Similarities in serum oxidative stress markers and inflammatory cytokines in patients with overt schizophrenia at early and late stages of chronicity

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\textbf{A B S T R A C T}

Schizophrenia (SZ) is a debilitating neurodevelopmental disorder that strikes at a critical period of a young person’s life. Its pathophysiology could be the result of deregulation of synaptic plasticity, with downstream alterations of inflammatory immune processes regulate by cytokines, impaired antioxidant defense and increased lipid peroxidation. The aim of this study was to examine serum oxidative stress markers and inflammatory cytokines in early and late phases of chronic SZ. Twenty-two patients at early stage (within first 10 years of a psychotic episode), 39 at late stage (minimum 10 years after diagnosis of SZ) and their respective matched controls were included. Each subject had 5 ml blood samples collected by venipuncture to examined thiobarbituric acid-reactive substances (TBARS), total reactive antioxidant potential (TRAP), protein carbonyl content (PCC), Interleukins 6 and 10 (IL-6, IL-10) and tumor necrosis factor alpha (TNF-alpha). TBARS, IL-6 and PCC levels were significantly higher in patients with SZ at early and late stages than in controls. There were no differences for TRAP and TNF-alpha levels in patients with SZ at early and late stages than in controls. IL-10 levels were decreased in patients at late stage and a decrease trend in early stage was found. Results provided evidence consistent with comparable biological markers across chronic SZ. The concept of biochemical staging proposed by others for bipolar disorder is not seen in this cohort of patients with SZ, at least for cytokines and oxidative stress markers. Our findings reinforce the need of assessment of individuals in ultra high risk to develop psychosis and first-episode population.

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1. Introduction

Schizophrenia (SZ) is a debilitating neurodevelopmental disorder that strikes at a critical period of a young person’s life (\textit{Kaur and Cadenhead, 2010}). It has been accepted that SZ originates from abnormalities occurring during the early stages of neural development (\textit{Lieberman, 1999}). The pathophysiology of SZ could be the result of deregulation of synaptic plasticity, with downstream alterations of neurotrophins, impaired antioxidant defense and increased lipid peroxidation (\textit{Gama et al., 2006, 2007, 2008a,b}).

Many lines of evidence support the hypothesis that inflammation-related pathways are involved in the pathophysiology of psychiatric disorders (\textit{Dean, 2011}). Inflammatory immune processes have been strongly implicated in the pathophysiological mechanisms of SZ (\textit{Reddy and Yao, 1996; Sasayama et al., 2011; Kunz et al., 2011; Drexhage et al., 2011; Cazzullo et al., 2001; Miller et al., 2011; Francesconi et al., 2011}). Cytokines regulate inflammation and coordinate both innate and adaptive arms of the immune system (\textit{Miller et al., 2011}), being important mediators of the cross-talk between the central nervous system (CNS) and the immune system, which might have implications for clinical psychiatry (\textit{Kapczinski et al., 2010, 2011}). Recent data have emerged
to suggest that changes in inflammation-related pathways are present in the CNS of subjects with psychiatric disorders (Kauer and Cadenhead, 2010). They can exert cellular effects that, if not adequately moderated or counteracted, ultimately lead to toxicities, physiological deregulation, and medical compromise (Kapczinski et al., 2010, 2011).

Examples of cytokines include interleukins (IL) and tumor necrosis factors (TNF). IL-1, IL-6 and TNF-alpha are considered pro-inflammatory, in the sense that they augment the immune response to infection and inflammation by promoting leukocyte recruitment to inflammatory sites and/or by activating inflammatory cells (Potvin et al., 2008). IL-10 is an anti-inflammatory cytokine that contributes to dampen the immune and inflammatory response (Potvin et al., 2008).

Oxidative damage is a mechanism of cellular injury in a number of conditions, including cancer, inflammatory states, and neurodegeneration (Kapczinski et al., 2011). Increased neuronal oxidative stress levels produce deleterious effects on signal transduction, structural plasticity and cellular resilience, mostly by inducing lipid peroxidation in membranes and direct damage in protein and genes (Gama et al., 2007, 2008a,b). Neurons and glia are particularly vulnerable to inflammatory processes and redox status, and are dependent on antioxidant defenses (Kapczinski et al., 2010, 2011; Kunz et al., 2008, 2011). Furthermore, oxidative stress has been identified as a possible element in the neuropathological processes of SZ (Riegel et al., 2010; Dietrich-Musalska and Kontek, 2010; Kunz et al., 2008; Gama et al., 2006, 2008a,b).

Thiobarbituric acid-reactive substances (TBARS) is one of the well-known secondary products of lipid peroxidation and was used as an indicator of oxidative damage for several diseases (Huang et al., 2010). TBARS has been studied in SZ, providing evidence of increased levels of lipid peroxidation (Gama et al., 2006, 2008a,b). Major molecular mechanisms induced by oxidative stress are protein oxidation. Structural changes by oxidative stress in proteins are characterized by carbonyl formation, so the protein carbonyl content (PCC) indicates oxidative stress (Dietrich-Musalska et al., 2009). Total reactive antioxidant potential (TRAP) is one of the methods most employed to estimate the antioxidant capacity of samples in vitro (Dresch et al., 2009).

A new approach to understanding severe mental disorders such as SZ is to adopt a clinical staging model (Wood et al., 2011). The clinical staging model is particularly useful as it differentiates early, milder clinical phenomena from those that accompany illness progression and chronicity (McGorry et al., 2010). A term called neuroprogression has been increasingly used to define the pathological reorganization of the CNS along the course of severe mental disorders (Berk et al., 2011). It could be a result of several insults, such as inflammation and oxidative stress (Berk et al., 2010).

Staging models for SZ (Agius et al., 2010; Wood et al., 2011) and bipolar disorder (BD) (Vieta et al., 2011; Kapczinski et al., 2009a,b; Berk et al., 2007) have been proposed in order to personalize and optimize treatments (Berk et al., 2009). The logic of staging is based on accessing people to give them different treatment approaches according to pathophysiological, symptomatic and structural changes (Franczy et al., 2010).

The clinical staging model in SZ consists on prodrome, first episode and chronic phases (Agius et al., 2010). Unlike BD, SZ present a unique and severe clinical deterioration pattern at the very beginning of the disease (Kauer-Sant’Anna et al., 2008; Lieberman, 1999). Episode dependant deterioration patterns have been widely described in BD by serum biomarkers (Kauer-Sant’Anna et al. 2008; Berk et al., 2011), brain imaging (Strakowski et al., 2002; Velakoulis et al., 2006) and functioning (Reinares et al., 2010; Scott et al., 2006). The overt BD would be staged in four categories, according to functioning and cognition (Kapczinski et al., 2009b). However, in SZ, the overt syndrome would not indicate clinical staging possibilities (Agius et al., 2010) and, as far as we aware, there is no data on serum biomarkers staging in this population.

In order to characterize several biological markers in patients with overt SZ at early and late phases of chronicity, and to test for evidence of progression in oxidative stress and inflammatory impairment, the present study examined TBARS, TRAP, PCC, IL-6, IL-10 and TNF-alpha. The study included separate control groups for early and late stages.

2. Methods

This study protocol was approved by the Ethical Committee of the Hospital de Clínicas de Porto Alegre, RS, Brazil (HCPC). In accordance with the Declaration of Helsinki, all subjects were advised about the procedure and signed the informed consent prior to participation. Sixty-one patients with SZ and fifty-seven healthy controls matched for age, gender and education were recruited. The double case–control design included 22 patients with SZ at early stage of chronicity (within first 10 years of a psychotic episode); 39 patients at late stage (minimum 10 years after diagnosis of SZ) and their respective matched controls (25 and 32 subjects). All patients had to fulfill the Statistical Manual of Mental Disorders Fourth Edition (DSM-IV) for SZ and their psychopathological state were assessed by the 18-item Brief Psychiatric Rating Scale (BPRS) (Romano and Elkins, 1996). The control group consisted of healthy volunteers who had no current or previous history as well as no first-degree family history of a major psychiatric disorder, including dementia or mental retardation assessed by the non-patient version of the Structured Clinical Interview for DSM-IV (SCID).

Patients were recruited from HCPC through the outpatients’ clinic. All subjects were required to be at least age 18 and no older than 60. None of them had any neurological disease, brain tumor, thyroid disease, severe hepatic disease, severe cardiac disease or any other psychiatric diagnosis. Included patients had body mass index at or below 27, they were non-smokers or smoked up to 10 cigarettes per day.

Each subject had 5 ml blood samples collected by venipuncture without anticoagulants, and serum was obtained by centrifugation at 300 × g for 5 min and kept frozen at −70 °C for up to 6 months, until the assay.

The levels of lipid peroxidation were measured using the TBARS method described by Wills (1966), and data were expressed as nmol/ml. The oxidative damage to proteins was measured by the determination of carbonyl groups (protein carbonyl content) based on the reaction with dinitrophenylhydrazine (DNPH), as previously described (Levine et al., 1990). The non-enzymatic antioxidant cellular defenses were estimated by the total radical-trapping antioxidant parameter (TRAP), which determines the non-enzymatic antioxidant potential, as previously described (Wayner et al., 1985). Serum cytokines (IL-6, IL-10 and TNF-alpha) were measured according to the procedures supplied by the manufacturer using highly sensitive sandwich–ELISA kits for TNF-alpha, IL-6 and IL-10 (Quantikine, R&D Systems, Minneapolis, Minn., USA). All samples were assayed in duplicates.

Analysis was performed using SPSS Version 18.0. Demographic and clinical characteristics were analyzed using Chi-Square, Mann–Whitney or T-test. Descriptive analyses are presented as mean ± SD or median (interquartile range) and p-values < 0.05 were considered significant. Appropriated tests for parametric or nonparametric distribution are indicated in Table 1.
3. Results

The subjects’ characteristics are summarized in Table 1. TBARS (p = 0.0001 for early and late-stage groups), IL-6 (p < 0.0001 for early, p = 0.003 for late stage) and PCC (p = 0.001 for early, p = 0.006 for late stage) levels were significantly higher in patients with SZ at early and late stages than in controls. There were no differences for TRAP (p = 0.083 for early, p = 0.731 for late stage) and TNF-alpha (p = 0.786 for early, p = 0.114 for late stage) levels in patients with SZ at early and late stages than in controls. IL-10 levels were decreased in patients at late stage (p = 0.006) and a decrease trend in early stage was found (p = 0.053) (Figs. 1 and 2).

4. Discussion

In our knowledge, this is the first study to examine oxidative stress markers and inflammatory cytokines levels in a sample of two groups of chronic patients with SZ, differing in illness duration: 7.25 (5.34) years for early and 21.19 (9.20) years for late stage.

The results suggest that SZ is associated with a chronic immune activation and the concept of biochemical staging proposed by Kapczinski et al., 2009a; Kauer-Sant’Anna et al., 2008; Berk et al., 2007) is not seen in this cohort of patients with overt SZ, at least for cytokines and oxidative stress markers. For instance, IL-6 was increased in the early and late stages of SZ and IL-10 was decreased in late stages, with a decrease trend in early stages. However, TNF-alpha levels were similar in patients with SZ at the early and late stages compared to controls. The results also show an increased redox status, indicated by higher serum levels of TBARS and PCC in the early and late stage of SZ, compared to controls. On the other hand, no differences found in TRAP levels at early and late stages compared to controls.

Increased IL-6 levels are one of the most robust findings in the study of inflammatory markers in SZ, as evidenced by a meta-analysis of 19 studies and 1219 patients (Sasayama et al., 2011; Potvin et al., 2008). Consistent with our findings, a recent study with patients with recent-onset SZ found activation of pro-inflammatory networks (Drexhage et al., 2011; Miller et al., 2011).

A recent study reports that patients with first episode of psychosis had increased levels of IL-6 gene expression when compared with controls, suggesting a pro-inflammatory state to be associated with decreased levels of BDNF and smaller hippocampus volume (Mondelli et al., 2011). In accordance with our findings, an elegant meta-analysis has reported that blood IL-10 levels were...
significantly decreased and IL-6 levels were significantly increased in acutely relapsed patients compared to control subjects (Miller et al., 2011). As reported by previous studies (Kunz et al., 2011; Francesconi et al., 2011) the fact that TNF-alpha levels were not different from those of the controls in our sample could be explained by the non-acute profile of the patients. In SZ, however, defining remission in a chronically ill and stable population may not allow for a clear differentiation of state and trait effects (Kunz et al., 2011).

In line with our findings, a recent meta-analysis showed that TBARS are significantly increased in SZ (Zhang et al., 2010). Previous studies have found increased levels of serum TBARS predominantly in never-medicated schizophrenia patients compared to controls (Arvindakshan et al., 2003; Grignon & Chianetta, 2007). Increased oxidative stress has been suggested in the pathophysiology of SZ, based on the increased peroxidation at the onset of psychosis in never-medicated patients (Arvindakshan et al., 2003). Gama et al., found elevated TBARS levels in chronically medicated SZ patients compared to controls, suggesting that the high level of TBARS is a sign of peroxidative injury to membrane phospholipids (Gama et al., 2006).

In our study, we presented elevated PCC levels in the early and late stage of chronic SZ, compared to controls. Corroborating our findings, Dietrich-Muszalska et al. found, in platelet proteins from patients with SZ, a statistically significant increase of the level of biomarkers of oxidative/nitrative stress such as carbonyl groups (Dietrich-Muszalska and Olas, 2009). Furthermore, we found total antioxidant defense, presented by TRAP levels, similar in both stages and controls. It seems that patients do not perform an antioxidant action reactive to the illness injury. In line with our findings, earlier results showed significantly increased TBARS levels in patients with SZ, whereas the activities of antioxidant defense enzymes were not increased (Dietrich-Muszalska and Kontek, 2010).

Our findings support the growing body of evidence corroborating the early central nervous system damage hypothesis in SZ, suggesting a different pattern of damage between SZ and BD, with an early degenerative component preceding the illness onset in SZ (Velakoulis et al., 2006) and an episode-dependent pattern of deterioration in BD (Strakowski et al., 2002). As reported by Kunz et al., this observation brings us back to the classic differentiation between SZ and BD, based on either an episodic or a chronic-deteriorating course (Kunz et al., 2011). In the same venue, while increased IL-6, TBARS and PCC seem to be a state effect in BD, it seems to be a trait effect in SZ (Kunz et al., 2011).

Our report must be interpreted in light of its limitations. The study design was cross-sectional; it did not allow us a direct examination of the course of oxidative stress markers and inflammatory cytokines in SZ. Nevertheless, the inclusion of two control groups matched to early and late stage groups allowed differentiation of age, sex and diagnosis effects. The effect of medication on serum biomarkers could not be excluded, however it has been reported that antipsychotics would decrease central and peripheral inflammation (Dean, 2011) as well as oxidative stress (Khan et al., 2002).

The present study provided evidence consistent with comparable biological markers across chronic SZ. It is conceivable that increased cytokines levels and impaired anti-oxidative stress defense may synergistically function in favor of neuronal degeneration in SZ. Overall, these clinical observations are consistent with differences reported in brain structure, metabolic and biochemical changes observed (Velakoulis et al., 2006; Gama et al., 2006, 2007, 2008a,b). As corroborated by Sponheim et al., cognitive deficits could be comparable from early in the course of SZ to the chronic phase of the disorder, without progressing beyond what is expected with normal aging (Sponheim et al., 2010).

In conclusion, our findings provide important information about biological markers in early and late stages of chronic SZ, a data that would be important to consider in interpreting findings of anomalous brain structure and function, reinforcing the need of assessment of individuals in ultra high risk to develop psychosis and first-episode population. Prospective studies of cytokines and oxidative stress markers in SZ and in at-risk/first-episode populations, together with neuroimaging techniques, should stratify patients by clinical status to better guide interventions.

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Contributors
MP designed the study, wrote the protocol, was responsible for the analysis and interpretation of data, participated in data interpretation, drafting the article and final approval of this version. RM, GRF, MABP, CES, JCFM, ALT, MIRL, JCW and, PSBA participated in study design and final approval of this version. CSG, MKS and FK were responsible for study design and interpretation of data, drafting the article and final approval of this version.

Conflicts of interest
The authors have declared no conflict of interest in this matter.

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