A Defect in the Antioxidant Defense System in Schizophrenia

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\section*{Introduction}

Reactive oxygen species (free radicals) are continuously produced by metabolism in the body and exert physiological and pathological effects by many different mechanisms, such as activation of phagocytes and the general immune system, lipid peroxidation, the electron transport system in mitochondria, ischemia and trauma [1]. Consequently, excess reactive oxygen species can cause oxidative damage in vulnerable targets such as polyunsaturated fatty acids in membranes, thiol groups in pro-
teins and nucleic acid bases in DNA [2, 3]. However, when oxidative stress occurs, enzymatic and nonenzymatic antioxidant systems of all organisms serve to protect them against the harmful oxidative reactions due to endogenous reactive oxygen species production [4].

There is increasing evidence that free radical-mediated central nervous system (CNS) neuronal dysfunction is involved in the pathophysiology of schizophrenia. Free radicals cause cell injury when they are generated in excess or the antioxidant defense system is impaired. Both of these processes seem to be affected in schizophrenia [5–17]. Plasma levels of the oxidants nitrite [6], nitric oxide and lipid peroxide [7] were found to be increased in patients with schizophrenia, and it has also been reported that nitric oxide radicals in the caudate region of post-mortem brain were increased in patients with schizophrenia [8]. The antioxidant defense status changes to cope with oxidative stress caused by free radicals. Increased [9, 10], unchanged [11] and decreased [12, 13] superoxide dismutase (SOD) activity, increased [10], unchanged [13, 14] and decreased [12] glutathione peroxidase (GSH-Px) activity and decreased [12] and increased [11] catalase (CAT) activity have all been reported in patients with schizophrenia. Antioxidant plasma proteins such as albumin, uric acid and bilirubin were found to be lower in haloperidol-managed [15] and first-episode schizophrenic patients [16].

Plasma concentrations of individual oxidants and antioxidants can already be measured separately in the laboratory, but these measurements are time-consuming, labor-intensive and costly. The number of different oxidants and antioxidants in plasma, serum, urine or other biological samples makes it difficult to measure each antioxidant separately. Therefore, several methods have been developed to determine the oxidative and antioxidative capacity of various biological samples [17]. Since the oxidative and antioxidative effects of the oxidant and antioxidant components of plasma are additive, only measurement of the total oxidant status (TOS) and total antioxidant status (TAS) can reflect the oxidative and antioxidative status of plasma [18–20]. Individual metabolites may not necessarily reflect the whole condition. Therefore, to explore a specific relationship between oxidative metabolism and particular diseases, an evaluation of total oxidant-antioxidant capacity is necessary. Novel automated colorimetric measurement methods for oxidative and antioxidative status developed by Erel [18, 19] provide us with the ability to evaluate TOS and TAS. The oxidative stress index (OSI) is calculated by dividing TOS by TAS and is a quantitative determinator of oxidative stress [21]. Although there are a few studies in the literature regarding the role of free radicals in schizophrenia from a general antioxidant point of view (i.e. TAS) [22–24], to the best of our knowledge, there are no studies evaluating TOS and OSI together with TAS in schizophrenia. Therefore, in the present study we aimed to evaluate the extent of the association between total oxidant-antioxidant status together with OSI and schizophrenia for the first time in the literature.

**Methods**

**Subjects**

Our sample consisted of 60 outpatients who presented to the Psychotic Disorders Unit of the Department of Psychiatry, School of Medicine, Gaziantep University, and were diagnosed with schizophrenia according to the 4th edition of the Diagnostic and Statistical Manual for Mental Disorders (DSM-IV) [25]. A DSM-IV diagnosis of schizophrenia was established on the basis of independent clinical interviews by a single senior psychiatrist (O.V.). Patients with any other kind of axis I psychiatric disorder were excluded. The protocol was approved by the local ethics committee. After the subjects were given a standard explanation and description of the study, all subjects gave informed written consent, in accordance with the Declaration of Helsinki [26]. The control group consisted of 40 healthy subjects matched to the patients with regard to age and gender and who had no history of any psychiatric disorder. The severity of schizophrenia symptoms in the patients was evaluated using the Positive and Negative Syndrome Scale (PANSS) [27] and the Clinical Global Impression-severity scale (CGI-S) [28]. Physical and neurological examinations were performed in each of the patients and controls. Liver and kidney function tests were also performed. Subjects who had normal results and did not meet any of the exclusion criteria were admitted to the study.

Alcohol and substance abuse or dependence or the presence of a severe organic condition such as Wilson’s disease, Down syndrome, malnutrition, pregnancy, diabetes mellitus, chronic renal failure, any cancer, liver cirrhosis and thyroid diseases resulted in exclusion from the study. Other exclusion criteria included the following: the use of glucocorticoids, oral contraceptives or any antioxidant agents such as vitamins (e.g. E and C), xanthine oxidase inhibitors (e.g. allopurinol, folic acid) and non-steroidal anti-inflammatory drugs; the presence of epilepsy or other severe neurological disorders such as Parkinson, Huntington or Alzheimer disease; the presence of any infectious disease, and excessive obesity.

**Blood Samples**

Venous blood samples were collected from the left forearm vein into heparinized tubes between 7.00 and 8.00 h after overnight fasting. The blood samples were centrifuged at 3,000 rpm for 10 min at 4°C in order to remove plasma. The Buffy coat on the erythrocyte sediment was separated carefully. Plasma samples were stored at −80°C until analysis.
Measurement of the TOS of Plasma

The TOS of the plasma was measured using a novel automated colorimetric measurement method for TOS developed by Erel [19]. In this method, oxidants present in the sample oxidize the ferrous ion-O-dianisidine complex to ferric ion. The oxidation reaction is enhanced by glycerol molecules, which are abundantly present in the reaction medium. The ferric ion makes a colored complex with xylene orange in an acidic medium. The color intensity, which can be measured spectrophotometrically, is related to the total amount of oxidant molecules present in the sample. The assay is calibrated with hydrogen peroxide and the results are expressed in terms of micromolar hydrogen peroxide equivalent per liter.

Measurement of the TAS of Plasma

The TAS of the plasma was measured using a novel automated colorimetric measurement method for TAS developed by Erel [18]. In this method, the hydroxyl radical, the most potent biological radical, is produced by the Fenton reaction and reacts with the colorless substrate O-dianisidine to produce the dianisyl radical, which is bright yellowish-brown in color. Upon the addition of a plasma sample, the oxidative reactions initiated by the hydroxyl radicals present in the reaction mix are suppressed by the antioxidant components of the plasma, preventing the color change and thereby providing an effective measure of the TAS of the plasma. The assay results are expressed as millimolar Trolox equivalent per liter, and the precision of this assay is considered excellent, being lower than 3% [29].

Determination of the OSI

The ratio of TOS to TAS was accepted as the OSI. To calculate the OSI, the unit for TAS values was changed to millimolar, and the OSI value was calculated according to the following formula: OSI (arbitrary unit) = TOS (mmol H2O2 Eq/l)/TAS (mmol Trolox Eq/l) [21]. For further details, see the studies of Erel [18, 19].

Apparatus

A Cecil 3000 spectrophotometer with a temperature-controlled cuvette holder (Cecil) and an Aeroset automated analyzer (Abbott) was used [18].

Statistical Analyses

The data were evaluated by SPSS 13.0 (SPSS Inc., Chicago, Ill., USA). Bivariate analyses were conducted using χ² statistics for categorical variables and 2-tailed t tests for continuous data. For correlation evaluations, Pearson’s correlation (2-tailed) was used. The 2-tailed significance level was set at 0.05. The Mann-Whitney U test was employed to compare oxidative values between paranoid and nonparanoid patients.

Results

There were no significant differences in age or the ratio of females to males between the patient and control groups, with a female to male ratio of 36/24 versus 23/17, respectively, and a mean age of 31.93 ± 9.37 years (range 19–55) versus 35.53 ± 9.86 years (range 20–58), respectively [χ² = 0.06, degrees of freedom (d.f.) = 1, p > 0.05; F = 0.01, p > 0.05]. The sociodemographic characteristics of the patients and controls are shown in table 1.

There was no significant difference between the patient and control groups in terms of the number of smokers (χ² = 0.66, d.f. = 1, p > 0.05). However, there were significantly more male patients who smoked than female patients (χ² = 7.54, d.f. = 1, p < 0.01). In contrast, in the control group there was no significant difference between males and females with regard to the number of smokers (χ² = 2.28, d.f. = 1, p > 0.05).

Although there were no significant differences between patients and controls with regard to TOS (F = 51.4, p > 0.05), the plasma TAS of the patients was significantly lower than that of the healthy controls (F = 27.4, p < 0.01). Thus, OSI values of the patients were significantly higher than those of the controls (F = 52.5, p < 0.01). Plasma TOS, TAS and OSI of the groups are shown in table 2 and figures 1, 2 and 3, respectively.

Oxidative parameters did not correlate with age and duration of schizophrenia (p > 0.05 for both), and they did not differ significantly between smoker and non-smoker patients (p > 0.05).

TAS values were higher in male patients than female patients (F = 2.48, p < 0.01), but there were no differences between the genders in the control group with regard to oxidative values (p > 0.05). In both the control and patient groups, no correlation was detected between smoking status and TAS or TOS (p > 0.05).

Of the 60 patients, 41 had paranoid, 16 disorganized and 3 catatonic schizophrenia (table 3). There was no significant difference between paranoid and nonparanoid patients in terms of TOS or TAS (p > 0.05). Both paranoid and nonparanoid patients had similar TOS but lower TAS compared to the healthy controls (table 2).

Table 1. Sociodemographics of patients and controls

<table>
<thead>
<tr>
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<th>Patients</th>
<th>Controls</th>
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<tbody>
<tr>
<td>Gender</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>24 (40)</td>
<td>17 (42.5)</td>
</tr>
<tr>
<td>Female</td>
<td>36 (60)</td>
<td>23 (57.5)</td>
</tr>
<tr>
<td>Age, years (range)</td>
<td>31.93 ± 9.37 (19–55)</td>
<td>35.53 ± 9.86 (20–58)</td>
</tr>
<tr>
<td>Smoking status</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Smokers</td>
<td>32 (53.3)</td>
<td>18 (45.0)</td>
</tr>
<tr>
<td>Nonsmokers</td>
<td>28 (46.7)</td>
<td>22 (55.0)</td>
</tr>
</tbody>
</table>

Values are numbers of patients with percentages in parentheses, except where indicated otherwise.
When we compared illness symptom severity and oxidative parameters, we found some differences. There was no significant correlation between TOS and PANSS total and subscale scores (p > 0.05). In contrast, we found weak to moderately significant negative correlations between TAS and PANSS total, positive and general psychopathology subscale scores (r = –0.37, p < 0.05, r = –0.28, p < 0.05, and r = –0.32, p < 0.05, respectively). However, there was no significant correlation between TAS and PANSS negative subscale scores (p > 0.05). We also found a weak to moderately significant negative correlation between CGI-S scores and TOS and TAS values (r = –0.26, p < 0.05 and r = –0.29, p < 0.05, respectively).

There were no significant differences in oxidative parameters among patients who used typical, atypical or a combination of antipsychotic medications (p > 0.05).

**Discussion**

Although this study demonstrates no difference for TOS, significantly lower plasma TAS and higher OSI were found in patients with schizophrenia in comparison to healthy controls. Recently, Ustundag et al. [22] investigated TAS in patients with schizophrenia using the same methods. Similar to our results, they found that patients with schizophrenia had lower plasma TAS than controls [22]. Also, Yao et al. [30] documented that the total antioxidant response was inadequate in schizophrenia. In a study of first-episode schizophrenics, Reddy et al. [16] reported that the major plasma antioxidants albumin, uric acid and bilirubin were all significantly lower in the schizophrenic patients than controls.

Some studies found differences in oxidative parameters among schizophrenia subtypes. Ustundag et al. [22]...
showed that although patients with all schizophrenia subtypes had lower TAS values than controls, paranoid schizophrenics had higher TAS compared to the patients with other schizophrenia subtypes. Herken et al. [11] reported that some individual antioxidant enzyme activities differ among schizophrenia subtypes. They identified a significant increase in SOD activity in the residual type compared to the paranoid type. CAT activity was significantly increased in disorganized, paranoid and residual types compared to the control groups. GSH-Px activity was found to be markedly increased in all study patients except for those with the paranoid type of schizophrenia [11]. In our study, the majority of patients had paranoid-type schizophrenia, and we found no significant difference in oxidative parameters between paranoid and nonparanoid patients.

We did not find a significant correlation between PANSS scores and TOS. While we did not find any correlation between TAS and the negative subscale of PANSS, we found a negative correlation between TAS and positive and general psychopathology subscale scores and total scores of PANSS. In contrast to our results, Ustundag et al. [22] reported that plasma TAS was significantly and negatively correlated with Negative Syndrome Scale scores, which is different from the scales we used. In addition, Yao et al. [30] reported that plasma TAS was significantly and inversely correlated with symptom severity in the drug-free condition. In addition, they emphasized that there were no significant differences between periods on and off haloperidol treatment. When patients returned to haloperidol treatment after a relapse, the plasma TAS remained fairly constant and was not significantly different in the same individuals during haloperidol stabilization or drug-free periods [30]. We detected a negative correlation between CGI-S scores and TAS values, which is compatible with the above-mentioned findings. As the deficiency in the antioxidant response increases, the severity of symptoms also increases. In the present study, we also found that TOS was negatively correlated with CGI-S scores.

Some clinical studies have indicated that antioxidant enzyme activities are associated with the particular antipsychotic medication taken by the patients with schizophrenia. It has been reported that typical antipsychotics such as haloperidol increased lipid peroxidation and decreased activities of antioxidant enzymes in rats, but similar changes were not observed with atypical antipsychotics such as risperidone, ziprasidone, clozapine and olanzapine. In addition, the lipid peroxidation products hydroxylalkenals are decreased with risperidone, ziprasidone and clozapine use [31, 32]. Atypical antipsychotics have also been associated with increased antioxidant enzyme activities according to some studies [33, 34]. On the other hand, several studies revealed that long-term treatment with typical and atypical antipsychotics may cause...
similar results with regard to antioxidant enzymes and lipid peroxidation and therefore does not lead to a significant change in total antioxidant capacity [10, 30, 35–37]. Zhang et al. [38] suggested that risperidone treatment significantly decreased the blood SOD levels of schizophrenia patients. Qing et al. [39] reported that atypical antipsychotic drugs slightly upregulated the expression of copper/zinc SOD mRNA, whereas haloperidol strongly increased the expression of copper/zinc SOD mRNA. In our study, all patients were stable and receiving either typical, atypical or a combination of typical and atypical antipsychotic medication(s) [39]. We did not find any difference among these medication groups with regard to TOS and TAS.

Reddy et al. [16] reported that a defect in the antioxidant defense system occurs early in the course of schizophrenia and is independent of treatment effects. In addition, they found that lower antioxidant levels in a patient group were independent of the smoking status of the patients, as was the case in our study [16].

In general, it may be stated that inadequacy of the antioxidant defense system in schizophrenia seems to be a trait feature rather than a status feature [12, 16, 30]. Ben Othmen et al. [12] found that in schizophrenia patients, CAT, SOD and GSH-Px activities were lower than in their siblings and healthy controls, and they emphasized inadequacy of the antioxidant defense system in schizophrenia as a trait feature. Also, lower levels of antioxidants were found in the caudate region and prefrontal cortex of postmortem brains from schizophrenic patients [40, 41].

Due to a high rate of oxidative metabolic activity (e.g. catecholamine degradation), a high ratio of membrane surface area to cytoplasmic volume, a vulnerable anatomic network, high concentrations of oxidizable membrane polyunsaturated fatty acids and low levels of protective antioxidant enzymes, the CNS cells are more vulnerable to the toxic effects of free radicals than other organs in the body. The polyunsaturated fatty acids in the membranes of CNS cells can undergo peroxidation by reacting with free radicals, and lipid peroxidation in the membranes can significantly impact on membrane functions. All of these mechanisms increase oxidative stress and can cause neuronal impairment, therefore making the brain selectively more susceptible to oxidative injury [13].

Some limitations of this study should be noted. Our sample size was relatively small. Different subtypes of schizophrenia, except the paranoid type, were limited and all patients were on antipsychotic treatment. Besides these limitations, it is worth mentioning that some confounding factors related to outpatient habits, such as exercise, lifestyle and dietary changes, may affect the levels of plasma oxidants and antioxidants. In addition, in this study, peripheral blood samples were used, and these samples possibly might not reflect the status in the CNS. On the other hand, peripheral blood samples were used in most of the studies published in this field. Also, in some studies conducted in postmortem brain tissue of schizophrenic patients, a decrease in antioxidant enzyme activities was demonstrated [40, 41].

In conclusion, this study confirmed the inadequate response of antioxidants in schizophrenia, as reported in many similar previous studies. The reasons for decreased antioxidant levels in schizophrenia are not clear. Increased oxidative stress due to a deficit in the antioxidant defense system leads to oxidative neuronal cell damage, which plays a role in the pathophysiology of schizophrenia and has an important negative effect on the course of schizophrenia.

Schizophrenia patients might have a better outcome if the impairment of the antioxidant defense system could be repaired by supplementation of antioxidants. It has already been reported that antioxidant supplementation has beneficial effects on positive and negative symptoms and antipsychotic medication-induced adverse events in schizophrenia [42–44]. Further studies are needed to better understand the association between oxidative metabolism and the etiology and clinical characteristics of schizophrenia.

References

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