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Oxidative stress and neopterin abnormalities in schizophrenia: A longitudinal study

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ABSTRACT

Oxidative stress abnormalities have been proposed to explain the pathogenesis of schizophrenia. The present study examined neopterin and oxidative stress abnormalities in schizophrenia patients before and after treatment. Serum neopterin, total anti-oxidants, nitrites and thiols in antipsychotic-naïve schizophrenia patients ($n = 45$) were assessed at baseline before treatment in comparison with healthy controls ($n = 43$). The schizophrenia patients on treatment were followed up for 3 months and these parameters were reassessed ($n = 32$). In comparison to healthy controls, schizophrenia patients had significantly higher levels of neopterin and nitrites and significantly lower levels of anti-oxidants before treatment. During follow-up assessments in schizophrenia patients after treatment with antipsychotics, there was a significant decrease in the neopterin levels and significant increase in anti-oxidant levels. Our study observations support increased oxidative stress in schizophrenia that improves with antipsychotic treatment.

1. Introduction

In schizophrenia, evidence for the dysregulation of free radical metabolism includes abnormal activities of critical anti-oxidant enzymes (Yao et al., 1998a,b); reduced levels of anti-oxidants (McCreadie et al., 1995; Yao et al., 2000); and other indices of lipid peroxidation in plasma, red blood cells, and cerebrospinal fluid (Mahadik et al., 1998; McCreadie et al., 1995). Such abnormalities have been associated with tardive dyskinesia, negative symptoms, neurological signs, poor premorbid function and computed tomography scan abnormalities (Reddy and Yao, 1996).

It has been hypothesized that immune mediated pathogenesis can result in increased free radicals leading to oxidative stress mediated damage in schizophrenia (Sirota et al., 2003). Indeed, recent study observations on oxidative stress and cytokines in schizophrenia supports this view (Zhang et al., 2009). Also, various lines of evidence suggest immune dysfunction in schizophrenia (Yolken and Torrey, 1995). These include detection of morphologically abnormal peripheral atypical lymphocytes similar to those found in infectious mononucleosis and other viral diseases, presence of auto-antibodies and increased levels of cytokines in schizophrenia. Recently, significant association between schizophrenia and autoimmune disorders (sjogren's syndrome, celiac disease, thyrotoxicosis, acquired hemolytic anemia and intestinal cystitis) has been reported (Eaton et al., 2006). The association between schizophrenia and autoimmune disorders suggests that assessment of immune parameters might be helpful in understanding the pathogenesis of this disorder.

Though various markers of immune activity have been examined in schizophrenia (Yolken and Torrey, 1995), limited studies have examined the status of neopterin in schizophrenia. Neopterin is especially important because of their established link with immune dysfunction as well oxidative stress. Higher neopterin levels might reflect increased oxidative stress potentially secondary to immune mediated pathogenesis (Murr et al., 1999). Importantly, concurrent examination of neopterin and other parameters of oxidative stress might potentially help one to elucidate the interaction between immune pathogenesis and oxidative stress abnormalities in schizophrenia. Apart from total anti-oxidants levels, assessment of thiols and nitrites would facilitate comprehensive assessment of oxidative stress status. Increased oxidative stress would be associated with decreased thiols; interestingly, a recent study has reported the same in schizophrenia patients (Dietrich-Muszalska et al., 2009). Similarly, examination of nitrites would help one to infer about the nitric oxide mediated oxidative stress in the pathogenesis of schizophrenia (Srivastava et al., 2001).

Previous studies on neopterin abnormalities in schizophrenia have reported conflicting findings. (Korte et al., 1998) reported increased neopterin concentration in acutely ill psychotic patients, which showed further elevation during re-assessment at 4–

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6 weeks. The increased neopterin concentration was associated with symptom improvement, which was interpreted as an indicator of dopamine turnover rather than marker of immunological processes. (Sperner-Unterweger et al., 1989) reported significantly lower neopterin in schizophrenia patients than healthy controls. Recently, a study examining the cerebrospinal fluid levels of neopterin in 11 schizophrenia patients reported no significant difference between patients and controls (Nikkila et al., 2002). It is possible that most of these previous studies might have been confounded because of possible inclusion of chronic schizophrenia patients who were on long-term antipsychotic treatment. Also, to the best of our knowledge, the relationship between neopterin status and oxidative stress in antipsychotic-naïve schizophrenia patients and the impact of neuroleptic treatment has not been reported previously.

In this study, we examined serum neopterin and bio-markers of oxidative stress in antipsychotic-naïve schizophrenia patients [$n = 45$] in comparison with healthy controls (HC) [$n = 43$]. The schizophrenia patients were followed up for 3 months and these biological parameters were reassessed [$n = 32$]. We hypothesized that schizophrenia patients will have significantly higher neopterin and oxidative stress that would improve with treatment.

2. Materials and methods

Forty-five patients who fulfilled DSM-IV criteria for schizophrenia were recruited from the clinical services of the National Institute of Mental Health and Neurosciences (India) for the study. The optimal sample size was calculated using the G*Power software 3 (Faul et al., 2007). For baseline assessments, it was found that a sample size of at least 42 patients and 42 controls would be optimal to detect a clinically significant large effect size of 0.8. For follow-up assessments, the optimal sample size was found to be at least 27 patients (i.e. minimum 27 patients should be assessed after follow-up). The diagnosis of schizophrenia (DSM-IV) was established using the Mini International Neuropsychiatric Interview Plus (MINI-Plus) (Sheehan et al., 1998), which was confirmed by a psychiatrist through an independent clinical interview. None of the patient was ever treated with any psychotropic medications including antipsychotics. These details related to the illness onset and antipsychotic-naïve status were carefully ascertained by reliable information obtained from the family members during the MINI-Plus interview. Clinical assessments were done to quantify the psychotic symptoms, depressive symptoms and to rule out any abnormal involuntary movements. Psychotic symptoms were assessed using the Schedule for Assessment of Negative Symptoms (Andreasen, 1983) and Schedule for Assessment of Positive Symptoms (Andreasen, 1984). Patients were also examined for co-existent depressive symptoms using the Montgomery-Asberg Depression Rating Scale.

Healthy Comparison [HC] subjects ($n = 43$), who volunteered for study, were recruited from among the hospital staff and their friends. Healthy controls were screened to rule out psychiatric disorder using General Health Questionnaire and comprehensive mental status examination. None of the control subject had family history of psychiatric disorder in first-degree relatives. Patients and controls did not score positive for alcohol use as examined by the CAGE (Cutting down, Annoyed, Guilty, Eye-opener) questionnaire. None used stimulant or opiate drug. No subject had history of neurological/medical disorder. None had involuntary movements as assessed by the Abnormal Involuntary Movements Scale (Guy, 1976). Clinical assessments and blood sample collection were performed on the same day before starting antipsychotics. After complete description of the study to the subjects, written informed consent was obtained. The Institute's ethics committee approved the study.

2.1. Specimen collection

Fasting blood specimens were collected during 08:00–09:00 h (A.M.) from the ante-cubital vein in vaccutainers (Becton and Dickinson, USA) and serum was separated within one hour by using refrigerated centrifuges (Hettich 32R, Germany) 4000 rpm/15 min. Serum was aliquoted in vials and stored at −80 °C till analysis. In order to rule out any accompanying disease affecting the blood glucose, hepato renal functions, and serum lipid profiles were studied by using commercially available reagents (Olympus AU 400).

2.2. Neurochemical assays

Assay of neopterin: The quantification of the Neopterin was carried out by using commercially available kits (IBL, Hamburg) based on competitive ELISA method with an analytical sensitivity of 0.7 nmol/L and the Inter and intra assay analytical C.V. is with in the range of 5–10%.

Assay of total anti-oxidants: The assay method is based on monitoring color changes, which are recalculated as a quantity of anti-oxidants to the standard Ferrous Sulfate. The FRAP method employs the ferric reducing/anti-oxidant power (FRAP) of total LMW anti-oxidants, collectively representing the non-enzymatic defenses in biological fluids (Benzie and Strain, 1999). At low pH, reduction of a ferric-tripyridyltriazine (Fe^{+3}-TPTZ) complex to ferrous form with an intense blue color λ_{max} at 595 nm, by the anti-oxidants in specimen.

Assay of total thiols: The assay of total thiols (−SH) is based on the ability of −SH groups to react with 5,5-dithiobys-2-nitrobenzoyc acid (DTNB) at alkaline pH (8.0), giving rise to a chromogen (λ_{max} 405 nm), which can be measured photometrically. The "titre" of thiols directly parallels with color intensity (Elman, 1959).

Assay of nitrites: The assay method for total nitrite involves the reduction of nitrate by vanadium (III) to nitrite, which in turn reacts with sulfanilic acid giving rise to diazo derivative. The diazo intermediate formed reacts with *N*-(1-naphthyl)-ethylenediamine results in chromogenic diazonium product (λ_{max} 540 nm), which is directly proportional to the concentration of total nitrite in biological specimens (Timothy et al., 2003).

2.3. Follow-up of patients

Subsequent to baseline assessments, all patients were started on antipsychotics as decided by the treating clinician. Of the 45 patients that were analyzed at baseline, follow-up assessments could be performed in 32 patients (the remaining 13 patients dropped out and were unavailable for follow-up) after about three months of treatment with antipsychotics [Mean ± SD – 92.1 ± 7.2 days]. Of these 32 patients, 13 were treated with olanzapine, 17 were treated with risperidone and two patients were treated with flupenthixol (intramuscular depot injection of 20 mg once in every two weeks). Patients who were treated with either risperidone or flupenthixol were also prescribed trihexyphenidyl (2–6 mg per day) to avoid extrapyramidal symptoms. During follow-up, the patients were re-examined using the same clinical rating scales as during baseline assessments; a comprehensive account of interval history was carefully ascertained and the adherence to antipsychotic treatment was ensured as per reliable information obtained from the care-giver. Also, blood investigations were repeated during follow-up. Data analysis was performed using the Statistical Package for Social Sciences [version-11.0] using the following statistics: chi-square test, student's *t*-test, paired sample *t*-test and spearman's correlation.