARS score	1 (0−3)	0.5 (0−1)	0.14	0.142

Values are presented as mean ± standard deviation, number (%), or median (interquartile range).

SCZ, schizophrenia; BMI, body mass index; BPRS, Brief Psychiatric Rating Scale; SAPS, Scale for the Assessment of Positive Symptoms; SANS, Scale for the Assessment of Negative Symptoms; CGI-S, Clinical Global Impression-Severity Scale; BARS, Barnes Akathisia Rating Scale; SAS, Simpson-Angus Scale.

[a]Unmarried category included single, divorced and widowed participants.

Table 2. Serum levels of each thiol-disulphide homeostasis parameter between acute SCZ and stable SCZ patients, after controlling for age, sex, BMI and smoking status

Parameters	Acute SCZ (n = 61)	Stable SCZ (n = 61)	F	p value[a]	η_p^2
NT (µmol/L)	405.49 ± 44.87	448.25 ± 56.77	15.02	< 0.001	0.220
TT (µmol/L)	451.34 ± 53.88	481.51 ± 53.76	9.54	0.003	0.150
SS (µmol/L)	18.63 ± 7.73	15.61 ± 8.63	3.74	0.058	0.065
SS/NT (%)	4.60 ± 1.86	3.64 ± 2.23	5.67	0.021	0.094
SS/TT (%)	4.09 ± 1.52	3.31 ± 1.89	5.72	0.020	0.096
NT/TT (%)	90.05 ± 4.39	93.01 ± 4.17	4.43	0.042	0.076

Values are presented as mean ± standard deviation.
SCZ, schizophrenia; BMI, body mass index; NT, native thiol; TT, total thiol; SS, disulphide.
[a]Bonferroni correction.
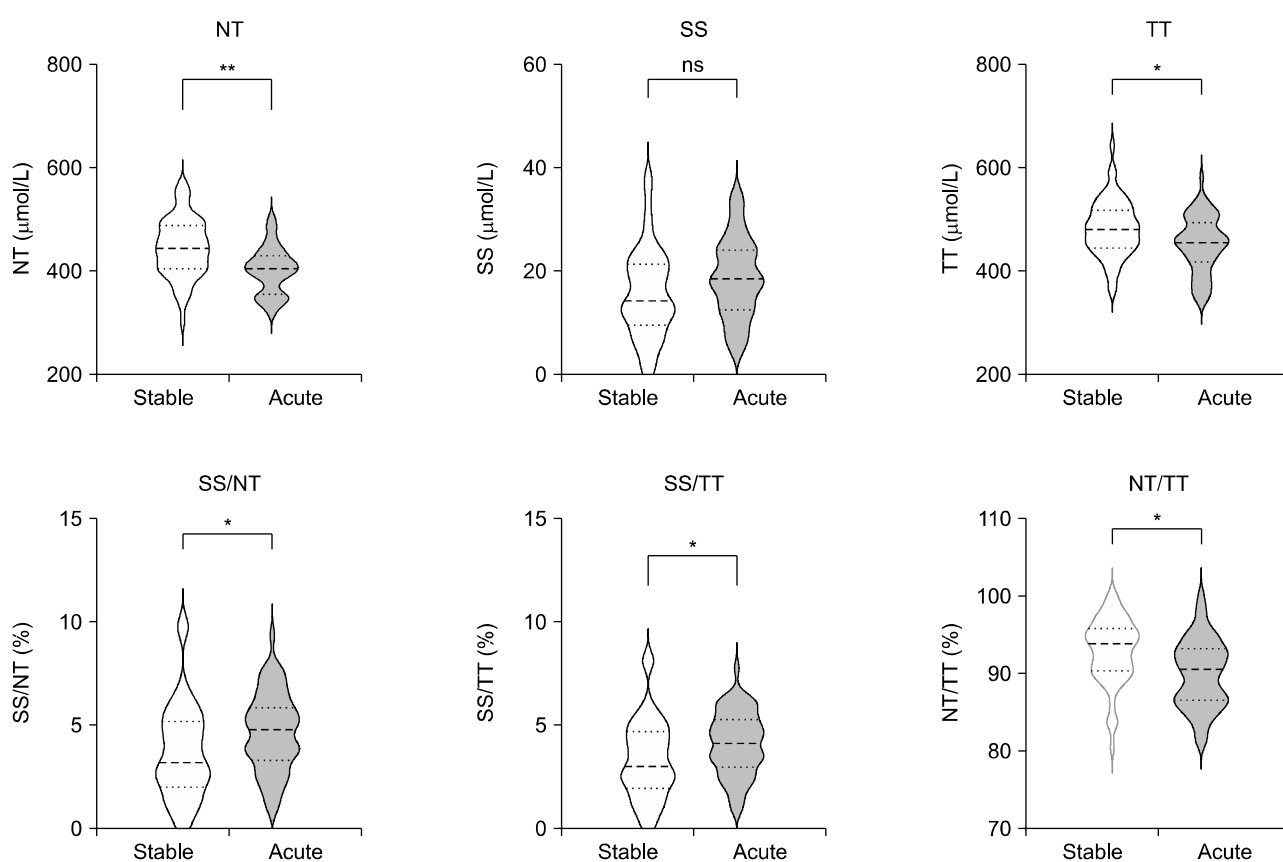
Fig. 1. The figure shows the difference in thiol-disulphide homeostasis parameters between schizophrenic patients with acute and remission phases when adjusted for age, sex, BMI and smoking with ANCOVA.
BMI, body mass index; NT, native thiols; SS, disulphide; TT, total thiol; SS/NT, disulphide/native thiol percents ratios; SS/TT, disulphide/total thiol percent ratios; NT/TT, native thiol/total thiol percents ratios; ns, non-significant; ANCOVA, analysis of covariance.
*p <0.05, **p < 0.001.

0.004), but not with others. The BARS scores were not significantly correlated with thiol-disulphide parameters (all p > 0.05). Moreover, there was no significant relationship between thiol-disulphide parameters and age at disorder onset or the total duration of the disorder when con-

trolling for age and sex.

Multiple regression analysis with a stepwise method was used to examine the relationship between thiol-disulphide parameters and clinical scale scores. The models contained eight independent variables (six thiol-disul-

Table 3. Correlation analysis results after controlling for age and sex

Variables	NT	SS	TT	SS/NT	SS/TT	NT/TT
BRPS	−0.328**	0.259*	−0.216*	0.304**	0.302**	−0.334**
SAPS	−0.408**	0.320**	−0.319**	0.370**	0.372**	−0.357**
SANS	−0.216*	0.078	−0.193*	0.107	0.107	−0.095
CGI-S	−0.511**	0.337**	−0.396**	0.395**	0.398**	−0.373**

NT, native thiol; TT, total thiol; SS, disulphide; BPRS, Brief Psychiatric Rating Scale; SAPS, Scale for the Assessment of Positive Symptoms; SANS, Scale for the Assessment of Negative Symptoms; CGI-S, Clinical Global Impression-Severity Scale.
$*p < 0.05$, $**p \leq 0.001$.
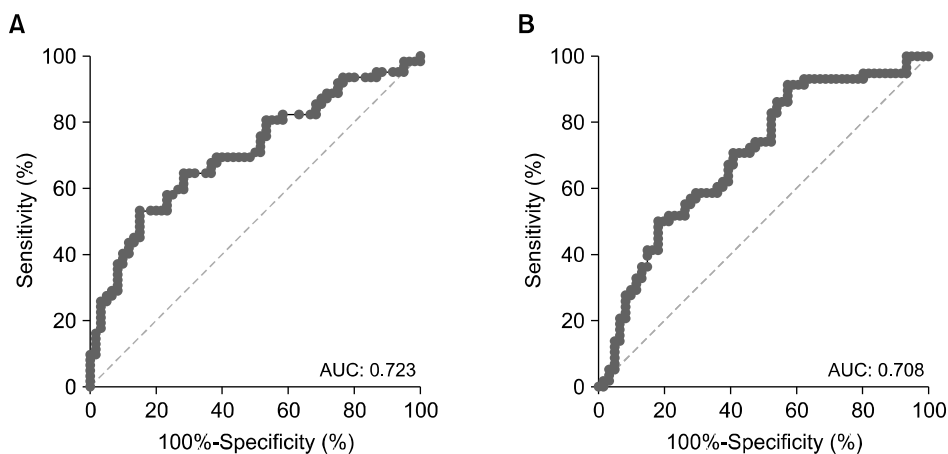
Fig. 2. (A) ROC curve analysis result of NT. (B) ROC curve analysis result of NT/TT.
NT, native thiols; NT/TT, native thiol/total thiol percents ratios; ROC, receiver operating characteristic; AUC, area under the curve.

phide parameters, age at disorder onset, and no treatment in the previous three months). Regression analysis identified NT (beta = −0.27, t = −2.86, p = 0.005), SS/TT (beta = 0.22, t = 2.48, p = 0.015), age at disorder onset (beta = −0.27, t = −3.12, p = 0.002), and not being treated in the previous three months (beta = −0.27, t = −3.27, p = 0.001) as the influencing factors for the SAPS total scores (model R^2 = 0.30). Furthermore, NT (beta = −0.47, t = −5.69, p < 0.001), SS (beta = 0.16, t = −2.0, p = 0.048), age at disorder onset (beta = −0.19, t = −2.29, p = 0.024) and no treatment in the previous three months (beta = −0.20, t = −2.50, p = 0.014) were the influencing factors for the CGI-S scores (model R^2 = 0.34). Similarly, TT (beta = −0.20, t = −2.34, p = 0.02) and NT/TT (beta = −0.32, t = −3.69, p < 0.001) were the predictors of BPRS scores (model R^2 = 0.144), and NT (beta = −0.21, t = −2.24, p = 0.027) was a weak predictor of the SANS total scores (model R^2 = 0.042).

ROC Curve and Discriminant Analyses

Significant ROC curves were found for all thiol-disulphide homeostasis parameters (all $p < 0.05$). The highest

AUC was observed for NT (AUC = 0.723, standart error [SE] = 0.046, 95% confidence interval [CI] = 0.633 − 0.813, $p < 0.001$), which can be considered good discrimination power [44]. The maximum sensitivity (65.6%) and specificity (72.1%) were reached at a cut-off value of $\geq$ 427.6 μmol/L for NT, predicting the acute phase of SCZ. Acceptable discrimination power was also observed for NT/TT (AUC = 0.708, SE = 0.047, 95% CI = 0.616 − 0.801, $p < 0.001$). When the maximum sensitivity (60%) and specificity (70.5%) were reached, the cut-off value for NT/TT was 92.68 μmol/L; higher values indicating a stable phase. However, AUC significantly differed from the reference AUC for SS, TT, SS/NT, and SS/TT, but these values were lower: AUCs ranged from 0.684 to 0.613. The ROC curves for NT and NT/TT are shown in Figure 2.

As a final step, we investigated the ability of the thiol-disulphide parameters (NT, SS, and NT/TT) to discriminate between patients with acute-phase and stable phases by discriminant analysis. The discriminant function was statistically significant (Wilks' Λ = 0.77, $p < 0.001$). The canonical correlation coefficient was 0.48, indicating that the predictors explained 23% of the variance. We found sen-

sitivity and specificity of 78.7% and 59%, respectively, when comparing the two groups. Moreover, 68.9% of originally grouped cases were correctly classified.

DISCUSSION

This study evaluated the differences in thiol-disulphide homeostasis in patients with acute-phase and stable phase SCZ. We found significantly lower levels of native thiol, total thiol, and native thiol/total thiol and higher levels of total disulphide, disulphide/total thiol, and disulphide/native thiol values in the patients with acute SCZ than in those with stable SCZ. While obtaining these results, we controlled for age, sex, BMI, and smoking status as many times as possible, which we thought would be confounding. The results showed significant changes in thiol-disulphide homeostasis, an essential indicator of oxidative stress, especially in patients with acute SCZ. Determining changes in thiol-disulphide homeostasis is important for oxidative stress. Thiols contain functional sulfhydryl groups and are non-enzymatic antioxidant molecules that prevent the damage caused by free radicals. Thiols undergo oxidation reactions in the presence of an oxidative environment and from their reversible disulphide forms. This form can be converted back to reduced thiols to maintain thiol-disulphide homeostasis [24]. Thiol-disulphide homeostasis thus reflects oxidant and antioxidant status [45]. These results emphasize that the differences in thiol-disulphide homeostasis in patients with acute SCZ increase the oxidant status.

Although the etiopathogenesis of schizophrenia has not yet been clarified, many biological pathways have been identified in recent studies. Oxidative stress is an important factor in its etiopathogenesis. An imbalance between reactive species and antioxidant capacity causes oxidative stress. There is strong evidence that oxidative stress is increased in schizophrenia [7,46,47]. Increased oxidative stress causes changes in neural cell signalling, mitochondrial dysfunction, oligodendrocyte abnormalities, and hypo/hyperactivation of receptors. Thus, it affects neuronal membrane functions, neurotransmission, and cell survival, and symptoms of schizophrenia appear [1,46].

Five studies have evaluated thiol-disulphide homeostasis, a crucial marker of oxidative stress in schizophrenia [29-32,48]. In all of these studies, it was determined that there were differences in thiol-disulphide homeostasis

compared to healthy controls. Native thiol and total thiol levels were significantly lower in patients with schizophrenia than in healthy controls, except in a study by Dogan *et al.* [29]. The levels of other thiol-disulphide parameters in this study also differed from those reported in other studies. The authors attributed this result to patients receiving antipsychotic medication; however, no information was provided about the clinical status of the patients. A significantly higher level of total disulphide levels in patients with schizophrenia was also determined compared to healthy controls, except in the study by Kulaksizoglu and Kulaksizoglu [30]. In none of these studies was the effect of possible confounding factors not controlled when measuring thiol-disulphide parameters. For this reason, studies are needed to control for potential confounding factors when comparing thiol-disulphide homeostasis in patients with schizophrenia and healthy controls.

Significant correlations were found between changes in thiol-disulphide homeostasis and the BPRS, SAPS, and CGI-S scores, which are widely used in clinical practice, but not the SANS scores. While BPRS, SAPS, and SANS scores were negatively correlated with NT, TT, and NT/TT, they were positively correlated with SS, SS/NT, and SS/TT, after adjusting for age and sex. BPRS, which is highly correlated with SAPS and SAPS, has a strong relationship with thiol-disulphide parameters, suggesting that thiol-disulphide homeostasis may be related to dopamine pathways. However, no study in the literature has investigated the interaction between thiol-disulphide homeostasis and dopamine pathways. It has been previously reported that there are changes in the dopamine pathway due to oxidative stress [49,50]. The results of our study suggest that a decrease in antioxidant capacity in thiol-disulphide homeostasis may be associated with positive psychotic symptoms. Similarly, a correlation was found between PANSS scores and thiol-disulphide parameters [30,32]. The fact that thiol-disulphide parameters are associated with positive rather than negative psychotic symptoms suggests that the oxidant-antioxidant status may be related to specific dopamine pathways (e.g., the mesolimbic dopaminergic pathway) in schizophrenia. In addition, it has been shown that there is a strong relationship between the global severity of disease symptoms and thiol-disulphide homeostasis in our study. Previous studies have also shown a relationship between symptom severity and oxidative stress biomarkers [11,51,52]. How-

ever, none of these findings suggest a causal relationship between schizophrenia and thiol-disulphide homeostasis. Further prospective longitudinal studies are needed to elucidate this phenomenon.

Thiol-disulphide homeostasis has also been studied in other mental disorders. In studies conducted with patients with autism spectrum disorder [53], attention deficit-hyperactivity disorder [54], bipolar disorder [55], major depressive disorder [56], and obsessive-compulsive disorder [57], it was determined that significant changes were observed in patients compared to healthy controls. The thiol-disulphide balance tended to change toward the side in the patients' groups. In this context, thiol-disulphide homeostasis is not specific to schizophrenia but appears to be a transdiagnostic feature. These results suggest that the consequences of chronic stress may represent a common pathway in mental disorders [58]. In addition, the results of our study showed that thiol-disulphide homeostasis had significant changes in favour of oxidation in patients with acute SCZ compared to those with stable SCZ. Therefore, these results provide an opportunity to evaluate the pathophysiology, risk of relapse, and severity of the mental disorders when certain biomarkers are used in concert.

This is the first study to investigate dynamic thiol/disulphide homeostasis, a specific oxidative stress marker, in patients with acute-phase and stable-phase SCZ using the automated, practical, and time-efficient method developed by Erel and Neselioglu [24] in 2014. Before this method, native thiol levels, which are only one aspect of thiol/disulphide homeostasis, could be determined using time-consuming, labour-intensive, and complicated techniques [24]. Thanks to this method, we will better understand dynamic thiol-disulphide homeostasis in mental disorders.

Our study has many limitations. First, the study had a cross-sectional design and was conducted in a single centre, and it was difficult to generalize the results. Second, the fact that all the patients were receiving antipsychotic treatment may have affected our results. Another limitation of this study is that the patients' lifestyles, activities, diets, and dietary supplements, which may affect oxidative stress, were not evaluated in detail. Third, the fact that we have examined oxidative stress only from the perspective of thiol-disulphide homeostasis cannot make a definitive conclusion about oxidative mechanisms and their effects on schizophrenia. Finally, the unmeasured

confounding effects are a weakness of the current study. One of the s strengths of this study is that when comparing thiol-disulphide homeostasis in patients with schizophrenia, confounding variables, such as age, sex, BMI, and smoking status were controlled. The large sample size and detailed evaluation of patients' symptoms are another strength of this study.

In conclusion, we showed that the thiol/disulphide balance shifted in favour of the oxidative side in patients with acute-phase SCZ compared with those with stable SCZ. These findings suggest that thiol-disulphide parameters can be used as biomarkers for acute exacerbation of schizophrenia. A Significant increase in general and psychotic symptoms severity of the disorder and the shift towards the oxidative side became more evident. Applications that reduce oxidative stress may cause a decrease in the symptoms of schizophrenia. Oxidative stress may be a good target for future pharmacotherapy of acute schizophrenia [59]. These treatments should increase antioxidant capacity and decrease oxidation. Future studies should investigate the oxidative stress balance in schizophrenia using longitudinal and prospective designs in which many biomarkers are evaluated together.

■ Conflicts of Interest

No potential conflict of interest relevant to this article was reported.

■ Author Contributions

Study design and Writing—protocol: Şükrü Alperen Korkmaz. Data acquisition: Şükrü Alperen Korkmaz, Semra Ulusoy Kaymak. Statistical analysis: Şükrü Alperen Korkmaz, Semra Ulusoy Kaymak. Design of the protocol: Şükrü Alperen Korkmaz, Salim Neşelioğlu, Özcan Erel. Writing—original draft: Şükrü Alperen Korkmaz, Semra

Ulusoy Kaymak, Salim Neşelioğlu, Özcan Erel. All the authors interpreted the results and revised the manuscript accordingly.

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