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Elevated serum thiobarbituric acid reactive substances in clinically symptomatic schizophrenic males

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Abstract

Impaired antioxidant defenses are suggested to participate in the pathophysiology of schizophrenia. Altered superoxide dismutase (SOD) and increased lipid peroxidation, measured by the thiobarbituric acid reactive substances (TBARS), are increased in schizophrenia. The aim of this study was to determine the effects of clinical course and subtype on oxidative stress parameters. In this study, 68 male patients, classified according to DSM-IV schizophrenia subtypes and clinical course (partial remission, marked symptoms, and deteriorated), were studied, and TBARS and SOD measured. Mean serum SOD and mean serum TBARS concentrations were similarly not significantly different among different subtypes (paranoid, disorganized and undifferentiated). However, marked symptoms status was associated with higher TBARS levels compared to the deteriorated group. This suggests a possible relationship between symptom acuity and oxidative stress in males.

Keywords: Schizophrenia; Oxidative stress; Peroxidative damage; Clinical course; Psychopathology

There is strong evidence that oxygen free radicals may play an important role in the pathophysiology of major mental illnesses, such as, schizophrenia (SZ) [25]. Increased neuronal oxidative stress levels produce deleterious effects on signal transduction, structural plasticity and cellular resilience, mostly by inducing lipid peroxidation in membranes, proteins and genes [16]. Thiobarbituric acid reactive substances (TBARS) are considered a direct index of cell lipid peroxidation, and the primary antioxidant system involves superoxide dismutase (SOD), catalase and glutathione peroxidase. Oxidative stress and impaired in antioxidant enzymes have long been in the pathophysiology of schizophrenia [5,13–15,19,25,26]. Lipid peroxidation, assessed, by TBARS levels, has been shown to be increased in the serum of drug free [2,16,22,28] and medicated [1,2,10,22] schizophrenic patients.

High levels of SOD, a key enzyme in the endogenous antioxidant defense pathways, were reported in neuroleptic-free schizophrenic patients [33] and were associated with positive symptoms. SOD activity seems to be increased in the residual schizophrenia subtype compared to the paranoid subtype [7]. We found high SOD and TBARS levels in chronically medicated schizophrenic patients [5].

To elucidate whether oxidative stress may have a pathophysiological role in schizophrenia subtypes and in clinical course,

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Table 1
Blood SOD and TBARS levels in males among DSM-IV schizophrenia subtypes

Schizophrenia forms	*N*	SOD (USOD/g)	*P*	TBARS (nmol/ml)	*P*
Paranoid	38	9.8 ± 5.1	0.500*	5.1 ± 1.7	0.837**
Disorganized	27	9.4 ± 4.1		5.0 ± 1.3	
Undifferentiated	3	12.7 ± 1.2		5.4 ± 1.4	

Mean ± S.D.
* ANOVA *P*-value for mean SOD levels between paranoid, disorganized and undifferentiated groups.
** ANOVA *P*-value for mean TBARS levels between paranoid, disorganized and undifferentiated groups.

the present study examined serum SOD and TBARS levels in different forms of schizophrenia, in chronically medicated male patients. This study also examined TBARS and SOD in individuals with different outcome patterns.

The study sample consisted of 68 males; all of them were on treatment with clozapine (mean daily dose 587.93 ± 211.59 mg). The cohort had a mean age of 35.15 ± 9.86 years, with a mean illness duration of 15.43 ± 9.13 years. Thirty-eight patients were classified as schizophrenic paranoid type, 27 as disorganized and 3 as undifferentiated. There were 18 subjects with partial remission, 19 with marked symptoms and 31 with deterioration. This study was approved by the Ethical Committee of the Hospital de Clínicas de Porto Alegre. Patients were recruited from the Schizophrenia Program of the Hospital de Clínicas de Porto Alegre (HCPA), RS and they had to fulfill DSM-IV [3,4,17] criteria for schizophrenia. Patients were classified according to DSM-IV schizophrenia subtypes. An additional classification, according to the presence of psychotic symptoms (partial remission, marked symptoms, and deteriorated) in the clinical illness course [20,23] was performed. All subjects were counseled about the study procedure and signed informed consent to participate in the study. Participants had 5 ml blood samples collected by venipuncture without anticoagulants, and serum was obtained by centrifugation at 3000 × *g* for 5 min and kept frozen at −70 °C for up to 6 months, until the assay.

Superoxide dismutase activity was determined spectrophotometrically in serum samples by measuring the inhibition of the rate of autocatalytic adrenochrome formation at 480 nm, in a reaction medium containing 1 mmol/l adrenaline (pH 2) and 50 mmol/l glycine (pH 10.2). This reaction was conduced at a constant temperature (30 °C) for 3 min. The enzymatic activity was expressed as superoxide dismutase units per gram of protein. One unit is defined as the amount of enzyme that inhibits the rate of adrenochrome formation by 50% [18].

Levels of serum malondialdehyde (MDA), a product of lipid peroxidation, were measured by the TBARS method, described by Wills [31]. Peroxidation was measured as the production of MDA, which in combination with TBARS forms a pink chromogen, i.e. a compound whose absorbance at 530 nm was measured. MDA results were expressed as nmol/ml.

Analysis was performed using Statistical Product and Service Solutions Version 15.0 (SPSS). Data were presented as mean ± S.D. Between-group comparisons were done using Student's *t*-test; ANOVA was performed to analyze variance between the groups and multiple comparisons between groups were assessed using the Tukey test. Relationships between variables were assessed using correlated Pearson's coefficient.

Mean serum SOD ($P = 0.500$, ANOVA) and mean serum TBARS ($P = 0.837$, ANOVA) concentrations were not significantly different among different subtypes (paranoid, disorganized and undifferentiated) (Table 1). Mean serum SOD concentrations ($P = 0.689$, ANOVA) were not different between the three different clinical course patterns (Table 2). Mean serum TBARS levels were significantly higher ($P = 0.37$, ANOVA and $P = 0.37$, Tukey test) in the subgroup with marked symptoms, compared to the deteriorated group (Table 2).

The present report provides evidence of changes in TBARS levels among acutely ill SZ male patients. Males with marked symptoms status had higher TBARS levels compared to the deteriorated group. The present findings do not provide evidence of differences in serum SOD and TBARS in individuals with paranoid, disorganized or undifferentiated schizophrenia.

Herken et al. [7] found higher levels of SOD activity in residual schizophrenia compared to paranoid subtype, we did not find that. Although, it is important to consider that none patient in our sample fulfilled criteria for residual schizophrenia and few patients ($n = 3$) fulfilled criteria for the undifferentiated form. Since patients with residual schizophrenia subtype present symptoms in an attenuated form, the higher levels of SOD in this subgroup, found by Herken [7], may reflect a pop-

Table 2
Blood SOD and TBARS levels in males among the three illness course patterns

Clinical course	*N*	SOD (USOD/g)	*P*	TBARS (nmol/ml)	*P*
Partial remission	18	10.4 ± 7.8	0.689*	4.9 ± 1.6	0.037**
Marked symptoms	19	10.1 ± 5.0		5.9 ± 2.0&	
Deteriorated	31	9.2 ± 4.5		4.7 ± 1.3&	

Mean ± S.D.
* ANOVA *P*-value for mean SOD levels between partial remission, marked symptoms and deteriorated groups.
** ANOVA *P*-value for mean TBARS levels between partial remission, marked symptoms and deteriorated groups.
& $P = 0.037$ (Tukey test) for mean TBARS levels between groups marked symptoms and deteriorated.

ulation of patients with SZ that have better superoxide radical detoxification and, as a result, a less severe behavioral alterations than other schizophrenia subtypes. However, this finding deserves additional replication in order to draw definitive conclusions about oxidative stress in schizophrenia subtypes. Our results provide additional evidence of similarity in SOD and TBARS among different subtypes (paranoid, disorganized and undifferentiated).

The idea of oxidative damage in the pathophysiology of schizophrenia as a result of oxidant/antioxidant imbalance has been extensively described in the literature [1,2,5,6,10,15,22,32]. We have previously found higher serum TBARS levels [5] in SZ compared to controls. Moreover, we found higher serum TBARS levels in SZ patients taking clozapine when compared to SZ patients on treatment with haloperidol [5], which might be indicative of greater illness severity in the clozapine group, since this drug is formally indicated for a specific group of schizophrenic patients whose symptoms are refractory to the other neuroleptics [9]. Parikh [21] found increased lipid peroxidation in the brain of rats chronically treated with haloperidol, but not in animals chronically treated with risperidone, olanzapine or clozapine, which is contrasting to our findings in plasma of schizophrenic subjects and supports the idea of clozapine as a illness severity marker in human samples.

Additionally, there was no evidence of difference between different subgroups (partial remission, marked symptoms, and deteriorated) in SOD and TBARS levels. Nevertheless, male patients presented higher TBARS levels in the marked symptoms group than in deteriorated group. This might suggests that greater clinical acuity might be associated with higher levels of oxidative stress.

A high level of TBARS is a sign of peroxidative injury to membrane phospholipids. Neuronal functioning is affected by this injury either by changes in membrane fluidity or by alterations in membrane receptors [16], which can cause altered neurotransmitter uptake and release, and even cell death. Elevated serum S100B protein, a sensitive marker of brain damage [8], was previously found in schizophrenia [11,27,30] and its elevated levels could indicate astrocyte activation or death, or blood-brain-barrier dysfunction. As pointed out by Theberge [29], an irreversible neurodegenerative process could characterize schizophrenia. Several neuroimaging and post-mortem findings, along with the behavioral and cognitive deterioration observed in schizophrenic patients, could reflect a limited, but significant neurodegenerative process probably most active in the early stages (5–10 years) of the disease [12].

There are some methodological characteristics of the present study. A strength of the study, is that all participants were on clozapine, controlling for the effects of pharmacotherapy on outcome. There were no comparisons to a control group. Our group and others have found previously high serum levels of SOD and TBARS in schizophrenic patients compared to controls [5]. Finally, none of the patients in our sample fulfilled criteria for residual schizophrenia and few patients ($n = 6$) fulfilled it for undifferentiated form.

In conclusion, we found no differences in SOD and TBARS serum levels among males with paranoid, disorganized or undifferentiated schizophrenia. The fact that males with marked symptoms status had higher TBARS levels compared to the deteriorated group supports an impact of illness acuity on oxidative stress. Our findings support the hypothesis of a state dependant process of oxidative stress in schizophrenia, which might be related to the known neurodegenerative process. These data support the notion that interventions at the level of oxidative stress may be of potential value in the treatment of schizophrenia.

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