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CLINICAL NEUROCHEMISTRY

Thiol/Disulfide Homeostasis in Schizophrenic Patients[1]

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Abstract—To determine serum thiol/disulfide homeostasis in schizophrenic patients. Serum native thiol, total thiol, and disulfide levels measuremented in the patients with 42 schizophrenia and 42 the healthy subjects. Serum native thiol, total thiol, and disulfide levels measuremented with a novel automated method. The thiol/disulfide ratio was also calculated. The Positive and Negative Syndrome Scale (PANSS) was used to assess the patients. The native thiol ($p < 0.001$) and total thiol ($p < 0.001$) levels, and the native thiol/total thiol ($p = 0.018$) ratio were significantly lower, whereas disulfide/native thiol ($p = 0.002$) and disulfide/total thiol ($p = 0.002$) ratio significantly increased in the schizophrenia patient group compared to the control group. A statistically significantly positive relationship was found between PANSS positive subscale with disulfide ($r = 0.43$, $p = 0.01$). Significantly positive relationships were found between PANSS total subscale with disulfide/total thiol ($r = 0.308$, $p = 0.047$). Our results suggest that the disulfide/thiol ratio is significantly greater in schizophrenia patients and disulfide/thiol ratio is closely related with the patients' clinical symptoms.

Keywords: schizophrenia, homocysteine, methionine, glutathione, vitamin B12, folate

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INTRODUCTION

Despite significant progress in studies on schizophrenia, specific etiologic factors and pathogenic processes cannot be clearly defined. One of the important processes in the pathogenesis of schizophrenia is oxidative stress [1]. Oxidative stress can be defined as the impairment of cell membrane functions due to the disequilibrium between the free oxygen radicals and the antioxidant mechanisms. The thiol groups are the primary targets of the reactive oxygen species. Thiol is an organic compound containing sulfhydryl (-SH) group which has a critical role in preventing the occurrence of oxidative stress. Thiol groups are oxidized by reactive oxygen species and reversible disulfide bonds are formed. This is the earliest sign of protein oxidation [2–5].

Thiol-disulfide homeostasis has an important role on antioxidant protection, detoxification, apoptosis, stabilization of protein structure, regulation of protein function, cell signaling and transcription [6].

The most of thiols in plasma consist of from albumin and other proteins, and the remaining part of the thiols consist of from low molecular weighted thiols such as cysteine, cysteinyl glycine, glutathione, homocysteine and γ-glutamylcysteine [7]. Thiol groups of proteins are oxidized in the medium by oxidant molecules and reversibly converted to disulfide bond structure. Disulfide bond structure can be again reduced to the thiol group and so that the thiol-disulfide balance can be maintained. When the native thiol levels decrease, disulfide levels increase [8]. While only the thiol levels were able to be measured since 1979, a novel and automated method was developed by Erel and Neselioglu, so that the thiol and also the disulfide levels can be measured one by one [8, 9].

Abnormal thiol-disulfide homeostasis state is related to the pathogenesis of different diseases, including diabetes mellitus [10], cardiovascular diseases [11], neurological diseases [12, 13], cancer [14], rheumatoid astride [15], liver diseases [16] and chronic renal failure [17]. There is no study in the literature showing the relationship between schizophrenia with the thiol-disulfide homeostasis. The aim of our study is to investigate the thiol-disulfide homeostasis in patients with schizophrenia and the relationship between thiol-disulfide homeostasis and the clinical status of schizophrenia patients.

MATERIAL AND METHODS

42 schizophrenic patients and 42 healthy controls aged between 18–65 years old, were participated into study. Informed consent was obtained from the patient and control groups. Control groups were assessed by a

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semistructured psychiatric interview. The schizophrenic patients had been diagnosed according to the diagnostic criteria of Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition, Text Revision (*DSM-IV-TR*) [18] and were followed for at least 2 years in the psychiatry clinic of Antalya Education and Research Hospital. The study complied with the Declaration of Helsinki, and was approved by institutional ethics committee of Antalya Training and Research Hospital.

Patients were excluded from the study if they met one or more of the following criteria: alcohol and substance abuse or dependence, hypertension, heart and cerebrovascular disease, diabetes mellitus, hepatic or renal failure, autoimmune diseases, active infection, active or chronic inflammatory diseases, smoking, obesity (BMI >30 kg/m^2), collagen tissue disease and treatment with antiinflammatory, antioxidant or immunosuppressive medications, malignity, vitamin supplements. Sociodemographic data form was completed to the participants. Positive and Negative Syndrome Scale (PANSS) scale which was developed by Kay and colleagues was applied to the patients [19].

Antecubital vein blood was taken after 12 hours of fasting from the participant for laboratory analysis. Vacutainer gel tubes were used. Serum was separated by centrifugation 10 min at 4000 *g* and rapidly serum fractions were stored at –80°C.

A new and fully automated method developed by Erel et al. was used for the measurement of plasma native thiol, total thiol and disulfide levels based on the reduction of dynamic disulfide bonds to functional thiol groups by sodium borohydrate ($NaBH_4$). Formaldehyde was used to remove all of the unused $NaBH_4$, in order to prevent extra reduction of the 5,5'-dithiobis-2-nitrobenzoic acid (DTNB) and further reduction of the formed disulfide bond, produced after the DTNB reaction. Native thiol content was subtracted from the total thiol content; half of the obtained difference provides the disulfide bond quantity. Disulfide/thiol, disulfide/total thiol and thiol/total thiol ratios were calculated automatically [8].

Statistical analysis. Continuous variables are presented as mean ± standard deviation, while categorical variables are given as percentages. Correlations were assessed with the Pearson/Spearman correlation coefficient. The Kolmogorov–Smirnov test was used to verify the normality of the distribution of continuous variables. Statistical analysis of clinical data between two groups consisted of unpaired *t*-tests for parametric data and Mann–Whitney *U*-test analysis for nonparametric data. Receiver operating characteristic (ROC) curve analysis was used to determine the optimum cut-off levels of native thiol, total thiol, disulfide/native thiol and disulfide/total thiol. Using the ROC curve, the responsiveness is described in terms of sensitivity and specificity. Values for sensitivity and for false-positive rates (1 – specificity) are plotted on the *y*- and the *x*-axes of the curve and the area under the curve represents the probability a measure correctly classifies patients as improved or unchanged. Analyses were performed with PASW 18 (SPSS Inc., Chicago, IL, USA) software and a *P*-value, 0.05 was considered statistically significant.

RESULTS

The mean age among the schizophrenic group (28 male, 66.7%) was 40 ± 11.6 years, and the mean age of the control group (25 male, 59.5%) was 38 ± 17.8 years. The age and gender distributions were similar in both main groups ($p = 0.525$, 0.273, respectively).

The laboratory findings of the control and schizophrenic groups are summarized in Table 1. Native and total thiol levels were significantly lower among the schizophrenic group relative to the control group ($p < 0.001$). There was not significant difference between the control and the schizophrenic groups in disulfide levels ($p = 0.488$). While the native thiol/total thiol ratio was decreased in the patient group ($p = 0.018$), disulfide/ native thiol and disulfide/total thiol ratios were elevated in the patient group ($p = 0.002$, $p = 0.002$ respectively).

Table 2 shows the relationship between laboratory findings and PANSS scale in the schizophrenia group. There was a significant positive correlation between the PANSS-P scores and serum disulfide levels and a positive correlation between the PANSS-T scores and disulfide/total thiol levels ($r = 0.43$, $p = 0.01$ and $r = 0.308$, $p = 0.047$, respectively).

ROC analysis of area under the curve, cut-off levels, and sensitivity and specificity values are given in Table 3, and ROC curves of all parameters are seen in Fig. 1. According to ROC analysis; *p* value of native thiol, total thiol, disulfide/native thiol, disulfide/total thiol levels and native thiol/total thiol were found to be statistically significant. Among these parameters the highest AUC value was found for native thiol (AUC = 0.857). The sensitivity and specificity of native thiol were 88.1 and 73.8%, respectively. The cut-off values are 415.55 μmol/L for native thiol, 444.20 μmol/L for total thiol, 3.62% for disulfide/native thiol, 3.41% for disulfide/total thiol.

DISCUSSION

There are ongoing studies related to oxidative stress in schizophrenia. Several studies conducted in patients with schizophrenia related to oxidative stress have evaluated molecules such as malondialdehyde (MDA), nitric oxide (NO), superoxide dismutase (SOD), glutathione (GSH), catalase, uric acid, and paraoxonase (PON); however, more recent studies have also evaluated distinct parameters, such as total anti-oxidant status (TAS), total oxidant status (TOS), and oxidative stress index (OSI) [20, 21]. These studies have yielded variable results. In a study, no signifi-

Table 1. Comparison of the laboratory findings between the control group and schizophrenic group

Biomarker	Patient group ($n = 42$) Mean ± SD	Control group ($n = 42$) Mean ± SD	p
Native thiol, μmol/L	348.91 ± 85.70	421.69 ± 30.70	<0.001*
Total thiol, μmol/L	377.55 ± 90.12	450 ± 33.82	<0.001*
Disulfide, μmol/L	14.93 ± 4.54	14.15 ± 5.68	0.488
Disulfide/native thiol, %	4.51 ± 1.81	3.36 ± 1.37	0.002*
Disulfide/total thiol, %	4.14 ± 1.63	3.12 ± 1.19	0.002*
Native thiol/total thiol, %	92.30 ± 3.14	93.76 ± 2.38	0.018*
PANSS-N	16.67 ± 3.73		
PANSS-P	17.17 ± 4.58		
PANSS-T	71.43 ± 19.21		

*Correlation is significant at the 0.05 level (2-tailed). Students t test were used.

Table 2. Relationship between PANSS and the laboratory findings

Biomarker	PANSS-N		PANSS-P		PANSS-T	
	r	p	r	p	r	p
Native thiol	–0.09	0.56	0.01	0.94	0.30	0.052
Total thiol	–0.08	0.60	0.05	0.75	0.30	0.056
Disulfide	0.05	0.75	0.43	0.01*	–0.10	0.531
Disulfide/native thiol	0.11	0.48	0.26	0.09	0.294	0.058
Disulfide/total thiol	0.10	0.52	0.26	0.09	0.308	0.047*
Native thiol/total thiol	–0.09	0.57	–0.24	0.12	0.061	0.683

*Correlation is significant at the 0.05 level (2-tailed). Pearson and Spearman correlation analysis were used

Table 3. ROC analysis data of native thiol, total thiol, disulfide, disulfide/native thiol, disulfide/total thiol, native thiol/total thiol parameters

Biomarker	Area Under Curve (AUC)	p Value	Cut-off level	Sensitivity, %	Specificity, %	+LR	–LR
Native thiol	0.857	<0.001	415.55	88.1	73.8	3.34	0.16
Total thiol	0.832	<0.001	444.20	88.1	71.4	3.08	0.17
Disulfide	0.449	0.421	–	–	–	–	–
Disulfide/native thiol	0.701	<0.001	3.62	72.5	59.5	1.79	0.46
Disulfide/total thiol	0.701	<0.001	3.41	69	59.5	1.70	0.52
Native thiol/total thiol	0.677	0.005	–	–	–	–	–

+LR = Positive likelihood ratio; –LR = Negative likelihood ratio. ROC analysis were used.

cant change in the total anti-oxidant potential (TOAP), total peroxide levels (TPEROX), and OSI was found in patients with schizophrenia, compared to the control group [21], whereas another study reported significantly elevated TOS and OSI in schizophrenia patients, compared to the control group, without no significant change in the TAS levels [22]. Although a meta-analysis in schizophrenia patients has reported increased levels of systemic oxidative mediators (i.e., MDA, NO), decreased levels of anti-oxidant substances (i.e., SOD, catalase, uric acid) and decreased lipid peroxidation in the erythrocytes, the actual effect of oxidative stress in schizophrenia patients has not been fully elucidated, yet [23].

The thiol-disulfide balance, which plays a role in the cellular structures and functions, is another important variable determining oxidative stress. The thiols are non-enzymatic anti-oxidant molecules taking an important part in preventing damage caused by free radicals. Thiol groups are primary target for reac-

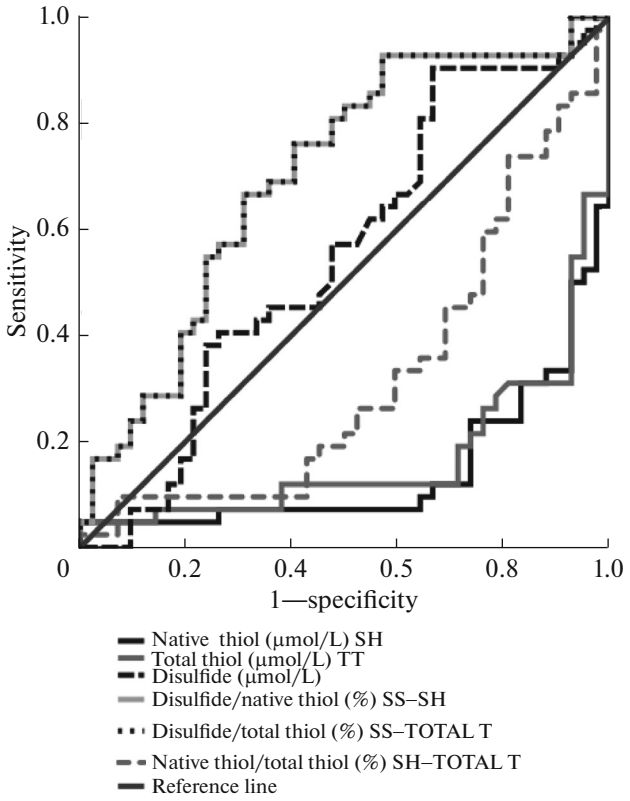

Fig. 1. ROC analysis of schizophrenic patients versus controls.

tive oxygen radicals [24]. The extent to which the proteins are affected from free radicals is related to their amino acid content. Molecules containing unsaturated bonds and SH groups and proteins releasing amino acids such as tryptophan, tyrosine, phenylalanine, histidine, methionine, and cysteine can be more easily affected by free radicals. The oxidation of proteins containing the vast amount of disulfide bonds, such as immunoglobulin produces sulfur-centered radicals, and this process disrupts the three-dimensional conformation of protein, thereby, rendering the molecule dysfunctional [25, 26].

Thiol-disulfide homeostasis, which was first measured in 1979, can be only measured in one direction [9], however, a novel, colorimetric and automated method developed in 2014 allows the measurement of the balance in either direction. Erel et al. reported that disulfide levels were higher in patients with degenerative diseases such as diabetes, in smokers, and obese patients, while the rate of infections were lower in patients with proliferative diseases, such as urinary bladder cancer, renal cancer, and multiple myeloma [8].

There is no study on thiol-disulfide homeostasis in patients with schizophrenia in the literature. The present study found decreased levels of native thiol, total thiol, and native thiol/total thiol and increased disulfide/native thiol and disulfide/total thiol levels in the patient group, compared to the control group. These data suggest that thiol-disulfide balance has shifted toward disulfide direction in schizophrenia patients. As an important component of serum anti-oxidant system [24], decreased thiol levels indicate a problem in anti-oxidant system in schizophrenia patients. Although the increase in disulfide molecules generated by oxidation of thiol groups in the patient group was not statistically significant, increased disulfide/native thiol and disulfide/total thiol levels suggest increased oxidative stress in schizophrenia patients. The lack of a significant increase in disulfide levels in the schizophrenia group can be explained by the lack of stability in the thiol-disulfide homeostasis and the fact that this balance is affected by liver and kidney functions [27]. According to our data, the cut-off value is 415.55 μmol/L for native thiol, 444.20 μmol/L for total thiol level, 3.62% for disulfide/native thiol, 3.41% for disulfide/total thiol. The patients with serum native thiol, total thiol levels under these thresholds and with serum disulfide/native thiol, disulfide/total thiol levels over these thresholds must be particularly evaluated for schizophrenia. The evaluation can be made by the automated measurement of these values.

Furtheremore, review of the literature revealed no study which compared thiol-disulfide homeostasis and Positive and Negative Syndrome Scale (PANSS) scale. However, the studies which evaluated the relationship between the PANSS scores and oxidative stress have yielded variable results. In a study including 50 schizophrenia patients, the authors did not found a significant relationship between the PANSS scores and the level of oxidative stress (MDA) and anti-oxidative enzymes (glutathione peroxidase [GPX] and SOD) [28], while another study found a negative correlation between the OSI and PANSS-total and PANSS-positive scores in 30 patients with schizophrenia [22]. Similarly, another study reported a significant negative correlation between TAS and PANSS-negative and PANSS-total scores [21]. To the best of our knowledge, the present study is the first in the literature to evaluate the relationship between the thiol-disulfide homeostasis and PANSS. According to our results, there was a significant positive correlation between serum disulfide levels indicating oxidative stress and PANSS-positive scores. In addition, there was a positive correlation between disulfide/total thiol and PANSS-total scores. These results suggest that oxidative stress is involved in psychopathology of schizophrenia. Therefore, meticulous assessment of psychopathological symptom severity may be beneficial in follow-up of schizophrenia patients with a disulfide/total thiol level above the pre-defined cut-off value (3.41%).

Nonetheless, there are some limitations to this study. Small sample size caused by the selection of non-smokers as the patient group is a limitation. Further large-scale studies with thiol-containing drugs

are, therefore, required to explore the psychopathological disease symptoms.

In conclusion, the present study investigated thiol-disulfide homeostasis using a new, colorimetric, and automated method in schizophrenia patients. Our study results showed that serum thiol levels were lower and disulfide/total thiol levels were higher in schizophrenia patients, compared to the controls. The increase in disulfide/total thiol ratio was correlated with increased psychopathological scores of patients. These results suggest an increase in oxidative stress in schizophrenia patients which is accompanied by worsening of the clinical symptoms. These findings also highlight the importance of oxidative stress in schizophrenia. We believe that further studies investigating the effects of SH-containing anti-oxidant components on thiol-disulfide homeostasis and clinical outcomes of schizophrenia treatment would make significant contribution to the treatment of this disease.

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